The Correlation of Enzymic Rates

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THIS Communication is a preliminary account of a correlation of the deacylation rates (k_3) of acyl-chymotrypsins; it is the first treatment which allows the prediction of enzyme rate constants for a range of derivatives conforming to the model R^1R^2CHCO–chymotrypsin.

Equation (1) correlates the deacylation rates of substituted benzoyl-, trimethylacetyl-, acetyl- and formyl-chymotrypsins;[1–4]

$$\log_{10}k_3 = 0{\cdot}85 \log_{10}k_{OH^-} - 1{\cdot}2 \qquad (1)$$

(The degree of fit[5] $\phi = 92\%$, with ten experimental points.)

where k_{OH^-} is the rate constant for the hydroxide ion-catalysed hydrolysis of the corresponding ethyl ester. The structures of deviant acyl groups are such that they would be expected to deviate; the deviation is a measure of a binding between acyl group and protein.

It can be shown[4] that

$$k_3 = \frac{\Sigma 1/K_i}{(1 + \Sigma 1/K_i + \Sigma 1/K_j)} \times k \qquad (2)$$

where K_i is the dissociation constant for a binding which aligns the acyl group favourably for reaction and K_j is a dissociation constant for an unfavourable binding. The hypothetical rate constant k for reaction of the acyl group when fixed in a favourable configuration is proportional to the rate constant for reactions of acyl groups with no binding complications (*e.g.*, k_{OH^-}). The dissociation constant of each configuration can be expressed as the multiple of the microscopic binding constants $K = K_1 \cdot K_2 \cdot K_C$ for the binding of each part (R^1, R^2 . . .) of the acyl group with a site (ρ_1, ρ_2 . . .) on the protein.[6,7] Assumptions are that $R^1\rho_1$, $R^2\rho_2$ interactions are mutually exclusive, and are respectively hydrogen bonding of an amido-group

TABLE 1.

R^1	S_1	R^2	S_2
acetamido-	1·63	*p*-hydroxybenzyl-	2·35
benzamido-	1·17	benzyl-	1·96
benzyloxycarbamido	1·2	3-methylbutyl-	1·22
		(3-indolyl)methyl-	1·83

(k_3's [1-5 9-11] and k_{OH^-}'s[4,12] refer to 25°, 0·1-M ionic concentration).

TABLE 2.

Acetyl-D-derivative	k_3(obs.) × 10^2sec.$^{-1}$	k_3(calc.) × 10^2sec.$^{-1}$
Phenylalanine	3·0	2·1
Leucine	6·75	5·1
Tryptophan	5·6	2·33

[k_3(calc.) derived from equation (5)].

and hydrophobic binding; if R^1 and R^2 are small (*e.g.*, hydrogen) there is negligible binding; there are no $R^1\rho_2$ or $R^2\rho_1$ interactions (complications can arise here[8] and are dealt with in a later publication); the binding of the rest of the acyl group in the reactive configuration is constant (K_c).

Equation (3) correlates k_3 for L-derivatives of amino-acids (ϕ = 87%, 22 experimental values).

$$\log_{10}k_3 = 0{\cdot}85\log_{10}k_{OH^-} - 1{\cdot}2 + S_1 + S_2 \quad (3)$$

This empirical equation can be derived from equation (2) if only one favourable configuration predominates and if $K_1{\cdot}K_2{\cdot}K_c > 1$.

An important corollary is that k_3's for D-derivatives of amino-acids fit equation (4) derived from the theoretical equation. Allowing the assumptions already stated, the D-acyl group has three predominant configurations represented by the interactions $R^1\rho_1$, $R^2\rho_2$ and $R^1\rho_1{\cdot}R^2\rho_2$. The last situation is unfavourable for reaction because the carbonyl must be distorted from the reactive configuration obtained with the corresponding L-compound.

$$k_3 = \frac{(1/K_1 + 1/K_2)}{(1 + 1/K_1{\cdot}K_c + 1/K_2K_c + 1/K_1K_2K_c')} \times \frac{k}{K_c} \quad (4)$$

K_c for the reactive configuration differs from that for the unreactive one; k_3 decreases as $K_1{\cdot}K_2K_c'$ decreases. The data for acetyl-D-derivatives[9] (Table 2) fit the empirical equation (5) which can be

$$k_3 = \frac{\text{antilog } S_1 + \text{antilog } S_2}{\text{antilog } S_1{\cdot}\text{ antilog } S_2} \times 61 \times 10^{-2}\,\text{sec.}^{-1} \quad (5)$$

derived from equation (4) using the assumptions given above.

The binding of derivatives not conforming to the prototype can be estimated using equation (1): cinnamoylchymotrypsin has $\log_{10}k_3(\text{obs.})/k_3(\text{calc.}) = 1{\cdot}2$ indicating less binding than in the fully saturated compound ($S_2 = 1{\cdot}96$). An explanation is that the cinnamoyl group is constrained from binding fully at the ρ_2 site.

The reactivity of acylchymotrypsins, represented by equation (1), allows the comparison of enzymic reactivity with model systems because binding effects, not directly concerned with reaction, are excluded.

That specificity in k_3 is largely entropy controlled[7] is supported by the good correlation obtained in this treatment.

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