Stabilizing Effect of Anti-Normal Erythrocyte Catalase Antibody on the Labile Catalase Variant Present in Swiss-Type Acatalasemia

It has been shown that residual catalase activity present in blood of individuals homozygous for Swiss-type acatalasemia is a mutant form of the enzyme. This catalase variant differs from the normal enzyme by its reduced thermostability, its accelerated inactivation in concentrated urea, as well as by its lower electrophoretic mobility[1,2]. Immunologically there is only partial cross reactivity between the normal and the mutant enzyme[3]. The reason for the instability of this mutant catalase is not known. It may be explained in various ways. One of the possibilities is, that the rapid loss of activity in vivo and in vitro is due to an accelerated decomposition of the active oligomeric form into inactive subunits.

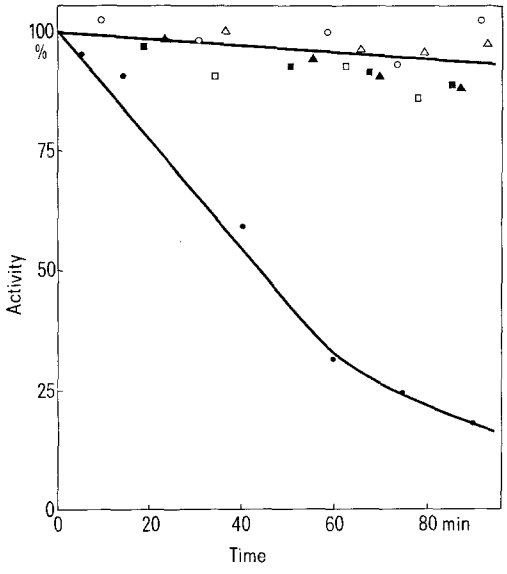
As demonstrated in a number of instances it is possible to enhance the stability of an enzyme considerably by complex formation with its specific antibody, e.g. penicillinase[4], acetylcholinesterase[5], β-D-galactosidase[6,7]. This procedure for stabilizing a given conformation of the molecule is particularly effective in unstable enzyme variants. Under suitable conditions the activity of such a defective enzyme can be brought to the level of its normal counterpart. Recently FEINSTEIN[8,9] has shown, that this is true for murine acatalasemia. In this communication evidence is presented that the heat stability of the human catalase variant, as present in blood of A.B., an individual homozygous for Swiss-type acatalasemia, is considerably increased in vitro by the addition of anti-normal erythrocyte catalase antibody. This effect is seen, whether the antibody is added as total IgG yielding an unsoluble complex, or is added as a monovalent Fab fragment preparation, thus giving rise to the formation of a soluble antigen-antibody-complex.

Methods. The specific anti-normal human erythrocyte catalase antibody as well as its IgG fraction were the same as those described previously[3,10]. The monovalent Fab fragments of IgG were prepared according to CEBRA et al.[11] by using an insoluble sepharose-papain conjugate[12]. The activated papain preparation was added to the IgG in a 1:100 molar ratio, incubated for 6 h at 37 °C, then removed by filtration. The Fc fragments were precipitated after overnight dialysis against distilled water at 4 °C and removed by centrifugation. The remaining Fab fragments were dialyzed against phosphate buffered saline. The IgG was determined spectrophotometrically assuming an E_{280} nm (1%) of 14.0[13]; the specific antibody concentration therein was calculated based on the quantitative precipitin test[14].

Catalase preparations from normal and acatalasemic erythrocytes were obtained and their activity determined by rapid titration technique as described previously[3,15]. The relative residual activity of both, normal and mutant catalase preparations incubated in presence or absence of antibodies was expressed as the percentage of the K_t-value (60 sec) measured before and at the end of the incubation at 47 °C.

Results. Normal (a) and mutant (b) erythrocyte catalase preparations were diluted to similar concentration (a) 6.21 μg/ml; b) 6.28 μg/ml) and preincubated at 0 °C in phosphate buffered saline for 90 min. This was done by dividing the batches (a) and (b) into 3 aliquots each. To aliquot 1, serving as a control, no addition was made. To aliquot 2 anticatalase IgG preparation (1.6 mg/ml) and to aliquot 3 an analogous amount of Fab-fragment (1.07 mg/ml) was added. The resulting molar antigen-antibody ratio was about 1:2.5. In all 6 samples there was no change in catalase activity throughout the preincubation period. After this, the samples were incubated at 47 °C, 25 μl aliquots were removed at different times and immediately used for the determination of (residual) catalase activity. The result of a typical experiment is presented in the Figure. The data indicate that, under the experimental conditions taken, normal catalase retains nearly all of its initial activity for as long as 90 min when kept at 47 °C. This is so whether antibodies are present or not.

