ON THE MODES OF INTERACTION BETWEEN COMPETITIVE INHIBITORS AND THE α-CHYMOTRYPSIN ACTIVE CENTRE

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1. Introduction

The "binding specificity" of α-chymotrypsin (CT) for different amino acid side-chains has been postulated to arise simply from a hydrophobic interaction between enzyme and substrate [1]. Any present day binding model of the enzyme-substrate or enzyme-inhibitor complex formation should take into account correlations between affinity of substrate or inhibitor for the enzyme and parameters relevant to the hydrophobic characteristics of these compounds [1–3]. The most correct approach to the study of hydrophobic interaction is that of comparing the free energy of process (1):

$$(I)_{\text{in water}} \overset{K_i}{\rightleftharpoons} (I)_{\text{in the active centre medium}} \qquad (1)$$

with free energy of inhibitor extraction (2) from water by the non-aqueous solvent:

$$(I)_{\text{in water}} \overset{P}{\rightleftharpoons} (I)_{\text{in non-aqueous solvent}} \qquad (2)$$

where $\Delta F_i = -2.3\ RT \log K_i$ and $\Delta F_{\text{extraction}} = -2.3\ RT \log P$. We used the above approach to study the nature of noncovalent interaction of some aromatic and aliphatic compounds with the CT active centre. For our work, hydrophobic binding is operationally defined by the water-octanol reference system [4]. Wildnauer and Canady [5] have recently shown that for certain α-chymotrypsin inhibitors, aliphatic hydrocarbons such as pentane, hexane, heptane and water serve as suitable reference systems.

2. Experimental

The purity of CT used was as previously described [6].

To study complex formation of various compounds with CT, use was made of competition between these inhibitors and proflavine for a binding site on the enzyme [7]. The equilibrium state in the system: CT-dye-competitive inhibitor was determined by a spectrophotometric method [8].

3. Results and discussion

Fig. 1 shows the relationship between inhibitor constants (K_i) and octanol-water partition coeffi-cients (P) for a number of aromatic compounds. The linearity of this relationship is a feature of such mono-substituted benzenes (and certain aromatic hydrocarbons) whose hydrophobic properties are stronger than those of benzene itself (line labelled a). For our work, hydrophobic properties of compounds studied were operationally defined by Hansch's reference system [4]. The slope of the straight line a (fig. 1) is approximately equal to 1. It reveals that the free energy increment of interaction of any hydrophobic group -X, in the inhibitor molecule RX, with the active centre is approximately equal to the free energy

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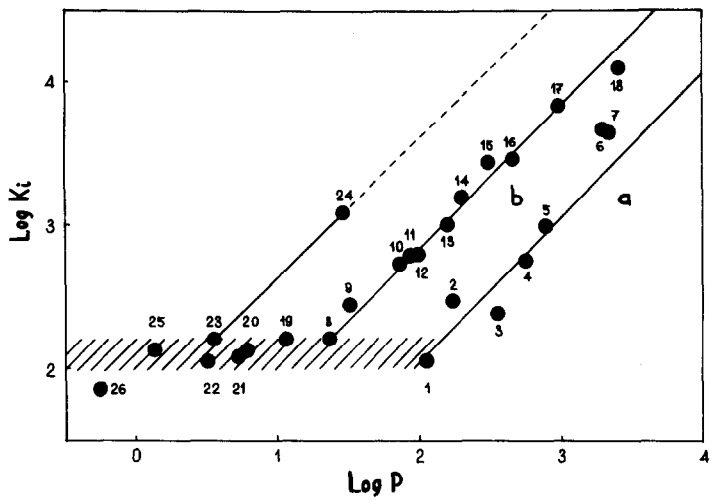

Fig. 1. Relationship between the binding constants (K_i, 1/mole) of aromatic compounds with the CT active centre and octanol-water partition coefficients *(P)*. Temperature 30°C, pH 8.0 (0.01 M tris-HCl), μ 0.1 (NaCl).
(1) benzene, (2) *N,N*-dimethylaniline, (3) toluene, (4) chlorobenzene, (5) bromobenzene, (6) naphthalene, (7) acridine, (8) phenol, (9) *m*-methoxyphenol, (10) *p*-cresol, (11) *m*-cresol, (12) *o*-chlorophenol, (13) *o*-bromophenol, (14) *p*-chlorophenol, (15) *p*-bromophenol, (16) 2-naphthol, (17) 2,4-dichlorophenol, (18) 2,4-dibromophenol, (19) acetanilide, (20) aniline, (21) resorcinol, (22) hydroquinone, (23) benzamide, (24) *p*-bromobenzamide, (25) *p*-hydroxybenzamide, (26) *p*-aminophenol. The log *P* values were measured by Hansch et al. [13].

increment required to transfer this group from water to the non-aqueous solvent:

$$\Delta\Delta F_i^X \approx \Delta\Delta F^X_{\text{extraction}}, \qquad (3)$$

where $\Delta\Delta F_i^X = \Delta F_i^{RX} - \Delta F_i^{RH}$

and $\Delta\Delta F^X_{\text{extraction}} = \Delta F^{RX}_{\text{extraction}} - \Delta F^{RH}_{\text{extraction}}$.

