Kinetic resolution of unnatural and rarely occurring amino acids: Enantioselective hydrolysis of N-acyl amino acids catalyzed by acylase I

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Kinetic Resolution of Unnatural and Rarely Occurring Amino Acids: Enantioselective Hydrolysis of *N*-Acyl Amino Acids Catalyzed by Acylase I[1]

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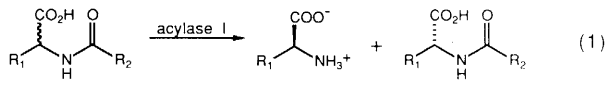
Abstract: Acylase I (aminoacylase; *N*-acylamino-acid amidohydrolase, EC 3.5.1.14, from porcine kidney and the fungus *Aspergillus*) is a broadly applicable enzymatic catalyst for the kinetic resolution of unnatural and rarely occurring α-amino acids. Its enantioselectivity for the hydrolysis of *N*-acyl L-α-amino acids is nearly absolute, yet it accepts substrates having a wide range of structure and functionality. This paper reports the initial rates of enzyme-catalyzed hydrolysis of over 50 *N*-acyl amino acids and analogues, the stabilities of the enzymes in aqueous and aqueous/organic solutions, and the effects of different acyl groups and metal ions on the rates of enzymatic hydrolysis. Eleven α-amino and α-methyl α-amino acids were resolved on a 2–29-g scale. Crude L- and D-amino acid products had generally >90% ee. The utility of resolved amino acids as chiral synthons was illustrated by the preparation of (*R*)- and (*S*)-1-butene oxide and the diastereoselective (cis:trans, 7–8:1) iodolactonization of three 2-amino-4-alkenoic acid derivatives.

Acylase I (aminoacylase; *N*-acylamino-acid amidohydrolase, EC 3.5.1.14) catalyzes the enantioselective hydrolysis of *N*-acyl L-amino acids (eq 1).[4-7] Acylase I enzymes isolated from porcine



(1)

kidney (PKA) and the fungus *Aspergillus* sp. (AA) are commercially available, inexpensive, stable in solution, and high in specific activity. Here we describe the use of these enzymes as catalysts for the kinetic resolution of α-amino acids. We report the range of substrates accepted by each enzyme, factors influencing the activities and stabilities of the enzymes, and methods for the preparative-scale resolution of representative compounds. Both L- and D-amino acid products were obtained with high (generally >90%) enantiomeric excesses.

Amino acids are important as biomedically active compounds[8] and as chiral directing auxiliaries[9,10] and chiral synthons[9,11-13] in organic synthesis. Both D- and L-amino acids[14] and α-methyl amino acids have important uses and are thus desirable in homochiral form. Methods for preparing enantiomerically enriched amino acids such as fermentation and enzymatic catalysis,[15]

Table I. Properties of Acylase I

	porcine kidney	*Aspergillus*
no. of subunits	2[a]	2[b]
MW (dimer)	85 500[a]	73 000[b]
optimum pH	7.0[c]–8.0[d]	6.0–8.5[b]
K_m (chloroacetyl-Ala), mM	6.6[d]	6.3[b]
Zn^{2+} per subunit	1[e]	3[b]
$K_{diss}(Zn^{2+})$, M	10^{-10}[e]	10^{-10}[f]
$K_{diss}(Co^{2+})$, M	10^{-7}[g]	$10^{-7.5}$[f]
essential Cys per subunit	1[a,h]	0[b]
dipeptidase activy	yes[d]	no[b]
sp activy,[i] U/mg	30	0.30
dollars/1000 U[j]	2.1	2.3

[a] Reference 31. [b] Reference 39. [c] Reference 24. [d] Reference 38. [e] Reference 32. [f] Reference 40. [g] Reference 35. [h] Reference 36. [i] Specific activity was determined as described in Results and Experimental Section. [j] Costs are for enzymes as research biochemicals (1988 price list, Sigma Chemical Co.) and thus are upper limits. Approximately 1000 U will catalyze the hydrolysis of 1 mol of acyl-L-amino acid per day.

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(2) NIH Training Grant Trainee, 1984–1985 (Grant 5-T32-GM-07598).

(3) Deutsche Forschungsgemeinschaft Postdoctoral Fellow, 1986–1987.

(4) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 2, pp 1753–1767.

(5) Greenstein, J. P. *Methods Enzymol.* **1957**, *3*, 554–570.

(6) Birnbaum, S. M.; Levintow, L.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *194*, 455–470.

(7) (a) Chibata, I.; Tosa, T.; Sato, T.; Mori, T. *Methods Enzymol.* **1976**, *44*, 746–759. (b) *Immobilized Enzymes: Research and Development*; Chibata, I., Ed.; Wiley: New York, 1978; pp 168–178. (c) Tosa, T.; Mori, T.; Fuse, N.; Chibata, I. *Biotechnol. Bioeng.* **1967**, *9*, 603–615.

(8) (a) Barrett, G. C., Ed. *Chemistry and Biochemistry of the Amino Acids*; Chapman and Hall: London, 1985. (b) Wagner, I.; Musso, H. *Angew. Chem., Int. Ed. Engl.* **1983**, *22*, 816–828. (c) Montreuil, J. In *Comprehensive Biochemistry*; Neuberger, A., van Deenen, L. L. M., Eds.; Elsevier: Amsterdam, The Netherlands, 1982; Vol. 19B, Part II, pp 1–188. (d) Païs, M.; Jarreau, F.-X. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1971; Vol. 1, pp 127–157. (e) *Amino Acids, Pept., Proteins (Spec. Period. Rep.)* **1976**, *8*, 326–327. (f) Davies, J. S. In *Chemistry and Biochemistry of Amino Acids, Peptides, and Proteins*; Weinstein, B., Ed.; Marcel Dekker: New York, 1977; Vol. 4, pp 1–27. (g) *Amino Acids, Pept., Proteins (Spec. Period. Rep.)* **1983**, *14*, 432–502.

(9) Coppola, G. M.; Schuster, H. F. *Asymmetric Synthesis: Construction of Chiral Molecules Using Amino Acids*; Wiley: New York, 1987.

(10) (a) Evans, D. A. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic: Orlando, FL, 1984; Vol. 3, pp 2–110. (b) Lutomski, K. A.; Meyers, A. I. *Ibid.* pp 213–274. (c) Enders, D. *Ibid.* pp 275–341. (d) Martens, J. *Top. Curr. Chem.* **1984**, *125*, 165–246.

(11) (a) Lubell, W. D.; Rapoport, H. *J. Am. Chem. Soc.* **1987**, *109*, 236–239. (b) Kunz, H.; Lerchen, H. G. *Tetrahedron Lett.* **1987**, *28*, 1873–1876; **1985**, *26*, 5257–5260. (c) Hernot, S.; Larcheveque, M.; Petit, Y. *Synth. Commun.* **1986**, *16*, 183–190. (d) Hagiwara, H.; Kimura, K.; Uda, H. *J. Chem. Soc., Chem. Commun.* **1986**, 860–861. (e) Mzengeza, S.; Yang, C. M.; Whitney, R. A. *J. Am. Chem. Soc.* **1987**, *109*, 276–277. (f) Schurig, V.; Leyrer, U.; Wistuba, D. *J. Org. Chem.* **1986**, *51*, 242–245. (g) Maurer, P. J.; Knudson, C. G.; Palkowitz, A. D.; Rapoport, H. *J. Org. Chem.* **1985**, *50*, 325–332. (h) Knudson, C. G.; Rapoport, H. *J. Org. Chem.* **1983**, *48*, 2260–2266. (i) Mori, K.; Sasaki, M.; Tamada, S.; Suguro, T.; Masuda, S. *Tetrahedron* **1979**, *35*, 1601–1605.

(12) Kurokawa, N.; Ohfune, Y. *J. Am. Chem. Soc.* **1986**, *108*, 6041–6043.

(13) Ohfune, Y.; Kurokawa, N. *Tetrahedron Lett.* **1985**, *26*, 5307–5308.

(14) By convention, we refer to the configuration at the α-carbon of amino acids as D or L. The more common, naturally occurring L-amino acids all have the (*S*) configuration, except for L-cysteine, which, because of prioritizing sulfur over oxygen, has the (*R*) configuration.

(15) (a) *Kirk-Othmer Encyclopedia of Chemical Technology*, 3rd ed.; Wiley: New York, 1978; Vol. 2, pp 376–410. (b) Considine, D. M., Ed. *Chemical and Process Technology Encyclopedia*; McGraw-Hill: New York, 1974; pp 93–106. (c) Aida, K., Chibata, I., Nakayama, K., Takinami, K., Yamada, H., Eds. *Biotechnology of Amino Acid Production*; Kodansha: Tokyo, 1986. (d) Hamilton, B. K.; Hsiao, H. Y.; Swann, W. E.; Anderson, D. M.; Delente, J. J. *Trends Biotechnol.* **1985**, *3*, 64–68. (e) Yamada, H.; Shimizu, S.; Shimada, H.; Tani, Y.; Takahashi, S.; Ohashi, T. *Biochimie* **1980**, *62*, 395–399. (f) Yamada, H.; Kumagai, H. *Pure Appl. Chem.* **1978**, *50*, 1117–1127. (g) Yonaha, K.; Soda, K. In *Advances in Biochemical Engineering Biotechnology*; Fiechter, A., Ed.; Springer-Verlag: Berlin, 1986; Vol. 33, pp 95–130. (h) Chibata, I.; Tosa, T.; Sato, T. *Methods Enzymol.* **1976**, *44*, 739–746.

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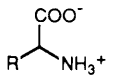
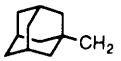
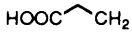
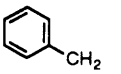
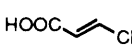
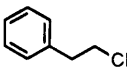
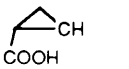
Chart I. Amino Acids Tested As Their Acyl Derivatives for Substrate Activity with Acylase I



R	compound	R	compound	R	compound
CH_3	1		17		29
CH_3CH_2	2				30
	3		18		31
$(CH_3)_2CH$	4				
$(CH_3)_3C$	5		19		32
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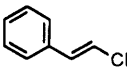
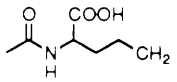
	7		20		33
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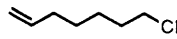
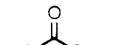
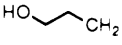
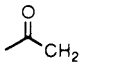
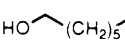
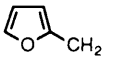
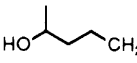
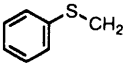
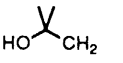
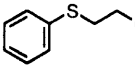
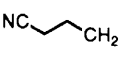
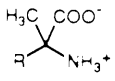
	9	$HOCH_2$	22		34
	10		23		
	11		24		35
	12		25		36
	13		26		37
	14		27		38
	15		28		
	16				

chemical resolution,[16] and asymmetric synthesis[17-22] all have limitations.

