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Heat Inactivation of Catalase in Deuterium Oxide

We have measured the kinetics of heat inactivation of the enzyme catalase in H_2O and D_2O over a range of temperatures. The results show a considerable difference in both the heat and the entropy of activation, both being higher for heating in D_2O.

The enzyme (*1*) was dissolved in 0.05*M* phosphate buffer, made up in either H_2O or D_2O (> 99.6 per cent) at *p*H 7.0 in H_2O. The D_2O buffer read 6.85 on the *p*H meter (*2*). The concentration of catalase during heating was 300 μg/ml. At appropriate intervals, 0.1-ml samples were taken, diluted to 15 μg/ml in buffer made in H_2O, and assayed by observing the breakdown of H_2O_2 by measurements of optical density at 2300 A in a Beckman model DU spectrophotometer (*3*). The D_2O concentration during assay was 1.4 percent. Standing in D_2O buffer for periods up to 2 weeks at 8°C had no measurable effect on the rate of subsequent inactivation in D_2O or on the absolute enzymatic activity as compared with fresh samples in H_2O.

The inactivation curves followed first-order kinetics within the precision of the data, with reaction times from a few seconds to several hours. From the observed rate constants, the free energies of activation were calculated from the theory of absolute reaction rates (*4*) according to the equation

$$k = \frac{\bar{k}T}{h} e^{-\Delta F^{\ddagger}/RT}$$

Figure 1 shows the temperature dependence of the free energy of activation ($\Delta F^{\ddagger}$). From the relation

$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$$

we find, for heating in H_2O, $\Delta H^{\ddagger} = 87$ kcal/mole and $\Delta S^{\ddagger} = 191$ cal/mole · deg, and for heating in D_2O, $\Delta H^{\ddagger} = 145$ kcal/mole, and $\Delta S^{\ddagger} = 360$ cal/ mole · deg.

Wiberg (*5*) and Morowitz and Brown (*6*) have recently reviewed the effect of deuterium on reaction rates. Since the zero-point energy of a covalent bond to deuterium is lower than that of the corresponding one to hydrogen by about 1.2 to 1.5 kcal/mole (because of the greater mass and consequent lower freqency), reactions which involve breaking such bonds are usually slower in D_2O, and deuterated compounds in general react more slowly than hydrogenated ones. Enzymatically catalyzed reactions have been observed to go both faster and slower in D_2O, and some of the reports are conflicting. Macht and Bryan (*7*) report a "noticeable acceleration" of the action of catalase on H_2O_2 in 0.05- to 1.0-percent D_2O, whereas Fox (*8*) reports no change in 1-percent D_2O. Our results tend to confirm Fox, although it is possible that a change of a few percent may have been unnoticed.

Caldwell, Doebbling, and Manion (*9*) reported that pancreatic amylase denatured more rapidly at 25°C in D_2O than in water, whereas Fox (*8*) reported no difference in the daily loss of catalase activity between D_2O and H_2O. It seems likely that neither of these cases represents thermal inactivation of the kind observed here. We found loss of catalase activity in H_2O at 25°C and 37°C to be much more rapid than consistent with the higher temperature data, and we suspect that bacterial growth was the cause. Extrapolation of the curve in Fig. 1 would yield a time of about 10 years at 25°C, for the same reaction in H_2O.

Morowitz and Chapman (*10*) reported that deuterium substitutes rapidly (within 20 minutes) in all bonds of proteins except C—H. Linderstrøm-Lang (*11*) found that in some proteins there is a continued slower exchange following the initial rapid one. Our evidence suggests no further effect on heat stability of catalase between about ½ hour and 2 weeks.

The mechanism of thermal inactivation of enzymes is still obscure, although it is presumably closely related, if not identical, to denaturation. The high values of the heat and entropy of activation observed here are typical of enzyme inactivations and have suggested a process involving the breaking of a number of weak interchain hydrogen bonds, with accompanying high entropy changes as the molecule becomes more disordered (*12*). It may be expected that deuterium bonds are related to hydrogen bonds qualitatively as the respective covalent bonds, and that they will be somewhat stronger. It is not surprising, therefore, to find $\Delta H^{\ddagger}$ higher for inactivation in D_2O. Further, since the vibrational energy levels of a deuterium bond are more closely spaced, the partition functions are affected, and thereby the entropy terms are expected to be changed in the free energies of reactants, products, and activated complex. It is difficult, however, to predict a priori the effect of this on the $\Delta S^{\ddagger}$ of the inactivation process, except to say that it is likely to be different (*13*).

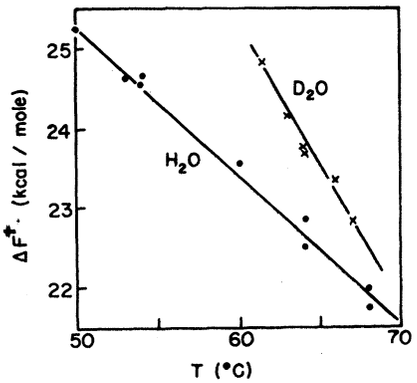

Fig. 1. The free energy of activation ($\Delta F^{\ddagger}$) for inactivation of catalase in H_2O and D_2O at various temperatures. The lines represent the equation $\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger}$, where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the heat and entropy of activation, respectively. For H_2O, $\Delta H^{\ddagger} = 87$ kcal/mole, $\Delta S^{\ddagger} = 191$ cal/mole · deg; for D_2O, $\Delta H^{\ddagger} = 145$ kcal/mole, $\Delta S^{\ddagger} = 360$ cal/mole · deg.

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