INTERACTION OF HYDROGEN PEROXIDE WITH SUPEROXIDE DISMUTASE FROM ERYTHROCYTES

M.A. SYMONYAN and R.M. NALBANDYAN
Institute of Biochemistry, Academy of Sciences of the Armenian SSR, Yerevan, USSR

Received 21 September 1972

1. Introduction

Copper-containing proteins without oxidase or electron-carrier activities have been detected in a number of animal tissues (brain, liver, heart, erythrocytes) [1–8]. McCord and Fridovich [9, 10] present evidence of these proteins possessing superoxide dismutase activity, catalysing the following process:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Thus, the physiological role of superoxide dismutase may perhaps be protection of aerobic organisms against damage by free radicals normally generated in the course of the one electron transfer from substrate to oxygen molecule.

In recent works [11, 12] it was shown that the dismutase activity in a large number of aerobic organisms correlates with the catalase activity and that not in all organisms the dismutases are copper-containing proteins. Investigation of the dismutase-hydrogen peroxide reaction is of certain interest because H_2O_2 is a product of the dismutase reaction. In this report some preliminary observations on the interaction of H_2O_2 with bovine erythrocyte dismutase are presented.

2. Materials and methods

The dismutase was isolated from bovine erythrocytes by a slight modification of the method of McCord and Fridovich [9]. The protein isolated was electrophoretically homogeneous and had A_{259}/A_{680} absorption ratio of about 30.

The hydrogen peroxide concentration was determined by potassium permanganate titration. Peroxide was added at room temp. to the dismutase solution in 0.05 M phosphate buffer, pH 8.0. After incubating the solution 3 min with H_2O_2, optical and EPR spectra were recorded. Spectra obtained after 30 min incubation were similar to those obtained after 3 min incubation.

Optical measurements were carried out at room temp. in 10 mm cells. The concentrations of the dismutase solutions were determined, using a molar extinction of 300 at 680 nm. EPR-spectra were recorded on a Varian E-4 instrument at 77°K. Quantitative data were obtained by comparing the double integrals of the dismutase low temperature EPR-spectra with the double integrals of frozen standards (Cu(II)–EDTA).

3. Results and discussion

Our results showed that addition of dilute hydrogen peroxide to a dismutase solution immediately caused irreversible bleaching of the protein. The extent of the bleaching depended on the concentrations of the H_2O_2, being linear up to 50% bleaching. After that a certain deviation from the linear dependence is observed. The results of the spectrophotometric titration of dismutase by H_2O_2 presented in fig. 1 showed that one mole of the dismutase is bleached to 50% by one mole of hydrogen peroxide.

Bleaching of the dismutase by H_2O_2 may be due to the reduction of the protein copper. This possibility is suggested by EPR-titrations of the dismutase by dilute solutions of H_2O_2 (see fig. 2). The EPR and optical data

North-Holland Publishing Company – Amsterdam

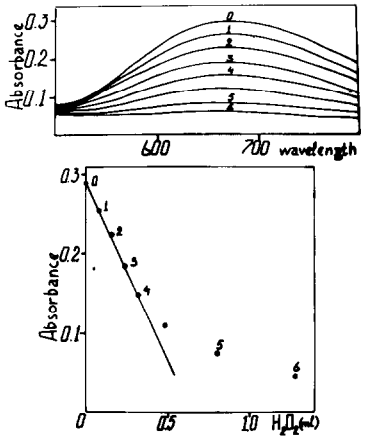

Fig. 1. Spectrophotometric titration of 3.5 ml superoxide dismutase by 1.1×10^{-2} M hydrogen peroxide. Spectral changes after addition of H_2O_2 (above) and titration curve (below).

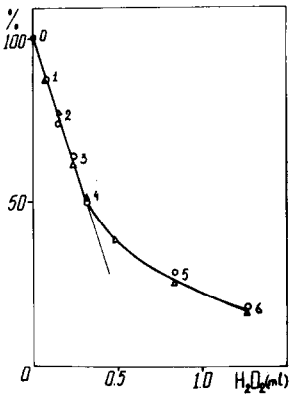

Fig. 3. Correlation between optical (△) and EPR (○) titration data.

shown in fig. 3 correlate well with each other. No significant changes in the shape of the EPR-signal of the dismutase were found in the course of the titration by dilute H_2O_2 solution. However, concentrated H_2O_2 solutions (10^{-1} M and more) lead to an EPR-spectrum of which the parameters differ from those of the native protein, while 680 nm band here also disappears. Hence in the titration with concentrated H_2O_2 there is no correlation between the EPR and optical data. Turbidity of the protein solutions on incubation with concentrated H_2O_2 has been observed. The EPR-spectra of native dismutase and of the dismutase following addition of concentrated H_2O_2 are shown in fig. 4.

