 Binding of D-Phenylalanine and D-Tyrosine to Carboxypeptidase A*

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The structures of the complexes of carboxypeptidase A with the amino acids D-phenylalanine and D-tyrosine are reported as determined by x-ray crystallographic methods to a resolution of 2.0 Å. In each individual study one molecule of amino acids binds to the enzyme in the COOH-terminal hydrophobic pocket: the carboxylate of the bound ligand salt links with Arg-145, and the α-amino group salt links with Glu-270. The carboxylate of Glu-270 must break its hydrogen bond with the native zinc-bound water molecule in order to exploit the latter interaction. This result is in accord with spectroscopic studies which indicate that the binding of D or L amino acids (or analogues thereof) allows for more facile displacement of the metal-bound water by anions (Bicknell, R., Schaffer, A., Bertini, I., Luchinat, C., Vallee, B. L., and Auld, D. S. (1988) Biochemistry 27, 1050–1057). Additionally, we observe a significant movement of the zinc-bound water molecule (~1 Å) upon the binding of D-ligands. We propose that this unanticipated movement also contributes to anion sensitivity. The structural results of the current x-ray study correct predictions made in an early model building study regarding the binding of D-phenylalanine (Lipscomb, W. N., Hartsuck, J. A., Reeeke, G. N., Jr., Quiocho, F. A., Bethge, P. H., Ludwig, M. L., Stetz, T. A., Muirhead, H., and Coppola, J. C. (1968) Brookhaven Symp. Biol. 21, 24–90).

Bovine carboxypeptidase A is an exopeptidase of molecular weight 34,472 containing a divalent zinc ion bound to a polypeptide chain of 307 amino acids (Lipscomb, 1982, 1983; Vallee et al., 1983; Vallee and Galdes, 1984). The complexes of carboxypeptidase A with various ligands have been investigated by x-ray crystallographic methods, and these structures have yielded important answers regarding the catalytic mechanism (Christianson and Lipscomb, 1985, 1986a, 1986b, 1986c, 1988; Christianson et al., 1985, 1987; Shoham et al., 1988). Of carboxypeptidase A substrates, D-amino acids do not comprise the biologically preferred COOH-terminal portion (i.e. P1' portion; for a description of S,P notation, see Schechter and Berger, 1967). Schechter and Berger (1966) have shown that the enzyme hydrolyzes substrates with P1' d-amino acids only very slowly. Interestingly, D-Phe is a much more potent inhibitor of enzyme activity than is L-Phe: respective K values are 2 and 18 mM (Elkins-Kaufman and Neurath, 1948, 1949; Neurath and DeMaria, 1950). Studies of enzyme-ligand complexes involving these compounds may prove finer structural details of the enzyme-active site and particularly details pertaining to the zinc-bound water molecule. For example, recent spectroscopic studies have shown that the function of this particular water molecule is significantly affected upon the binding of zwitterionic L- or D-amino acids and small, anionic carboxylate-containing inhibitors (Bicknell et al., 1988). The zinc-bound water molecule of carboxypeptidase A is more easily displaced by anions when the enzyme is first treated with single amino acids or carboxylate inhibitors. The postulated feature of anion sensitivity, the breaking of an enzyme hydrogen bond with the zinc-bound molecule, is revealed in the current x-ray crystallographic study. Additionally, the observed binding mode of D-Phe reported herein is remarkably different from that predicted in an early model building study (Lipscomb et al., 1968).

Important residues for binding and catalysis in the carboxypeptidase A active site include Glu-270, Arg-127, Arg-145, Arg-71, Tyr-248, the zinc ion (liganded by His-69, His-196, and the two carboxy oxygens of Glu-72), and its bound water molecule. Of these components, the most functionally enigmatic remains the zinc-bound water molecule. X-ray studies of the native enzyme result in electron density, corresponding to this water molecule, which has been described as disordered (Rees et al., 1983) or reasonably ordered (Hardman and Lipscomb, 1984). Furthermore, the pK of this zinc-bound water molecule continues to be the subject of much study and conjecture. The enzyme displays an acidic pK at about 6 and a basic pK at about 9 (Auld and Vallee, 1971; Auld et al., 1986; Hall et al., 1969). It is often though that the lower pK reflects the ionization of the zinc-bound water during substrate turnover (Wooley, 1975; Markinen et al., 1979). However, the upper pK, too, has been considered to reflect the ionization of the zinc-bound water molecule (Kaiser and Kiser, 1972; Suh and Kaiser, 1976; Spratt et al., 1983), and this consideration has, in the free enzyme, some support from x-ray diffraction studies at basic pH values (Shoham et al., 1984). Studies of a mutant carboxypeptidase A from the rat are also interpretable to this end (Hi level et al., 1986; Gardell et al., 1987).