We have examined kinetic resolutions using acylase I as a convenient route to unnatural and uncommon amino acids having high (>95%) enantiomeric excesses.[23] Greenstein et al.[4,24-27] and Chibata et al.[7,28,29] have studied acylase I from porcine kidney and (*Aspergillus oryzae*), respectively. The physical and kinetic properties of both enzymes have been studied.[30-40] Properties relevant to their applications in synthesis are summarized in Table I.

Our purpose in this work was to establish the breadth of utility of acylase I for resolving amino acids and analogues. We have measured both initial rates and enantioselectivities of hydrolyses catalyzed by acylase I. Only isolated resolutions of unnatural amino acids using acylase I have been reported previously.[41]

Chart II. α-Methyl Amino Acids Tested As Their Acyl Derivatives For Substrate Activity with Acylase I



R	compound
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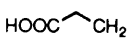
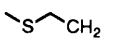
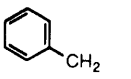
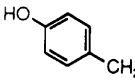
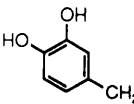
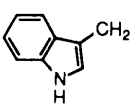
$HOCH_2$	40
	41
	42
	43
	44
	45
	46

(16) (a) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; Wiley: New York, 1961; Vol. 3, pp 715–760. (b) Egusa, S.; Sisido, M.; Imanishi, Y. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 3175–3178. (c) Bollinger, F. W. *J. Med. Chem.* **1971**, *14*, 373–374. (d) Bajgrowicz, J. A.; Cossec, B.; Pigiere, C.; Jacquier, R.; Viallefont, P. *Tetrahedron Lett.* **1984**, *25*, 1789–1792. (e) Terashima, S.; Achiwa, K.; Yamada, S. *Chem. Pharm. Bull.* **1966**, *14*, 1138–1143. (f) Yamaguchi, T.; Nishimura, K.; Shinbo, T.; Sugiura, M. *Chem. Lett.* **1985**, 1549–1552. (g) Viret, J.; Patzelt, H.; Collet, A. *Tetrahedron Lett.* **1986**, *27*, 5865–5868.

(17) (a) Morrison, J. D., Ed. *Asymmetric Synthesis, Chiral Catalysis*; Academic: Orlando, FL, 1985; Vol. 5. (b) Knowles, W. S. *Acc. Chem. Res.* **1983**, *16*, 106–112. (c) Vineyard, B. D.; Knowles, W. S.; Sabacky, M. J. *J. Mol. Catal.* **1983**, *19*, 159–169. (d) Collman, J. P.; Hegedus, L. S. *Principles and Applications of Organotransition Metal Chemistry*; University Science: Mill Valley, CA, 1980; pp 341–349. (e) Vigneron, J. P.; Kagan, H.; Horeau, A. *Tetrahedron Lett.* **1968**, 5681–5683. (f) Corey, E. J.; McCaully, R. J.; Sachdev, H. S. *J. Am. Chem. Soc.* **1970**, *92*, 2476–2488. (g) Corey, E. J.; Sachdev, H. S.; Gougoutas, J. Z.; Saenger, W. *Ibid.* 2488–2501.

(18) (a) Schöllkopf, U. *Top. Curr. Chem.* **1983**, *109*, 65–84. (b) Schöllkopf, U. *Pure Appl. Chem.* **1983**, *55*, 1799–1806. (c) Schöllkopf, U. *Tetrahedron* **1983**, *39*, 2085–2091. (d) Schöllkopf, U.; Busse, U.; Lonsky, R.; Hinrichs, R. *Liebigs Ann. Chem.* **1986**, 2150–2163.

(19) (a) Seebach, D.; Miller, D. D.; Muller, S.; Weber, T. *Helv. Chim. Acta* **1985**, *68*, 949–952. (b) Seebach, D.; Aebi, J. D.; Naef, R.; Weber, T. *Helv. Chim. Acta* **1985**, *68*, 144–1559 (c) Ikegami, S.; Hayama, T.; Katsuki, T.; Yamaguchi, M.; *Tetrahedron Lett.* **1986**, *27*, 3403–3406.

(20) (a) Evans, D. A.; Weber, A. E. *J. Am. Chem. Soc.* **1986**, *108*, 6757–6761. (b) Evans, D. A.; Sjogren, E. B.; Weber, A. E.; Conn, R. E. *Tetrahedron Lett.* **1987**, *28*, 39–42. (c) Seebach, D.; Juaristi, E.; Miller, D. D.; Schickli, C.; Weber, T. *Helv. Chim. Acta* **1987**, *70*, 237–261. (d) Ito, Y.; Sawamura, M.; Hayashi, T. *J. Am. Chem. Soc.* **1986**, *108*, 6405–6406.

(21) (a) Williams, R. M.; Sinclair, P. J.; Zhai, D.; Chen, D. *J. Am. Chem. Soc.* **1988**, *110*, 1547–1557. (b) Sinclair, P.; Zhai, D.; Reibenspies, J.; Williams, R. M. *J. Am. Chem. Soc.* **1986**, *108*, 1103–1104. (c) Oppolzer, W.; Pederosa, R.; Moretti, R. *Tetrahedron Lett.* **1986**, *27*, 831–834. (d) Schöllkopf, U.; Neubauer, H. J.; Hauptrief, M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 1066–1067.

Results

Potential Substrates. Charts I–III list compounds examined as potential substrates. These compounds comprise acyl derivatives of simple α-amino acids (**1–38**), α-methyl α-amino acids (**39–46**), β-amino acids (**47**, **48**), an α-aminophosphonic acid (**49**), and two α-hydroxy acids (**50**, **51**), as well as heterocyclic compounds **52** and **53**. Acyl groups employed were acetyl (A), chloroacetyl (CA), and methoxyacetyl (MA).[42]

Many of the amino acids (**8**, **9**, **12–14**, **19**, **20**, **23–29**, **31**, **35**, **37**, **38**) were made by the phase-transfer-catalyzed (PTC) alkylation[43] of ethyl *N*-(diphenylmethylene)glycinate (**54**)[44] (Chart IV). This procedure worked well and generally gave free amino acid in 30–55% yield. Ketimine **54** was important as the glycine enolate equivalent. In our hands, PTC alkylation of the aldimine, ethyl *N*-[(4-chlorophenyl)methylene]glycinate (**55**),[43] led only to hydrolysis of **55**.

Diethyl acetamidomalonate (**56**) is another useful glycine equivalent. Alkylation of **56** in sodium ethoxide/ethanol, followed by saponification and decarboxylation, gave acetyl amino acids **10-A**, **11-A**,[45] **16-A**, and **36-A** in 40–65% yields. This procedure was especially useful for preparing compounds for enzymatic resolution, because the product *N*-acetyl α-amino acids were substrates of acylase I.

Free amino acids were generally acylated with the appropriate acyl chloride in 4 N NaOH.[25] Alternatively, acetylations were performed with acetic anhydride/acetic acid. We found, as have others,[6,46] that chloroacetylations of amino acids under Schot-

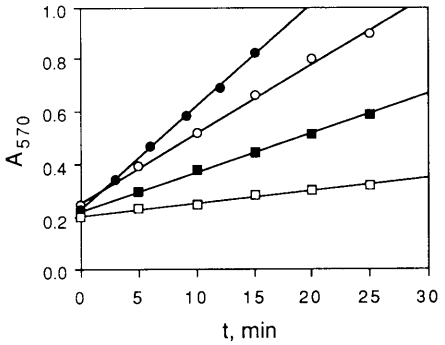


Figure 1. Colorimetric determination of the initial rates of hydrolysis of *N*-acetylmethionine (●), *N*-chloroacetyl-α-cyclopropylglycine (○), *N*-acetyl-*cis*-α-crotylglycine (■), and *N*-acetyl-α-propargylglycine (□) catalyzed by PKA.

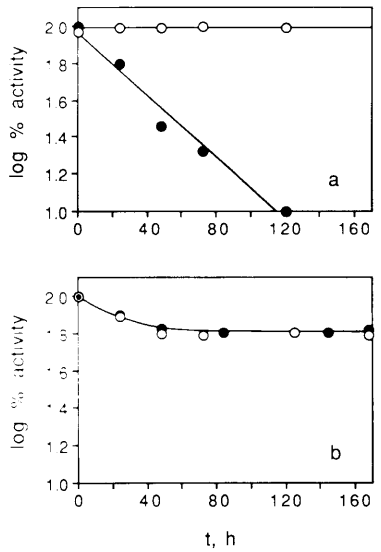


Figure 2. Stability of soluble acylase I from (a) porcine kidney and from (b) *Aspergillus*. Enzymes were incubated in 0.10 M phosphate buffer, pH 7.5, at 25 °C, under ambient atmosphere (●) and under nitrogen (○).

Chart III. Analogues of α-Amino Acids Tested for Substrate Activity with Acylase I

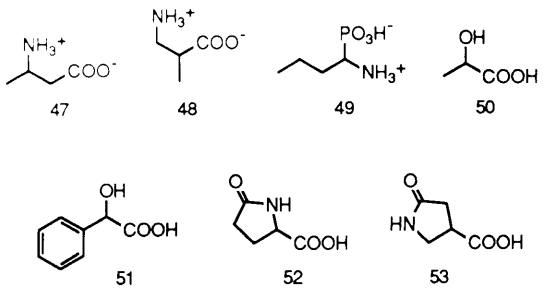
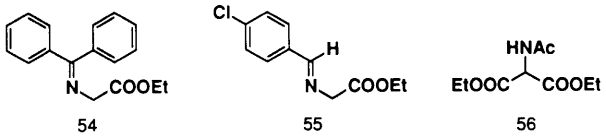


Chart IV. Glycine Enolate Equivalents



(22) (a) Evans, D. A.; Britton, T. C.; Dorow, R. L.; Dellaria, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 6395–6397. (b) Gennari, C.; Colombo, L.; Berolini, G. *J. Am. Chem. Soc.* **1986**, *108*, 6394–6395. (c) Trimble, L. A.; Vederas, J. C. *J. Am. Chem. Soc.* **1986**, *108*, 6397–6399. (d) Oppolzer, W.; Moretti, R. *Helv. Chim. Acta* **1986**, *69*, 1923–1926. (e) Evans, D. A.; Ellman, J. A.; Dorow, R. L. *Tetrahedron Lett.* **1987**, *28*, 1123–1126.

