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A semiempirical study on inhibition of catechol *O*-methyltransferase by substituted catechols

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Summary

Catechol and endogenous catechol derivatives are readily methylated by catechol *O*-methyltransferase (COMT). In contrast, many catechol derivatives possessing electronegative substituents are potent COMT inhibitors. The X-ray structure of the active site of COMT suggests that the methylation involves a lysine as a general base. The lysine can activate one of the catecholic hydroxyl groups for a nucleophilic attack on the active methyl group of the coenzyme S-adenosyl-L-methionine (AdoMet). We studied the effect of dinitrosubstitution of the catecholic ring at the semiempirical PM3 level on the methylation reaction catalysed by COMT. The electronegative nitro groups make the ionized catechol hydroxyls less nucleophilic than the corresponding hydroxyl groups of the non-substituted catechol. As a consequence, dinitrocatechol is not methylated but is instead a potent COMT inhibitor. The implications of this mechanism to the design of COMT inhibitors are discussed.

Introduction

Catechol *O*-methyltransferase (COMT, EC 2.1.1.6) belongs to the mammalian small molecule methyltransferases, which catalyse the transfer of the methyl group from S-adenosyl-L-methionine (AdoMet) to acceptor molecules. COMT methylates hydroxyl groups of substrates with a catechol structure. Physiological substrates of COMT include the catecholamine neurotransmitters dopamine, adrenaline and noradrenaline and catecholic steroids such as 2-hydroxyestradiol. The general function of COMT is the elimination of biologically active or toxic catechols [1].

The kinetic mechanism of COMT enzyme has been studied extensively with partially purified enzyme preparations [2–5], and recently with recombinant enzymes [6, 7]. COMT is active only in the presence of Mg^{2+} ions. It binds first AdoMet, then magnesium, and as the last ligand the catechol substrate. The methylated product dissociates first, followed by magnesium and S-adenosyl-L-homocysteine (AdoHcy) [7].

The methyl transfer has been proposed to proceed through a trigonal-bipyramidal transition-state structure as a direct S_N2-like nucleophilic attack by one of the hydroxyl groups of the catechol substrate on the methyl group of AdoMet [5, 8]. A general base catalysed proton abstraction from one (or both) of the catechol hydroxyl groups has been suggested to activate the reaction in the enzyme [3, 9, 10]. The Mg^{2+} ion was suggested to participate in the binding of the substrates to the enzyme [11, 12], but its central role was not clear until the crystal structure of soluble rat COMT was solved [13]. A simple catechol derivative inhibitor 3,5-dinitrocatechol (DNC) was bound at the active site, revealing the exact position of the catechol structure with respect to the catalytic machinery of the enzyme [13]. The essential features of the active site of COMT are illustrated in Figure 1. These can be summarized as follows: (1) The two hydroxyl oxygens of the catechol structure are directly bound to the Mg^{2+} ion. (2) The active methyl group of AdoMet is near

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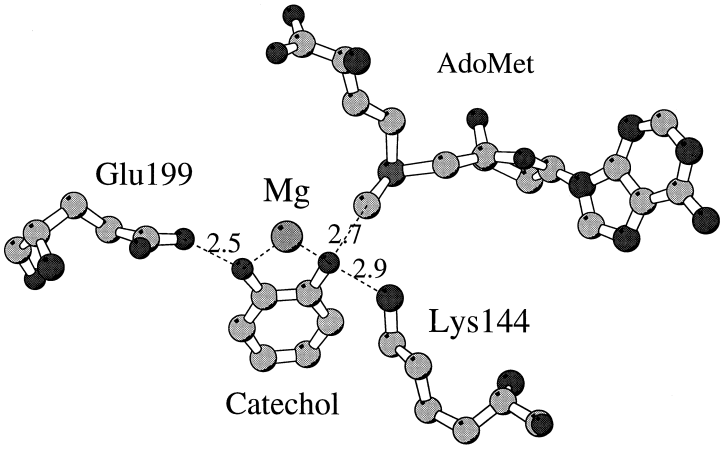

Figure 1. Part of the active site of COMT taken from the X-ray structure [13]. The groups which are near the two catecholic hydroxyls are shown. The distances are in Ångstroms. The figure was generated using MOLSCRIPT [30].

one of the hydroxyl groups (OH1), on one side of the catechol ring. The distance to the other hydroxyl group (OH2) is considerably larger. (3) The amino group of Lys^{144} is also near the OH1 hydroxyl group, on the other side of the catechol ring. (4) Glu^{199} is near the other hydroxyl group (OH2).