A wealth of experimental results support the former assignment, including those results obtained with model complexes (Wooley, 1975; Groves and Olsen, 1985). Most notably, Groves and Olsen (1985) report a model (but non-catalytic) complex with a zinc-bound water of pH 7.0. The pKa of the zinc-bound water in native carboxypeptidase A may be depressed further by a strong hydrogen bond with the gamma-carboxylate of residue Glu-270, the proposed intermediate proton donor in proteolysis (Rees and Lipscomb, 1982; Monzino and Matthews, 1984). The strength of the association of this water molecule in carboxypeptidase A with the metal ion is remarkable; it is quite difficult to displace by other ligands such as chloride or...
azide unless carboxylate-bearing compounds, such as 3-phenylpropionic acid or D- or L-phenylalanine, are present (Bicknell et al., 1988; Bertini et al., 1988). Alternatively, chloride can displace zinc-bound water from carboxypeptidase A at low pH due to protonation of Glu-270 (Stephens et al., 1974), but if Glu-270 is chemically modified the enzyme is anion sensitive at low as well as neutral pH values (Geoghegan et al., 1983). The current x-ray study shows that the following two factors are responsible for anion sensitivity of zinc-bound water: the breaking of its hydrogen bond with Glu-270, and the subsequent movement of this water molecule by as much as 1 Å.

MATERIALS AND METHODS

Carboxypeptidase A, D-Phe, and D-Tyr were purchased from Sigma and used without further purification. Carboxypeptidase A was crystallized in space group P2₁ (a = 51.60 Å, b = 60.27 Å, c = 47.25 Å, β = 97.27°, crystal habit elongated along the a axis) by dialysis of the enzyme (solubilized in 1.2 M LiCl, 0.02 M Tris-HCl, pH 7.4) against 0.15 M LiCl, 0.02 M Tris-HCl, pH 7.4. Crystals of typical dimensions 0.3 × 0.3 × 0.9 mm appeared within ~1-2 days. Harvested crystals were transferred to a buffer solution of 0.15 M LiCl, 0.02 M Veronal-LiOH, pH 7.4, where they were lightly cross-linked with 0.1% glutaraldehyde (ν/v) for 6 h. These crystals were then soaked in similar buffer solutions for 2 weeks containing either 5 mM D-Phe or 5 mM D-Tyr. Initial experiments at 1 and 2 mM D-Phe showed amino acid binding with less than satisfactory occupancy. Crystals deteriorated badly upon soaking in buffer solutions containing greater than 5 mM D-Phe.

Final data on D-Phe- and D-Tyr-soaked carboxypeptidase A crystals were collected to 2.0 Å resolution on a Syntax (Nicollet; Madison, WI) automated four-circle x-ray diffractometer. Integrated intensities, estimated using the Wyckoff step scan (Wyckoff et al., 1967), were corrected for Lorentz and polarization effects; additionally, a linear correction for crystal decay in the x-ray beam was applied based upon the average decay of four standard check reflections. After scaling and merging, R factors based on intensities were 0.049 and 0.078 for D-Phe and D-Tyr data sets, respectively. Model building was performed on an Evans and Sutherland PS300 interfaced with a VAX 11/780 with graphics software developed by Jones (1982). The enzyme-amino acid complexes were refined by using the reciprocal least squares method employing the stereochemically restrained least squares algorithm of Hendrickson and Konnert (1981). The crystallographic R factor for the carboxypeptidase A/D-Phe model is 0.168, and that for the carboxypeptidase A/D-Tyr model is 0.177. The highest peaks in final electron density maps, calculated for each model with Fourier coefficients |Fᵣ| - |Fₛ|, and phases derived from each final model, were less than 4°.

RESULTS AND DISCUSSION

It is interesting to note that although the side chains of D-Phe and D-Tyr differ by a phenolic hydroxyl group, the two amino acids bind in identical fashion within the Sᵢ hydrophobic pocket. Distances of interactions between carboxypeptidase A and D-amino acids are recorded in Table I. The root mean square (rms) deviation between corresponding atomic coordinates of D-Phe and D-Tyr is 0.2 Å; this deviation is the same as that resulting from the experimental rms error in each set of atomic coordinates (about 0.2 Å). We propose that the hydroxyl or other similarly sized para-substituents on a Pᵢ benzyl side chain will perturb favorable enzyme-ligand association. A stereoview comparing the two binary complexes is presented in Fig. 1. The binding of the two amino acids differs in one respect, however, within the hydrophobic pocket: the phenolic hydroxyl of D-Tyr displaces a water molecule, whereas this water molecule is not displaced by the side chain of D-Phe. The phenolic hydroxyl of D-Tyr makes hydrogen bond contacts with two additional water molecules in the hydrophobic pocket which are not displaced by the ligand.

