A THERMODYNAMIC COMPARISON OF SOME OXIDATIONS OF FERROCYTOCHROME *C*

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The physiological function of ferrocytochrome *c* requires that it should resist direct oxidation by oxygen under cellular conditions. It is, however, readily oxidized by hydrogen peroxide [1], or by oxygen at low pH [2] or in the presence of copper complexes [3], organic solvents [4], or the enzymes cytochrome oxidase (EC 1.9.3.1) [5] or cytochrome *c* peroxidase (EC 1.11.1.5) [6]. Studies of effects of temperature, pH and ionic strength reported here suggest that the main factor determining the relative rates of oxidation of ferrocytochrome *c* in these various systems is the magnitude of the entropy of activation.

Autoxidation of ferrocytochrome *c* was followed by adding 0.1 ml of ferrocytochrome *c* (H_2/Pd reduced) to a cuvette containing Teorell-Stenhagen buffer of the stated pH and ionic strength, and the absorbance at 550 nm was followed.

Figures 1 and 2 show that in spite of the decrease in the autoxidation rate as pH is increased, there is a decrease in the Arrhenius activation energy. At pH 2.5, 3.0, 3.4, 4.0, 4.4, and 5.5 activation energies were respectively 33.8, 28.9, 26.0, 22.2, 18.8, and 9.3 kcal/mole.

The effect on the first order rate constant of increasing the buffer concentration is shown in fig. 2, where the slope of 14.5 indicates the creation of considerable charge in the formation of the transition state in the rate determining step of the uncatalysed autoxidation, in partial agreement with a previous report [7].

Activation energies were determined for the autoxidation catalysed by copper histidine, or accelerated by non-aqueous solvents at pH 5 in the assay systems previously described [3, 4]. Values of 17.2 and 48.1 kcal/mole were obtained. In both cases acceleration of the reaction is accomplished in the face of an increase in activation energy. (Compare the value of 12.3 kcal/mole for the uncatalysed reaction at pH 5). One must conclude that the relative rates of oxidation in all the above systems are determined primarily by entropy considerations.

The effect of temperature on the maximum velocity of cytochrome oxidase was studied using the assay system of Wainio [8], and the results (fig. 4) show an activation energy for further reaction of the cytochrome *c*–cytochrome oxidase complex equal to 8.5 kcal/mole. This corresponds to the Q_{10} of 1.84 over the temperature range 20–30°. These values can be compared with the Q_{10} of 1.78 reported by Minnaert [9] for an unspecified temperature range, and with the activation energies of 8.2 and 12.6 kcal/mole corresponding to Q_{10} values of 2.01 and 1.58 over the temperature ranges 20–30° and 30–40° reported by Smith [10]. In the absence of enzyme, the activation energy (extrapolated to pH 6.0) is 4.0 ± 0.2 kcal/mole.

If the uncatalysed value at pH 5.5 is used for comparison, it is still clear that the acceleration caused by the enzyme is produced primarily by entropy effects.

A similar series of experiments was carried out in which hydrogen peroxide was the oxidizing agent. To initiate the reaction, peroxide was added to a final concentration of 0.30 mM at final pH 7.0, at which oxidation by oxygen was negligible. The effect of temperature on the maximum rate of this reaction is shown in fig. 5. Although the reaction is much faster than the autoxidation at pH 5.5, the activation energy is 33.5 kcal, i.e., much higher.

