Determination of a Constant for a Specific Conformational Transition

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There is increasing evidence that the specific complex formation presumably inherent in the biological activity of a protein is often accompanied by detectable changes in the conformation of the protein. Most of the evidence concerns enzymes and their interaction with substrates or allosteric effectors. The mechanism and the functional significance of the conformational changes have been correlated in two widely accepted models, the 'induced fit' model (Koshland, 1959) for enzyme-substrate interactions, and the allosteric model (Monod, Changeux and Jacob, 1963) for protein-effector interactions. The effect of cofactors (Grisolia, 1964) or competitive inhibitors (Garber and Citri, 1962) on the conformation of enzymes, and indeed analogous conformational shifts associated with specific complex formation in other proteins (antibodies, carrier proteins, hormones) may also be related to their respective functions.

It seems desirable to make a clear distinction between the effect of conformation-disrupting agents (e.g. urea, heat) which is generalized and nonspecific, and the limited, reversible conformational transition accompanying specific complex formation. We suggest that the latter be termed <u>conformative response</u>. This term is meant to stress the

specificity of the change in conformation and to indicate the possibility of functional significance.

For quantitative expression of a conformative response we suggest a constant, termed conformative response constant (K_{cr}), which is defined as numerically equal to the concentration (M) of the substrate (sensu lato) which causes half the maximal change in the property taken as a measure of the conformative response. The following example illustrates the determination of K_{cr} and its relation to the relevant functional (catalytic) constants.

Exposure of penicillinase to certain analogs of the substrate (penicillin) causes unmasking of reactive groups in the active site (Citri, Garber and Kalkstein, 1964). Such analogs sensitize the enzyme to iodination in a way suggesting the following scheme:-

(1) $E + A \rightleftharpoons E'A \longrightarrow E + P$

(2) $E'A + I \longrightarrow EI + A$ (or EIA)

where E' is the enzyme in the iodine-sensitive conformation (A = analog; P = product; I = iodine).

We found that at 0^{o} the equilibration of $E + A \rightleftharpoons E'A$ is much more rapid than the rate of inactivation by iodine. The rate of product formation is negligible at 0^{o} and under these conditions equation (1) becomes:

(3) $E + A \rightleftharpoons E'A$

It will be noted that in this scheme the concentration of the enzyme in the iodine-sensitive conformation (E') is equal to the concentration of bound enzyme. The scheme also implies that the rate of inactivation of the enzyme (step 2) is proportional to the concentration of the enzyme-analog complex.

The rate of inactivation has been measured and found to follow

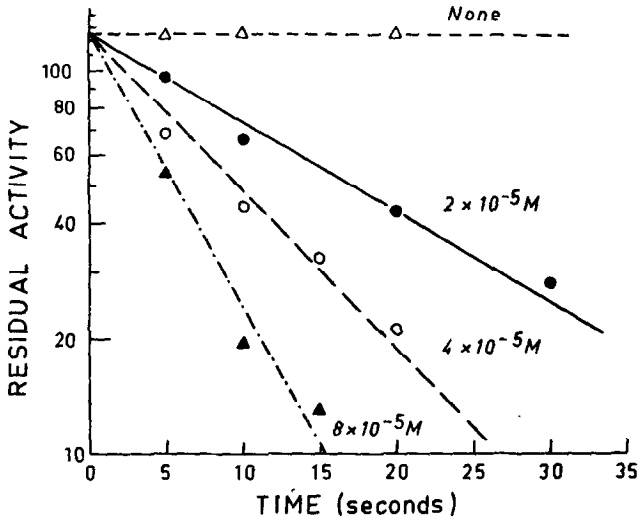

Fig. 1. Effect of analog concentration on the rate of inactivation of the enzyme. Samples of crystalline exopenicillinase of Bacillus cereus strain 5/B (125 units in 0.2 ml of 0.5% gelatin) were injected into tubes containing 0.15 ml of the iodinating reagent (0.025M I_2 and 0.125M KI) and varying amounts of oxacillin (dissolved in 0.1 ml of 0.25M phosphate buffer, pH 7.3). All reagents and the reaction mixture were maintained at 0°. Iodination was terminated at the indicated time intervals, and the residual activity assayed, as described by Citri et al. (1964).

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first order kinetics (Fig. 1). The expected dependence of the rate of inactivation on the concentration of the analog is evident from Fig. 1. For quantitative expression of this dependence the rate constant (k_a) for each concentration of the analog can be determined from the data in Fig. 1, by applying the integrated equation for first order kinetics

$$k_a = \frac{1}{t} \ln \frac{E_o}{E_t}$$

where E_o = initial enzyme activity and E_t = residual activity at time t. In Fig. 2 the reciprocal values of k_a are plotted against the reciprocal values of the concentrations of the analog. The straight line obtained is extrapolated to intersect the vertical and horizontal axis. It will be obvious that the vertical intercept gives the reciprocal value for

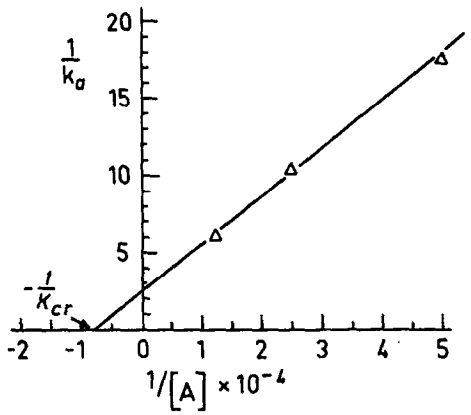

Fig. 2. Plot of reciprocal of the rate constant of inactivation (k_a) of the enzyme versus the reciprocal of the concentration of the analog (oxacillin). Data were taken from Fig. 1, and calculated as described in the text.

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the maximal rate constant of inactivation.

The molar concentration of the analog which causes half-maximal inactivation can be determined from the intercept with the horizontal axis. The treatment here is analogous to that employed for the graphic determination of K_m (Lineweaver and Burk, 1934), and the intercept is equal to $-\frac{1}{K_{cr}}$

The method illustrated above has been applied to several other systems consisting of four distinct molecular species of penicillinase and two substrate analogs. In all cases the K_{cr} values obtained were distinctively characteristic of the enzyme-analog system.

The relationship between the conformative response constant (K_{cr}) and the corresponding catalytic constants, the kinetic constant (K_m) and the competitive inhibitor constant (K_i), was next investigated. According to equation (3), K_{cr} represents the true equilibrium constant, hence the following relationship was to be expected:

$$K_{cr} = K_i \ll K_m$$

The K_{cr} values obtained for all the systems tested were found to be significantly lower than anticipated (Zyk and Citri, to be published). These results are consistent with the following modified scheme of conformative response:

$$(4)\quad E + A \longrightarrow E'A \rightleftharpoons E' + A \longrightarrow E + A$$

It will be noted that equation (4) differs from equations (1) and (3) in that the time required for the termination of the conformative response after the dissociation of E'A is taken into consideration. Consequently equation (2) is replaced by

$$(5)\quad (E'A + E') + I \longrightarrow EI + A \text{ (or } EI + A + EIA)$$

and the rate of inactivation of the enzyme is assumed to be proportional to the concentration of (E'A + E').

It will be obvious that unless the concentration of (E') is negligible, the conformative response constant (K_{cr}) will be lower than the equilibrium constant of dissociation of the enzyme-analog complex.

In conclusion, we have illustrated a method for the derivation of a conformative response constant and discussed its relation to the corresponding catalytic constants. We have shown that a comparison of the values obtained for the respective constants may serve to detect the persistence of the conformative response after the dissociation of the enzyme-ligand complex.

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