In each complex, the side chain of residue Glu-270 is observed to move away from its position in the native enzyme, primarily by a rotation about the Cα-Cα bond. By virtue of this rotation, Glu-270 breaks a hydrogen bond with the zinc-bound water. Distances of relevant interactions in each complex are recorded in Table II. The movement of Glu-270, probably driven by a stronger, hydrogen-bonded salt link between Glu-270 and the positively charged amino group of the bound D-amino acid, contributes to the anion sensitivity of the active site metal ion in the presence of amino acids (or their analogues) demonstrated in recent spectroscopic studies (Bicknell et al., 1988). We judge this interaction to be strong and likely to involve an ion pair due to the short distance of the interaction; the amino-carboxylate distance for D-Phe/Glu-270 is 2.3 Å, and that for D-Tyr/Glu-270 is 2.4 Å (although these distances appear anomalously short, carboxylic oxygens are known to engage in strong hydrogen bonds with nonhydrogen atom separations of 2.4 to 2.5 Å; see Jeffrey and Maluszynka, 1982). The proposal of Bicknell and colleagues (1988) is supported in these crystal structures: by breaking the hydrogen bond between Glu-270 and zinc-bound water, the water molecule is more readily displaced by anions.

We observe that the water molecule also moves from its position in the native enzyme, although it remains coordinated to zinc (the zinc-water distance in each complex is 2.3 Å, compared with 2.1 Å in the native enzyme). The zinc-bound water moves 1.0 Å in the carboxypeptidase A-D-Phe complex and 0.5 Å in the carboxypeptidase A-D-Tyr complex.

We propose that the actual motion of zinc-bound water subsequent to ligand binding also contributes to its anion sensitivity.

The binding of single amino acids will provide a strong, favorable charge-charge interaction with Glu-270. We illustrate the nature of this interaction schematically in Fig. 4. The interaction of Glu-270 with the amino group of a single L-Phe molecule in the Sᵢ subsite would be less optimal than that observed for D-Phe. Crystallographic investigation of the binary carboxypeptidase A-L-Phe complex has yet been unsuccessful, so these conclusions regarding L-Phe remain partially speculative until a satisfactory structure determination is made. However, a carboxypeptidase A/L-Phe interaction was observed in a ternary enzyme-substrate-product complex (Christianson and Lipscomb, 1987) in which the amino nitrogen of L-Phe was 3.3 ± 0.2 Å away from the nearest carboxylate oxygen of Glu-270. This distance is longer than that observed for the interaction of D-Phe with Glu-270, and it is thus indicative of a weaker hydrogen bond interaction with L-Phe.
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FIG. 1. Coordinates of the carboxypeptidase A/D-Phe structure (thick bonds) superimposed on those of the carboxypeptidase A/D-Tyr structure (thin bonds). Residues displayed are the D-amino acids, Glu-270, Tyr-248, Arg-145, zinc, and the zinc-bound water molecule. The zinc ion and its bound water in the carboxypeptidase A-D-Phe complex are drawn as larger spheres relative to those in the carboxypeptidase A-D-Tyr complex. Both D-Phe and D-Tyr bind nearly identically in the S1 pocket.

Two binding sites for D-Phe (the "metal" and "non-metal" sites) were observed in the spectroscopic studies (Bicknell et al., 1988), but only one is observed occupied in each of the current crystal structures. We conclude that the protein-amino acid interaction we observe is that of the non-metal site, and the metal-carboxylate carbon distance of $4.8 \pm 0.2$ Å is in accord with the distance of $4.2 \pm 0.4$ Å observed in a recent spectroscopic study (Luchinat et al., 1988). Since higher concentrations of amino acids caused severe crystal deterioration, we were unable to observe saturation of the metal site in the crystal. This lack of saturation is due solely to ligand concentration and not to crystal lattice obstructions, since the metal site is observed occupied by larger inhibitors spanning several enzyme subsites (Christianson and Lipscomb, 1989).