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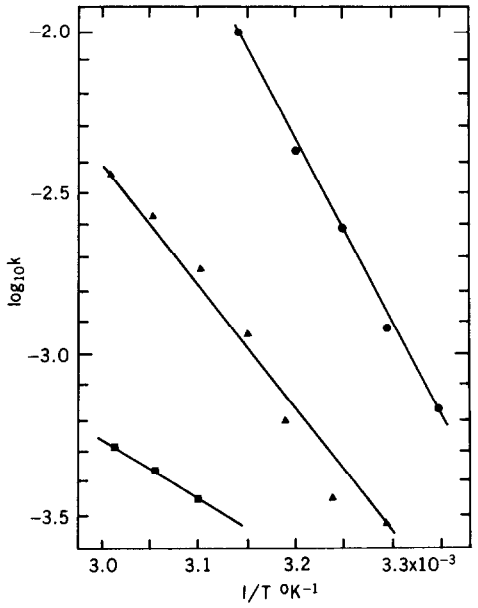

Fig. 1. The effect of temperature on the first order rate constant (k) for the uncatalysed reaction of ferrocytochrome c with oxygen at pH = 3.4, 4.4, and 5.5. Activation energies were: 26.0, 18.8, and 9.3 kcal.

The corresponding activation energy for cytochrome c peroxidase was calculated from data of Beetlestone [6] to be 9.5 kcal. The enzyme cytochrome c peroxidase thus produces a very significant decrease in the activation energy for the peroxidation of ferrocytochrome c. Clearly also, the overall entropy of activation for uncatalysed peroxidation at pH 6.8 is less unfavourable than for oxidation by oxygen. Increasing ionic strength decreased the peroxidation rate, and decreasing pH below 7 slowed this reaction. Evidently autoxidation, but not peroxidation, involves creation of charge having a kinetically significant requirement for protonation.

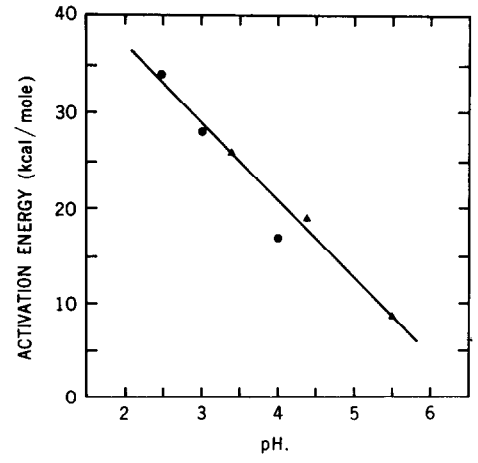

Fig. 2. The effect of pH on the Arrhenius activation energy for the uncatalysed autoxidation of ferrocytochrome c.

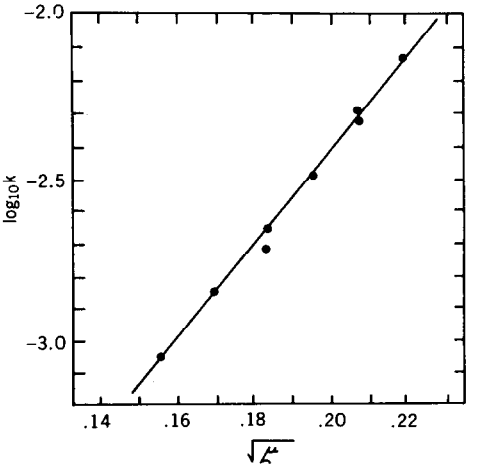

Fig. 3. The effect of ionic strength (μ) on the first order rate constant (k) for the uncatalysed reaction of ferrycytochrome c with oxygen. pH = 2.9, temperature = 21.9°, slope = 14.5.

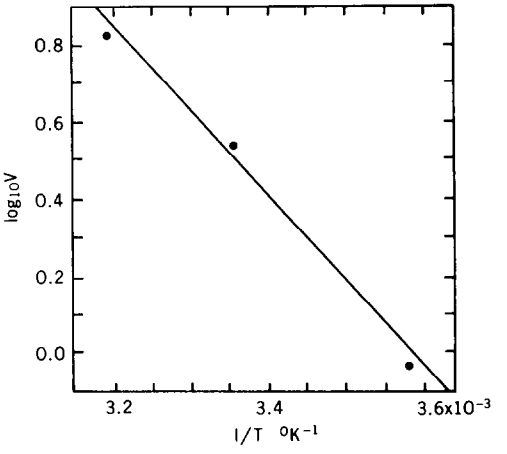

Fig. 4. The effect of temperature on the velocity extrapolated to infinite ferrocytochrome c concentration (V), for the reaction of cytochrome c with oxygen, catalysed by cytochrome oxidase. Conditions: 0.1 M phosphate buffer, pH = 6.0. Activation energy = 8.5 kcal.