In the late 1980s potent and selective COMT inhibitors were developed [14, 15]. The first clinical use of COMT inhibitors was in the treatment of Parkinson's disease, as adjuncts in L-dopa therapy [1, 16]. QSAR studies of catechol-type COMT inhibitors have shown that the key determinant of the potency is the electronegativity of the substituents [17, 18]. The knowledge on the atomic structure of the COMT active site makes it possible to understand both the methylation reaction mechanism and the criteria for potent inhibition [19]. In the present study we describe semiempirical PM3 calculations on COMT–catechol and COMT–DNC complexes.

Methods

Kinetic data give useful information on the general course of a reaction, but are often difficult to interpret in terms of a detailed reaction pathway. Electronic redistribution occurs in chemical reactions, i.e. bonds are broken and formed. Any model for a chemical reaction must be able to describe such electronic redistributions correctly, and thus generally quantum mechanical methods are used [20].

A major problem of using quantum chemical methods to study enzyme catalysis is the large size of the structures involved. Instead of complete structures, calculations are performed on model systems, which represent the essential features of the enzyme active site. Ideally, a model is based on the enzyme's X-ray or NMR structure.

In this study a model assembly approach is used. This method has been used with success to study proton transfer reactions in a receptor-ligand system [21]. The coordinates of the COMT structure are available from the Brookhaven Protein Data Bank (structure 1VID) [22]. The model of the active site of COMT includes amino acid residues Asp^{141}, Lys^{144}, Asp^{169}, Asn^{170} and Glu^{199}, the Mg^{2+} ion, one water molecule, which completes the octahedral coordination of Mg^{2+}, the co-substrate AdoMet and the substrate catechol. Lys^{144} and Glu^{199} can make hydrogen bonds to the hydroxyls of the catechol, while Asp^{141}, Asp^{169} and Asn^{171} coordinate the Mg^{2+} ion. The glutamate and aspartates are modeled using propanoate, the asparagine using propionamide and the lysine using ethylammonium cation. AdoMet is modeled using trimethylsulphonium cation. The net charge of the system is zero. The model assembly of the COMT–catechol complex is shown in Figure 2.

All complexes studied were optimized using the semiempirical PM3 model [23] included in the SPARTAN software (SPARTAN 3.1, Wavefunction Inc., Irvine, CA, 1994). The PM3 approximation has been shown to give good results for hydrogen bonded complexes [24, 25] and a good accuracy for the hydration energies of various kinds of metal cationic species, including Mg^{2+} [26]. During optimization the outermost methyl groups of the model amino acid residues, the four non-hydroxyl-bound carbons of the catechol

Table 1. Absolute and relative energies for the PM3 optimized assemblies of the different protonation states of COMT-catechol and COMT-methoxyphenol complexes

Ligand	Protonation state[a]	PM3 optimized E (kJ mol^{-1})	Relative energy ΔE (kJ mol^{-1})
Catechol	CatI	−1971.83	77.8
	CatII	−1859.86	190
	CatIII	−2049.67	0
	CatIV	−1876.03	174
2-Methoxyphenol	MeCatI	−2047.93	0
	MeCatII	−1893.09	155
	MeCatIII	−1883.90	164
	MeCatIV	Unstable	–

[a]For the definition of the protonation states see Figure 3.

ring, and the sulphur atom of the trimethylsulphonium cation were fixed in space, while the rest of the complexes were fully optimized. This prevents the unnatural translation of the model residues in space during the optimization procedure.

Results

COMT–catechol complex

The pK_a of the catecholic hydroxyl is about 9.8. Thus, the hydroxyl groups of catechol-type substrates of COMT are unionized at physiological pH. However, when bound to COMT, one or both of the hydroxyls can be ionized. This is especially due to the Mg^{2+} ion interacting directly with the hydroxyl oxygens. The amino acid residues Lys^{144} and Glu^{199} can participate in proton transfer due to their orientation in the active site (Figure 1). Thus four possible protonation states of the bound catechol in the active site of COMT can be depicted, as shown in Figure 3. These four states will be denoted as CatI–CatIV. After methylation, the methylated product can in principle exist in four corresponding states denoted MeCatI–MeCatIV (Figure 3). All these states are sterically reasonable, due to the position of the AdoMet and Lys^{144} at the opposite sides of the catechol ring.