The correlation obtained (equation 3) is in agreement with the data of Canady and coworkers [5, 9]. These authors have compared the inhibiting capacity of some aromatic hydrocarbons with surface area of these compounds [3, 9] and with their solubility in water [5].

On the contrary, benzene derivatives containing substituents -X, whose free energy increment of extraction process (2) is larger than zero, do not differ in their inhibiting capacity (fig. 1, the points labelled 8, 19, 20, 23). This evidence is in agreement with the data of Niemann and coworkers [10]. On the other hand, their respective $\Delta F^{RX}_{\text{extraction}}$ values (measured in the octanol-water reference system) change significantly. Proceeding from this, it is possible conclude that on enzyme-inhibitor complex formation the

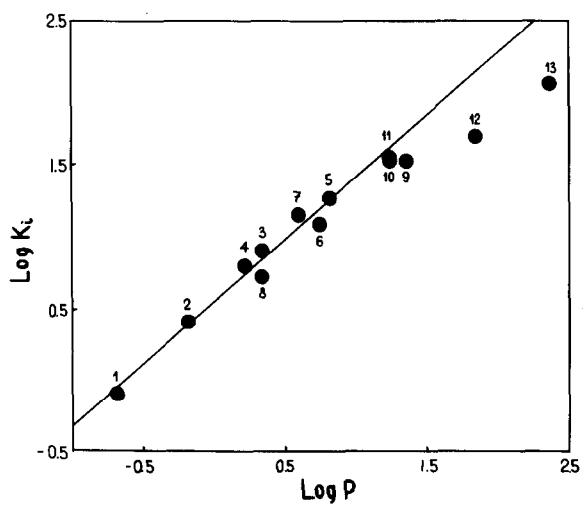

Fig. 2. Relationship between the binding constants (K_i, 1/mole) of aliphatic alcohols with the CT active centre and respective ocatanol-water partition coefficients *(P)*. The experimental conditions were similar to those described in fig. 1.
Alcohols: (1) methyl, (2) ethyl, (3) *n*-propyl, (4) isopropyl, (5) *n*-butyl, (6) isobutyl, (7) sec-butyl, (8) tert-butyl, (9) pri-*n*-amyl, (10) isoamyl, (11) cyclohexyl, (12) *n*-hexyl, (13) *n*-heptyl. The log *P* values are taken from Hansch et al. [14, 15].

polar groups are not transferred into a nonpolar environment of the active centre.

Further evidence in favour of this pattern comes from the studies with substituted phenols. In the case of hydrophobic substituents (i.e. those substituents with free energy increments of extraction (2) less than zero) complete extraction of these groups by the CT active centre takes place on enzyme-inhibitor complex formation. This follows from equation (4):

$$\Delta\Delta F_i^X = 0.95\ \Delta\Delta F^X_{\text{extraction}} \qquad (4)$$

which describes the slope of the straight line b in fig. 1. Furthermore inhibiting capacities (log K_i) of phenol and *p*-hydroxybenzamide (fig. 1, the points labelled 8, 25) are similar, though the difference in corresponding log P values is considerable.

While studying reversible binding of aliphatic alcohols to CT active centre we also came to the conclusion that the main factor in enzyme-inhibitor complex formation is hydrophobic interaction. The data given in fig. 2 show that in case of C_1-C_4 aliphatic alcohols ROH the enzyme-inhibitor binding (as defined log K_i) depends linearly on the hydrophobic character of R group (as defined by the octanol-water reference system). In case of C_5-C_7 *n*-alkanols a slight deviation from the linearity was observed. The free energy increment $\Delta\Delta F_i^{CH_2}$ of interaction of every CH_2-group in the inhibitor molecule with the CT active centre is equal to –700 cal/mole (for the lower members of the homologous series of *n*-alkanols). This value is characteristic of the "liquid" CH_2-group transfer from water into the apolar (non-aqueous) environment [11, 12].

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