Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 7–10

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

ASSESSMENT OF THE STERIC TOLERANCE OF THE *P* SECTOR IN THE CATALYTIC SITE OF PORCINE LIVER ESTERASE

Raúl E. Rojas-Garbanzo and Julio F. Mata-Segreda*

School of Chemistry, University of Costa Rica, 2060 Costa Rica

Received 15 August 1997; accepted 3 November 1997

Abstract: Porcine liver esterase catalyzes the hydrolysis of simple esters capable of hydrogen bonding more rapidly than the reaction for small hydrophobic acetates. The result can be understood in terms of the high steric tolerance of the *P* sector in the catalytic site.

Carboxyl esterases (EC 3.1.1.1) are widely distributed in the tissues and blood of vertebrates. The highest mammalian activities are found in blood plasma, liver, kidney, and duodenum. For this reason, considerable effort is devoted to the development of ester prodrugs.

Many studies on liver esterase have been published regarding the molecular features of these enzymes from different mammalian sources.[1a] A high degree of genetic homology of carboxyl esterase isozymes among various animal species is a known fact.[1b] Nevertheless, these enzymes of microsomal localization share a lesser degree of homology with acetylcholinesterases (EC. 3.1.1.7) and cholinesterases (EC 3.1.1.8).[1c] Of particular interest is porcine liver esterase (PLE), which is an inexpensive and commercially available enzyme that has become a biochemical model for enzymic ester hydrolysis as well as a synthetic tool in organic chemistry. Commercial PLE is a mixture of six isoenzymes that behave as if it were a single species.[2a]

Jones[2] has proposed a model for the catalytic site of this serine hydrolase, since no X-ray crystallographic data is currently available. The model is based on the enzyme behavior upon different ester substrates. Two cavities are thought to exist (6 A^3 and 90 A^3) for the binding of hydrophobic substrates (*H* sector).[3] A two-cavity polar sector (*P* sector) is also proposed, for substrates capable of hydrogen bonding to polar amino acid side chains. *P* sector appears to have less stringent steric requirements than the *H* cavities. For example, relatively bulky substrates such as 3,4-(isopropylidenedioxy)-2,5-tetrahydrofuranyl diesters are sterically tolerated by the catalytic site.[4]

A useful strategy for ester prodrug development might thus be the synthesis of derivatives with hydrogen bonding groups near the carboxyl moiety, in order to favor substrate binding into the *P* sector and further successful catalysis.

Herein we provide kinetic data (Table I) under saturation conditions (zeroth-order kinetics), for the PLE-catalyzed hydrolysis of soluble hydrogen-bonding esters, in order to assess the minimal steric tolerance of *P* sites for transition-state complex formation.

Rate measurements were done at 37 °C and pH 7.50 (10 mM Tris buffer in 0.15 M NaCl), by following the release of free acid by titration with a 2-mL microburet (NaOH, phenolphtalein). Substrate concentrations were 3% per volume. All enzymic activities are given relative to tributyroyl-glycerol (3% aqueous suspension

0960-894X/98/$19.00
PII: S0960-894X(97)10169-X

with 5% gum Arabic), in order to normalize the activity of different enzyme solutions from the same stock preparation (Sigma). All esters were common chemicals from the shelf and were distilled prior to use.

The rate data indicate that the hydrogen-bonding substrates undergo hydrolysis at faster rates than the alkyl esters,[5] and no other obvious structure–reactivity relationship is evident, except for the fact that the enzyme fails to perform significant catalysis on the cationic substrate acetylcholine and the two small oxalate and formate esters.

Figure 1 shows a lack of correlation between the enzyme activities and the corresponding rates for aqueous hydrogen-ion catalyzed hydrolysis.[6] Therefore, the results shown in Table I are indeed of microenvironmental origin.

These data point to a greater degree of stabilization of transition-state (TS) complexes in the *P* microenvironment of the enzyme, than in either of the two *H* cavities. This effect probably occurs at the expense of hydrogen bonds formed more strongly in the TS than in the Michaelis complex, since kinetic measurements under k_{cat} regime imply that the enzyme–substrate complex is the initial state.

The kinetic solvation pressure[7] (KSP) is an extrathermodynamic quantity that measures the amount of isothermal work, per unit volume expansion, that is done in reorganizing the molecular microenvironment upon TS complex formation. It is formally defined as $(\partial\ \Delta G^*/\partial V)_T$, where V is the substrate partial molal volume.[8] For the case of enzyme reactions, this quantity relates to the steric tolerance of catalytic sites.

