EFFECT OF IONIC STRENGTH ON THE ACTIVITY OF BOVINE SUPEROXIDE DISMUTASE

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1. Introduction

In studies of activity of superoxide dismutase (SOD), see [1], two kinds of methods have been used. For routine detection of activity, the inhibition by the enzyme of some O_2^- dependent reactions has been utilized [2–4]. For studies of the catalytic mechanism and when a precise evaluation of rate constants is needed, pulse radiolytic methods have been the only successful ones so far [5–8]. Recently we have applied the polarographic method of the kinetic currents to kinetic studies of the dismutation of O_2^-, which allows the determination of the rate constants by a very simple procedure [9]. Moreover it presents some advantages with respect to pulse radiolysis in so far that the composition of the solution under study can be varied in a way which would disturb straightforward pulse radiolysis experiments. For example, variation of ionic strength and type of ions of the solution, can be somewhat limited in pulse radiolysis, because of the possible production of secondary radicals. In this report we present results on the effect of ionic strength and different species of anions and cations on the activity of SOD as a part a program aimed to study SOD activity by the kinetic currents method systematically.

2. Materials and methods

Superoxide dismutase was prepared from bovine blood according to McCord and Fridovich [2]. The protein concentration was determined by the extinction coefficient at 680 nm (ϵ:300 M^{-1} cm^{-1}; see [2]). Catalytic rate constants were measured in different conditions as described elsewhere [9]. The activity of SOD was tested at 25°C in air saturated buffered solutions (borax 0.025 M, pH 9.90), in which were added different salts to realize the desired ionic strength. All the solutions were prepared from analytical grade chemical dissolved in twice distilled water. An AMEL mod. 461 polarograph and AMEL mod. 331 pH meter were employed for the polarographic and pH measurements respectively.

3. Results

Fig.1 shows the effect of increasing ionic strength

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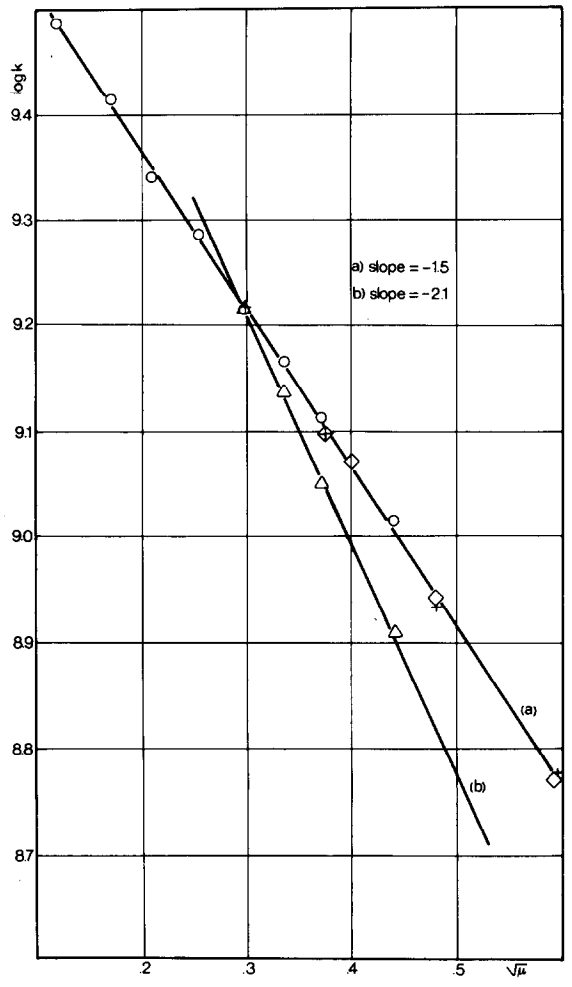

Fig. 1. Effect of ionic strength on the SOD activity: (o—o—o) $NaClO_4$; (+ + +) Na_2HPO_4; (◇—◇—◇) Na_2SO_4; (△—△—△) NaCl. Borax 0.0025 M, pH 9.80; T = 25°C.

by varying concentrations of different sodium salts. The data are plotted in the usual way for ionic strength effects on ionic reactions, that is according the Brönsted's equation [10] which at low concentration predicts that the plot of log k vs the square root of the ionic strength is a straight line, with the slope nearly equal to the product of the ionic charges of the ions involved in the formation of the activated complex. From fig.1 it is evident that in all cases the rate constant decreases with the ionic strength. The slope is very near to −1.5 for ClO_4^-, SO_4^{2-} and HPO_4^{2-} while

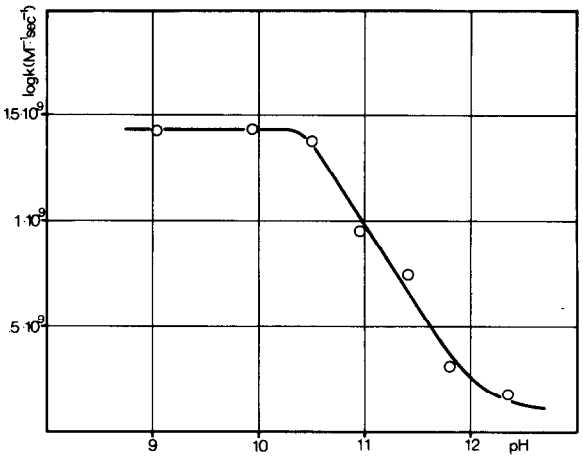

Fig. 2. pH dependence of SOD activity at constant ionic strength: borax 0.025 M; T = 25°C; μ = 0.16 adjusted by addition of $NaClO_4$.

it is −2.23 for Cl^-. The inhibition by Br^- is very strong and the plot is not linear. No changes were observed by varying the cationic species: Li^+, Na^+, K^+, Mg^{++}, Ca^{++}, Ba^{++}.

