A Linear Free Energy Relationship for Inhibitor Binding to Carboxypeptidase A

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Summary The binding of carboxylate anion competitive inhibitors of esterase activity to carboxypeptidase A correlates with Hansch's π-parameter.

CARBOXYPEPTIDASE A specifically catalyses the hydrolysis of the *C*-terminal amide bonds of peptides (and analogous ester bonds) with a preference for *C*-terminal amino-acids (or α-hydroxycarboxylic acids) with aromatic or large aliphatic side chains.[1] Carboxylate anions with similar types of hydrocarbon moieties are reversible inhibitors of the enzyme. *X*-Ray crystallographic studies of crystals of this enzyme have indicated that the active site contains a convenient hydrophobic pocket for the acceptance of such entities.[2]

As part of a comprehensive quantitative survey of the substrate and inhibitor specificity of this enzyme, we have measured inhibition constants (K_I) for a number of carboxylate anion competitive inhibitors of esterase activity. In the Figure, the log K_I values for eleven of these inhibitors are plotted against Hansch's π-parameter[3] which quantitatively reflects the hydrophobic character of the hydrocarbon residue of the carboxylate anion. The data appears to fall on to two linear plots of similar slope, with aliphatic carboxylate anions all being accommodated on one line, and all inhibitors having an aromatic ring lying on the other line. Least-squares fitting of these data gives the relationships in equations (1) (correlation coefficient = 0·981) and (2) (correlation coefficient = 0·980) for the aliphatic and aromatic compounds, respectively.

$$-\log K_I \text{ (aliph)} = 1{\cdot}7\pi + 0{\cdot}50 \quad (1)$$

$$-\log K_I \text{ (arom)} = 1{\cdot}5\pi - 0{\cdot}90 \quad (2)$$

Inhibitors with longer hydrocarbon side-chains than those presented in the Figure do not fit the relationships (1) and (2), and apparently are too large to allow the hydrocarbon moiety to fit into the hydrophobic pocket in its energetically most favourable conformation.

Since the pre-π factors in equations (1) and (2) are approximately the same within experimental error, these equations may be combined to give equation (3) for aliphatic and aromatic inhibitors with equal π values.

$$\log K_I \text{ (arom)} = \log K_I \text{ (aliph)} + 1{\cdot}40 \quad (3)$$

Equation (3) suggests that there is a fundamental difference in the binding of aromatic and aliphatic hydrocarbon moieties to the enzyme.

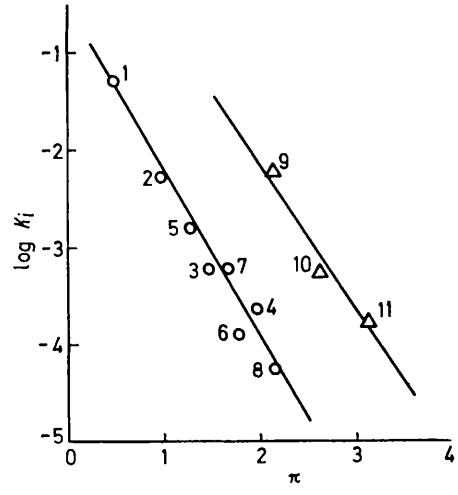

FIGURE. *Dependence of log* K_I *on* π. K_I *measured at* pH 7·5, 25°, *ionic strength* 0·2 (NaCl) *with* O-*hippuryl*-L-3-*phenyllactic acid as substrate. Inhibitors* (RCO_2^-): (1) Me, (2) Et, (3) Pr^n, (4) Bu^n, (5) Pr^i, (6) Bu^i, (7) Pu^t, (8) Me_3CCH_2, (9) Ph, (10) $PhCH_2$, (11) $Ph(CH_2)_2$.

Equations (1) and (2) represent linear free energy relationships for the binding of carboxylate anion inhibitors in the active site of carboxypeptidase A. The close correlation of K_I with π further substantiates the importance of hydrophobic interactions for the binding of substrates and inhibitors in the active site of this enzyme. Our preliminary data[4] on the K_m values for hydrolysis of hippuric acid esters by this enzyme suggest that substrate binding also may be correlated with π.

This research was supported by a grant from the National Research Council of Canada and an NRCC Scholarship (C.D.M.).

(*Received*, 10*th April* 1972; *Com.* 608.)

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