Stoichiometry of Antibody Stabilization of a Labile Enzyme (36194)

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The phenomenon of antibody stabilization or enhancement of the activity of an enzyme antigen is by now well established. The subject was most recently reviewed by Cinader in 1966 (1), and several additional contributions have since appeared (2–10). The distinction between "stabilization" on the one hand, and "activation" or "enhancement" on the other, is difficult to define and may eventually prove to be entirely semantic. In the catalase–anticatalase system, we consider "stabilization" the appropriate designation (9), and in this work we have examined the stoichiometry of the antigen–antibody reaction in order to explain the mechanism of stabilization.

We have developed in this laboratory a mutant strain of "acatalasemic" mice (11). The mutation is a structural one; blood and tissues contain normal amounts of catalase protein, which is less active and less stable than normal catalase (12); in particular, mutant blood and liver catalases are extremely sensitive to slight increases in temperature and pH. It has been known since 1921 (13) that the combination of catalase with its antibody is catalatically active; this has permitted the demonstration (9) that if a rabbit is immunized against *normal* mouse blood lysate, antibodies are produced which will not only precipitate both normal and mutant catalase but will stabilize the mutant enzyme against the 37° temperature employed for the catalase assay (14). If the assay is conducted in an ice bath, the mutant catalase shows the same activity as the normal form, and the antiserum is without effect on its catalatic activity (9). This is part of our argument that we are dealing with a stabilization and not an activation.

The mechanism whereby an antibody can stabilize or actually enhance the activity of an enzyme antigen is a fascinating question. The present paper provides some data on the stoichiometric relationships between stabilization and the antigen–antibody reaction; such information is needed to understand the mechanisms.

Materials and Methods. Two strains of mice were used: the acatalasemic strain, Cs^b, and the normal, wild type strain, Cs^a from which the mutant line was derived (11). Cs^b, though called acatalasemic, actually retains a measurable 1–2% of normal blood catalase activity; this residual activity is, as noted above, very sensitive to heat and alkali.

Rabbits were immunized against normal mouse blood lysate as described earlier (9). Because liver and blood catalase are largely cross-reactive in the mouse, as in other species (15, 16), and because liver catalase in the mutant, Cs^b, mouse is much more active than is blood catalase, the experiments to be described used mutant liver homogenate supernatant (2% liver homogenate centrifuged at 48,000*g* for 15 min) as test antigen. Cs^a (normal) liver, when used as antigen, was prepared in the same fashion but was diluted as necessary immediately before assay.

The experiments were performed as follows: Antiserum (undiluted) and antigen, diluted as desired, were incubated separately and together at 37° for 30 min. Phosphate buffer (0.022 *M*, pH 6.8) was present in all mixtures, because of the extreme sensitivity of mutant catalase to mild alkalinity. Mixtures were incubated at 37° for 30 min, then treated in one of two ways, to measure either total activity or separate supernatant and precipitate activity:

a. The whole mixture was briefly stirred vigorously, to suspend the antigen–antibody precipitate, and aliquots of the suspensions were added to perborate flasks for catalase assay (14). The catalatic activity observed was corrected for the small amount of catalatic activity sometimes present in the rabbit

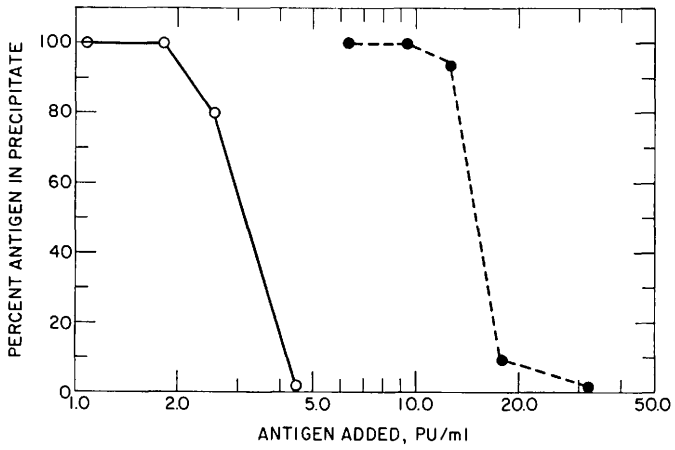

FIG. 1. Antigen titration curves, normal and acatalasemic liver homogenate supernatants as antigen: Antiserum is rabbit-antinormal mouse blood lysate antiserum; (○) Cs^b (acatalasemic) liver; (●) Cs^a (normal) liver; PU = perborate units of catalase activity (14).

sera, and the resulting value was divided by the activity added, to obtain a "percentage recovery." This was, of course, never appreciably less than 100%, and if stabilization had been demonstrated, it was much more than 100%.

b. After 30 min incubation at 37°, the mixtures were centrifuged. The supernatant was drawn off and maintained in ice until assayed. The precipitate was washed once with cold saline, then resuspended in the original volume of cold saline, and assayed. In this case, only the supernatant activity was corrected for catalatic activity added with the antiserum.

