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A Structure–Activity Relationship for the Hydrolysis of Acetylamino Acids by Porcine Aminoacylase

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A structure–activity relationship is presented that satisfactorily predicts the rates of hydrolysis of a series of acetylglycine derivatives by porcine aminoacylase. It is apparent that the substrate specificity of aminoacylase is mainly kinetic in origin, the observed correlation with Taft's E_s parameter supporting the notion that enzymolysis proceeds through a mechanism that is analogous to chemical hydrolysis. It is suggested that the α-CH_2CH group of those substrates that possess this moiety is conformationally immobile upon binding. This lock facilitates rapid hydrolysis and results from steric interactions between the enzyme and substrate. The incorporation of α-methyl amino acid derivatives in the structure-activity relationship is consistent with a flexible active site model and it is concluded that the α-methyl effect in this system is a binding phenomenon. It is evident that the active center of porcine aminoacylase can comfortably accommodate amino acid derivatives with side chains containing less than six carbon atoms, contrary to previous assertions. It is suggested that the binding of bulkier derivatives necessitates the distortion of the active site. Derivatives possessing β-hydroxyl groups are found to deviate from expected behavior and a nonproductive binding model is presented.

Key Words: aminoacylase; acetylamino acids; structure–activity relationship; steric effects.

INTRODUCTION

Aminoacylase (*N*-acetylamino-acid amidohydrolase, EC 3.5.1.14) catalyses the hydrolysis of peptide linkages with a high specificity (*1–3*). It is reported that L-substrates hydrolyse at a rate some 10,000 to 40,000 times that of their D-enantiomorphs (*2*) and the purified enzyme is used extensively in preparative organic chemistry. For experimental reasons, much attention has been directed towards the hydrolysis of acetylamino acids (Scheme 1).

It has been concluded that reaction rates conform to Michaelis–Menten kinetics (*4*). The mechanism is believed to proceed through nucleophilic attack on the carbonyl carbon of the acylamino group (*4*), in a similar fashion to chemical hydrolysis. In spite of the large number of kinetic data that have been collected; however, little attempt has been made to draw quantitative conclusions regarding the relationship between substrate structure and catalytic efficiency. Undoubtedly, this is due to the

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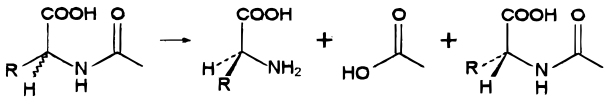
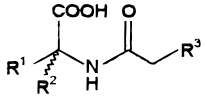


Scheme 1



1 $R^1 = H, R^3 = H$

2 $R^1 = H, R^3 = Cl$

3 $R^1 = Me, R^3 = Cl$

4 $R^1 = Et, R^3 = Cl$

difficulty of applying physical organic methodology to systems where interactions are complicated by the heterogeneous and topological nature of bioreceptors (*5*). Thus, Ötvös has shown that the specificity constants of a series of *N*-acyl-L-norvaline derivatives with aminoacylase may be predicted if the spatial orientation of the acyl substituent is taken into consideration (*6*).

Of greater interest to this study is Birnbaum's observation that the rates of hydrolysis of *N*-acetylamino acids by hog kidney aminoacylase correlate with a parameter that measures the polar effect of the amino acid side chain (*1*). The observed biphasic relationship is attributed to the dependence of the hydrolysis reaction on both electronic and steric effects (*1*). Problems remain with this interpretation, however. It is not clear why the rate of hydrolysis is reported to rise as the electron-releasing ability of the side chain increases, contrary to the generally accepted mechanism (*4*). Moreover, while the α-hydrogen derivatives possessing bulky side chains exhibit an excellent correspondence to the correlation that is ascribed to steric influences, the α-methyl derivatives exhibit significant scatter (*1*).

The purpose of this work is to present a revised analysis of Birnbaum's rate data that is both in accord with the established mechanism and provides novel insights into the catalytic mechanism.