(23) Other enzymatic kinetic resolutions of amino acids or amino acid precursors: (a) Chen, S.-T.; Wang, K.-T.; Wong, C.-H. *J. Chem. Soc., Chem. Commun.* **1986**, 1514–1516. (b) Lalonde, J. J.; Bergbreiter, D. E.; Wong, C.-H. *J. Org. Chem.* **1988**, *53*, 2323–2327. (c) Roper, J. M.; Bauer, D. P. *Synthesis* **1983**, 1041–1043. (d) Anantharamaiah, G. M.; Roeske, R. W. *Tetrahedron Lett.* **1982**, *23*, 3335–3336. (e) Turk, J.; Panse, G. T.; Marshall, G. R. *J. Org. Chem.* **1975**, *40*, 953–955. (f) Bjorkling, F.; Boutelje, J.; Hjalmarsson, M.; Hult, K.; Norin, T. *J. Chem. Soc., Chem. Commun.* **1987**, 1041–1042.

(24) Rao, K. R.; Birnbaum, S. M.; Kingsley, R. B.; Greenstein, J. P. *J. Biol. Chem.* **1952**, *198*, 507–524.

(25) Fu, S.-C. J.; Birnbaum, S. M. *J. Am. Chem. Soc.* **1953**, *75*, 918–920.

(26) Fu, S.-C. J.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1954**, *76*, 6054–6058.

(27) Marshall, R.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1956**, *78*, 4636–4642.

(28) Tosa, T.; Mori, T.; Fuse, N.; Chibata, I. *Enzymologia* **1967**, *32*, 153–168.

(29) Tosa, T.; Mori, T.; Chibata, I. *Enzymologia* **1971**, *40*, 49–63.

(30) Kördel, W.; Schneider, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1976**, *357*, 1109–1115.

(31) Kördel, W.; Schneider, F. *Biochim. Biophys. Acta* **1976**, *445*, 446–457.

(32) Kördel, W.; Schneider, F. *Z. Naturforsch., C* **1977**, *32C*, 337–341.

(33) Kördel, W.; Schneider, F. *Z. Naturforsch., C* **1977**, *32C*, 342–344.

(34) Frey, J.; Kördel, W.; Schneider, F. *Z. Naturforsch., C* **1977**, *32C*, 769–776.

(35) Kumpe, E.; Löffler, H.-G.; Schneider, F. *Z. Naturforsch., C* **1981**, *36C*, 951–955.

(36) Szajáni, B.; Kiss, A.; Boross, L. *Acta Biochim. Biophys. Acad. Sci. Hung.* **1980**, *15*, 29–37.

(37) Löffler, H.-G.; Schneider, F. *Biol. Chem. Hoppe-Seyler* **1987**, *368*, 481–485.

(38) Kördel, W.; Schneider, F. *Hoppe-Seyler's Z. Physiol. Chem.* **1975**, *356*, 915–920.

(39) Gentzen, I.; Löffler, H.-G.; Schneider, F. *Z. Naturforsch., C* **1980**, *35C*, 544–550.

(40) Gilles, I.; Löffler, H.-G.; Schneider, F. *Z. Naturforsch., C* **1981**, *36C*, 751–754.

(41) (a) Keller, J. W.; Hamilton, B. J. *Tetrahedron Lett.* **1986**, *27*, 1249–1250. (b) Schwyzer, R.; Karlaganis, G. *Ann. Chem.* **1973**, 1298–1309. (c) Jansen, A. C. A.; Weustnik, R. J. M.; Kerling, K. E. T.; Havinga, E. *Recl. Trav. Chim. Pays-Bas* **1969**, *88*, 819–827. (d) Baldwin, J. E.; Haber, S. B.; Hoskins, C.; Kruse, L. I. *J. Org. Chem.* **1977**, *42*, 1239–1241. (e) Morishima, H.; Yoshizawa, J.; Ushijima, R.; Takeuchi, T.; Umezawa, H. *J. Antibiot.* **1982**, *35*, 1500–1506. (f) Scannell, J. P.; Pruess, D. L.; Demny, T. C.; Sello, L. H.; Williams, T.; Stempel, A. *J. Antibiot.* **1972**, *25*, 122–127. (g) Sahn, U.; Knobloch, G.; Wagner, F. *J. Antibiot.* **1973**, *26*, 389–390.

(42) Acyl derivatives of amino acids and amino acid analogues **1–51** are designated by the appropriate number followed by suffixes indicating the acyl group: A (acetyl), CA (chloroacetyl), and MA (methoxyacetyl).

(43) O'Donnell, M. J.; Wojciechowski, K. *J. Chem. Soc., Chem. Commun.* **1984**, 313–315.

(44) O'Donnell, M. J.; Polt, R. L. *J. Org. Chem.* **1982**, *47*, 2663–2666.

(45) Coulter, A. W.; Talalay, P. *J. Biol. Chem.* **1986**, *243*, 3238–3247.

(46) (a) Ronwin, E. *J. Org. Chem.* **1953**, *18*, 127–132. (b) Ronwin, E. *Ibid.* 1546–1553.

ten–Baumann type conditions with either chloroacetyl chloride or chloroacetic anhydride suffer from side reactions, low yields (often 10–20%), and difficult purifications. Ronwin reported the chloroacetylation of amino acids in ethyl acetate,[46] but yields were still variable and low. We found that a variety of amino acids gave satisfactory (50–90%) yields of chloroacetylated product in dry, refluxing acetonitrile. The method worked particularly well with α-methyl α-amino acids and other hydrophobic amino acids, but failed with hydrophilic amino acids such as alanine and glutamic acid.

Sources and Specific Activity of Acylase I. Although acylase enzymes are present in various mammalian tissues and microorganisms, commercial preparations of acylase I (or aminoacylase) are isolated from porcine kidney or from *Aspergillus*. Both enzymes may be purified to a specific activity of around 300 U/mg,[39] but commercial preparations vary widely in their specific activities.[47] The results presented in this paper were obtained with enzymes from Sigma (Table I).

Kinetic Analysis. The activity of acylase I with potential substrates was determined by measuring the initial rate of enzyme-catalyzed hydrolysis of racemic acyl amino acid (40 mM in 0.10 M potassium phosphate buffer, pH 7.5, 40 °C). Aliquots of the hydrolysis reactions were removed periodically, quenched with perchloric acid, and assayed for free amino acid by colorimetric determination with ninhydrin (Figure 1).[48] The rates of hydrolysis were calibrated by measuring the colorimetric response of the free amino acids with ninhydrin and were normalized to the rate of hydrolysis of *N*-acetyl-DL-methionine (AcMet; V_r = 100). AcMet is commercially available, inexpensive, and an excellent substrate for both enzymes.[6,27]

Control experiments included incubating potential substrates in the absence of enzyme and incubating the enzymes in the absence of potential substrate. Only **22-CA**[42] and **40-CA** exhibited spontaneous hydrolysis under the conditions of the assay, and so **22-MA** and **40-MA** were used for measurements of enzymatic hydrolysis. PKA (1 mg mL^{-1}) showed no increase in colorimetric response after 10 h. AA, however, always exhibited a small background increase in absorbance or "false activity". The absorbance always increased more sharply initially and then leveled off. Dialysis of the enzyme did not eliminate the phenomenon. Therefore, when slowly reacting substrates were assayed with AA, the enzyme was allowed to rest in buffer solution for 6 h before assaying, and a blank reaction containing only AA was run in parallel. The differences in absorption between the assay reaction and the control were used to calculate the rate of enzymatic hydrolysis. In practice, all compounds that exhibited activity with AA were hydrolyzed at rates at least 10 times greater than the background reaction, but the background reaction does limit the sensitivity of the assay.

Stability of Acylase I. PKA and AA were stable as lyophilized powders (the form in which they are bought) when stored desiccated at 4 °C and showed no loss of activity after storage for 6 months to 2 years. Soluble PKA was sensitive to autoxidation[49] under ambient atmosphere (first-order half life, 36 h) but lost no activity when kept under an atmosphere of nitrogen (Figure 2). Reducing agents, such as mercaptoethanol or dithiothreitol, are not helpful in preventing oxidative inactivation of PKA. At low concentrations, they, themselves, deactivate PKA, presumably by reducing cystine linkages.[31]

Soluble AA showed no sensitivity toward autoxidation (Figure 2).[50] The initial loss of activity was reproducible but was not a result of proteolytic degradation of the enzyme or of partial absorption of protein to the inner surfaces of the incubation vessels. It was independent of the concentration of AA and was not affected by the presence of soluble bovine serum albumin or by precoating the vessels with denatured protein.

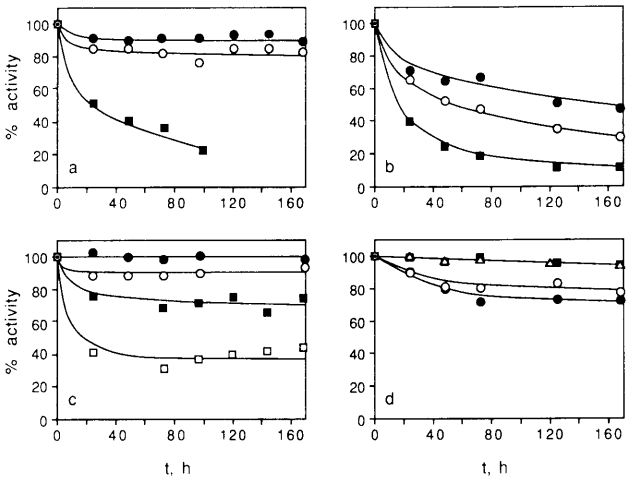


Figure 3. Stability of soluble acylase I in the presence of organic cosolvents. Acylase I from porcine kidney (a, c) and from *Aspergillus* (b, d) were incubated in 0.10 M phosphate buffer, pH 7.5, containing ethanol (a, b) or DMSO (c, d), at 25 °C, under nitrogen. Concentrations of organic cosolvents were 10% (●), 20% (○), 30% (■), 40% (□), and 50% (△).

Both enzymes exhibited moderate stability in the presence of organic cosolvents (Figure 3) and were catalytically active in the mixed-solvent systems. They retained approximately 50% of their aqueous specific activities in 30% DMSO.

Substrate Specificity. Chart V summarizes data from the initial rates of hydrolysis of a broad range of substrates catalyzed by PKA and AA. It is a general guide to our results and those of others[24,28,39,51,52] and is useful as a predictive tool. Chart V breaks the activities of substrates down into component reactivities of individual functional groups in each of the three variable domains (R_1, R_2, R_3) of substrates. As a first approximation, these component reactivities are cumulative. Thus, the reactivity of alanylalanine with PKA is approximately 1 order of magnitude less than that of *N*-acetylalanine, but the rate of hydrolysis of *N*-(α-chloropropionyl)-α-cyclohexylglycine is 2–3 orders of magnitude less than that of *N*-chloroacetylvaline. Relative rates of initial hydrolysis (V_r) of individual compounds are tabulated in the Experimental Section.