No significant dependence of rate on V is evidenced for the substrates capable of hydrogen bonding to the polar side chains in the *P* sector, at least up to 190 cm^3/mol. On the contrary, the *H* sector imposes steric restrictions of a magnitude that $(\partial\ \Delta G^*/\partial V)_T = -\ RT\ (\partial\ \ln k_{cat}/\partial V)_T = 53 \pm 8$ J/cm^3. The KSP of aqueous solvation shells is equal to 26 ± 4 J/cm^3 for the acid-catalyzed hydrolysis and saponification of alkyl acetates in water solvent.[7] A value of zero for KSP has been observed for the aqueous hydrogen-ion catalyzed hydrolysis of 2-alcoxyethyl acetates.[9] This result was interpreted as a minimal degree of rearrangment in the solvation sphere of these relatively hydrophylic esters, as the TS is reached.

The present enzymic data suggest a greater degree of atomic reorganization in the *H* sector upon activation, than in the *P* sector. This view agrees with the model of Jones[2], in which it is thought that substrate (and TS-complex) with hydrogen-bonding groups in the *P* cavities may extend beyond the enzymic realm, and into the surrounding aqueous region. This situation would certainly involve less microenvironmental reorganization when going from the Michaelis complex to the TS complex, thus showing a high degree of steric tolerance of the enzyme for substrates capable of hydrogen bonding, that is, a low or zero value of KSP.

The catalytic sites of other mammalian esterases seem to have higher degrees of plasticity toward the hydrolysis of *n*-alkyl acetates. The activity of isozyme ES-1A of rabbit liver esterase rises with increasing alkyl chain length, at least up to four carbon atoms,[10] whereas the esterolytic activity of rat liver ES-10 is highest with *n*-propyl acetate. The rate profiles of the two enzymes are similar, although rabbit liver ES-1A shows its maximum activity with the substrate having one more carbon atom in the alkyl chain.

Since the commercial preparation of PLE used in this work is a mixture of six isozymes, the point of molecular multiplicity must be addressed.

Lam and Jones[11] reported differences of up to 40% within the activities of the isozymes of PLE on the hydrolysis of the two non-hydrogen bonding methyl 4-*t*-butyl-cyclohexylcarboxylates. Differences in the specific activities of the isozymes ES-1A and ES-1B of rabbit liver esterase are also reported on the hydrolysis of *p*-nitrophenyl propionate and acetate,[10] although the steric tolerances are similar for both isozymes (propionate/acetate is 1.8 for ES-1A and 1.6 for ES-1B). A difference of greater magnitude has been reported by the same Dutch group[10] on the hydrolysis of the hydrogen bonding substrate aspirin. The esterolytic activity of the isozyme ES-1B is two times higher than the observed value for ES-1A.

The difference in the activities reported in the present work between the two types of substrates is of a much larger magnitude thus indicating that, despite the intrinsic difference between the PLE isozymes, the activity is greater for substrates capable of forming hydrogen bonds.

An interesting finding of T. Cynkowski et al.[12] is that many oxa-alcanoic ester prodrugs are good substrates for serum esterases. The hydrogen-bonding nature of the acyl chains in the prodrugs probably favors the binding of these substrates in the *P* sectors of the enzymes.

Another point that deserves a brief comment is the identical rates of hydrolysis of ethyl acetate and γ-butyrolactone. The result can be interpreted as a cisoid conformation of the substrate imposed by the enzyme in the *H* sector.

Thus, we conclude that it is anticipated that ester prodrugs with hydrogen-bonding groups near the ester moiety should be better substrates for liver esterase (and probably for other carboxyl esterases) than more hydrophobic ester analogs.

Table I. Relative rates of PLE-catalyzed hydrolysis of simple esters at 37 °C and pH 7.50

Substrate	Rel d(RCO_2H)/dt	$V°/cm^3\ mol^{-1}$
1. Tributyroyl-glycerol (emulsion)	1	293.0
Hydrogen-bonding substrates		
2. Triacetyl-glycerol	0.65 ± 0.03	188.2
3. 1-Monoacetyl-glycerol	0.70 ± 0.09	111.2
4. Ethyleneglycol diacetate	0.58 ± 0.05	132.1
5. 2-Ethoxyethyl acetate	0.37 ± 0.04	135.7
6. 2-Butoxyethyl acetate	1.8 ± 0.1	167.7
7. Ethyl lactate	0.87 ± 0.08	114.7
Non hydrogen-bonding substrates		
8. Methyl acetate	0.46 ± 0.03	79.4
9. Ethyl acetate	0.16 ± 0.01	97.9
10. *n*-Propyl acetate	0.23 ± 0.02	115.0
11. *n*-Butyl acetate	0.062 ± 0.005	131.6
12. γ-Butyrolactone	0.15 ± 0.01	76.3
13. Acetyl choline chloride	0.031 ± 0.04	-
14. Ethyl formate	0.17 ± 0.02	80.8
15. Diethyl oxalate	0.017 ± 0.002	135.5