Results and Discussion. Figure 1 compares antigen titration curves of Cs^a and Cs^b liver homogenate supernatants. The antiserum is rabbit-anti-Cs^a mouse blood lysate antiserum. Observe that the curves are very similar in shape, but in the zone of optimal proportion approximately five times the catalatic activity is carried into the precipitate from Cs^a liver as from Cs^b. Assuming that a constant quantity of antibody will precipitate a constant amount of catalase protein of comparable antigenic activity, regardless of the enzymatic activity of that protein, this result may be considered to confirm, by a more direct procedure, our earlier statements that; (a) the mutation is a structural one, with mutant tissues containing approximately normal amounts of a catalatically less active (less stable) catalase protein (12); and (b) Cs^b liver contains approximately 25% of the catalatic activity of Cs^a liver (17). If these curves are plotted as milligrams of liver added, rather than in terms of perborate units of catalatic activity added, the two curves very nearly coincide; the maximum deviation is about 35%, as contrasted with the nearly fivefold differences in Fig. 1.

To determine whether or not the stabilization was dependent upon a fixed ratio of antigen to antibody, we did an antigen titration in which we measured the amount precipitated and the percentage "activation" (stabilization). Figure 2 shows that the amount of stabilization is relatively constant well into the zone of antigen excess. These results indicate that stabilization occurs at a 2:1 ratio of antigen:antibody, from the following reasoning: (a) We know (9) that heat and pH inactivation cannot be reversed by the stabilizing antibody; (b) it is commonly accepted that the zone of optimal proportions, the inflection point in the precipitation curve, represents a 1:1 molar ratio of antigen:antibody, and that the antibody molecule contains only two combining sites; and (c) stabilization (Fig. 2) continues well beyond the zone of optimal proportions for precipitation. The reason for the rapid decline in percentage maximum "activation" is that, in the zone of antibody excess, a larger proportion of the mutant catalase molecules are stabilized before they lose their activity

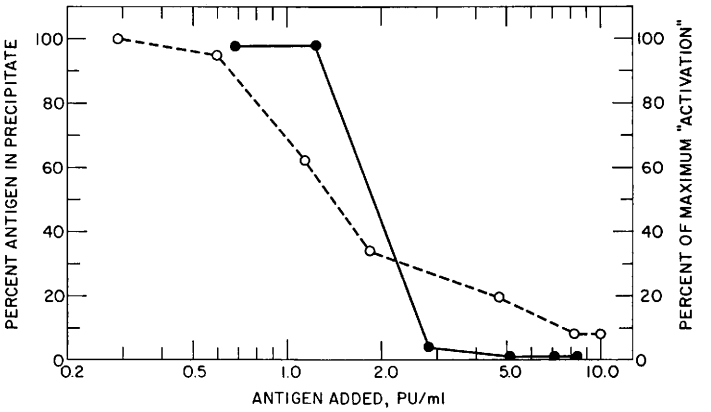

FIG. 2. Antigen titration curves for precipitation and for "activation" (stabilization): Antigen is Cs^b (acatalasemic) liver homogenate supernatant; antiserum is rabbit-antinormal mouse blood lysate antiserum; (●) percentage antigen in precipitate; (○) percentage of maximum "activation" (stabilization); PU = perborate units of catalase activity (14).

from thermal inactivation (37°). In the zone of antigen excess, a smaller portion of the original inoculum is stabilized so that percentage maximum stabilization is less, but still significant to dilutions 5- to 10-fold beyond the zone of equivalence. The data shown are compatible with the concept that all antigen molecules which are stabilized, whether in precipitate or in solution, are *equally* stabilized; the curve of stabilization ("activation") falls off in antigen excess because of simple dilution of stabilized antigen molecules with free antigen molecules for which no stabilizing antibody is available.

These results suggest that insolubilization is not a requisite of stabilization, and that stabilization occurs at a 2:1 ratio of antigen: antibody.

Summary. Antibody to normal mouse catalase will precipitate both normal and mutant mouse catalase. It will also stabilize the mutant catalase against loss of activity at 37°. The stabilization phenomenon is evidently not dependent on insolubilization but occurs also in antigen excess, where little antigen exists in precipitate form.

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