Acylase I was quite specific for the hydrolysis of *N*-acyl α-amino carboxylic acids. Within that constraint, however, PKA and AA accepted a broad range of structure and functionality in both the amino acid (R_1) and acyl (R_3) moieties of substrates. The two enzymes were complementary in their substrate specificities. Both enzymes showed an affinity for straight-chain alkyl, alkenyl, or alkynyl amino acids of moderate length (C_3–C_9). Cis and trans double bonds were tolerated equally well. AA showed higher relative activities with aromatic and β-branched amino acids than PKA. PKA showed preferential activity with ω-carboxylate amino acids and accepted substrates in which the α-hydrogen had been replaced by a methyl group ($R_2 = CH_3$). AA did not accept α-methyl amino acids.

Productive binding and turnover of substrates required the terminal carboxylate group. Acylamines, *N*-acyl amino acid esters and amides,[25] and *N*-acetyl-α-aminophosphonic acid **49-A** were not substrates ($V_r < 0.005$ and 0.05 with PKA and AA, respectively). The failure of PKA to hydrolyze **49-A** contradicted the reported use of PKA to resolve α-aminophosphonic acids.[53]

(47) It is important to note that some manufacturers and authors cite units of acylase activity as micromoles of substrate hydrolyzed *per hour* instead of per minute. Different manufacturers and authors also use different temperatures, pH, substrates, and concentrations to measure specific activity. Throughout this paper, a unit (U) designates the amount of enzyme required to hydrolyze 1 μmol of substrate *per minute* under the standard assay conditions outlined in the Experimental Section. *N*-Acetyl-DL-methionine is the standard substrate unless another is specified.

(48) Rosen, H. *Arch. Biochem. Biophys.* **1957**, *67*, 10–15.

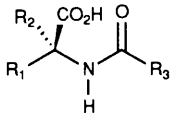
(49) Autoxidation probably involves oxidation of an essential cysteine residue. See: References 31 and 36.

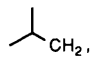
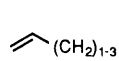
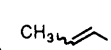
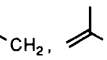
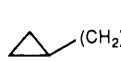
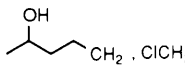
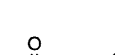
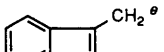
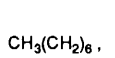
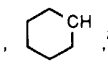
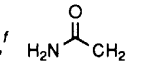
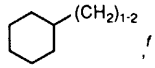
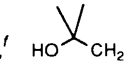
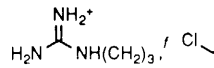
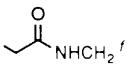
(50) AA lacks free thiol groups.[39]

(51) Ötvos, L.; Moravcsik, E.; Mády, Gy. *Biochem. Biophys. Res. Commun.* **1971**, *44*, 1056–1064.

(52) (a) Sato, T.; Mori, T.; Tosa, T.; Chibata, I. *Arch. Biochem. Biophys.* **1971**, *147*, 788–796. (b) Birnbaum, S. M.; Fu, S.-C. J.; Greenstein, J. P. *J. Biol. Chem.* **1953**, *203*, 333–338.

Chart V. Reactivities of Substituents in Substrates of Acylase I



R_1	R_2	R_3	reactivity[a]
$CH_3(CH_2)_{0-5}$, $(CH_3)_2CH$,  [b]			
 ,  , 		CH_3, C_2H_5	
 , $HOCH_2$, 		XCH_2 (X = Cl, Br, CH_3O)	
	H	XCH_2CH_2 (X = Cl, Br)	good, > 10 %
$NC(CH_2)_{3-4}$, $HOOC(CH_2)_{2-3}$,[c] $CH_3(CH_2)_{0-1}S$ 		H,[b] C_6H_5,[d]	
 ,[d]  ,[b] [d]		H_2NCH_2[d]	
 , , 			
$CH_3(CH_2)_6$,  ,  ,[f] $HO(CH_2)_{3-8}$		$CH_3(CH_2)_{0-1}CH(Cl)$[g]	fair, 1-10 %
 ,[f] 		L-$RCH(NH_2)$[g]	
$CH_3(CH_2)_{7-8}$,[f] $(CH_3)_3C$,  ,[f] 			
 ,[f]  ,[f] 	CH_3[g]	$CH_3CH_2CH(CH_3)$[g]	poor, 0.01-1 %
			

[a] Reactivities of substituents (R_1, R_2, R_3) are expressed relative to the reactivities of the corresponding substituents of the model substrate, *N*-acetylmethionine (40 mM racemic; $R_1 = CH_3SCH_2CH_2$, $R_2 = H$, $R_3 = CH_3$). Contains data from ref 6, 48, 52, 62, 66, and 78. See Results and Experimental Section for details. [b] AA only; reactivity with PKA is fair. [c] PKA only; reactivity with AA is fair. [d] AA only; reactivity with PKA is poor. [e] AA only; no reactivity with PKA. [f] Data for PKA only. [g] PKA only; no reactivity with AA.

Acylase I was specific for the position and chemical nature of the amide bond in substrates. *N*-Acyl β-amino acids **47-A** and **53** were not substrates. Although **53** was an *N*-acyl β-amino acid, it also resembled an *N*-acyl α-amino acid in that the amide carbonyl resided γ to the carboxylate group. Compound **48-A** was hydrolyzed by PKA ($V_r = 0.06$) but, apparently, with little or no enantioselectivity. The rate of hydrolysis of **48-A** (initial concentration, 40 mM) remained constant through 72% conversion and showed no curvature around 50% conversion. An inflection around 50% conversion would have been expected if the enzymatic catalysis had been appreciably enantioselective. Replacing the hydrogen on the amide nitrogen with an alkyl group destroyed substrate activity: *N*-Methyl and *N*-ethyl *N*-acyl amino acids (tertiary amides) were not substrates.[25] Likewise, replacing the amide nitrogen by oxygen destroyed substrate activity: *O*-Acetyllactic acid (**50-A**) and *O*-acetylmandelic acid (**51-A**), ester analogues of acyl amino acids, were not substrates.

Other compounds not accepted as substrates included acyl derivatives of proline and aspartic acid. Failure of acylase I to hydrolyze acyl aspartates must result from unfavorable charge interactions. Acyl derivatives of asparagine and aspartate β-esters were substrates ($V_r \sim 1$), and **35-MA**, a methyl ketone analogue of acyl aspartate, reacted with PKA at a relative rate of 41%. Pyroglutamic acid (**52**) was not hydrolyzed by acylase I.

Effect of Acyl Groups on the Rates of Enzymatic Hydrolysis. PKA and AA accept a range of unsubstituted and ω-substituted acetyl and propionyl moieties as the acyl group in substrates (Chart V).[7,24,26,51,54] Both enzymes hydrolyze formyl, benzoyl, and glycyl amino acids, although AA does so at relative rates higher than those of PKA. PKA but not AA tolerates branching at the α position of acyl groups. PKA therefore hydrolyzes dipeptides and α-chloroacyl or α-methylacyl amino acids; AA does not.

We examined acetyl, chloroacetyl, and methoxyacetyl derivatives for use in preparative resolutions of amino acids. Acetyl amino acids are good substrates of acylase I[4-6,28] and are readily prepared. Chloroacetyl amino acids have particularly high en-

(53) Telegdi, J.; Moravcsik, E.; Ötvös, L. In *Proceedings of the International Conference on the Chemistry and Biotechnology of Biologically Active Natural Products, 1st*; Atanasova, B., Ed.; Bulgarian Academy of Science: Sofia, Bulgaria, 1981; Vol. 3, pp 221–225.

(54) Price, V. E.; Gilbert, J. B.; Greenstein, J. P. *J. Biol. Chem.* **1949**, *179*, 1169–1174.

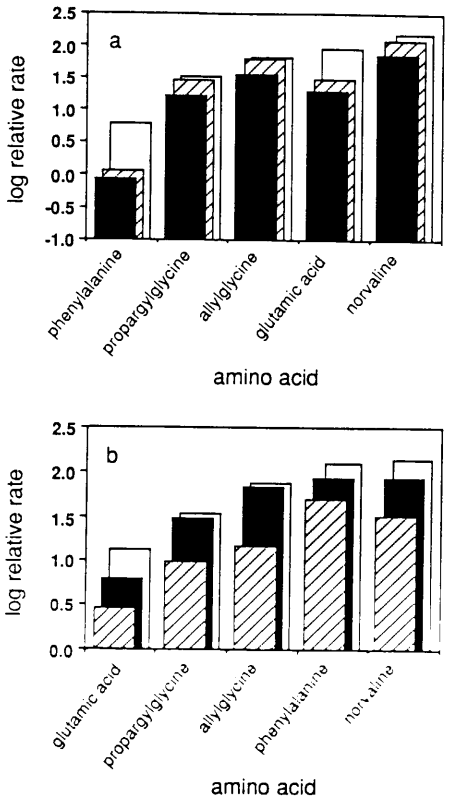


Figure 4. Effect of acyl groups on the rates of hydrolysis catalyzed by (a) PKA and (b) AA. Acyl groups are acetyl (black), methoxyacetyl (striped), and chloroacetyl (white).

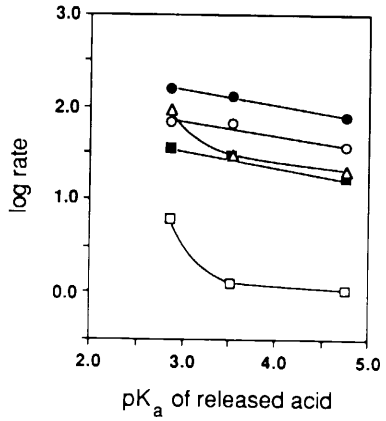


Figure 5. Effect of the pK_a of the released carboxylic acid on the rates of PKA-catalyzed hydrolysis of acylnorvaline (●), -α-allylglycine (○), -α-propargylglycine (■), -glutamic acid (△), and -phenylalanine (□). The pK_a values of the free acids that form the acyl groups are 2.86 (chloroacetic), 3.53 (methoxyacetic), and 4.76 (acetic).

zymatic reactivities, are generally stable against spontaneous hydrolysis, and have the added advantage that they may be deprotected under milder and more varied conditions than acetyl amino acids.[55,56] We expected methoxyacetyl amino acids to have electronic and hence reactive properties intermediate between those of acetyl and chloroacetyl derivatives. We also found them easier to prepare and recrystallize than chloroacetyl amino acids.