The PM3 optimized energies of the complexes CatI–CatIV and MeCatI–MeCatIII are shown in Table 1. MeCatIV was not stable during PM3 optimization: this complex optimized to give MeCatII. All other complexes could be fully optimized at the PM3 level.

The protonation state CatIII, with one of the hydroxyls and the nearby lysine amino group neutral,

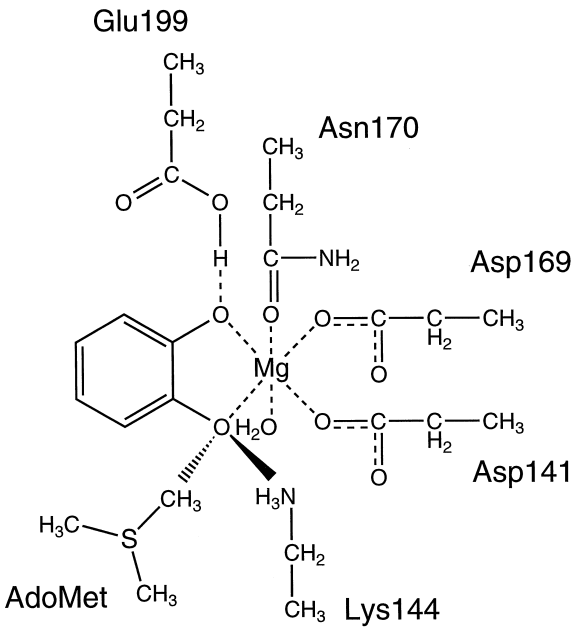

Figure 2. A schematic representation of the model assembly of the COMT–catechol complex used in the calculations. The protonation state CatI is shown (for definitions see Figure 3).

has the lowest energy. CatI, with both hydroxyls ionized, has about 78 kJ mol^{-1} higher energy while the two forms with the neutral hydroxyl near Glu^{199} have considerably higher energy. The result infers that the reaction coordinate for the methylation of catechols by COMT consists of a proton transfer from a hydroxyl group to Lys^{144} and a subsequent methyl transfer from AdoMet to the hydroxyl group. The activation of the hydroxyl group to methyl attack after the proton transfer can be readily seen from the molecular

