Heat and Urea Stability of Blood Catalase of Catalase-Mutant Mouse Strains[1]

The development of acatalasemic and hypocatalasemic mouse strains[2] has permitted an indirect study of the combination of catalase subunits and has led to some unexpected conclusions regarding the nature of heterozygote enzyme molecules. The mutation is a structural one[3]; the amount of blood catalase protein appears to be unchanged by the mutation[4]. In such a situation, it would be expected that the heterozygote catalase would display more than one molecular species. Since catalase is a tetramer[5], the most probable composition of the heterozygote would be 5 molecular forms[6], one of each parental species and 3 different species combining subunits from both parents in varying ratios. In fact, SCANDALIOS[7] has demonstrated exactly this result in a study of maize strains bearing catalase of differing electrophoretic mobilities.

Unfortunately, the catalase of our mutant strains does not differ from normal catalase in its electrophoretic mobility[8]. Because of this fact, we have laid emphasis on the characterization of blood catalase by stability to urea and to heat; these have proven to be sensitive, reproducible parameters[8,9]. Using these techniques, we earlier demonstrated[8] that blood catalase of a hypocatalasemic heterozygote differs from that of both parents and consists of a single molecular species. As explanation of this unexpected finding, we suggested that the combination of subunits is non-random; by some unknown mechanism, a certain specified number of subunits of each parental form are combined to form an invariant heterozygote molecule.

We now wish to report that we have performed similar experiments on other heterozygotes, including the mixed heterozygote between the acatalasemic and the hypocatalasemic strains, and all results are the same: the heterozygotes produce single molecular species of blood catalase, and this species differs from both parental forms. It is of further interest that the stability of a heterozygote molecule is not directly related to its level of catalatic activity.

Materials and methods. The following strain designations are employed: Cs^a Cs^a is the normal, wild type homozygote; Cs^bCs^b is the acatalasemic homozygote (this strain was not used in the experiments here reported, because its blood catalase activity is already so low as to limit the accuracy of assay of further degraded samples); Cs^dCs^d is one of the hypocatalasemic homozygotes; Cs^aCs^b and Cs^aCs^d are the heterozygotes of the acatalasemic and hypocatalasemic strains, respectively; and Cs^bCs^d is the mixed acatalasemic-hypocatalasemic heterozygote. Blood was drawn from the orbital sinus[10] into heparinized tubes and lysed by 50-fold dilution (for the urea experiments) or 100-fold dilution (for thermal studies) with cold distilled water. Lysed bloods were stored in an ice bath until use. For the urea experiments, a series of small test tubes, each containing 1.0 ml of water or of a given urea solution, was warmed to 37 °C. Small aliquots of blood lysate were also brought to 37 °C. At zero time, 1.0 ml of blood was added to 1.0 ml of urea (or water, as control), the tube was briefly mixed, and the mixture was incubated at 37 °C for exactly 10 min, at which time 1.0 ml of the mixture was pipetted directly into a flask of perborate for catalase assay[11]. For the heating experiments, small empty test tubes were pre-warmed to the desired temperature. At zero time, about 1.5 ml of blood lysate was added to the test tube and was then incubated at that temperature for exactly 10 min before being plunged back into the ice bath, where it was stored until assay. Changes in catalase activity during this period in the ice bath have been shown to be negligible.

Results. Urea and heat stability curves of the 5 sorts of blood tested are shown in Figures 1 and 2, respectively. It is clear that the normal homozygote, Cs^aCs^a, is most stable in both respects. Blood of the 2 heterozygotes carrying the Cs^a gamete, namely Cs^aCs^b and Cs^aCs^d, are less stable than Cs^aCs^a but very similar to each other. Of least stability are

