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Enantioseparation of the Esters of α-*N*-acetyl Amino Acids by Lipase in Ionic Liquid

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ABSTRACT Chiral α-amino acids have been obtained via enzymatic resolution in ionic liquid medium. The acetyl esters of a series of amino acids were separated by enzyme porcine pancreas lipase (PPL) in an aqueous solution of *N*-ethyl pyridinium trifluoroacetate, $[EtPy]^+[CF_3OO]^-$. A comparative study with organic solvent acetonitrile shows that the ionic liquids provide a better medium for enzymatic resolution. *Chirality 17:S240–S242, 2005.* © 2005 Wiley-Liss, Inc.

KEY WORDS: amino acids; enzymatic resolution; lipases; ionic liquid; *N*-ethyl pyridinium trifluoroacetate

α-Amino acids are important intermediates for many pharmaceutical and biological applications. For example, L-(+)-homophenylalanine ((*S*)-2-amino-4-phenylbutanoic acid) is a vital component of angiotensin-converting enzyme (ACE) and renin inhibitors.[1,2] Many ACE inhibitors, such as Benazepril, Enalapril, and Lisinopril, have been intensely studied as medicinal targets for the treatment of hypertension and heart failure.[3] Therefore, it has been a challenge for chemists to come up with new methods to obtain optically pure amino acids. Enzymatic resolution of amino acid derivatives is one of the simplest and most efficient methods of producing enantiomerically enriched amino acids. Traditionally, mixtures of organic solvents and water have been used as reaction media for the kinetic resolution. For example, enzymatic resolution of different amino acids by enzyme alcalase has been studied in acetone–water,[4] acetonitrile–water,[4] ethanol–water,[4] 1-propanol–water,[4] tetrahydrofuran–water,[4] dioxane–water,[4] *tert*-butanol–water,[5] 2-methyl-2-propanol–water,[6] DMF,[7] etc.

Ionic liquids are a new class of solvents that offer alternatives to conventional molecular solvents in organic synthesis and other chemical processes.[8,9] These solvents are often fluid at room temperature and commonly consist of organic cationic and inorganic anionic species. They have no measurable vapor pressure and hence can emit no volatile organic compounds (VOCs). Also, with many favorable characteristics such as being good solvents for a wide range of inorganic, organic, and polymeric materials, having adjustable polarity, and possessing catalytic effects, they have been investigated as reaction media for organic and organometallic syntheses.[10] More recently, ionic liquids have been also used in the studies of enzymatic systems, such as lipase-catalyzed kinetic resolution of 1-phenylethanol,[11] enzymatic catalysis in the formation of *Z*-aspartame,[12] and as catalysts in alcoholysis, ammoniolysis, and perhydrolysis reactions.[13] Similarly, enhanced enantioselectivity has been achieved in ionic liquids for lipase-catalyzed transesterifications.[14]

Recently, we achieved the synthesis of homophenylalanine ester and piperazine-2-carboxylic acid ester and obtained single enantiomers through enzymatic resolution.[15,16] A report in the literature showed that organic solvents have significant effect on the activity and enantioselectivity of protease in hydrolyzing esters or peptides.[4] However, based on our earlier successful studies in ionic liquids[17,18] and with the foreseeable advantages and goal of finding an environmentally friendly, i.e., "green" medium to substitute organic solvents, we investigated the resolution of a series of amino acid esters using porcine pancreas lipase (PPL). In this article, we report the results of this study.

MATERIALS AND METHODS

Materials

The *N*-acetyl homophenylalanine ethyl ester was prepared through a three-step reaction strategy,[15] while other amino acids and the enzyme porcine pancreas lipase (PPL), also named Lipase-3126, were obtained from Sigma-Aldrich and used as received. One unit of PPL will hydrolyze 1.0 microequivalent of fatty acid from a triglyceride in 1 h at pH 7.4 and 37°C. The N-acetylation of amino acids was carried out in our laboratory following the procedure given below. Organic solvents were purchased from Aldrich Chemical Co. (Milwaukee, WI), purified, and dried before use. All solvents have purity higher than 99%. Ionic

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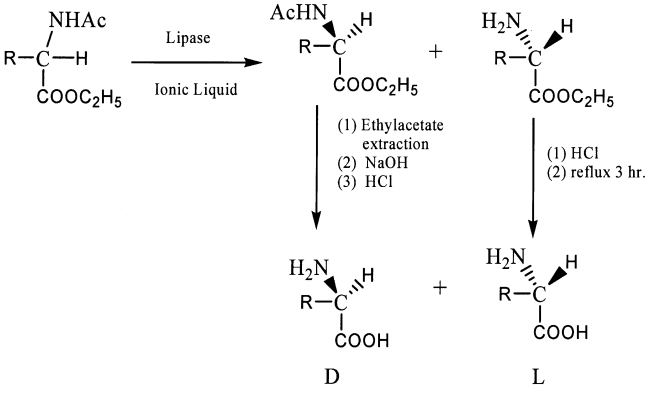


Scheme 1.

liquid *N*-ethyl pyridinium trifluoroacetate ([EtPy][CF_3OO]) was prepared according to literature method.[19]

Acetylation of Amino Acid Esters

General procedure: preparation of *N*-acetyl amino acid esters was based on a literature method.[20] One gram of amino acid ester was dissolved in 20 mL glacial acetic acid, followed by addition of 1.2 molar equivalent of acetic anhydride. The mixture was stirred at room temperature for 1 h. The solvent was removed by rotary evaporation under vacuum, and the residue was taken into acetone and filtered. Rotary evaporation of the filtrate gave *N*-acetyl amino acid ester.