The initial rates of enzymatic hydrolysis of the three acyl groups are depicted in Figure 4. The five amino acids used in these tests reflected a range of functionality and intrinsic reactivity with acylase I. With PKA, the rates of reaction of the three acyl groups

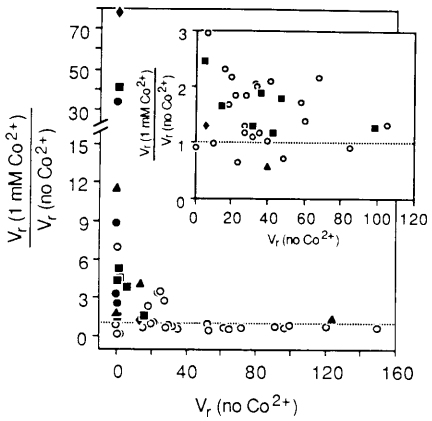


Figure 6. Effect of Co^{2+} on the rates of hydrolysis catalyzed by PKA and AA (inset). Amino acid residues contain α-methyl (●), aromatic (■), β-branched (▲), long-chain (>C_7, ◆), or other functionality (○). Data were taken from the initial rates of enzymatic hydrolysis tabulated in the Experimental Section.

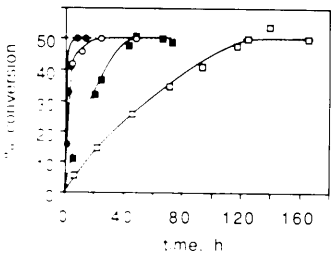


Figure 7. Progress of resolutions of α-*cis*-crotylglycine (●), α-aminobutyric acid (○), α-methylmethionine (■), and α-methyltyrosine (□) catalyzed by acylase I.

correlated with the electron deficiency of the carbonyl carbon as represented by the pK_a of the free acids (Figure 5). For derivatives of **3**, **7**, and **13**, the free energy relationships were linear and had slopes of −0.2. They suggest hydrolysis by the same mechanism, involving rate-determining base-catalyzed attack of the carbonyl carbon by water.[57,58]

With AA, the rates of hydrolysis did not correlate to the pK_a of the free acids: Methoxyacetyl amino acids reacted with AA more slowly than acetyl analogues. We presume that the initial rates of hydrolysis, which were not strictly measurements of k_{cat}, reflected steric hindrance to the binding of the methoxyacetyl group.

Effects of Metal Ions on Acylase I. PKA and AA are zinc metalloproteins[27,34] and are generally unaffected or inhibited by other divalent metal cations in solution. Cobalt(II) accelerates the rates of enzymatic hydrolysis of at least some substrates.[27,28,39] Cobalt almost invariably activates AA but affects PKA less consistently. The mechanism of activation and inhibition by Co^{2+} is unknown, but it appears not to involve simple substitution of Co^{2+} for Zn^{2+} in the enzyme active sites.[27,40]

We measured the initial rates of enzymatic hydrolysis of potential substrates in both the absence and presence of 1 mM $CoCl_2$ in order to identify trends in the effects of Co^{2+} on acylase I. Cobalt generally activated AA, increasing the rates of reaction by 15–200%. Exceptional substrates included derivatives of **7**, **13**, and **15**, whose rates of hydrolysis were reduced by 11–43%. In all cases, however, Co^{2+} enhanced the rates of hydrolysis of acetyl amino acids.

(55) Greene, T. W. *Protective Groups in Organic Synthesis*; Wiley: New York, 1981; pp 251–255, 328.

(56) Nucleophilicity assisted deprotection of **42-CA** by thiourea led to clean formation of **42**. In contrast, acidic hydrolysis of **42-CA** caused nearly complete decomposition to α-methylhomoserine.

(57) For previous evidence that the rate-determining step is attack of water on the carbonyl carbon of the substrate, see: (a) Röhm, K. H.; Van Etten, R. L. *Eur. J. Biochem.* **1986**, *160*, 327–332. (b) Röhm, K. H. *Adv. Biosci.* **1987**, 249–256. (c) Reference 51.

(58) For a discussion of free energy electronic effects on aliphatic acids, see: Lowry, T. H.; Richardson, K. S. *Mechanism and Theory in Organic Chemistry*, 3rd ed.; Harper and Row: New York, 1987; pp 152–154. March, J. *Advanced Organic Chemistry*, 3rd ed.; Wiley: New York, 1985; pp 245–249.

Table II. Resolutions of Amino Acids Using Acylase I

racemic substrate (mmol)	enzyme	Co^{2+}	isolation	% yield L	% yield D	% ee L	% ee D
2-A (200)	PKA	no	EtOH ppt/EtOAc extr	40	32	>99.5	>99.5
2-CA (70)	PKA	no	ion exch	40	41	>99.5	>99.5
3-A (31)	AA	yes	ion exch	33	32	>99.5	>99.5
7-CA (52)	PKA	no	EtOAc extr/EtOH ppt	41	33	>99.5	>99.5
10-A (17)	AA	yes	ion exch	33	38	99	93
11-A (57)	PKA	no	EtOH ppt/EtOAc extr	44	47	>99.5	>99.5
15-CA (8)	PKA	yes	EtOAc extr/ion exch	37	42	99	84
16-A (24)	AA	yes	EtOAc extr/ion exch	50	50	95	98
36-A (14)	AA	yes	EtOAc extr/ion exch	45	41	99	*a*
42-CA (19)	PKA	yes	EtOAc extr/ion exch	51	31	93	*a*
43-CA (15)	PKA	yes	EtOAc extr/ion exch	43	46	91	80
44-CA (15)	PKA	yes	EtOH ppt/EtOAc extr	17	64	95	47
44-CA (7)	PKA	yes	EtOAc extr/ion exch	30	63	97	48

[a] The free amino acid was not isolated.

With PKA, the effects of Co^{2+} were less consistent and often more dramatic than with AA. Cobalt generally inhibited the reactions of fair-to-good substrates but enhanced the reactivities of poor-to-fair substrates (Figure 6). In particular, Co^{2+} activated PKA toward acyl derivatives of hydrophobic or sterically hindered amino acids. Aromatic, long-chain (>C_7), β-branched and α-methyl amino acids all showed dramatically increased reactivities in the presence of Co^{2+}. No such trend existed for AA.

Preparative Resolutions of Amino Acids Using Acylase I. To demonstrate the use of PKA and AA for preparative resolutions of unnatural and uncommon amino acids, we subjected 11 compounds representing the full range of reactivity with acylase I (V_r = 0.005–169) to enzymatic hydrolysis (Table II). Resolutions were performed on 2–29 g of starting material at pH 7–8. Initial concentrations of racemic substrate were 0.1–0.5 M. When needed, 1 mM $CoCl_2$ was included in reaction mixtures to enhance the rates of reaction. Progress of the reactions was monitored by assaying aliquots for free amino acid by colorimetric determination with ninhydrin (Figure 7).[48] Periodic, manual addition of a few drops of base maintained the pH of reactions at 7–8.

Resolutions of good substrates with PKA ran about 24 h and were usually performed under ambient atmosphere. Under these conditions, PKA retained approximately 50% of its original activity. Under anaerobic conditions, however, PKA maintained full activity. To protect PKA from autoxidation in resolutions lasting longer than 24 h, substrate solutions were sparged with nitrogen before adding PKA and then kept under an atmosphere of nitrogen during the reactions. Resolutions using AA did not require anaerobic conditions.

After resolutions were complete, acylase I was denatured and the products were separated by differential solubility or ion-exchange chromatography. We used various protocols, depending on the solubilities of the products and the size of the reactions (see Experimental Section). The most general, simple, and high-yielding protocol, however, involved acidifying the product mixture and extracting the acyl D-amino acid into ethyl acetate. The crude acyl D-amino acid, which was contaminated by free carboxylic acid cleaved from the L enantiomer, was hydrolyzed in refluxing 2 N hydrochloric acid to recover free D-amino acid or washed with ether to obtain reasonably pure *N*-acyl D-amino acid. The acidic reaction mixture was applied to a column of Dowex-50 (H^+). Elution of the column with 1 N aqueous ammonia provided the neutral, salt-free L-amino acid. Another protocol involved precipitating the L-amino acid by adding ethanol to the near-neutral (pH 6) reaction mixture. Filtration removed the L-amino acid, and acidification followed by extraction gave crude *N*-acyl D-amino acid.

Other protocols were used as times. Sequential application of a neutral product solution to columns of Dowex-50 (H^+) and then Dowex-1 (OH^-) followed by elution with ammonia and formic acid, respectively, gave L-amino and crude acyl D-amino acids, respectively. In an alternative to ion-exchange chromatography, amino acids were freed from salt and carboxylic acids by dissolving the dry, amino acid hydrochloride in methanol, filtering, and adding excess propylene oxide (warning: cancer suspect agent). Propylene oxide consumed hydrochloric acid, and the neutral amino acid precipitated from solution. In general, neutral (zwitterionic) amino acids were water soluble but not organic soluble, and *N*-acyl amino acids were soluble in ethanol, acetone, and ethyl acetate.

Resolutions of α-Methyl α-Amino Acids. To test the practical limits of resolutions of very low activity substrates with acylase I, we used membrane-enclosed acylase I[59] to resolve α-methylmethionine (**42**), α-methylphenylalanine (**43**), and α-methyltyrosine (**44**). The technique of membrane-enclosed catalysis (MEEC)[59] allowed the use of large quantities of protein in small reaction volumes and the recovery and reuse of the soluble enzyme. PKA (5 g) was enclosed in dialysis tubing and used successively to catalyze the hydrolyses of **44-CA** (2.0 g), **43-CA** (3.9 g), and **42-CA** (4.4 g). Reactions were kept under an atmosphere of nitrogen. After completion of each resolution, the reaction was terminated by removing the dialysis tubing containing enzyme from the reaction mixture. Products remaining within the membrane were recovered by dialysis, and products from the reaction mixture and dialyzates were separated by ion-exchange chromatography. After catalyzing three reactions over a period of 36 days, the enzyme retained 55% of its original activity.

Chemical yields and enantiomeric purities of products were fair to good. In general, the enantioselectivity for the hydrolysis of the L enantiomer was good, but unfavorable Michaelis constants led to incomplete conversion of the L enantiomer and subsequent enantiomeric contamination of the D isomer. A separate resolution of **44** under similar conditions gave results virtually identical with the first.