The observation of the ionic strength effect prompted us to reevaluate the pH dependance of the enzyme activity in the alkaline range, where a reversible loss of activity was shown to occur by a pulse radiolytic investigation [11].

Also when the ionic strength of the solution was carefully kept constant, a reversible decrease of activity was observed (fig.2).

4. Discussion

The effect of ionic strength on superoxide dismutase activity allows further considerations on the nature of the catalytic reaction. In fact Brönsted's theory predicts that a reaction proceeding via the formation of a transition state complex involving charged species, should be affected by ionic strength in a way depending by the number and the sign of the charges. On the basis of results from pulse radiolysis measurements [5,6] the reaction catalysed by superoxide dismutase has been suggested to occur via a ping-pong mechanism involving two reactions with similar rate constants:

1) $E\text{–}Cu^{2+} + O_2^- \longrightarrow E\text{–}Cu^{+} + O_2$

2) $E\text{–}Cu^{+} + O_2^- \xrightarrow{H^+} E\text{–}Cu^{2+} + H_2O_2$

where $E\text{–}Cu^{2+}$ and $E\text{–}Cu^{+}$ represent the active site of the enzyme in the oxidized and reduced state respectively. If it is assumed that reactions 1 and 2 require the formation of activated state complexes between copper and O_2^-, the product of the charges is -2 for reaction 1 and -1 for reaction 2. The value of -1.5 obtained from the dependence of the overall reaction on ionic strength is a confirmatory evidence that the mechanistic scheme obtained from pulse radiolytic measurements is correct. Moreover it points to a direct interaction of charges at the active site with possible formation of intermediate complexes for which no evidence was obtained so far [5,6]. It has been confirmed in this work that the enzyme reversibly loses its activity in the range pH 10–12 showing that the decrease observed by pulse radiolysis [11] was not simply an ionic strength effect. This is further evidence that the active site reversibly changes its geometry in this pH range as already indicated by EPR [12] and pulse NMR [13] measurements.

Results which are presently object of further investigation are the effects of Cl^- and Br^- which do not fit in with the scheme presented above. Br^- does not give a linear plot, while Cl^- still give a linear plot, but in both cases the slope is always higher than 1.50 indicating that beside the ionic interaction should exist a specific inhibition by the halide ion.

A marginal consideration which arises from these observations is that the inhibitory effect on bovine SOD reported by Forman and Fridovich [14] for guanidinium chloride is at least partly due to ionic stength and Cl^- effects, as evidenced by a simple comparison of their data with the plots of fig. 1.

References

[1] Fridovich, I. (1972) Arch. Chem. Res. 5, 321–326.
[2] McCord, J. M. and Fridovich, I. (1969) J. Biol. Chem. 244, 6049–6055.
[3] Misra, M. P. and Fridovich, I. (1972) J. Biol. Chem. 247, 3170–3175.
[4] Nishikimi, M., Ammaji Rao, N. and Yagi, K. (1972) Biochem. Biophys. Res. Commun. 46, 849–853.
[5] Rotilio, G., Bray, R. C. and Fielden, E. M. (1972) Biochim. Biophys. Acta, 268, 605–609.
[6] Klug, D., Rabani, J. and Fridovich, I. (1972) J. Biol. Chem. 247, 4839–4842.
[7] Klug, D., Roth, D., Fridovich, I. and Rabani, I. (1973) J. Amer. Chem. Soc. 95, 2786–2790.
[8] Fielden, E. M., Roberts, P. B., Bray, R. C., Lowe, D. J., Mautner, G. N., Rotilio, G. and Calabrese, L. (1974) Biochem. J. 139, 49–60.
[9] Rigo, A., Tomat, R. and Rotilio, G. (1975) J. Electranal. Chem. in press.
[10] Brönsted, J. N. (1925) Z. physik Chem. 118, 251.
[11] Roberts, P. B., Fielden, E. M., Rotilio, G., Calabrese, L., Bannister, J. V. and Bannister, W. M. (1974) Radiation Res. in press.
[12] Rotilio, G., Finazzi-Agrò, A., Calabrese, L., Bossa, F., Guerrieri, P. and Mondovì, B. (1971) Biochemistry 10, 616–621.
[13] Terenzi, M., Rigo, A., Franconi, C., Mondovì, B., Calabrese, L. and Rotilio, G. (1974) Biochim. Biophys. Acta, 230–236.
[14] Forman, H. and Fridovich, I. (1973) J. Biol. Chem. 248, 2645–2649.