THE INFLUENCE OF THE GEOMETRIC PROPERTIES OF THE ACTIVE CENTRE ON THE SPECIFICITY OF α-CHYMOTRYPSIN CATALYSIS

V.N. DOROVSKA, S.D. VARFOLOMEYEV, N.F. KAZANSKAYA, A.A. KLYOSOV and Karel MARTINEK

Laboratory of Bio-organic Chemistry ("A" Building), Lomonosov State University, Moscow 117 234, USSR

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1. Introduction

The Taft-Ingold relationship modified to include a specificity constant, S, has proved fruitful for analysing rates of α-chymotrypsin (CT) catalysed reactions [1, 2]:

$$\log k = \rho\sigma^* + \delta E_s + S + \text{constant.} \quad (1)$$

Since the specific protein–substrate interaction is primarily hydrophobic (see [3] and references therein), function S of eq. (1) should relate to substrate hydrophobicity. For this purpose the Hansch constant, π, can be used [2].

As an extension to previous works [1–4] we should like here to discuss the following characteristics of CT catalysis in the framework of eq. (1)*:
(i) The complex (non-linear) character of S–π profiles is a *quantitative* reflection of the geometric properties of the hydrophobic cavity in the CT active centre.
(ii) The dependence of reaction rate on steric, inductive and specific (hydrophobic) factors is different, for different classes of substrates, as well as for different stages in the enzymatic reaction.

We confine ourselves to a discussion of the following:
(a) Data on the rate of hydrolysis of the intermediate acyl-enzymes, derivatives of straight-chain (our data, for details see [5]) and branched [1, 4] aliphatic as well as phenylalkyl [1] carboxylic acids;

(b) The separation of polar, steric and specific effects in CT hydrolysis of the virtual substrates, derivatives of *N*-acylated-L-amino acids is of special interest. Unfortunately, the inductive (σ^*) and steric (E_s) constants relating to side chains of amino acids have not yet been estimated with sufficient reliability. Therefore, to elucidate the importance of polar and steric effects we have determined the rate constants for alkaline hydrolysis of a number of *N*-acetyl-L-amino acid methyl esters.

2. Results and discussion

From the extensive data of Dupaix et al. [1] on deacylation of acyl-chymotrypsin derivatives of aliphatic carboxylic acids with sufficiently short or branched side chains (the compounds listed in fig. 1) eq. (2) has been derived (regression coefficient, $r = 0.993$):

$$\log k_3 = -(0.03 \pm 0.05)\pi + (2.29 \pm 0.06)\sigma^* + (0.72 \pm 0.04)E_s - (1.84 \pm 0.07) \quad (2)$$

As can be seen, the influence of hydrophobicity of the acyl group on the deacylation rate constant is in this case negligibly small. On the contrary, enzyme–substrate hydrophobic interaction has a predominant influence on the deacylation rate constants for the acyl-enzymes with comparatively long *n*-alkyl chains, such as butyryl, valeryl, hexanoyl and heptanoyl (our data, for details see [5]). Omitting for these

* When this paper was being prepared a preprint of a similar work by Hansch [8] came to our attention.

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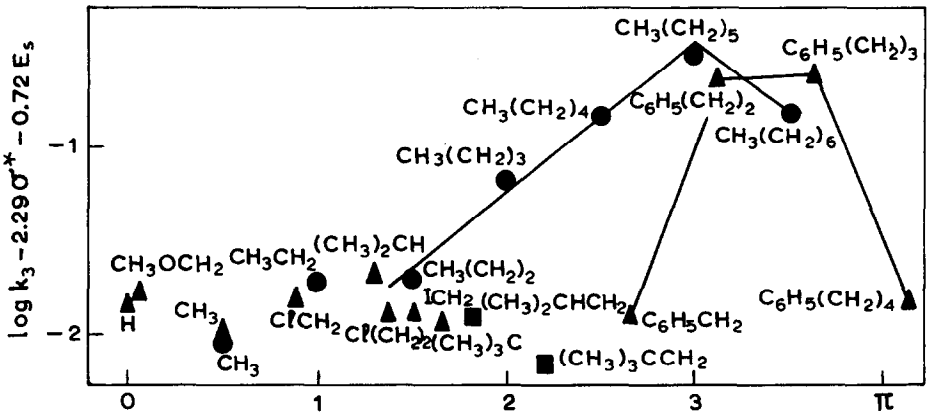

Fig. 1. The deacylation rate data for acylchymotrypsins, RC(O)–enz, plotted in the coordinates of eq. (2). The k_3 values were measured by Fife and Milstien [4] (■); by Dupaix et al. [1] (▲–▲–▲); and in our investigation [5] (●–●–●). Experimental conditions: 25°, pH > 8. Values σ* and E_s are the Taft's aliphatic constants for the acyl groups. The π values are the Hansch constants for the R substituents [2].

Table 1
Kinetic parameters for hydrolysis of *N*-acetyl-L-amino acid methyl esters.