Enantioselectivity and Absolute Stereoselectivity of Acylase I. The enantiomeric purities and absolute configurations of the products of preparative resolutions catalyzed by acylase I were determined by ^1H NMR analysis of the (*R*)-(+)-MTPA amides of the corresponding amino acid methyl esters.[60,61] In all cases, measurements of enantiomeric excess were performed on crude, unrecrystallized products. Thus, the measurements reflect the intrinsic enantioselectivity of the resolution process. Methyl esters of amino acids were prepared without racemization by the action of thionyl chloride/methanol or diazomethane/methanol on the free amino acids.

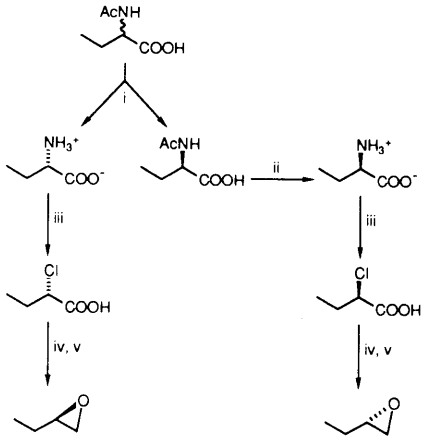
The ^1H NMR signals of the (+)-MTPA methoxy groups of L and D-amino acid derivatives appeared at approximately 3.35 and 3.50 ppm, respectively, in agreement with previous stereochemical correlations.[61] With derivatives of α-methyl α-amino acids, the α-methyl signals could also be analyzed. Again, in agreement with previous stereochemical correlations,[60,61] the

(59) Membrane-enclosed enzymatic catalysis (MEEC): Bednarski, M. D.; Chenault, H. K.; Simon, E. S.; Whitesides, G. M. *J. Am. Chem. Soc.* **1987**, *109*, 1283–1285.

(60) Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

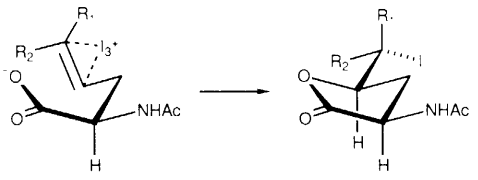
(61) (a) Yamaguchi, S. In *Asymmetric Synthesis*; Morrison, J. D., Ed.; Academic Press: New York, 1983; Vol. 1, pp 125–152. (b) Yasahara, F.; Yamaguchi, S. *Tetrahedron Lett.* **1980**, *21*, 2827–2830.

Scheme I. Conversion of α-Aminobutyric Acid to (*R*)- and (*S*)-Butene Oxide[a]



[a] Reaction conditions: (i) acylase I, 40% yield of each product (theoretical maximum is 50%); (ii) 2 N HCl, reflux, 90%; (iii) $NaNO_2$, HCl, 91%; (iv) BH_3/THF, 90%; (v) KOH, 75%, 95–96% ee

Scheme II. Iodolactonization of α-Allylglycine Derivatives



cis : trans = 7-8:1

7-A, $R_1 = R_2 = H$
10-A, $R_1 = H$, $R_2 = CH_3$
11-A, $R_1 = CH_3$, $R_2 = H$

57, $R_1 = R_2 = H$
58, $R_1 = H$, $R_2 = CH_3$
59 $R_1 = CH_3$, $R_2 = H$

α-methyl signal of L-amino acids appeared downfield of that of the D enantiomorphs. When possible, optical rotations of products were compared to those of known compounds. In all cases, acylase I catalyzed the hydrolysis of the *N*-acyl L-amino acid.[62]

Enantiomeric excess measurements were calibrated by adding 0.5–5.0% of the (+)-MTPA derivative of the corresponding racemic amino acid to the derivative of resolved amino acid. A diastereomeric impurity of 0.25% could be detected.

Use of Resolved Amino Acids as Chiral Synthons. To demonstrate the use of resolved unnatural amino acids as chiral synthons, we prepared (*R*)- and (*S*)-1-butene oxide from α-aminobutyric acid (Scheme I) and three chiral lactones via iodolactonization of substituted α-allylglycines (Scheme II).

L- and D-Aminobutyric acids (ee >99.5%), obtained by resolution (Table II), were converted in three chemical steps to (*R*)- and (*S*)-1-butene oxide, respectively, in 61% overall yield. The optical purities of the epoxides were determined by ^1H NMR spectroscopy in the presence of $Eu(hfc)_3$.[63] Both had a 96% ee.

Kinetically controlled iodolactonizations[64] of D-**7-A**, D-**10-CA**, and D-**11-A**, recovered from the resolutions of the corresponding racemates, gave lactones **58–60** (cis:trans, 7–8:1). The diastereomeric ratios of the products were measured by ^1H NMR. Assignment of the configurations of the major and minor diastereomers was performed by NOE difference spectroscopy and by correlation of the ^1H NMR spectral data of both diastereomers to that of known cis- and trans-2,4-disubstituted γ-butyrolactones.[65,66] Although little precedent exists, in general, for 1,3 asymmetric induction in the electrophilic cyclizations of 2-substituted 4-alkenoic acids, cis selectivity for the cyclizations of 2-amino-4-alkenoic acid derivatives is well documented.[12,13,66] The role of nitrogen in stereodirection is unknown.

Discussion

Acylase I is a useful catalyst for the resolution of unnatural amino acids on a multigram scale. Its general stability in solution allows it to be used in soluble form, and membrane-enclosure of the enzyme allows large quantities of protein to be used to catalyze the reactions of even very low activity substrates. The practical laboratory application of PKA extends to the resolution of substrates having >1% of the activity of AcMet on a 10–100-g scale and to the resolution of substrates having 0.001–1% activity on a 1–5-g scale. AA exhibits a somewhat more limited substrate specificity than PKA. The specific activity of AA, which is 100-fold lower than that of PKA, limits the scale of its applicability proportionately.

Although both PKA and AA have been used to resolve many natural and some unnatural amino acids, enantiomeric purities, if reported at all, have been reported for the (often repeatedly) recrystallized products. Many of the determinations of enantiomeric purity have depended on values of optical rotation and have therefore been approximations at best. Here, we have shown that the intrinsic enantioselectivity of the resolution step, itself, is high. (An exception is our resolution of **43**.) Furthermore, the absolute sense of the enantioselectivity remains constant, even with unnatural and α-methyl amino acids.

A problem more prevalent than nonspecific hydrolysis is the incomplete hydrolysis of the L enantiomer, causing enantiomeric contamination of the D product. Our measurements of initial rates of hydrolysis did not evaluate the Michaelis constants (K_m) of substrates explicitly. Substrates having high K_m (>10 mM, as apparently **15-CA**, **43-CA**, and **44-CA** do) will not afford highly enantiomerically enriched D products without recrystallization.

The syntheses of butene oxide and compounds **57–59** represent only two general procedures by which the resolved α-amino center may be transformed to different functionality or used to induce asymmetry at newly created stereocenters. In particular, other forms of electrophilic cyclization or oxidation of double bonds near the amino group may be induced by the α-chiral center.[67]

We believe the use of acylase I is a practical method for the preparation of enantiomerically pure, unnatural amino acids *of known absolute configuration*. It is practical even for the preparation of research quantities (1–5 g) of very low activity substrates, such as the α-methyl α-amino acids (at least the L enantiomer). We note that the resolutions described here have not been specifically optimized and that the optical purities of already enantiomerically enriched amino acids are readily improved by recrystallization. In general, though, these resolutions provide, *in a single process*, both enantiomers of an amino acid having high enantiomeric purities. Like any resolution, a disadvantage of the method is that the maximum chemical yield of any one enantiomer is 50%.

Experimental Section

Materials and Methods. Organic chemicals were from Aldrich and Fluka. Biochemicals were from Sigma. Crotyl bromide was distilled from calcium hydride. Oxalyl chloride was distilled and stored under nitrogen. Diisopropylamine and triethylamine were distilled from calcium hydride and stored under nitrogen. Methylene chloride and acetonitrile were freshly distilled from calcium hydride. THF was freshly distilled from sodium/benzophenone. Ethanol was dried over 2-Å molecular sieves. Reactions requiring anhydrous conditions were performed in oven- or flame-dried glassware under an atmosphere of nitrogen.

^1H and ^{13}C NMR spectra were recorded on Bruker AM-300 and AM-250 instruments with peaks referenced to $CHCl_3$ (^1H δ 7.26, ^{13}C δ

(62) With α-methyl amino acids, the L enantiomer refers to that enantiomer which would normally be considered L if the α-methyl group were in fact a hydrogen. L enantiomers of the α-methyl amino acids discussed in this paper are (*S*) in absolute configuration.

(63) Kim, M.-J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1988**, *110*, 2959–2964.

(64) Gonzalez, F. R.; Bartlett, P. A. *Org. Synth.* **1985**, *64*, 175–181.

(65) Hussain, S. A. M. T.; Ollis, W. D.; Smith, C.; Stoddart, J. F. *J. Chem. Soc., Perkin Trans. 1* **1975**, 1480–1492.

(66) Ohfune, Y.; Hori, K.; Sakaitani, M. *Tetrahedron Lett.* **1986**, *27*, 6079–6082.

(67) (a) Tamaru, Y.; Hojo, M.; Higashimura, H.; Yoshida, Z. *J. Am. Chem. Soc.* **1988**, *110*, 3994–4002. (b) Garner, P.; Park, J. M. *J. Org. Chem.* **1988**, *53*, 2979–2984. (c) Cardillo, G.; Orena, M.; Sandri, S. *J. Org. Chem.* **1986**, *51*, 713–717.

Table III. Direct Chloroacetylation of Amino Acids in Acetonitrile

amino acid	yield,[a] %
alanine	*b*
valine	75
norvaline	77
α-allylglycine	58
phenylalanine	67
α-methylphenylalanine	76
α-methyltyrosine	89
α-methyl-DOPA	60
serine	*c*
glutamic acid	*b*
α,ε-diaminopimelic acid	*b*

[a] Yield is of isolated, recrystallized product. [b] Only starting material was recovered. [c] Reaction of serine gave a complex product mixture that was not characterized.

77.0; $CDCl_3$), acetone-d_5 (^1H δ 2.04; acetone-d_6), or the methyl signal of sodium 3-(trimethylsilyl)propanesulfonate (^1H δ 0.00, ^{13}C δ 0.0; D_2O). Elemental analyses were performed by Galbraith and Spang Microanalytical Laboratories. Mass spectral data were obtained by H.K.C. and Drs. Todd Williams and Sheri Ogden (Mass Spectrometry Laboratory, University of California, Berkeley).

Enzymes. All of the results in this paper were obtained with acylase I from Sigma: porcine kidney (grade I) and *Aspergillus*. PKA and AA are also available from Biozyme and Amano, respectively. A preliminary survey of the activities of these enzymes indicated that both had activity profiles similar to their Sigma counterparts. The specific activities of both enzymes were about half of the corresponding Sigma enzymes, but their costs per unit of activity were 25 and 50%, respectively, of the Sigma enzymes.