The results suggest that in the presence of dilute hydrogen peroxide solutions, reduction (bleaching) of the dismutase proceeds according to the scheme:

$$2Cu^{2+} + H_2O_2 \rightarrow 2Cu^{1+} + 2H^+ + O_2$$

In concentrated peroxide solutions, however, inactivation of the protein takes place with retention of

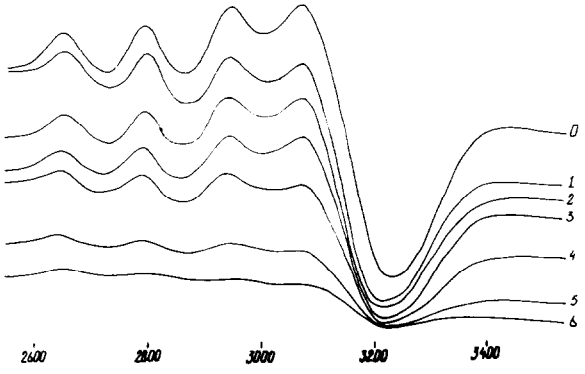

Fig. 2. EPR-titration of 3.5 ml superoxide dismutase ($A^{init.}_{680\,nm}$ = 0.29) by 1.1×10^{-2} M hydrogen peroxide. Recording conditions: receiver gain, 3.2×10^{-2}; time constant, 3 sec; scan time, 4 min; $T = 77°K$; modulation amplitude, 6.3 gauss.

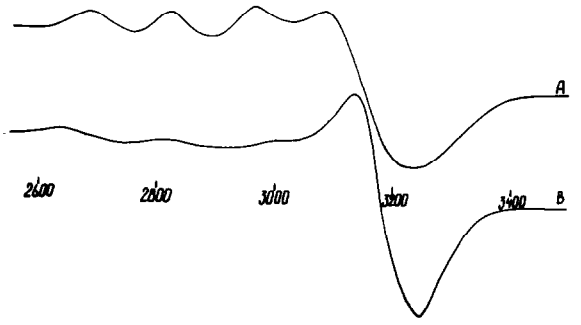

Fig. 4. EPR-spectra of native (A) and H_2O_2-inactivated (B) dismutases. The native protein sample was in 0.025 M phosphate buffer, pH. 7.0. Inactivation was carried out by addition of 0.2 ml 10% H_2O_2 to 1 ml dismutase solution. After 10 min incubation at room temp. the EPR-spectrum was recorded. The recording conditions were the same as those for the case shown in fig. 2 with the exception of receiver gain being 1.6×10^2. EPR-spectral parameters of native dismutase: $A_{\parallel} = 140\kappa$; $g_{\parallel} = 2.26_8$; $g_m = 2.07_8$. EPR-spectral parameters of inactivated dismutase: $A_{\parallel} = 172\kappa$; $g_{\parallel} = 2.25_4$; $g_m = 2.04_8$.

the valency of the copper atoms but with change in the environment of the cupric copper in the protein.

Recent studies [13] have shown that H_2O_2 partially reduces the so-called "blue" copper atoms of laccase and a complex of the H_2O_2 with the "non-blue" copper atoms of this protein are formed. We have also observed the reduction of this "blue" atom in other blue copper-containing proteins such as ceruloplasmin and plastocyanine. However, this occurs slowly whereas bleaching of the dismutase is a rapid reaction.

Detailed kinetic studies of the reduction of dismutase copper by hydrogen peroxide are in progress.

References

[1] T. Mann and D. Keilin Proc. Roy. Soc., B126 (1939) 303.
[2] H. Porter and J. Folch. J. Neurochem. 1 (1957) 260.
[3] H. Markowitz, G.E. Cartwright and M.M. Wintrobe, J. Biol. Chem. 234 (1959) 40.
[4] J.R. Kimmel, G. Markowitz and D.M. Brown, J. Biol. Chem. 234 (1959) 46.
[5] M.J. Stansell and H.A. Deutch J. Biol. Chem. 240 (1965) 4306.
[6] H. Porter, M. Sweeney and E. Porter, Arch. Biochem. Biophys..
[7] J. Bannister, W. Bannister and E. Wood, European J. Biochem. 18 (1971) 178.
[8] B.B. Keele, J.M. McCord and I. Fridovich, J. Biol. Chem. 246 (1971) 2875.
[9] J.M. McCord and I. Fridovich, J. Biol. Chem. 244 (1969) 6049.
[10] J.M. McCord and I. Fridovich, J. Biol. Chem. 244 (1969) 6056.
[11] J.M. McCord, B.B. Keele and I. Fridovich, Proc. Natl. Acad. Sci. U.S. 68 (1971) 1024.
[12] B.B. Keele, J.M. McCord and I. Fridovich, J. Biol. Chem. 245 (1970) 6176.
[13] R. Brändén, B.G. Malmström and T. Vänngård, European J. Biochem. 18 (1971) 238.