On the other hand the activity of the labile catalase variant has fallen as much as 50% after 45 min and to 20% of the initial activity after 90 min, if no additions are made. However, this rapid thermal inactivation can be overcome almost completely by catalase-anticatalase



Effect of anti-catalase on normal (○ □ △) and variant erythrocyte catalase (● ■ ▲) incubated at 47 °C. 1. In absence of antibodies (○ ●); 2. in presence of anticatalase IgG-preparation (□ ■); 3. in presence of antibody-Fab-fragments (△ ▲). Detailed experimental conditions see text.

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complex formation. This can be achieved not only by precipitating catalase by anticatalase IgG-preparation, but to the same extent also by Fab-fragments forming a soluble complex with the catalase mutant. Analogous experiments with essentially the same result were performed at 37 °C and 52 °C, indicating that this stabilizing effect can be observed in a rather broad temperature range[16]. Furthermore, in order to verify the specificity of the stabilizing effect of anticatalase, the same type of experiment was performed using an anti-carbonic anhydrase B-IgG-fraction and its corresponding Fab-preparation instead. These antibodies, prepared by the same procedure, were used in the same concentration range. In this control experiment no stabilization effect could be observed[16]. In another series of experiments the effect of the antigen-antibody ratio was investigated. Using the anticatalase IgG-preparation, a lower catalase activity is observed in the zone of antigen-antibody equivalence. This interference is no longer observed if a moderate excess of antibody (2.5 molar ratio) is used. No such effect could be detected when the Fab-preparation was used as a stabilizing agent.

Discussion. Catalase is one of the few enzymes retaining full enzymatic activity when bound in an antigen-antibody complex. This complex formation stabilizes the active conformation of the enzyme, without interfering with its active site. The effect is particularly striking in a labile enzyme variant crossreacting with the antibody specific for its normal counterpart. The stabilization of a human catalase mutant is essentially the same phenomenon as that observed by FEINSTEIN et al.[8,9] in acatalasemic mice. This enzyme defect resembles that observed in Swiss type acatalasemia in as much both are due to the synthesis of an unstable enzyme variant. In both instances insolubilization is not a requisite of stabilization. As shown here Fab-fragments are as efficient as the IgG-preparation in stabilizing the labile catalase mutant.

This stabilization effect by complex formation lends further support to the assumption that the fixation of a particular conformation of the subunits counteracts the disintegration of an oligomer-enzyme. It may be anticipated that this action is especially pronounced if the interacting forces are altered as a result of a mutation. Therefore, it is probable that the mechanism of stabilization of variant catalase resembles that of β-D-galactosidase in *E. coli*, where, even with an inactive enzyme from a mutant strain full activity could be restored by means of antibodies specific for the wild type enzyme[6].

These observations are of theoretical as well as of practical interest. On one hand, the preparation of active antigen-antibody complexes of labile enzyme variants may provide a valuable tool for their determination and isolation. On the other hand, experimental evidence has been presented recently by FEINSTEIN[17] that upon injection of catalase antibodies to acatalasemic mice it seems possible to counteract the inactivation of labile enzyme variants in vivo also. Although this preliminary result does not permit an extrapolation, the idea of an in vivo stabilization of labile mutants deserves further consideration[18].

Zusammenfassung. Die instabile Katalase-Variante, welche in den Erythrocyten des Akatalasie-Falles A.B. vorkommt, lässt sich in vitro durch Zusatz von Anti-Katalase IgG-Fraktion oder von Fab-Fragmenten ohne Aktivitätseinbusse stabilisieren. Es wird angenommen, dass dies auf eine Fixierung der aktiven Konformation des Enzyms zurückzuführen ist.

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Immunochemical Relationships Among Lysosomal Acid Phosphohydrolases: DNase II and Nonspecific Phosphodiesterase

Among the lysosomal enzymes are a group of acid phosphohydrolases. These include both phosphomono- and diesterases. All chromatograph on CM-cellulose[1], and it has been reported that DNase II possesses activity against *bis-p*-nitrophenyl phosphate in addition to its nucleolytic activity[2,3]. These findings of common location and certain closely related chemical properties led us to conjecture that lysosomal acid phosphohydrolases may have arisen by the processes of gene duplication and mutation from a common ancestral, rather nonspecific phosphohydrolase. Proteins with common origins often have the same antigenic determinants[4]. For this reason we have started an immunological comparison of the acid phosphohydrolases. We report here a comparison of the immunoprecipitation reactions of cow and hog spleen DNase II and hog spleen nonspecific phosphodiesterase (PDE) with rabbit anti-hog spleen antiserum.

For these experiments the enzymes were purified as previously described for beef spleen DNase II[5]. After chromatography on CM-cellulose and Sephadex G-100 the specific activities of the enzymes in units/mg were: Beef spleen DNase II, 216; hog spleen DNase II, 68; hog spleen phosphodiesterase, 1.71. Antisera were prepared in New Zealand white rabbits by immunization with 3 mg of purified enzyme in Freund's complete adjuvant followed twice at weekly intervals by injection of 3 mg of enzyme in incomplete adjuvant. Blood was obtained after the 4th week. IgG was prepared by ammonium sulfate fractionation and chromatography on DEAE cellulose[6].

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