Synthesis of Amino Acids and Analogues. Compounds **8**, **9**, **12**, **14**, **19**, **20**, **23–29**, **31**, **35**, **37**, and **38** were prepared by phase-transfer alkylation of **55**.[43,44] Compounds **10-A**, **16-A**, and **36-A** were prepared by alkylation[68] of **56** in sodium ethoxide/ethanol, followed by saponification and decarboxylation.[45] Hydrolysis in refluxing 2 N HCl for 2 h (or 2.5 N NaOH for 4 h with **36-A**) gave the free amino acids. Compounds **15** and **17** were prepared by modified Strecker reactions[69] of cyclopropanecarboxaldehyde and 2-adamantylethanal,[70] respectively. 4-Carboxy-2-pyrrolidone (**53**) was made by reducing *N*-benzyl-4-carboxy-2-pyrrolidone[71] in sodium/liquid ammonia. α-Methyl-α-vinylglycine (**39**) was made from DL-α-methylglutamic acid by a method analogous to that for preparing L-α-vinylglycine.[72] Compounds **11** and **11-A**,[45] **32**,[73] **49**,[74] and **50-A**[75] were prepared by literature methods.

Acylation of Amino Acids (Schotten–Baumann Conditions). The amino acid was dissolved in 3 equiv of 4 N NaOH (4 equiv if acylating a dicarboxylic acid), and acyl chloride (1.1 equiv) was added in five portions and with vigorous shaking to the chilled (<0 °C) solution over 50 min. Acidification to pH 1.5 with concentrated HCl, extraction with ethyl acetate, drying the organic layer ($MgSO_4$), and rotary evaporation gave the product as an oil. Trituration with petroleum ether caused the *N*-acyl amino acid to crystallize. The crystals were washed with ether and recrystallized from ethyl acetate/hexane.

Acetylation of Amino Acids (Acetic Anhydride/Acetic Acid). To 2 g of amino acid slurried in 25 mL of glacial acetic acid was added 1.2 molar equiv of acetic anhydride. The mixture was stirred at room temperature until it became homogeneous. After removal of the solvent by rotary evaporation, the residue was taken up in acetone and filtered. Rotary evaporation of the filtrate gave acetyl amino acid. This method was especially useful for N-acetylation of hydroxy amino acids and aminophosphonic acid **49**.

(68) Preparation of (bromomethyl)cyclopropane: Meek, J. S.; Rowe, J. W. *J. Am. Chem. Soc.* **1955**, *77*, 6675–6677. Preparation of (chloromethyl)furan: Kirner, W. R. *J. Am. Chem. Soc.* **1928**, *50*, 1955–1961. Divald, S.; Chun, M. C.; Joullie, M. M. *J. Org. Chem.* **1976**, *41*, 2835–2846.
(69) Gaudry, R. *Can. J. Chem., B* **1946**, *24*, 301–307.
(70) Prepared from cyclopropyl- and 1-adamantylmethanol, respectively, by Swern oxidation: Omura, K.; Swern, D. *Tetrahedron* **1978**, *34*, 1651–1660. Aldehydes were previously reported: Smith, L. I.; Rogier, E. R. *J. Am. Chem. Soc.* **1951**, *73*, 4047–4049. Do, K. Q.; Thanei, P.; Caviezel, M.; Schwyzer, R. *Helv. Chim. Acta* **1979**, *62*, 956–964.
(71) Paytash, P. L.; Sparrow, E.; Gathe, J. C. *J. Am. Chem. Soc.* **1950**, *72*, 1415–1416.
(72) Hanessian, S.; Sahoo, S. P. *Tetrahedron Lett.* **1984**, *25*, 1425–1428.
(73) Joucla, M.; El Goumzili, M.; Fouchet, B. *Tetrahedron Lett.* **1986**, *27*, 1677–1680.
(74) Oleksyszyn, J.; Tyka, R.; Mastalerz, P. *Synthesis* **1978**, 479–480.
(75) Filachione, E. M.; Lengel, J. H.; Fisher, C. H. *J. Am. Chem. Soc.* **1944**, *66*, 494–496.

Table IV. Relative Initial Rates of Hydrolysis[a] of *N*-Acyl Amino Acids Catalyzed by Acylase I

	porcine kidney		*Aspergillus*	
compd	no Co^{2+}	1 mM Co^{2+}	no Co^{2+}	1 mM Co^{2+}
AcMet	100	86	58	100
N-benzoylmethionine	0.23			
1-A			33	68
1-CA				
2-A	53	53	22	40
3-A	72	50	41	86
3-CA	150	103	105	137
3-MA	121	94	27	32
4-A	14	58		
5-CA	7.7×10^{-3}	1.4×10^{-2}		*c*
6-A	40			
7-A	35	23	34	68
7-CA	65	36	85	76
7-MA	62	42	23	15
8-MA	22	23	32	35
9-MA	13	17		
10-A	35	29	40	41
10-CA	66			56
11-A	36	28	28	51
11-CA	97	61	68	148
12-MA	28	78	20	43
13-A	16	10	18	30
13-CA	33	26	48	35
13-MA	29	20	9.9	9.7
14-CA	54	27	35	41
15-CA	124	169	40	23
16-A	36	22	61	84
17-CA	*b*	*b*	*c*	*c*
18-A	0.85	3.9	47	84
18-CA	5.8	23	98	123
18-MA	1.2	6.4	43	50
19-MA	0.48		43	
20-MA	6.3×10^{-2}	2.6	4.9	12
21-A	46			
22-A	24	81	16	37
22-MA	26	90	6.1	18
23-MA	2.4	0.38	1.2	
24-MA	2.0	0.24	7.5	
25-MA	5.4×10^{-2}	4.2	6.2	7.9
26-MA	24		10	
27-MA	0.14	0.13	0.2	0.18
28-MA	96		13	
29-MA	19	44	27	35
30-A	20	19		6.6
30-CA	92	69		14
30-MA	30	28		3.1
31-CA	0.9	6.3		
32-MA	0.19	2.2	0.12	1.0
33-A	*b*	*b*	*c*	*c*
34-A	1.5			
35-MA	41			
36-A	16	28	36	68
37-CA	0.44	0.71	32	41
38-MA	0.2	0.9	14	23
39-MA	5.1×10^{-2}	0.17		
40-A	*b*	*b*		
40-MA		0.026		
41-CA	2.8×10^{-3}	9.3×10^{-2}	*c*	*c*
41-MA	*b*	2.2×10^{-3}		
42-CA	0.5	1.3	*c*	*c*
43-CA	1.7×10^{-3}	1.5×10^{-2}	*c*	*c*
44-CA	*b*	4.7×10^{-3}	*c*	*c*
45-CA	*b*	*b*	*c*	*c*
46-CA	*b*	*b*	*c*	*c*
47-CA	*b*	*b*		*c*
48-A		0.06		*c*
49		*b*		*c*
50-A		*b*		*c*
51-A				
52-A		*d*		*d*

[a] Initial rates of hydrolysis of 40 mM racemic substrate, pH 7.5, 40 °C, are reported as a percentage of the rate of hydrolysis of *N*-acetylmethionine: for PKA, 30 ± 5 μmol min^{-1} mg^{-1} (no Co^{2+}); for AA, 0.30 ± 0.02 μmol min^{-1} mg^{-1} (1 mM Co^{2+}). [b] Relative rate is $<5 \times 10^{-3}$%. [c] Relative rate $<5 \times 10^{-2}$%. [d] Relative rate is $<1 \times 10^{-3}$%.

Direct Chloroacetylation of Amino Acids. Chloroacetyl chloride (1.05 equiv) was added to a dry, round-bottom flask containing 20–100 mmol of amino acid and enough acetonitrile to make the final solution 0.5 M

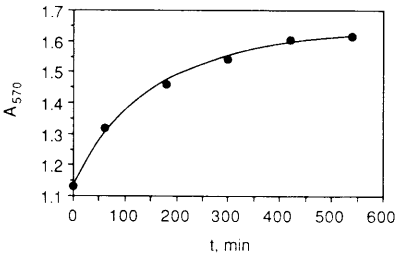


Figure 8. Colorimetric response with ninhydrin intrinsic to acylase I isolated from *Aspergillus* (Sigma; 10 mg in 2.00 mL of buffer). Enzyme was incubated under assay conditions, and aliquots (0.20 mL) were quenched and reacted with ninhydrin.

in acid chloride. The flask was fitted with a reflux condenser and a nitrogen source. The mixture was heated at reflux under nitrogen until the amino acid dissolved completely (generally, 1–1.5 h). Occasionally, a small amount of the amino acid did not dissolve. The colorless to orange solution was cooled and evaporated under reduced pressure. The residue was stirred in acetone or ethyl acetate for about 15 min to allow complete solution of the acyl amino acid. The solution was filtered and the filtrate evaporated under reduced pressure. The residue was recrystallized from ethyl acetate/hexane (Table III).

Enzymatic Assays. Initial rates of enzymatic hydrolysis (Table IV) were determined by combining 80 μmol of racemic acyl amino acid or analogue, 20 μL of 4 N KOH (40 μL for dicarboxylic acids), and acylase I (0.01–1.0 mg of PKA or 1.0–10 mg of AA) in 0.10 M potassium phosphate buffer (with or without 1 mM $CoCl_2$), pH 7.5, 40 °C. The enzyme was added last to initiate reaction. The final assay volume was 2.00 mL. The reaction proceeded at 40 °C, and six aliquots (0.20 mL) were periodically removed and quenched in 0.20 mL of 14% perchloric acid. Portions (0.16 mL) of the acidified aliquots were combined with 0.84 mL of water, 0.50 mL of sodium cyanide/acetate buffer, and 0.50 mL of 3% ninhydrin, heated to 100 °C for 15 min, and quenched with 5.00 mL of 50% aqueous 2-propanol, according to the method of Rosen.[48] The absorbances (570 nm) of the solutions were measured after allowing them to cool for 15–30 min.

The acidified reaction aliquots were stable when stored at 4 °C and showed no significant hydrolysis of acyl amino acid or the protein. The absolute intensity of the colorimetric response generated by acidified aliquots stored in the cold remained virtually constant for 24 h, and the relative intensities of aliquots collected and stored together remained constant for at least 3 days.

Rates of hydrolysis were calibrated by reacting known amounts (0.00–0.20 μmol) of each amino acid by the procedure above (see Supplementary Material for details). The activity of acylase I itself was determined in a similar manner, with AcMet (40 mM racemic) as the substrate. The activities of PKA and AA were measured in the absence and presence of 1 mM $CoCl_2$, respectively. Relative rates (V_r) of hydrolysis of substrates were expressed as percentages of the rate observed with AcMet.