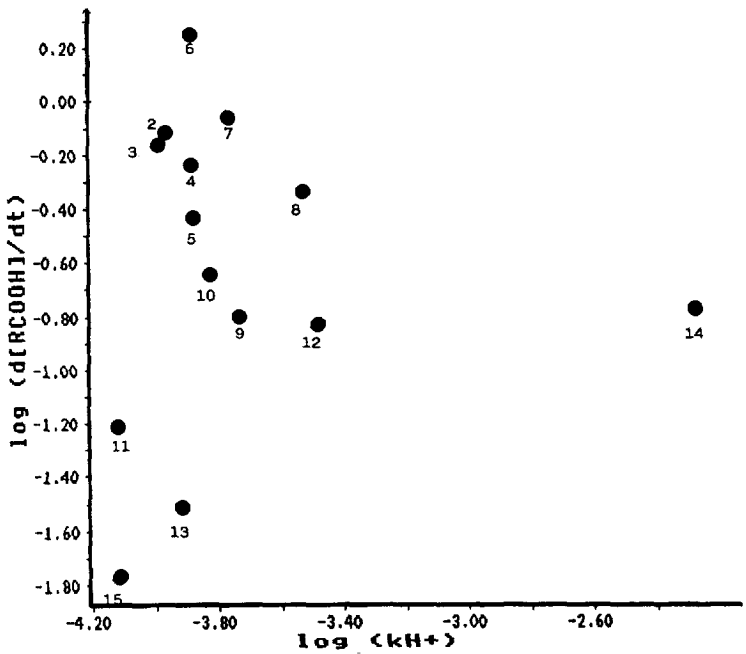

Figure 1. Lack of correlation between PLE activity and the specific rates of hydrogen-ion catalyzed hydrolysis of the substrate esters.

References and Notes

1. (a) Junge, W.; Krisch, K. *CRC Crit. Rev. Toxicol.* **1975**, *3*, 371; (b) Van Lith, H. A.; Haller, M.; Van Hoof, I. J. M.; Van der Wouw, M. J. A.; Van Zutphen, B. F. M.; Beynen, A. C., *Arch. Biochem. Biophys.* **1993**, *301*, 265; (c) Taylor, P.; Gibney, G.; Camp, S.; Maulet, Y.; Eksstrom, T.; Rachinsky, T.; Li, Y. In *Cholinesterases. Structure, Function, Mechanism, Genetics, and Cell Biology*; Massoulié, J; Bacou, F.; Barnard, E.; Chatonnet, A.; Doctor, B. P.; Quinn, D. M., Eds.; American Chemical Society Conference Proceedings: Washington, D. C., 1991; p 179.
2. (a) Jones, J. B. *Aldrichimica Acta* **1993**, *26*, 105; (b) Toone, E. J.; Werth, M. J.; Jones, J. B. *J. Am. Chem. Soc.* **1990**, *112*, 4946.
3. Provencher, L.; Jones, J. B. *J. Org. Chem.* **1994**, *59*, 2729.
4. Hultin, P. G.; Mueseler, F.-J.; Jones, J. B. *Ibid.* **1991**, *56*, 5375.
5. The average reaction rate in the hydrogen-bonding substrate group is four times faster, at the 99.5% certainty level: 0.8 ± 0.2, N = 6 vs. 0.16 ± 0.05, N = 8; t (calc) = 3.48 and t (p = 0.995) = 3.06.
6. Hydrogen-ion catalyzed reactions (0.1 M H_2SO_4) were followed by titration of the free RCO_2H with NaOH/phenolphtalein.
7. Mata-Segreda, J. F. *J. Am. Chem. Soc.* **1988**, *110*, 6224.
8. As a first approximation, V can be equated with the molar volume of the substrate, V°
9. Mata-Segreda, J. F. In *Atual. Fisico-Quim. Org.*; Humeres, J. J., Ed.; 1991; p 246.
10. Van Lith, H. A.; Haller, M.; Van Zutphen, B. F. M.; Beynen, A. C. *Eur. J. Biochem.* **1992**, *206*, 527.
11. Lam, L. K. P.; Jones, J. B. *J. Org. Chem.* **1988**, *53*, 2637.
12. Cynkowski, T.; Cynkowska, G.; Crooks, P. A.; Guo, H.; Ashton, P., *213th ACS National Meeting* 1997, abstract No. 118.