GENETIC REGULATION OF ENZYME ACTIVITY IN MAMMALIAN SYSTEM BY THE ALTERATION OF THE RATES OF ENZYME DEGRADATION

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Using 3-amino-1,2,4-triazole (AT) and allylisopropylacetamide (AIA), either singly or in combination, Price, Rechcigl and Hartley (1961), Price et al., (1962) and Rechcigl and Price (1963) developed simple but sensitive techniques for measuring the kinetics of catalase synthesis and destruction in vivo. AT acts by irreversibly inhibiting catalase without interfering with its resynthesis while AIA acts by blocking the synthesis of new enzyme without interfering with the activity of previously formed catalase.

After administration of AT, the return of catalase activity is paralleled by a corresponding uptake of Fe^{59} into the catalase, indicating that the return of catalase activity results from the formation of new enzyme rather than a reversal of the inhibitory process which led to initial fall in the enzyme activity. These data are in agreement with the findings of Margoliash, Novogrodsky and Schejter (1960) which demonstrated that AT binds to the protein moiety of catalase to form an irreversible complex.

From kinetic studies on the rate of return of catalase activity after administration of AT, it was calculated that the observed data could be accounted for by the synthesis of 4.8 units of catalase per hour per gram of liver if 2.25% of the catalase molecules were being destroyed each hour. By use of AIA to block the formation of new catalase, it was shown that the rate of catalase disappearance was nearly the same as that calculated from the AT data.

By isolating catalase from the liver under these conditions it was shown (Rechcigl and Price, 1963; and unpublished observations) that AIA almost completely blocks the incorporation of Fe^{59} into liver catalase.

The above techniques have found useful application in biology, including such fields as molecular physiology (Price et al., 1962), nutrition (Rechcigl, 1964a; Rechcigl and Price, 1961; Rechcigl and Price, 1963), pharmacology (Rechcigl, 1964b), and pathology (Rechcigl and Price, 1962).

The finding of different levels of catalase activity in certain substrains of C57BL mice (Rechcigl and Heston, 1963), which are under genetic control (Heston, Hoffman and Rechcigl, 1965) provided an excellent model for pursuing fundamental research in biochemical genetics in the mammalian system. An analyses of the F_1,F_2, and backcross generations between high-liver-catalase substrain C57BL/He and low-liver-catalase substrain C57BL/6 showed that the difference was due to a single autosomal gene pair with low dominant to high. From the history of the substrains it appeared that the original mutation had been to the dominant gene for low level activity. There is a question concerning the mechanism by which the mutant gene brings about the observed reduction in enzyme activity. The present communication presents preliminary observations on the kinetics of catalase synthesis and destruction in these two substrains of mice. The results provide evidence for the gene regulation of enzyme activity by the alteration of the rate constant of enzyme destruction.

Experimental: Male C57BL/He (B/He) and C57BL/6 (B/6) mice, 6 weeks of age, were used. Half of the animals of both substrains were injected intraperitoneally with AT, at a level of one gram per kg of body weight, the others being kept as uninjected controls. At various points thereafter 5 animals each of the control and the AT-treated groups were killed and their liver catalase activity determined, using previously described techniques (Rechcigl, Price and Morris, 1962).

Fig. 1 depicts the changes in liver catalase activity following the administration of AT. After reaching the minimal point, within 2 hours after

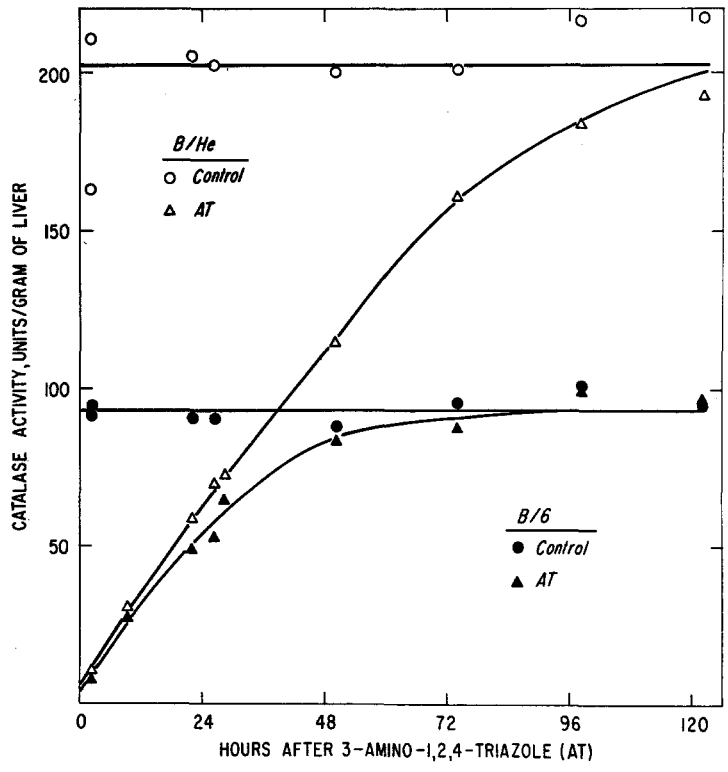

Fig. 1. Liver catalase activity in two substrains of C57BL mice after injection of 3-amino-1,2,4-triazole (AT), 1 gram per kg of body weight, intraperitoneally. Each point represents the average of five animals.