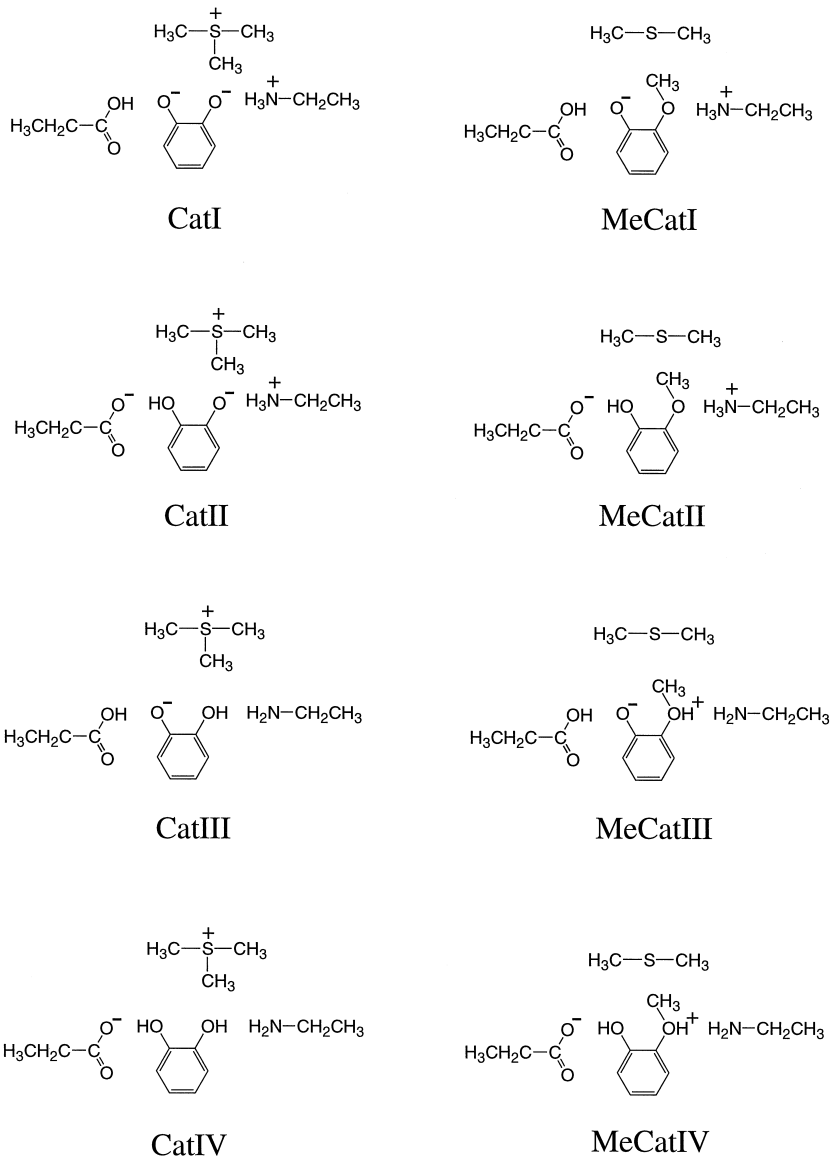

Figure 3. The possible protonation states I–IV of catechol and *O*-methylated catechol at the active site of COMT. Only the part of the model assembly which participates in the proton transfer or methyl transfer reactions is shown for clarity.

electrostatic potential (MEP) surfaces of the different protonation states of the model assembly. The MEP surfaces were calculated at the PM3 level and plotted at -84 kJ mol^{-1} (-20 kcal mol^{-1}) for the complexes CatIII and CatI (Figure 4a,b). When the hydroxyl group is neutral, most of the negative MEP of the system lies on the opposite side of the catechol ring to AdoMet. After the proton transfer the negative potential becomes much stronger on the side of AdoMet, and especially between the hydroxyl oxygen and the active methyl group.

COMT–3,5–dinitrocatechol complex

It has been known for some time that catechols with strong electronegative substituents are potent COMT inhibitors [7, 17]. An example of such an inhibitor is 3,5-dinitrocatechol (DNC) which was co-crystallized with COMT in the determination of the structure of the enzyme [13].

The protonation states I-IV (compare Figure 3) of the COMT–DNC complex (DNCatI–DNCatIV), and of the corresponding methylated product complexes (MeDNCatI–MeDNCatIV) were fully optimized at

Table 2. Absolute and relative energies for the PM3 optimized assemblies of the different protonation states of COMT-dinitrocatechol and COMT-methoxydinitrophenol complexes

Ligand	Protonation state[a]	PM3 optimized E (kJ mol^{-1})	Relative energy ΔE (kJ mol^{-1})
3,5-Dinitrocatechol	DNCatI	−2067.89	0
	DNCatII	−1927.64	140
	DNCatIII	−2066.55	1.3
	DNCatIV	−1873.75	194
2-Methoxy-3,5-dinitrophenol	MeDNCatI	−2053.48	0
	MeDNCatII	−1872.84	181
	MeDNCatIII	−1852.10	201
	MeDNCatIV	Unstable	–

[a]For the definition of the protonation states see Figure 3.

the PM3 level. (Note that only the binding mode with the *ortho*-nitro at the side of AdoMet is sterically feasible and found in the X-ray structure of the COMT–DNC complex [13].) The results are shown in Table 2.

The electronegative nitro groups strongly stabilize the doubly ionized form DNCatI, which has now roughly the same energy as DNCatIII. The methylation activation barriers were approximated using the reaction coordinate driving method. The distance between the catechol oxygen and the methyl carbon was reduced using a 0.05 Å step size, while the other degrees of freedom (except the frozen ones) were optimized. The PM3 barrier for the catechol methylation step was estimated to be about 90 kJ mol^{-1}, while for the dinitrocatechol methylation it was approximately 150 kJ mol^{-1}. Since the model assemblies possessed several frozen degrees of freedom, the exact transition state structures were not calculated. Besides, the MEP surface of DNCatI shows that the electronegative nitro groups drain much of the negative potential of the hydroxyl groups, making them less nucleophilic (Figure 4c). Thus the methylation reaction is predicted to happen very slowly, if at all. In fact, it has been shown with a nitrocatechol type inhibitor, nitecapone, in a recombinant rat COMT assay *in vitro*, that the rate of nitecapone methylation is equivalent to only about 1% of the rate of dopamine methylation [27].

