SPECIFICITY OF α-CHYMOTRYSPIN

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The specificity of the proteolytic enzyme α-chymotrypsin for different amino acid side chains has been postulated to arise from hydrophobic interaction between the enzyme and substrate [1]. For quantitative verification of this conception it would have been useful to consider the free energy profile (fig. 1) of enzyme reactions for isochemical series of substrates in terms of the following scheme:

$$E + S \overset{K_s}{\rightleftharpoons} ES \underset{P_1}{\overset{k_2}{\rightleftharpoons}} EA \xrightarrow{k_3} E + P_2 \qquad (1)$$

where E, ES and EA stand, as usual, for free enzyme, Michaelis complex and acylenzyme, and P_1 and P_2 for the products of hydrolysis of substrate (S), respectively.

At present, however, such an approach seems hardly possible because the thermodynamic dissociation constant of ES (K_s) and the catalytic rate constants (k_2, k_3) for substrates of α-chymotrypsin are practically unavailable. In terms of the above scheme the measured steady-state parameters are $K_{m\,(app)} = K_s k_3/(k_2 + k_3)$, amd $k_{cat} = k_2 k_3/(k_2 + k_3)$. It follows from these expressions that $k_{cat}/K_{m\,(app)} = k_2/K_s$ and hence it is not difficult to evaluate the overall free energy of activation $\Delta F^{\ddagger}$ for the process of formation of acylenzyme EA as the sum of $\Delta F_2^{\ddagger}$ and ΔF_s, as is also shown in fig. 1.

It might also be suggested that the value of k_{cat} is a first approximation for the rate constant of the step that limits the steady-state rate of the enzyme reaction. For instance, when a number of ester substrates is hydrolysed by α-chymotrypsin the rate limiting step is that of enzyme deacylation [2]. In this case $k_{cat} \sim k_3$.

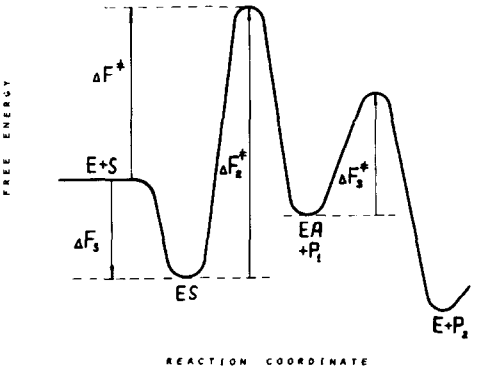

Fig. 1. Diagrammatic representation of the free energy profile of scheme (1).

In fig. 2 are given the values of $\Delta F^{\ddagger}$ (relating to $k_{cat}/K_{m\,(app)}$) and $\Delta F_{cat}^{\ddagger}$ (relating to k_{cat}) that describe the kinetics of hydrolysis by α-chymotrypsin of the series of substrates: $RCH(NHCOCH_3)C(O)OCH_3$, calculated from the data reported by Niemann and coworkers [3]. The substrates in question differ only in the size of the chemically inert hydrocarbon group R. Along the X-axis are plotted the values of the free energy increment of binding of this group R on the α-chymotrypsin active centre. These values of $\Delta\Delta F_s^{(R)}$ were estimated from studies of the binding of aliphatic alcohols ROH at the α-chymotrypsin active centre [4, 5]. The value of $\Delta\Delta F_s^{(R)}$ for the $C_6H_5CH_2$ –group, corresponding to the phenylalanine derivative (point 7 of fig. 2) was derived from data on inhibition of catalytic activity of α-chymotrypsin by substituted benzenes [5].

The linear correlations presented in fig. 2 show

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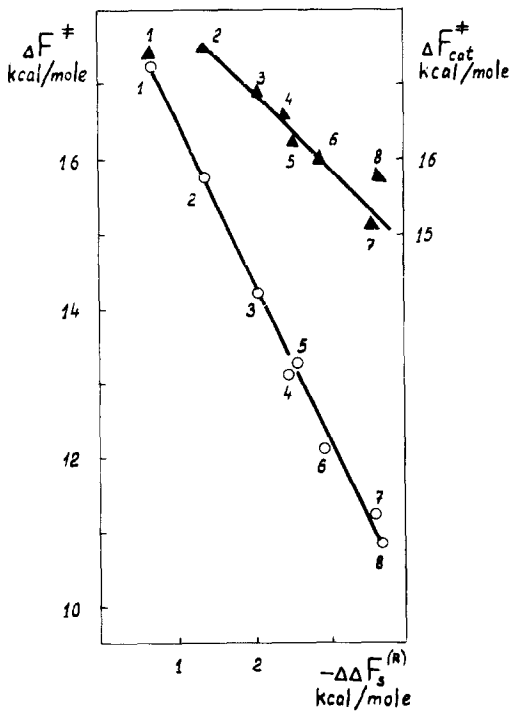

Fig. 2. Dependence of the free energy of activation $\Delta F^{\ddagger}$ and $\Delta F^{\ddagger}_{cat}$ observed on hydrolysis of substrates $RCH(NHCOCH_3)C(O)OCH_3$ (in terms of data reported by Nimenn and coworkers [3]) upon the increment of the free energy of noncovalent binding on the α-chymotrypsin active centre of the hydrocarbon group R [4, 5]. The substrates considered were methyl esters of the following *N*-acetyl-L-aminoacids:

(1) alanine, (2) α-aminobutyric acid, (3) norvaline, (4) leucine, (5) norleucine, (6) α-aminoheptanoic acid, (7) phenylalanine, (8) cyclohexylalanine.

that similar types of forces are involved in determining both binding and kinetic specificity displayed by α-chymotrypsin. That is to say, these correlations (fig. 2) suggest that the formation both of the enzyme-inhibitor complex (and of the Michaelis complex) and of the transition states of the catalytic steps involve the same type of interaction, that is, the hydrophobic binding of the side group R of substrate to the active centre [1, 3, 6]. Our analysis presented in figs. 1 and 2 illustrates quantitatively the postulate advanced by Knowles: "Better binding – better analysis" [1].

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