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the injection of AT, catalase activity starts to return, reaching a maximal rate at about 12 hours. Initially the rates of catalase return were almost identical in the two C57BL substrains. Following the 12 hour point, however, the catalase return of C57BL/6 mice begins to slow down, reaching the maximal value of 93 units/gram, approximately 3 days after the injection of AT. In C57BL/He mice, on the other hand, the initial rapid rate of catalase return remains uninterrupted, for at least the first 36 hours after AT. From this point on the catalase activity mounts at slower pace, until the maximal value of 202 units/gram is reached, 5 days after the administration of AT.

On the basis of our previous studies (Price, Rechcigl and Hartley, 1961; Price et al., 1962; Rechcigl and Price, 1963) it was postulated that catalase

is being synthesized at a constant rate and that catalase molecules are being destroyed in a random fashion without regard to their age. Similar conclusions have been subsequently reached in other enzyme systems (Berlin and Schimke, 1965; Schimke, 1964; Schimke, Sweeney and Berlin, 1965; Segal, 1965).

These relationships can be expressed mathematically as follows:

$$\frac{dC}{dt} = k_S - k_D C$$

where C is the enzyme activity at any time t, k_S is the rate constant for enzyme synthesis (i.e., the amount of enzyme activity synthesized per unit time) and k_D, the first-order rate constant for enzyme degradation (i.e., the fraction of enzyme molecules destroyed per unit time).

If C = 0 at t = 0 then:

$$C_t = \frac{k_S}{k_D} (1 - \exp^{-k_D t})$$

At the plateau level, C_N the amount of catalase being synthesized per unit time equals the amount being destroyed. Hence, if k_D is known, k_S can readily be calculated from the equation:

$$k_S = k_D C_N$$

Fig. 2 presents the semilogarithmic plot of the reappearance of catalase activity in the two C57BL substrains of mice, expressed as the difference between the C_N, the normal catalase level, and C_{AT}, the catalase level in the AT-treated animals. From the slope of the lines and from the previously established mathematical relationships (vide supra), the rate constants for enzyme synthesis and destruction, k_S and k_D, respectively, were determined.

It was calculated that in C57BL/He substrain there were 3.60 units liver catalase synthesized per hour as compared with 3.79 units in C57BL/6 substrain. The calculated rate constant for catalase destruction in the former group was 1.9% per hour, while in the latter group it was 4.5%.

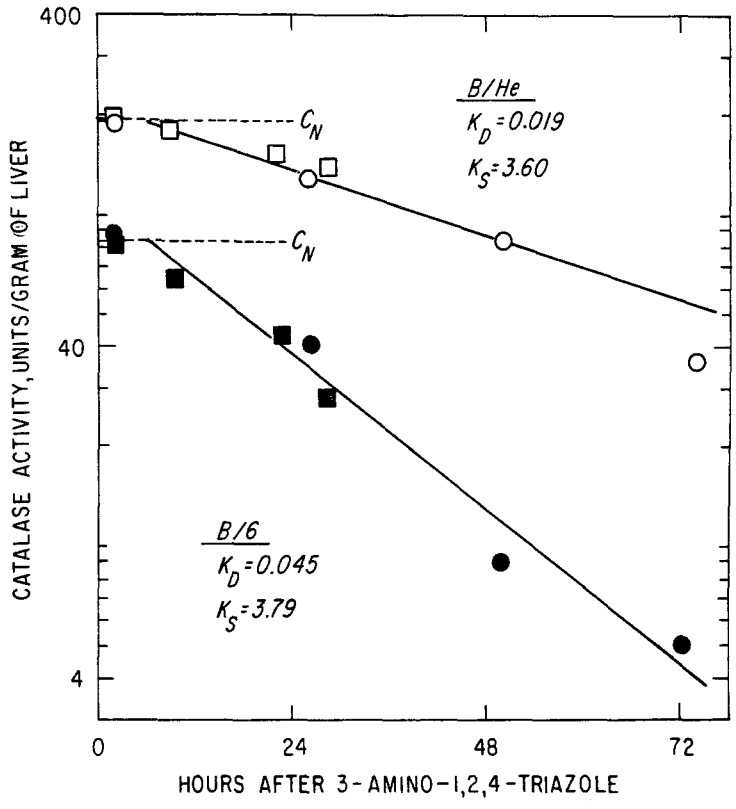

Fig. 2. Kinetics of liver catalase destruction in two substrains of C57BL mice. Semilogarithmic plot of $C_N - C_{AT}$ versus time, where C_N is the normal level of liver catalase, C_{AT} is the catalase level at various times after the administration of AT, 1 gram per kg of body weight, intraperitoneally. Circles and squares denote data from two separate experiments.

Discussion: It is evident from these studies that an alteration in the rate of degradation, as well as in the rate of enzyme synthesis, may play a significant role in controlling the level of catalase. The alteration in the rate of catalase destruction is a unique finding since in all rats and mice, examined so far in our laboratory, the rates of catalase destruction seemed to be almost constant.

Although alteration in the rate of enzyme degradation has been observed under certain physiological conditions with other enzyme systems (Schimke, 1964;

Schimke, Sweeney and Berlin, 1965), the present communication is believed to be the first demonstration of such regulatory mechanism under genetic control.

In view of the previous finding that the dominant low-catalase-gene reduced enzyme activity only to one-half the normal rather than eliminating it completely, and the fact that the action of the gene is probably limited to the liver, it was postulated that the action of the gene may be indirect. The present demonstration of the alteration of the catalase turnover rate gives further support to the idea that the gene may be providing a "regulator" of the amount of catalase activity in the liver.

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