Discussion and conclusions

A key feature in many enzyme catalysed reactions is the ability of the enzyme to change pK_a values of ligands or substrates, when these are bound at the enzyme's active site. In the *O*-methylation reaction of catechols, a general base catalysis means an abstraction of a proton from the catechol hydroxyl by the enzyme. The X-ray structure of COMT reveals that the only amino acid which can act as a catalytic base near the hydroxyl group that is methylated is Lys^{144}. PM3 calculations suggest that the pK_a of Lys^{144} in the enzyme environment near Mg^{2+} and the positively charged AdoMet may be unusually low. Thus, if the catechol substrate is removed from the model assembly and the two hydroxyl binding sites are occupied by water molecules, a protonation state in which Lys^{144} is in its neutral form has the lowest energy (at the PM3 level 26 kJ mol^{-1} below the corresponding state in which the lysine is protonated). Thus, in the free enzyme Lys^{144} would be in the NH_2 form and ready to take a proton from the catecholic substrate when this binds to the active site. This proton transfer activates the hydroxyl group for the final methyl transfer step. The role of Lys^{144} as a catalytic base in COMT is similar to the catalytic mechanism of aspartate aminotransferase, where a lysine residue functions as a catalytic base in the amino acid transamination reaction [28]. This reaction mechanism for COMT was also suggested in a recent paper by Zheng and Bruice [29].

The known potent inhibitors of COMT have a catecholic structure with electronegative substituents. Quantum chemical energy calculations show that such catechol-type inhibitors have a low energy at the active site of COMT in a doubly ionized form. Electronegative substituents withdraw electrons from the ionized hydroxyl groups, decreasing their nucleophilicity. As

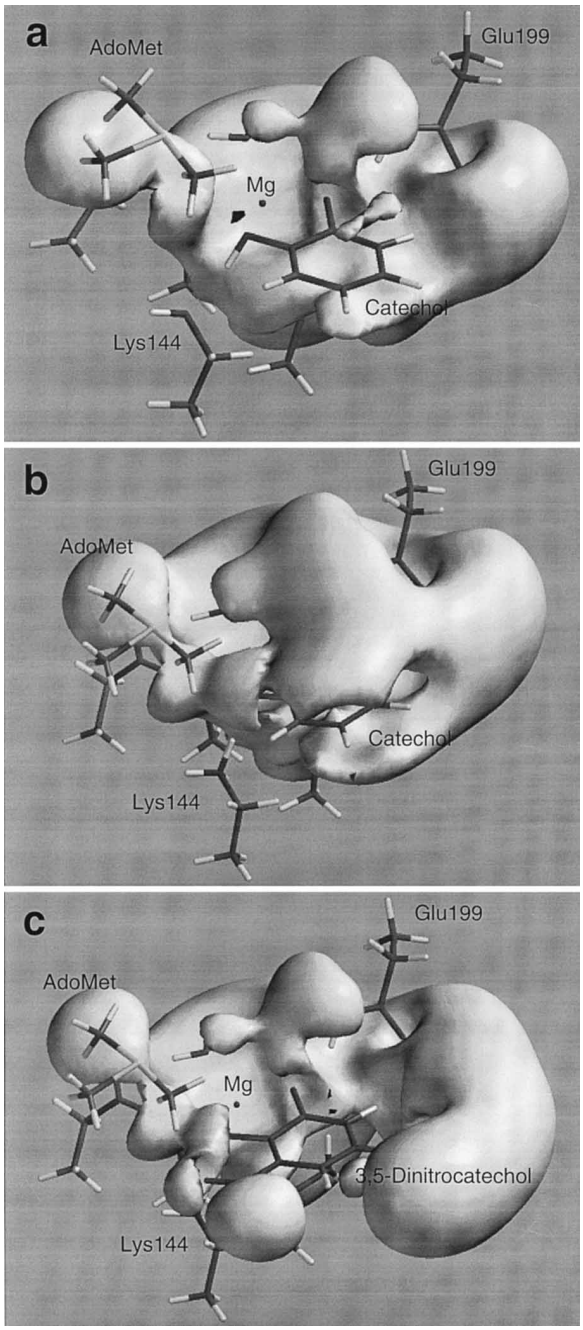

Figure 4. Molecular electrostatic potentials at -84 kJ mol^{-1} (-20 kcal mol^{-1}) of the model assemblies CatIII (a), CatI (b), and DNCatI (c).

a consequence, such molecules are not substrates for COMT, but potent inhibitors.

The design of non-catecholic COMT inhibitors is of some interest, because pharmacokinetic properties such as metabolic reactions and penetration of the blood-brain barrier could be modified with such structures. Comparison of calculated electronic properties of COMT–ligand complexes for the designed compounds and for the known potent catecholic inhibitors (e.g. DNC) may be used to guide the design process.