Control experiments involved incubating the enzymes (1.0 mg of PKA and 10 mg of AA) in the absence of substrates and potential substrates (40 mM) in the absence of enzyme under assay conditions. Aliquots were periodically removed and tested for response with ninhydrin by the method above. AA exhibited an intrinsic "false activity" (Figure 8). Consequently, when slowly reacting substrates were assayed with AA, the enzyme was allowed to rest in buffer solution for 6 h before assaying, and a blank reaction containing only AA was run in parallel. The differences in absorption between the assay reaction and the control were used to calculate the rate of enzymatic hydrolysis.

Acidified aliquots from assays of PKA and AA with AcMet were incubated at 4 °C and periodically assayed for response with ninhydrin to determine the stability of the acidified aliquots during storage.

Compounds **21-A**, which itself reacted with ninhydrin, and **51-A** were assayed similarly to other potential substrates except that the rates of hydrolysis were determined by ^1H NMR. Each acidified aliquot was evaporated under reduced pressure, and the residue was taken up in D_2O. Peaks for the α-methine protons of the acylated and free acids were integrated to measure the amount of hydrolysis. Hydrolysis of **50-A** was monitored by assaying aliquots for L- and D-lactic acids.[76]

Stability of Acylase I. Acylase I (1.0 mg of PKA or 10 mg of AA) was incubated at 25 °C in 0.10 M potassium phosphate buffer, pH 7.5, with or without organic cosolvents. Buffer for AA also contained 1 mM

(76) (a) Noll, F. In *Methods of Enzymatic Analysis*, 3rd ed.; Bergmeyer, H. U., Ed.; Verlag, Chemie: Weinheim, Germany, 1983; Vol. 6, pp 582–588. (b) Gawehn, K. *Ibid.* pp 588–596.

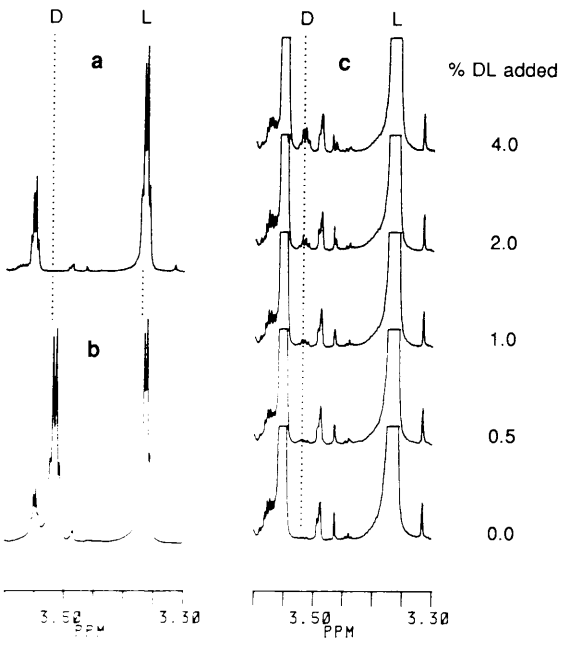


Figure 9. ^1H NMR spectra (300 MHz) at 3.30–3.60 ppm of the methoxy ether peaks of the (*R*)-(+)-MTPA derivatives of (a) methyl L-α-*cis*-crotylglycinate, (b) methyl DL-α-*cis*-crotylglycinate, and (c) a + 0.0–4.0% of b (base-line detail).

$CoCl_2$. Final volume was 2.00 mL. Unless the enzyme was to be exposed to atmospheric oxygen, all solutions were made in buffer previously degassed with nitrogen, and the incubating enzyme solution was kept under an atmosphere of nitrogen, sealed with a septum. Aliquots of the enzyme solutions were periodically removed via syringe and assayed for activity.

Resolution of Amino Acids. To distilled water were added racemic acyl amino acid and enough lithium or potassium hydroxide to make the final solution pH 7.5–8.0.[77] Solutions also contained 1 mM $CoCl_2$ and were deoxygenated, if necessary. Final concentration of racemic acyl amino acid was 0.1–0.5 M. With good substrates, addition of 8.3 U of acylase I (mmol of acyl L-amino acid initiated the reaction (units based on the initial rate of hydrolysis of the substrate being resolved and measured as described above). With poor substrates, relatively fewer units of enzymatic activity were used. Progress of the reaction was monitored by assaying aliquots for free amino acid with ninhydrin.[48] Resolutions proceeded for 24 h or until the release of free amino acid leveled off. Periodic, manual addition of base maintained the pH of the reaction at approximately 7.5.

The reaction was adjusted to pH 5.0 with concentrated hydrochloric acid, heated to 60 °C with Norit, and filtered. The filtrate was acidified to pH 1.5 with concentrated hydrochloric acid and extracted with ethyl acetate. The aqueous layer was applied to a column of Dowex-50 (H^+), and the column was rinsed with water until neutral and then eluted with 1 N aqueous ammonia. Alternatively, the aqueous layer was evaporated to dryness by rotary evaporation. The residue was taken up in methanol, filtered, and treated with excess propylene oxide (warning: cancer suspect agent) to give the free L-amino acid as a precipitate.

The ethyl acetate layer was dried ($MgSO_4$), filtered, and evaporated by rotary evaporation. Trituration of the oily residue with ether or ether/petroleum ether mixtures gave the acyl D-amino acid as a solid. Alternatively, the crude oil could by hydrolyzed by refluxing for 2 h in 2 N HCl.[78] The D-amino acid was recovered from the hydrolysis mixture by cation-exchange chromatography or precipitation from methanol with propylene oxide, similar to the recovery of the L-amino acid.

Resolutions of **42**–**44** were performed as described above except that 5 g of PKA in approximately 40 mL of degassed 0.1 M potassium phosphate buffer, pH 7.5, was enclosed in 13.5 × 2.1 cm of dialysis tubing (Spectropor 2; MW cutoff, 12000–14000). After dialytic removal of the buffer salts, the enclosed enzyme was placed in a degassed

(77) Because of its high solubility, lithium hydroxide was used in resolutions of which the L-amino acid product was precipitated from neutral aqueous solution by the addition of ethanol. Both lithium and potassium cations functioned equally well in purifications by ion-exchange chromatography.

(78) Compounds **36-A** and **42-CA** were hydrolyzed by refluxing for 4 h in 2.5 N NaOH and in ethanol with 2 equiv of thiourea, respectively. Acidic hydrolysis destroyed both compounds. Hydrolyses of *N*-acyl α-methyl amino acids required more than 2 h for completion.

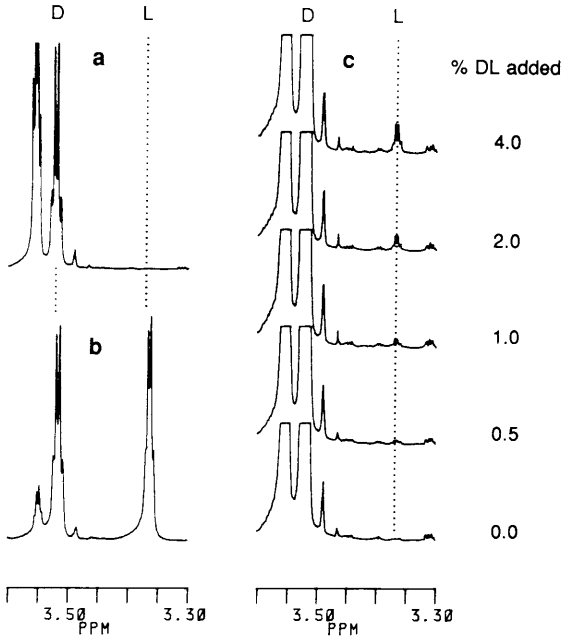


Figure 10. ^{1}H NMR spectra (300 MHz) at 3.30–3.60 ppm of the methoxy ether peaks of the (*R*)-(+)-MTPA derivatives of (a) methyl D-α-*cis*-crotylglycinate, (b) methyl DL-α-*cis*-crotylglycinate, and (c) a + 0.0–4.0% of b (base-line detail).

solution of **44-CA**, pH 7.5, and stirred gently under nitrogen. After the reaction had leveled off at 50% conversion, the dialysis tubing was removed and dialyzed against distilled, degassed water to remove products. The same enzyme was used subsequently to hydrolyze **43-CA** and **42-CA**.

Determination of the Enantiomeric Purities of Amino Acids. The enantiomeric purities of amino acids were determined by ^{1}H NMR analysis of the (+)-MTPA amides[60] of the amino acid methyl esters. Methyl esters were prepared without racemization with methanol/thionyl chloride[79] or diazomethane/methanol. The (+)-MTPA methoxy groups of L- and D-amino acid derivatives appeared as narrow quartets (J_{HF} = 1.0–1.5 Hz) at approximately δ 3.35 and 3.50, respectively. The α-methyl singlets of α-methyl amino acid derivatives (δ 1.69 and 1.65 for D- and L-amino acids, respectively) were also distinguishable. Calibration by the addition of known amounts of the (+)-MTPA derivative of the appropriate racemic amino acid showed that a 0.25% diastereomeric impurity (99.5% ee for the amino acid) could be detected (Figures 9 and 10).

Conversion of D- and L-α-Aminobutyric Acid to (*S*)- and (*R*)-Butene Oxide. Resolved α-aminobutyric acid was converted in three steps to enantiomerically enriched (96% ee) butene oxide as described previously.[80]

Iodolactonization of 7-A, 10-A, and 11-A. Allylglycine derivatives **7-A**, **10-A**, and **11-A** were iodolactonized under kinetic-controlled conditions.[64] Analysis by ^{1}H NMR and NOED spectroscopy indicated that **57**, **58**, and **59** were formed with cis to trans ratios of 8:1, 8:1, and 7:1, respectively.

Acknowledgment. We thank Jaesang Kim for preparing **10-A** and for performing some of the direct chloroacetylations and the resolution of **3-A**.

Supplementary Material Available: Calibration of the variable colorimetric response of amino acids with ninhydrin and tables of spectroscopic and analytical data for potential substrates of acylase I and compounds **57**–**59** (25 pages). Ordering information is given on any current masthead page.

(79) Uhle, F. C.; Harris, L. S. *J. Am. Chem. Soc.* **1956**, *78*, 381–384.
(80) Chenault, H. K.; Kim, M.-J.; Akiyama, A.; Miyazawa, T.; Simon, E. S.; Whitesides, G. M. *J. Org. Chem.* **1987**, *52*, 2608–2611.