An interesting difference is noted when comparing the two electron density maps of Figs. 2 and 3. Although the zinc-bound water is well-ordered and its electron density spherically symmetric in the carboxypeptidase A/D-Phe structure (with thermal $B = 13$ Å$^2$), this water molecule is not similarly well behaved in the carboxypeptidase A-D-Tyr complex. The shape of the electron density above the zinc ion is rather elongated in the latter complex; this feature is reminiscent of the phenomenon described for the zinc-bound water of the native enzyme (Rees et al., 1983). In native carboxypeptidase A, the odd-shaped electron density above the zinc ion was interpreted as a disordered water molecule (with thermal $B = 26$ Å$^2$). Our interpretation is the same for the elongated density found in the carboxypeptidase A-D-Tyr complex (the peak above zinc displays a thermal $B = 25$ Å$^2$). This anomaly for zinc-bound water between the D-Phe and D-Tyr complexes could be artifactual in origin. The poorly shaped electron density may reflect the quality of diffraction data acquired for the D-Tyr complex relative to that acquired for the D-Phe complex. The higher $R$ factor for scaling and merging replicate reflections in the carboxypeptidase A/D-Tyr data set (0.078) relative to that for the carboxypeptidase A/D-Phe data set (0.049), and the slightly higher crystallographic $R$ factor for the carboxypeptidase A-D-Tyr complex (0.177) relative to that for the carboxypeptidase A-D-Phe complex (0.168) indicate
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An asterisk denotes a possible hydrogen bond as judged from both distance and geometric criteria. The rms error in atomic coordinates is about ±0.2 Å. CPA, carboxypeptidase A.

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SUMMARY

The binding of D-amino acids to carboxypeptidase A confers anion sensitivity upon the function of the enzyme by breaking the hydrogen bond between the active site base, Glu-270, and the zinc-bound water molecule. An additional factor contributing to anion sensitivity is the substantial movement (~1 Å) of the zinc-bound water molecule which accompanies ligand binding. To be sure, the surviving zinc-water interaction may be strong, but spectroscopic studies indicate that it is sufficiently weak to be displaced by anions such as chloride, azide, or the carboxylate of a second amino acid. Based on prior crystallographic experiments, the less potent anion sensitivity conferred by simple carboxylate inhibitors which lack an α-amino group arises from a carboxyl-carboxylate interaction with Glu-270. The binding mode for D-Phe does not differ much from that observed for L-Phe as observed in the ternary complex of carboxypeptidase A with benzoyl-L-phenylalanine plus L-phenylalanine (Christianson and Lipscomb, 1987). However, the α-amino group of D-Phe makes a stronger salt link with Glu-270 than that observed for L-Phe. Finally, we note that the results of the current study correct the predictions of an early model building study regarding the carboxypeptidase A-D-Phe complex (Lipscomb et al., 1968).

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REFERENCES

Auld, D. S. and Vallee, B. L. (1971) *Biochemistry* 10, 2892-2897

Auld, D. S., Larson, K. and Vallee, B. L. (1986) in *Zinc Enzymes* (Berti, I., Luchinat, C., Mare, W. and Zepperauzer, M., eds) pp. 131-154, Birkhauser, Boston

Berti, I., Monnanni, R., Pellacani, G. C., Soia, M., Vallee, B. L. and Auld, D. S. (1988) *J. Inorg. Biochem.* 32, 12-26

Bicknell, R., Schäffer, A., Berti, I., Luchinat, C., Vallee, B. L. and Auld, D. S. (1988) *Biochemistry* 27, 1050-1057

Christianson, D. W. and Lipscomb, W. N. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 6840-6844

Christianson, D. W. and Lipscomb, W. N. (1986a) *Proc. Natl. Acad. Sci. U. S. A.* 83, 7565-7572

Christianson, D. W. and Lipscomb, W. N. (1986b) *J. Am. Chem. Soc.* 108, 545-546

Christianson, D. W. and Lipscomb, W. N. (1986c) *J. Am. Chem. Soc.* 108, 4998-5003

Christianson, D. W. and Lipscomb, W. N. (1987) *J. Am. Chem. Soc.* 109, 5536-5538

Christianson, D. W. and Lipscomb, W. N. (1988) *J. Am. Chem. Soc.* 110, 5560-5565

Christianson, D. W. and Lipscomb, W. N. (1989) *Acc. Chem. Res.* 22, 62-89

Christianson, D. W., Kuo, L. C. and Lipscomb, W. N. (1985) *J. Am. Chem. Soc.* 107, 8281-8283

Christianson, D. W., David, P. R. and Lipscomb, W. N. (1987) *Proc. Natl. Acad. Sci. U. S. A.* 84, 1512-1515

