Parallel electrostatic effects in the interactions of superoxide with cytochrome *c* and with superoxide dismutase

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Cytochrome c *Superoxide dismutase* *Ionic strength* *Dielectric constant* *Lysine residues* *Electrostatic facilitation*

1. INTRODUCTION

The bovine erythrocyte superoxide dismutase [1] exhibits an isoelectric point of 5.0 [2] and therefore has a net negative charge at neutral pH. Its rate of reaction with O_2^- decreases with increasing ionic strength, indicating an electrostatic facilitation of this reaction. Acylation of lysine residues destroys this electrostatic assistance to the catalytic process [3]. Cytochrome *c* contains lysine residues in the vicinity of the exposed heme edge and these have also been shown to facilitate the reaction of this hemoprotein with anionic reductants [4–7]. If the electrostatic effects on the reaction of O_2^- with SOD and with cytochrome *c* were parallel and of the same magnitude, then the cytochrome *c* reduction assay of superoxide dismutase activity should be unresponsive to changes in ionic strength or dielectric constant. The following data demonstrate this.

2. MATERIALS AND METHODS

2.1. *General*

Cytochrome *c* reduction assays were done as in [8,9]. Ionic strength was varied by adding $NaClO_4$. Dielectric constant was varied by the addition of ethanol. When necessary, the assay mix was titrated back to pH 7.8, prior to the addition of xanthine oxidase. The stability of native and glyoxal-modified BESOD were determined by incubation at high $NaClO_4$ and EtOH for 4 h at room temperature. Subsequent assays indicated no change in activity for either sample.

Abbreviations: SOD, superoxide dismutase; BESOD, bovine erythrocyte superoxide dismutase

2.2. *Glyoxal modification*

BESOD (1.6 μM) was incubated with glyoxal (40 mM) in 0.125 M sodium bicarbonate solution pH 8.8 at 25°C for 3 h. The sample was then dialyzed against 50 mM potassium phosphate, 0.1 mM EDTA (pH 7.8) at 4°C.

3. RESULTS

The ionic strength of the medium was increased by the addition of $NaClO_4$. Control measurements demonstrated that $NaClO_4$, up to 0.25 M, did not affect the molar extinction coefficient at 550 nm associated with the reduction of cytochrome *c*. This level of $NaClO_4$ was also without effect on the rate of reduction of cytochrome *c* by the xanthine oxidase reaction. It was thus clear that the perchlorate was not interfering with the cytochrome *c* reduction assay. Fig.1 demonstrates further that perchlorate, up to 0.20 M, had no effect on the apparent activity of native SOD (line 1) as measured in the cytochrome *c* reduction assay. The apparent activity of glyoxal-modified enzyme was weakly diminished with increasing perchlorate (line 2).

The dielectric constant of the medium was lowered by the admixture of ethanol. Ethanol, up to 3.0

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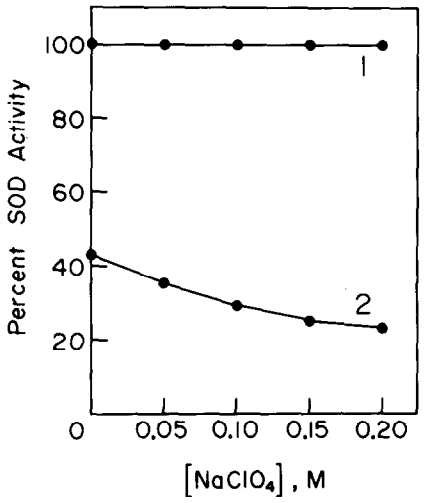

Fig.1. Variation of SOD activity with ionic strength using the cytochrome *c* assay. Buffers contained 50 mM phosphate and 0.1 mM EDTA, at pH 7.8. Activity is reported as the % of specific activity of native BESOD assayed in the absence of $NaClO_4$: (1) native BESOD; (2) glyoxal-modified BESOD.

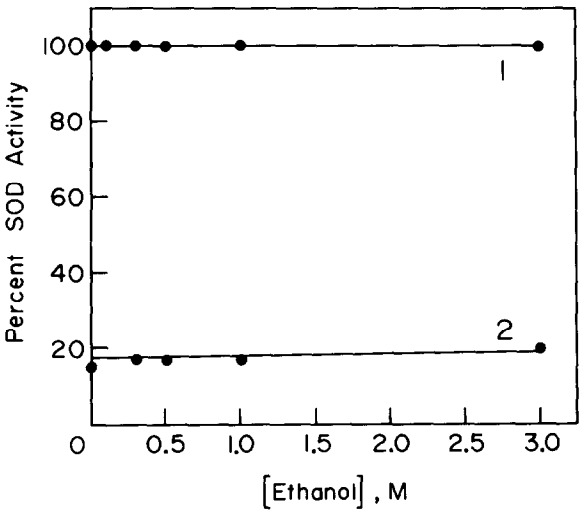

Fig.2. Variation of SOD activity with dielectric constant using the cytochrome *c* assay. Buffers contained 50 mM phosphate and 0.1 mM EDTA, at pH 7.8. Activity is reported as the % of specific activity of native BESOD assayed in the absence of ethanol: (1) native BESOD; (2) glyoxal-modified BESOD.

M, had no effect on the apparent activities of the native (line 1) or of the glyoxal-modified (line 2) enzyme (fig.2).

4. DISCUSSION

The above results are of interest from 2 points of view:

(i) Given the competition between SOD and cytochrome *c* for O_2^-, which is the basis of the assay used, and given the known importance of electrostatic factors in the reaction of cytochrome *c* with anionic reductants, we can conclude that electrostatic facilitation is as important for the interaction of SOD with O_2^-, as it is for the reduction of cytochrome *c* by this species.

(ii) The apparent unresponsiveness of the cytochrome *c* reduction assay for SOD activity to changes in ionic strength and dielectric constant makes it a very convenient assay when one wishes to work over a range of salt or miscible solvent concentrations. Further, comparisons of SOD activity in samples of different ionic strength or dielectric constant are valid.

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