RESULTS AND DISCUSSION

An extrathermodynamic approach has been used to correlate the rates of hydrolysis, *v*, measured under closely analogous conditions by Birnbaum using the ninhydrin

method (*1,2*). Appropriate physicochemical parameters are available for 33 acetylamino acid derivatives **1–4**. The rates of hydrolysis of 27 substrates are found to conform to Eq. [1], determined by multiparameter regression analysis;

$$\log v = (1.067 - 0.467\, I_1)\, E_s - 3.106\, I_2 + 0.528\, I_3 - 0.934\, I_4 + 4.963$$
$$r^2 = 0.989 \quad s = 0.129 \quad F = 387.8 \qquad [1]$$

Five parameters are required to ensure a satisfactory correspondence between experiment and expectation (Table 1). E_s is the Taft steric parameter for the R^2 substituent (*5,7*). The four *I* parameters are indicator variables that can adopt a value of 0 or 1. In other studies, such variables have been used to code for the key structural features of a substrate that influence activity (*8*), facilitating the study of complex substrate–receptor interactions. This analysis of Birnbaum's experimental data reveals that the enzyme discriminates between those substrates that posses the $-CH_2CH<$ moiety attached to the α-carbon atom ($I_1 = 1$) and those that do not ($I_1 = 0$). This specificity has not been reported previously and is evident in a plot of log *v* versus $E_s(R^2)$ (Fig. 1). The other indicator variables separate the α-hydrogen derivatives ($I_2 = 0$) from the α-alkyl derivatives ($I_2 = 1$), the acetyl derivatives ($I_3 = 0$) from the chloroacetyl derivatives ($I_3 = 1$), and the substrates that possess less than six carbon atoms in their side chains ($I_4 = 0$) from the other substrates ($I_4 = 1$).

The rate of hydrolysis is dependent on contributions from both binding, K_m, and the chemical steps, k_{cat}, according to Eq. [2],

$$\log v = \log k_{cat} + \log [E]_o[S] - \log (K_m + [S]). \qquad [2]$$

K_m values of 10, 7, 10, and 7 mM have been reported for those acetylamino acid derivatives with Me, Et, Pr, and Bu^i as the respective side chains (*9*). In Birnbaum's studies, the concentration of the L-substrates was 8 mM (*1,2*). As log (K_m + [*S*]) remains broadly constant for these substrates, the simplest rationale for the observed behavior is to suppose that log k_{cat} is linearly related to E_s for those α-H derivatives with less than six carbon atoms in their side chains. In accord with previous work (*10*), therefore, we propose that aminoacylase specificity is mainly of a kinetic nature, with exceptions discussed subsequently. The correlation of catalytic constants with E_s is in accord with the established mechanism (*4*). Although Taft's steric parameter has been criticized for including an electronic component (*5*), it exhibits an excellent correlation against minimum van der Waals radii (*11*), indicating that it is a reliable indicator of both bulk and shape.

Birnbaum has suggested that interactions between the polar regions of both the enzyme and substrate are important in establishing the Michaelis complex (*1*). Accordingly, it has been suggested that the substrate is bound to the enzyme by hydrogen bonding to the amino group (*10*). A model of the active site has been proposed in which the hydrogen bond donor is the thiol group of a cysteine residue (*12*). This binding is augmented by an electrostatic interaction between the amido carbonyl group and a closely bound Zn^{2+} ion that increases the susceptibility of C=O to nucleophilic attack (Fig. 2) (*12*). The identity of the nucleophile remains unclear,

TABLE 1

Enzymolysis Data and Physicochemical Parameters Used to Derive Equation 1 for Acetylamino Acid Derivatives.