References

1. Männistö, P.T., Ulmanen, I., Lundström, K., Taskinen, J., Tenhunen, J., Tilgmann, C. and Kaakkola, S., In Juner, E. (Ed.) Progress in Drug Research, Birkhäuser Verlag, Basel, 1992, pp. 291–350.
2. Flohe, L. and Schwabe, K., Biochim. Biophys. Acta, 220 (1970) 469.
3. Coward, J.K., Slisz, E.P. and Wu, F.Y.-H., Biochemistry, 12 (1973) 2291.
4. Borchardt, R.T., J. Med. Chem., 16 (1973) 377.
5. Woodard, R.W., Tsai, M.-D., Floss, H.G., Crooks, P.A. and Coward, J.K., J. Biol. Chem., 255 (1980) 9124.
6. Malherbe, P., Bertocci, B., Caspers, P., Zürcher, G. and Da Prada, M., J. Neurochem., 58 (1992) 1782.
7. Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melén, K., Julkunen, I. and Taskinen, J., Biochemistry, 34 (1995) 4202.
8. Borchardt, R.T., In Usdin, E. (Ed.) Structure and Function of Monoamine Enzymes, Marcel Dekker, New York, NY, 1977, pp. 707–726.
9. Knipe, J.O., Vasquez, P.J. and Coward, J.K., J. Am. Chem. Soc., 104 (1982) 3202.
10. Thakker, D.R., Boehlert, C., Kirk, K.L., Antkowiak, R. and Creveling, C.R., J. Biol. Chem., 261 (1986) 178.
11. Creveling, C.R., Dalgard, N., Shimizu, H. and Daly, J.W., Mol. Pharmacol., 6 (1970) 691.
12. Borchardt, R.T. and Huber, J.A., J. Med. Chem., 25 (1982) 321.
13. Vidgren, J., Svensson, L.A. and Liljas, A., Nature, 368 (1994) 354.
14. Bäckström, R., Honkanen, E., Pippuri, A., Kairisalo, P., Pystynen, J., Heinola, K., Nissinen, E., Lindén, I.-B., Männistö, P., Kaakkola, S. and Pohto, P., J. Med. Chem., 32 (1989) 841.
15. Borgulya, J., Bruderer, H., Bernauer, K., Zurcher, G. and Da Prada, M., Helv. Chim. Acta, 72 (1989) 952.
16. Kaakkola, S., Gordin, A. and Männistö, P.T., Gen. Pharmacol., 25 (1994) 813.
17. Taskinen, J., Vidgren, J., Ovaska, M., Bäckström, R., Pippuri, A. and Nissinen, E., Quant. Struct.-Act. Relatsh., 8 (1989) 210.
18. Lotta, T., Taskinen, J., Bäckström, R. and Nissinen, E., J. Comput.-Aided Mol. Design, 6 (1992) 253.
19. Vidgren, J. and Ovaska, M., In Veerapandian, P. (Ed.) Structure-Based Drug Design, Marcel Dekker, New York, NY, 1997, pp. 343–363.
20. Mulholland, A.J., Grant, G.H. and Richards, W.G., Protein Eng., 6 (1993) 133.
21. Peräkylä, M. and Pakkanen, T.A., J. Am. Chem. Soc., 115 (1993)10958.

22. Bernstein, F.C., Koetzle, T.F., Williams, G.J.B., Meyer Jr., E.F., Brice, M.D., Rodgers, J.R., Kennard, O., Shimanouchi, T. and Tasumi, M., J. Mol. Biol., 112 (1977) 535.
23. Stewart, J.J.P., J. Comput. Chem., 10 (1989) 209.
24. Schröder, S., Daggett, V. and Kollman, P.A., J. Am. Chem. Soc., 113 (1991) 8922.
25. Rzepa, H.S. and Yi, M.Y., J. Chem. Soc. Perkin Trans., 2 (1991) 531.
26. Furuki, F., Sakurai, M. and Inoue, Y., J. Comput. Chem., 16 (1995) 378.
27. Wikberg, T., Biotransformation and Bioanalytics of Nitecapone and Entacapone, Ph.D. Thesis, University of Helsinki, Helsinki, Finland, 1993, p. 29.
28. Toney, M.D. and Kirsch, J.F., Science, 243 (1989) 1485.
29. Zheng, Y.-J. and Bruice, T.C., J. Am. Chem. Soc., 119 (1997) 8137.
30. Kraulis, P.J., J. Appl. Crystallogr., 24 (1991) 946.