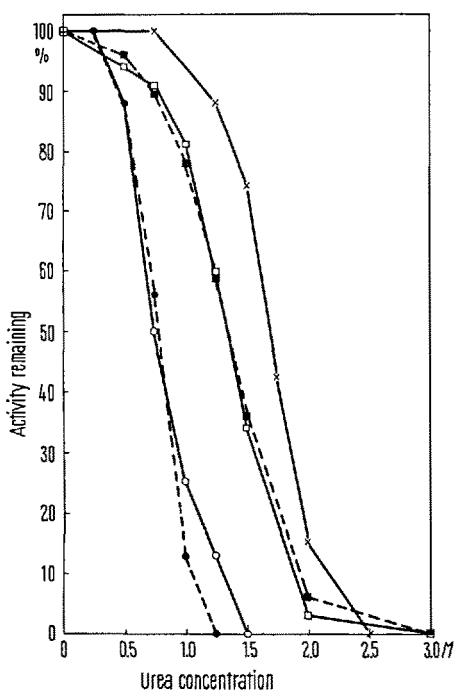

Fig. 1. Urea stability of blood lysates. For definition of mouse strains, see text. ×—×, Cs^aCs^a; □—□, Cs^aCs^d; ■---■, Cs^aCs^b; ●---●, Cs^dCs^d; and ○—○, Cs^bCs^d.

Table I. Distinguishing between similar urea stabilities

Strain	Urea concentration (M)	Activity remaining (%)	Student's t-test	P
Cs^aCs^d	2.0	3.7 ± 0.3		
Cs^aCs^b	2.0	6.2 ± 0.4	4.87	< 0.001
Cs^bCs^d	1.25	12.9 ± 1.8		
Cs^dCs^d	1.25	4.4 ± 0.2	4.70	< 0.001

For definition of strains, see text. Urea concentration is that in the final mixture of urea solution and blood lysate. Figures for % activity remaining are mean ± standard error. They are based on 6 completely separate blood samples of each strain.

Table II. Distinguishing between similar heat stabilities

Strain	Temperature (°C)	Activity remaining (%)	Student's t-test	P
Cs^aCs^d	48.0	18.0 ± 1.2		
Cs^aCs^b	48.0	16.3 ± 1.5	0.86	N.S.
Cs^bCs^d	42.0	65.7 ± 2.4		
Cs^dCs^d	42.0	75.5 ± 1.8	3.23	< 0.01

For definitions of strains, see text. Figures for % activity remaining are mean ± standard error. They are based on 6 completely separate blood samples of each strain.

the 2 strains lacking the Cs^a gamete, namely the hypocatalasemic homozygote, Cs^dCs^d, and the mixed heterozygote, Cs^bCs^d; the members of this pair are also very similar to each other. The 2 sets of curves, urea and heat stability, are in all respects extraordinarily similar. As in our earlier work[8], all curves are smoothly S-shaped, with no suggestion of separate denaturation of variant species within a given blood. Again we conclude that each sample, heterozygote as well as homozygote, contains but a single molecular species.

The Cs^aCs^b and Cs^aCs^d curves, though very similar, are not identical; the same is true of Cs^dCs^d vs Cs^bCs^d. This was demonstrated by selecting single urea concentrations, and single temperatures, where greatest differences appeared to exist in the curve pairs, and replicating these points with 6 separate blood samples. Table I and II show that these curve pairs differ significantly, though not greatly, from each other; urea stability data are much more conclusive than heat stability.

It was shown earlier[9] that a convenient figure for characterizing heat stability of a given blood sample is the T_{50}, that temperature at which 50% of the catalase activity is lost. Of comparable convenience is the U_{50}, the urea concentration at which 50% of the catalase activity is lost. Table III lists the T_{50} and the U_{50} for the 5 strains tested, as well as the catalase activity of the untreated blood. The table makes 2 points evident: a) sensitivities to heat and to urea are very closely related, and b) these sensitivities are not related to the catalatic activity of the untreated lysate.

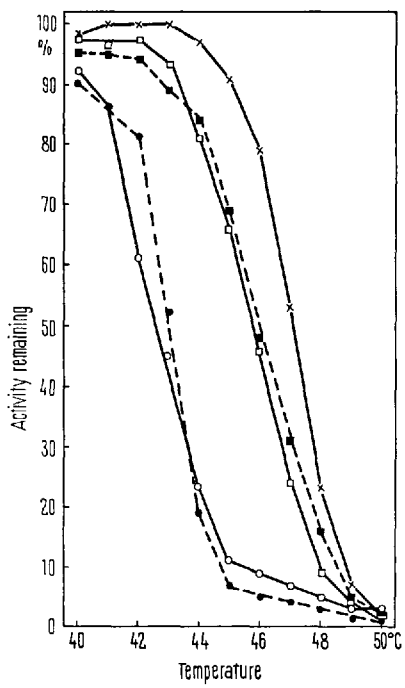

Fig. 2. Heat stability of blood lysates. Legend as for Figure 1.