N-acetyl-DL-	R^1	R^2	R^3	$E_s(R^2)$[a]	I_1[b]	I_2[b]	I_3[b]	I_4[b]	log (v/μmol h^{-1}) Exp.[c]	Calc.[d]	Resid
1 a Alanine	H	Me	H	−1.24	0	0	0	0	3.51	3.64	−0.13
b Valine	H	iso-Pr	H	−1.71	0	0	0	0	3.22	3.14	0.08
c Alloisoleucine	H	s-Bu	H	−2.37	0	0	0	0	2.40	2.43	−0.03
d Isoleucine	H	s-Bu	H	−2.37	0	0	0	0	2.58	2.43	0.15
e Phenylalanine	H	Bzl	H	−1.61	0	0	0	1	2.14	2.31	−0.17
f Leucine	H	iso-Bu	H	−2.17	1	0	0	0	3.73	3.66	0.07
g Norleucine	H	Bu	H	−1.63	1	0	0	0	4.16	3.98	0.18
h Glutamic acid	H	$CH_2CH_2CO_2H$	H	−2.21	1	0	0	0	3.49	3.64	−0.15
2 a Alanine	H	Me	Cl	−1.24	0	0	1	0	4.06	4.17	−0.11
b Valine	H	iso-Pr	Cl	−1.71	0	0	1	0	3.70	3.67	0.03
c Alloisoleucine	H	s-Bu	Cl	−2.37	0	0	1	0	3.00	2.96	0.04
d Isoleucine	H	s-Bu	Cl	−2.37	0	0	1	0	3.08	2.96	0.12
e *Tert*-leucine	H	t-Bu	Cl	−2.78	0	0	1	0	2.48	2.52	−0.04
f Aminophenylethanoic acid	H	Ph	Cl	−1.01	0	0	1	1	3.65	3.48	0.17
g Phenylalanine	H	Bzl	Cl	−1.61	0	0	1	1	2.66	2.84	−0.18
h 2-Aminobutanoic acid	H	Et	Cl	−1.31	1	0	1	0	4.53	4.70	−0.17
i Norvaline	H	Pr	Cl	−1.60	1	0	1	0	4.61	4.53	0.08
j Leucine	H	iso-Bu	Cl	−2.17	1	0	1	0	4.22	4.19	0.03
k Norleucine	H	Bu	Cl	−1.63	1	0	1	0	4.48	4.51	−0.03
l 2-Aminoheptanoic acid	H	Pe	Cl	−1.64	1	0	1	0	4.45	4.51	−0.06
m 2-Aminooctanoic acid	H	Hx	Cl	−1.54	1	0	1	1	3.89	3.63	0.26
n 2-Amino-3-cyclohexyl propanoic acid	H	CH_2cHx	Cl	−3.22	1	0	1	1	2.54	2.62	−0.08
o Glutamic acid	H	$CH_2CH_2CO_2H$	Cl	−2.21	1	0	1	0	4.10	4.16	−0.06
p Aminocyclohexylethanoic acid[e]	H	*c*Hx	Cl	−2.03	0	0	1	1	3.66	2.39	1.27
q Glycine[e]	H	H	Cl	0	0	0	1	0	3.42	5.49	−2.07
r Serine[e]	H	CH_2OH	Cl	−1.21	1	0	1	0	4.06	4.76	−0.70
s Threonine[e]	H	CH(OH)Me	Cl	−1.15	0	0	1	0	2.86	4.26	−1.40
t Allothreonine[e]	H	CH(OH)Me	Cl	−1.15	0	0	1	0	3.41	4.26	−0.85
3 a Dimethylglycine	Me	Me	Cl	−1.24	0	1	1	0	1.04	1.06	−0.02
b α-methyl valine	Me	iso-Pr	Cl	−1.71	0	1	1	0	0.54	0.56	−0.02
c Isovaline	Me	Et	Cl	−1.31	1	1	1	0	1.57	1.60	−0.03
d α-Methyl norvaline	Me	Pr	Cl	−1.60	1	1	1	0	1.49	1.42	0.07
4 Diethylglycine[e]	Et	Et	Cl	−1.31	1	1	1	0	f	1.60	

[a] Ref. 5.
[b] Indicator variable, see text.
[c] Experimental data recorded at pH 7.0 and 38 °C from Refs. *1* and *2*.
[d] Calculated using Eq. [1]
[e] Not included in the derivation of Eq. [1]
[f] Not hydrolyzed by aminoacylase under the experimental conditions.