Elkins-Kaufman, E. and Neurath, H. (1948) *J. Biol. Chem.* 175, 893-911

Elkins-Kaufman, E. and Neurath, H. (1949) *J. Biol. Chem.* 178, 645-654

Gardell, S. J., Hilvert, D., Barnett, J., Kaiser, E. T. and Rutter, W. J. (1987) *J. Biol. Chem.* 262, 576-582

Georghegan, K. F., Holmquist, B., Spilburg, C. A. and Vallee, B. L. (1983) *Biochemistry* 22, 1847-1852

Goves, J. T. and Olson, J. R. (1985) *Inorg. Chem.* 24, 2715-2717

Hall, P. L., Kaiser, B. L. and Kaiser, E. T. (1969) *J. Am. Chem. Soc.* 91, 485-491

Hardman, K. D. and Lipscomb, W. N. (1984) *J. Am. Chem. Soc.* 106, 463-464

Hendrickson, W. A. and Konnert, J. (1981) in *Biomolecular Structure, Function, Conformation, and Evolution* (Srinivasan, R., ed) Vol. 1, pp. 43-47, Pergamon, Oxford

Hilvert, D., Gardell, S. J., Rutter, W. J. and Kaiser, E. T. (1986) *J. Am. Chem. Soc.* 108, 5288-5304

Jeffrey, G. A. and Malusynska, H. (1982) *Int. J. Biol. Macromol.* 4, 173-185

Jones, T. A. (1982) in *Computational Crystallography* (Sayre, D., ed) pp. 303-317, Oxford University Press, London

Kaiser, E. T. and Kaiser, B. L. (1972) *Acc. Chem. Res.* 5, 219-224

Lipscomb, W. N. (1982) *Acc. Chem. Res.* 15, 232-233

Lipscomb, W. N. (1983) *Annu. Rev. Biochem.* 52, 17-34

Lipscomb, W. N., Hartsuck, J. A., Reeke, G. N., Jr., Quiocio, F. A., Bethge, P. H., Ludvig, M. L., Stetiz, T. A., Muirhead, H. and Coppola, J. C. (1968) *Brookhaven Symp. Biol.* 21, 24-60

Luchinat, C., Monnanni, R., Roelens, S., Vallee, B. L. and Auld, D. S. (1988) *J. Inorg. Biochem.* 32, 1-6

Makinen, M. W., Kuo, L. C., Dymowski, J. J. and Jaffer, S. (1979) *J. Biol. Chem.* 254, 356-366

Monzingo, A. F. and Matthews, B. W. (1984) *Biochemistry* 23, 5724-5729

Neurath, H. and DeMaria, G. (1950) *J. Biol. Chem.* 186, 653-666

Rees, D. C. and Lipscomb, W. N. (1982) *J. Mol. Biol.* 160, 475-498

Rees, D. C., Lewis, M. and Lipscomb, W. N. (1983) *J. Mol. Biol.* 168, 367-387

Sawyer, L. and James, M. N. G. (1982) *Nature* 295, 79-80

Schechter, I. and Berger, A. (1958) *Biochemistry* 5, 3371-3375

Schechter, I. and Berger, A. (1987) *Biochem. Biophys. Res. Commun.* 27, 157-162

Shoaham, G., Rees, D. C. and Lipscomb, W. N. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 7767-7771

Shoaham, G., Christianson, D. W. and Oren, D. A. (1988) *Proc. Natl. Acad. Sci. U. S. A.* 85, 684-688

Spratt, T. E., Sugimoto, T. and Kaiser, E. T. (1983) *J. Am. Chem. Soc.* 105, 3679-3683

Stephens, R. S., Jontoft, J. E. and Bryant, R. G. (1974) *J. Am. Chem. Soc.* 96, 8041-8045

Suh, J. and Kaiser, E. T. (1976) *J. Am. Chem. Soc.* 98, 1940-1947

Vallee, B. L. and Galdes, A. (1984) *Adv. Enzymol.* 56, 283-430

Vallee, B. L., Galdes, A., Auld, D. S. and Riordan, J. F. (1983) in *Metal Ions in Biology* (Sapiro, T. G., ed) Vol. 5, pp. 25-27, Wiley, New York

Wooley, P. (1975) *Nature* 258, 677-682

Wyckoff, H. W., Doscher, M., Tsarenoglu, D., Inagami, T., Johnson, L. N., Hardman, K. D., Allwell, N. M., Kelly, D. M. and Richards, F. M. (1967) *J. Mol. Biol.* 27, 563-578