APPENDIX

Kinetics of Irreversible Inhibition of Catalase

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The inhibitors of the 3-amino-1:2:4-triazole series will cause an irreversible inhibition of catalase in the presence of hydrogen peroxide (Margoliash & Novogrodsky, 1958; Margoliash, Novogrodsky & Schejter, 1960). Assuming that the irreversible inhibition reaction was a second-order reaction between these inhibitors and catalase-H₂O₂ complex I, and under experimental conditions in which H₂O₂ is continuously supplied in excess, so that steady-state conditions may be assumed for the catalytic reaction (Chance, Greenstein & Roughton, 1952), the following three sets of reactions had to be considered.

(1) The reactions between catalase and hydrogen peroxide involving catalase-H₂O₂ I, which can be represented by the following equations, according to Chance *et al.* (1952):

in which E represents the enzyme, S the substrate, hydrogen peroxide, and ES the primary complex (catalase-H₂O₂ I). The expressions in parentheses represent the concentrations of the reactants under which they are marked and the symbols the concentrations of the total original enzyme concentration (*E*) in terms of catalase haematin, the primary complex (*p*), the enzyme haematin bound reversibly by the inhibitor (*q'*) and the enzyme haematin irreversibly reacted with the inhibitor (*q''*).

(2) The reversible inhibition of catalase by the inhibitors of the aminotriazole series, first reported for 3-amino-1:2:4-triazole by Heim, Appleman & Pyfrom (1956), may be considered to be due to reversible reactions between the inhibitor and either the free enzyme or its primary complex, as follows:

in which I represents the inhibitor, *i* its concentration and EI' and ESI' the reversibly inhibited enzyme complexes produced by reactions (C) and (D) respectively; *K₅* will be used to represent the equilibrium constant for reaction (C) and *K₆* that for reaction (D). Different symbols were not used for the two possible types of reversible inhibitions since they will be considered separately as alternative possibilities.

(3) The irreversible reaction between the primary complex and the inhibitor, which may be written:

in which EI'' represents the irreversibly inhibited enzyme and *q''* its concentration in terms of irreversibly inhibited catalase haematin. Since it is not known whether in the irreversible inhibition reaction peroxide or a product of peroxide is liberated or remains bound to the irreversibly inhibited catalase the symbol (?) has been added to equation (E). None of the symbols for the various enzyme-inhibitor complexes was intended to imply any particular structure or composition.

Under steady-state conditions for the catalytic reaction the ratio of the concentration of haematin bound by hydrogen peroxide in complex I and the total active enzyme haematin is a constant (Chance *et al.* 1952). With the reactions postulated above for the present system this ratio was:

$$\frac{p}{E-p-q'-q''} = n. \quad (1)$$

The reversible reaction of the inhibitors with either the free enzyme (reaction C) or the primary complex (reaction D) could be formulated respectively:

$$(E-p-q'-q'')i = q'K_5, \quad (2)$$

$$\text{and} \quad pi = q'K_6. \quad (3)$$

First case. The reversible inhibition reaction occurred between the inhibitor and the free enzyme (reactions *A*, *B*, *C* and *E* took place).

From equations (1) and (2), after suitable transformations between them, q' can be eliminated, and solving for p gave

$$p = \frac{[(E - q'')K_s]/i}{[(1 + \{K_s/i\})/n] - 1}. \quad (4)$$

Reaction (*E*) could be formulated

$$\frac{dq''}{dt} = k_7 p i. \quad (5)$$

Introducing the expression for p given in (4) into (5) and integrating between the limits of 0 and t for the time, and 0 and q'' for the inhibited enzyme concentration gave

$$2.3 \log \frac{E - q''}{E} = - \frac{k_7 K_s}{[(1 + \{K_s/i\})/n] - 1} t. \quad (6)$$

Defining the coefficient of t in (6) as λ , equation (6) gave

$$2.3 \log \frac{E - q''}{E} = - \lambda t, \quad (7)$$

in which λ is the first-order reaction constant for the irreversible inhibition of the enzyme and may be determined experimentally by plotting $\log (E - q'')/E$ against t .

From equation (6):

$$\lambda = \frac{k_7 K_s}{[(1 + \{K_s/i\})/n] - 1}. \quad (8)$$

Transformation of (8) gave

$$\frac{1}{\lambda} = \frac{1 - n}{k_7 K_s n} + \frac{1}{k_7 n i}. \quad (9)$$

It may be readily seen from (9) that plotting $1/\lambda$ against $1/i$ should give a straight line, the slope of which $(1/k_7 n)$ could permit the calculation of k_7 assuming a value for n , and the intercept of that line with the $1/\lambda$ axis $[(1 - n)/k_7 K_s n]$ could permit the calculation of K_s by using the value of k_7 obtained from the slope.

Moreover, K_s may be independently estimated from the equation developed by Beers (1955):

$$K_s = \frac{R_K k_0^* i}{(R_K + 1)(k_0^e - k_0^*)}, \quad (10)$$

in which R_K is related to n by the equation

$$n = \frac{1}{R_K + 1}, \quad (11)$$

and k_0^e and k_0^* are respectively the first-order reaction constants for the decomposition of H_2O_2 in the absence and in the presence of a concentration of inhibitor equal to i . Introducing equations (10) and (11) into (8), after suitable transformations the following equation could be obtained:

$$\lambda = \frac{k_7 n}{k_0^e} k_0^* i. \quad (12)$$

k_0^e and k_0^* can be determined experimentally for different values of i , and plotting λ against $k_0^* i$ should give a straight line passing through the origin, the slope of which $(k_7 n/k_0^e)$ could permit the calculation of k_7 .

Second case. The reversible inhibition reaction occurred between the inhibitor and the primary complex (reactions *A*, *B*, *D* and *E* took place).

By the use of equations (1) and (3) and treating them in the same way as for the first case gave

$$\frac{1}{\lambda} = \frac{1}{k_7 K_s} + \frac{1}{k_7 n i}. \quad (13)$$

Introducing in (13) the expression for K_s given by Beers (1955):

$$K_s = \frac{(k_0^* i)}{(R_K + 1)(k_0^e - k_0^*)} \quad (14)$$

gave, after suitable transformations, equation (12).

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