Table III. Comparison of catalatic activities with heat and urea stabilities

Strain	Catalase activity (PU/ml of blood)	T_{50} (°C)	U_{50} (*M*)
Cs^aCs^a	168	47.1	1.675
Cs^aCs^d	94	45.8	1.325
Cs^aCs^b	69	46.0	1.350
Cs^dCs^d	16	43.0	0.800
Cs^bCs^d	8	42.6	0.750

For definition of strains, see text. Catalase activity is expressed as perborate units[11] per ml whole blood. T_{50} is that temperature, in degrees C, at which 50% of the catalase activity is lost under the conditions of the experiment; U_{50} is that urea concentration, in molarity, at which 50% of the catalase activity is lost under the conditions of the experiment.

Discussion. KREMER[12] has recently presented evidence that catalase hematins are equivalent and non-interacting during catalysis. JONES and SUGGETT[13] have suggested that monohematin subunits of catalase are catalatically inactive because in the catalase tetramer an amino acid from a different subunit is co-ordinated around each hematin iron atom. We have postulated[14] that the amino acid modifications caused by mutation lead to an unstable molecular configuration of the catalase. On these bases it is understandable that the presence of the normal, Cs^a, gamete stabilizes the molecule, while its absence causes a decreased stability to the next level, stabilized by the Cs^d gamete, although at the same time there are considerable differences in the blood catalase level of the several strains. For the explanation of the existence of single molecular forms of catalase in heterozygote blood, we again suggest a non-random combination of subunits. We have no evidence as to the nature of the mechanism bringing about a non-random combination.

The possibility must be considered that one or more of the strains examined does contain catalase of more than one more molecular species, so nearly identical in their denaturing properties that they will not be detected by the methods employed. However, the 3 parental homozygotes (Cs^aCs^a, Cs^dCs^d, and Cs^bCs^b) are so dissimilar in these properties that separate denaturation would almost certainly be evident, as demonstrated earlier[8] with mixtures of homozygote blood.

Zusammenfassung. Normale und anomale Katalase-Varianten mit identischer elektrophoretischer Wanderungsgeschwindigkeit konnten durch unterschiedliche Harnstoff- und Temperatur-Wirkung voneinander unterschieden werden.

R. N. FEINSTEIN, J. B. HOWARD and R. SAVOL

Division of Biological and Medical Research, Argonne National Laboratory, 9700 South Cass Avenue, Argonne (Illinois 60439, USA), 1 June 1971.

[1] This work was performed under the auspices of the U.S. Atomic Energy Commission.

[2] R. N. FEINSTEIN, J. B. HOWARD, J. T. BRAUN and J. E. SEAHOLM, Genetics *53*, 923 (1966).
[3] R. N. FEINSTEIN, J. T. BRAUN and J. B. HOWARD, Arch. Biochem. Biophys. *120*, 165 (1967).
[4] R. N. FEINSTEIN, H. SUTER and B. N. JAROSLOW, Science *159*, 638 (1968).
[5] W. A. SCHROEDER, J. R. SHELTON, J. B. SHELTON and B. M. OLSON, Biochim. biophys. Acta *89*, 47 (1964).
[6] C. R. SHAW, Brookhaven Symp. Biol. *17*, 117 (1964).
[7] J. G. SCANDALIOS, Proc. natn. Acad. Sci., USA *53*, 1035 (1965).
[8] R. N. FEINSTEIN, J. T. BRAUN and J. B. HOWARD, Biochem. Genet. *1*, 277 (1968).
[9] R. N. FEINSTEIN, G. A. SACHER, J. B. HOWARD and J. T. BRAUN, Arch. Biochem. Biophys. *122*, 338 (1967).
[10] V. RILEY, Proc. Soc. exp. Biol. Med. *104*, 754 (1960).
[11] R. N. FEINSTEIN, J. biol. Chem. *180*, 1197 (1949).
[12] M. KREMER, J. theor. Biol. *29*, 387 (1970).
[13] P. JONES and A. SUGGETT, Biochem. J. *108*, 833 (1968).
[14] R. N. FEINSTEIN, B. N. JAROSLOW, J. B. HOWARD and J. T. FAULHABER, J. Immunol., *106*, 1316 (1971).