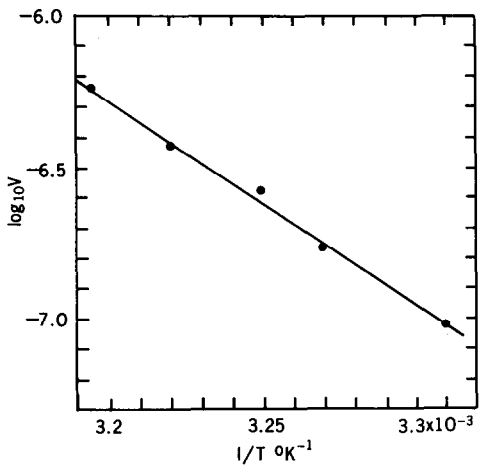

Fig. 5. The effect of temperature on the maximum rate (k) of the uncatalysed reaction of ferrocytochrome c with hydrogen peroxide. pH = 6.8, μ = 0.03. Activation energy = 33.5 kcal.

The first intermediate in the transfer of successive electrons to oxygen is the perhydroxyl radical $HO_2\cdot$ or the superoxide ion O_2^- [11].

The pK of the perhydroxyl radical is 4.5 [12], and over the pH range examined the first product of this step would be HO_2 at low pH and O_2^- at high pH. The entropy of formation of O_2^- (aq.) is particularly unfavourable due to its requirement for solvation. Both this fact and changes in the conformation of cytochrome c at low pH values and in the presence of organic solvents undoubtedly contribute to the observed thermodynamics, and our data do not distinguish between these different effects. From whatever cause however, the activation enthalpy for the reaction of ferrocytochrome c with oxygen near neutral pH is rather small, and the high catalytic efficiency of cytochrome oxidase resides primarily in its ability to remove an unfavourable entropy of activation, perhaps by by-passing the crucial initial step, as suggested by George [11].

When this initial step is avoided, as when hydrogen peroxide is used as the electron acceptor, our experimental data show that $T\Delta S^{\neq}$ for the oxidation of ferrocytochrome c is more favourable by at least 25 kcal/mole, although the activation energy is higher. In contrast to cytochrome oxidase, cytochrome c peroxidase has a marked effect on the enthalpy of activation. Further studies are in progress to answer some of the questions raised by the above findings.

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References

[1] E. Shashoua, Arch. Biochem. Biophys. 111 (1965) 550.
[2] H. Teorell and J. Åkesson, J. Am. Chem. Soc. 63 (1941) 1804.
[3] A.J. Davison and R.T. Hamilton, J. Biol. Chem. 243 (1968) 6064.
[4] L. Kaminsky, R. Wright and A.J. Davison, Biochemistry 10 (1971) 458.
[5] D. Keilin and E.F. Hartree, Nature 171 (1953) 413.
[6] J. Beetlestone, Arch. Biochem. Biophys. 89 (1960) 35.
[7] E. Boeri and L. Tosi, Arch. Biochem. Biophys. 52 (1954) 83.
[8] W.W. Wainio, J. Biol. Chem. 216 (1955) 593.
[9] K. Minnaert, Biochem. Biophys. Acta 50 (1961) 23.
[10] L. Smith and N. Newton, Symposium on Cytochromes, Osaka, 1967 (University of Tokyo Press, 1967) p. 153.
[11] P. George, T.E. King, H.S. Mason and M. Morrison, Oxidases and Related Redox Systems, Vol. 1 (John Wiley and Sons, New York, 1965) p. 3.
[12] G. Czapaki and L. Dorfman, J. Phys. Chem. 68 (1964) 1169.