	Amino acid	k_2/K_s* $sec^{-1}\ M^{-1}$	k_{OH}** $sec^{-1}\ M^{-1}$
1	Gly	0.126	2.48
2	Ala	1.78	1.31
3	α-amino But	21.1	0.71
4	nor-Val	355	0.58
5	nor-Leu	3000	–
6	Phe	104000	1.94
7	Val	1.97	0.16
8	iso-Leu	2.47	0.09

* Over-all rate constants in CT catalyzed hydrolysis, pH 7.8 (pH-stat), 0.1 M KCl, 25°. For experimental details see [3].

** Rate constants for alkaline hydrolysis, pH 10 (pH-stat), 0.1 M KCl, 25°.

compounds functions σ* and E_s (which have approximately constant values in this series), eq. (3) was obtained ($r = 0.994$):

$$\log k_3 = (0.72 \pm 0.09)\pi - (3.28 \pm 0.21) \qquad (3)$$

Comparing eqs. (2) and (3), we can see that the geometry of the substrate side group produces a strong effect on the relationship between hydrophobicity and reactivity of CT substrates (compare the coefficients before π in both equations). This is apparently connected with the fact that with branched side groups the strength of enzyme–substrate interaction is reduced due to geometric incongruence of the substrate acyl group with respect to the cavity of the active centre. In general, the specific effects resulting from geometric features of the active centre give rise to a complex dependence of the specificity constant, *S*, upon π, as can be seen from fig. 1.

The model also accommodates the data on the rates of chymotryptic hydrolysis of *N*-acetyl-L-amino acid methyl esters (see table 1). Fig. 2 shows that the compounds with branched aliphatic side chains display large deviations from linear dependence of reactivity upon hydrophobicity of the substrate side group.

For a certain size of the substrate side chain the specific effects reach a maximum (fig. 1), the existence of which has been discussed qualitatively [6, 7]. Here it is shown for the first time that the maximum occurs at almost the same value of hydrophobicity, π, both for aliphatic and phenylalkyl derivatives. The most important conclusion from the present work is that the maximum specificity effect, *S*, is also the same for both sets of substrates (fig. 1). These findings may be very important in elucidating the intimate mechanism of CT specificity [9].

Of particular interest is the observation that for different classes of substrates the dependence of reac-

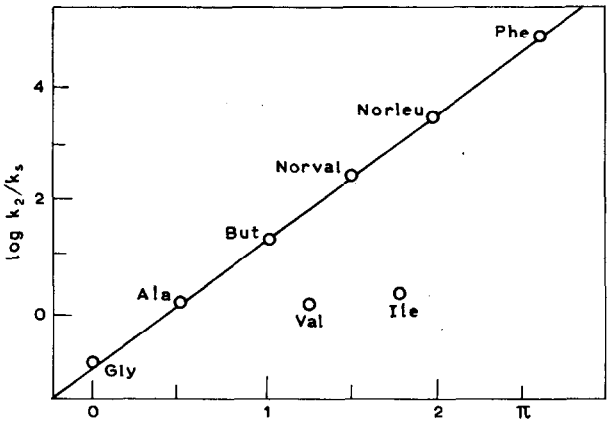

Fig. 2. The values of rate constants (k_2/K_s) for chymotryptic hydrolysis of methyl esters of *N*-acetyl-L-amino acids, $RCH(NHCOCH_3)C(O)OCH_3$, against the hydrophobicity π of corresponding R substituents. Experimental conditions are given in the table. The π values are the Hansch constants for the R substituents [2].

tion rates upon steric and inductive effects is essentially different. Derivatives of aliphatic carboxylic acids with branched or sufficiently short alkyl chains (which do not interact with a hydrophobic part of the enzyme active centre) display the usual order of reactivity with respect to steric and inductive factors [1, 4]. However, this is not so for the virtual substrates, derivatives of *N*-acylated-L-amino acids. Comparing the rate constants of enzymatic and spontaneous (alkaline) hydrolysis of methyl esters of *N*-acylated-L-amino acids (compounds 1–6 in table 1), the following equation was obtained ($r = 0.999$):

$$\log (k_2/K_s) = (2.25 \pm 0.05)\pi - (0.06 \pm 0.1)\log k_{OH} - (0.88 \pm 0.07) \quad (4)$$

As can be seen, the rate constant of the enzymatic reaction depends practically solely on hydrophobicity of the side group, R, compare [3]. The absence of the steric effect with the virtual substrates can easily be accounted for by the suggestion that the side group is firmly set in a "chink" in the active centre so that there is no hindrance to catalytically functional groups of the enzyme attacking the susceptible carbonyl group of the substrate.

The steric limitations of the specificity cavity in CT, discussed in the present paper, are consistent with the proposed crystal structure of the enzyme. Thus, Steitz et al. [10] believe that the hydrophobic slit which binds the substrate side chains is a flattened shape whose dimensions are 10–12 by 5.5–6.5 by 3.5–4.0 Å, the narrowness of the slit allowing only one orientation of the plane of bound aromatic side chains.

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