Kinetic Resolution

General procedure: 0.5 g of racemic *N*-acetyl amino acid ethyl ester was suspended or dissolved in 60 ml of the mixed solvent (15% solution of ionic liquid in water); 0.5 g $NaHCO_3$ was dissolved in the reaction mixture, and the pH of solution was about 7.5. Following this, 1.0 ml of PPL-(lipase) was added to the reaction mixture. The reaction mixture was gently stirred at 25°C for 24 h under nitrogen atmosphere. The *N*-acetyl D-ester was extracted by ethyl acetate three times. HCl (6 N) was added, and the pH decreased to 2–3. The remaining trace ethyl acetate in aqueous solution was evaporated, and the solution was further concentrated until the precipitate appeared. Removal of water gave *N*-acetyl L-acid. The *N*-acetyl D-acid was obtained by hydrolyzing *N*-acetyl D-ester in 6 N NaOH solution for 2 h. The *N*-acetyl group in the L- and D-acid was taken off by refluxing them in 3 N HCl for 3 hr.

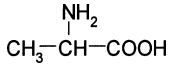
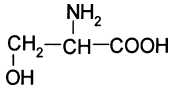
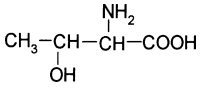
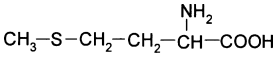
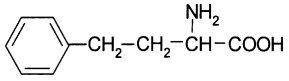
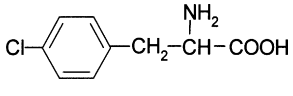
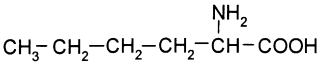
Analysis of Enantiomeric Excess (ee)

The enantiomeric excess (ee) was calculated from the specific optical rotation which was measured in 3 N HCl using AUTOPOL IV polarimeter (Rudolph Research Analytical, Flanders, NJ). Also, the ee measurements were confirmed by HPLC with Chiralpak WH column.

RESULTS AND DISCUSSION

Lipases have been widely investigated in the synthesis of chiral synthons and optically pure compounds including amino acids.[21–23] Among various lipases, PPL was shown

TABLE 1. Kinetic resolution of *N*-acetyl amino acids in the ionic liquid [$EtPy^+$][CF_3COO^-] (0.5 g ester, 0.5g $NaHCO_3$, 15% ionic liquid or acetonitrile in water, 3.0 ml *PPL*, 25 °C, 24 hours)

Amino acid		Acetonitrile		[EtPy][CF_3COO]	
Racemic ester (each with *N*-acetyl)	Acid structure	ee (%)	Yield (%)	ee (%)	Yield (%)
Alanine ethyl ester		63	26	81	30
Serine methyl ester		35	21	78	28
Threonine methyl ester		36	19	89	24
Methionine methyl ester		62	31	86	29
Homophenylalanine ethyl ester		92	33	95	39
4-Chlorophenylalanine ethyl ester		95	26	98	41
Norleucine methyl ester		18	32	73	30

to give best results in the enantioselective resolution of amino acids.[21] It has also been reported that enzymatic resolution revealed that optically pure product could be obtained in acetone, acetonitrile, and 1,4-dioxane while poor resolution was observed in methanol and ethanol.[4] On screening a range of solvents, we also observed polar solvents such as acetonitrile, 1-propanol, and ethanol are good media. However, better results are obtained using ionic liquid *N*-ethyl pyridinium trifluoroacetate $[EtPy]^+[CF_3COO]^-$. Inspired with these results, we studied the enzymatic resolution of a series of amino acid esters using enzyme porcine pancreas lipase (PPL) in $[EtPy]^+[CF_3COO]^-$. Our aim was to investigate if organic solvents could be replaced with the environmentally friendly medium of ionic liquid. A representative reaction procedure is shown in Scheme 1.

The kinetic resolution of amino acids is influenced by solvent nature, polarity, and concentration.[17] It was observed that improved enzyme activity could be obtained by adjusting these solvent parameters. A systematic study with variation in ionic liquid concentration in the reaction mixture showed that best results in terms of enantiomeric excess and product yields are obtained with 15% ionic liquid solution in water. Based on the preliminary data, a reaction procedure for the current study was developed using enzymes PPL and *N*-ethyl pyridinium trifluoroacetate $[EtPy]^+[CF_3COO]^-$. The protocol was tested for a host of amino acid esters to obtain optically enriched amino acids. In order to evaluate the effect of ionic liquids in comparison with an organic solvent, we also studied the enzymatic resolution in 15% acetonitrile solution in water. Results are shown in Table 1. As data shows, highly enantio-enriched products are obtained when an ionic liquid system is used compared to an acetonitrile system. PPL shows more specific resolution for esters of amino acids with an aromatic side chain, while other amino acids show moderate yields and ee. It is also noteworthy that the esters of serine, threonine, and norleucine have no significant resolution in acetonitrile. Use of ionic liquids tremendously increases the enantioselectivity of the resolution process, and these results and earlier data demonstrate that ionic liquids could provide a viable alternative to organic solvents for kinetic separation and chiral synthesis of organic molecules.

CONCLUSION

The ionic liquid *N*-ethyl pyridinium trifluoroacetate $[EtPy]^+[CF_3OO]^-$ could be a good substitute for organic solvents in the kinetic resolution of the esters of amino acids using the enzyme PPL. It can enhance the activity and selectivity of the enzymes, thereby providing highly enatiomerically enriched amino acids.

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