An estimate of the dipole moment of superoxide dismutase

Johan W. van Leeuwen

Department of Biochemistry, Agricultural University, De Dreijen 11, 6703 BC Wageningen, The Netherlands

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A lower limit for the value of the dipole moment of superoxide dismutase (SOD) is calculated to be 485 Debye. This limit follows from the observation that the rate constant of the reaction between superoxide (O_2^-) and SOD decreases upon increasing the ionic strength, and the fact that at pH > 5 SOD has a net negative charge.

Superoxide dismutase *Dipole moment* *Electrostatic interaction* *Ionic strength*

1. INTRODUCTION

Recently authors in [1] came to the conclusion that the electrostatic interaction of O_2^- with cytochrome *c* is of the same nature as the interaction of O_2^- with superoxide dismutase (SOD). This seems surprising because the isoelectric point of SOD is 5.0 [2] and therefore SOD has a net negative charge at pH > 5.0. These authors also showed that the activity of SOD decreased with increasing ionic strength. This was also observed when arginine 141 was modified with phenylglyoxal [3]. However, after modification of lysine residues by acetylation or succinylation the SOD activity increases with increasing ionic strength. They conclude that the lysines are important in providing electrostatic guidance to the anionic substrate. To explain why modification of arginine 141 does not change the response of the enzyme to ionic strength they suggest that arginine 141 is buried in the protein and is not easily accessible from the solvent. In my opinion this last argument is contradicted by the fact that the ion O_2^- reacts very fast with SOD, so the copper which is close to the arginine must be easy to reach. Furthermore, since arginine 141 can be easily modified it must be reasonably accessible. It was also found that the p*K* of arginine 141 is hardly shifted [3] which is another argument supporting the access of the solvent. As will be shown in this letter, the electrostatic facilitation by the lysines can be represented quantitatively in terms of the dipole moment of the charge distribution. The ionic strength dependence is determined by the electrostatic interaction of the O_2^- with the dipole moment of SOD. This can explain why modification of arginine 141 hardly influences the ionic strength dependence, whereas lysine modification reverses the effect of ionic strength. The method used here to obtain an estimate of the dipole moment can also be applied to other enzymes and substrates.

Abbreviation: SOD, superoxide dismutase

2. RESULTS AND DISCUSSION

To explain the ionic strength dependence of the rate constant of the reaction between a protein and an ion (substrate) it is normally only the net charge on a protein that is taken into account. However, sometimes the effect of the ionic strength dependence is the opposite of what one expects on the basis of the net charge. This is attributed to the interactions of the substrate with the charged groups near the active site on the protein [3]. The preference of a substrate for a certain site on the protein can be ascribed to the dipole moment of the charge distribution. In first approximation the real charge distribution on the protein is given by a homogenous charge distribution with a net

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charge of Z_1. In the second order approach the asymmetry of the charge distribution is expressed in terms of a dipole moment P_1. Normally the ionic strength dependence of the rate constant depends exponentially on the electrostatic interaction energy at the closest approach of the two reactants. The electrostatic interaction energy and its ionic strength dependence have been calculated before [4,5] and are given by equations (1) and (2):

$$V(R) = \frac{Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon R} \cdot f(\varkappa) \quad (1)$$

$$V_P(R) = \frac{Z_2 e P_1 (1 + \varkappa R)\cos\theta}{4\pi\epsilon_0\epsilon R^2} \cdot f(\varkappa) \quad (2)$$

where:

$V(R)$ = the monopole–monopole interaction energy;
$V_P(R)$ = the monopole–dipole interaction energy;
Z_i, R_i = the charge and radius of the protein (i = 1) and substrate (i = 2);
$R = R_1 + R_2$,
P_1 = the dipole moment of the protein;
ϵ = the dielectric permittivity;
$\varkappa$ = 0.33 $\sqrt{I}$ Å^{-1}, where I is the ionic strength.

The function $f(\varkappa)$ depends on the ionic strength, R_1 and R_2. The function $f(\varkappa)$ varies continuously between 1 (for $\varkappa = 0$) and 0 ($\varkappa \longrightarrow \infty$). Expressions for $f(\varkappa)$ are given in [4] for the case $R_2 < R_1$ and in [5] if $R_2 \simeq R_1$. The exact nature of $f(\varkappa)$ is not relevant here. In eq. (2) θ is the angle between the direction of the dipole moment and the direction of the interaction site relative to the centre of mass of the protein. For example, if an ion interacts with the positive site of the dipole, then $\cos\theta = 1$, and for the interaction with the negative site of the dipole $\cos\theta = -1$.

The total interaction energy ($V(R) + V_P(R)$) determines the ionic strength dependence. If ($V(R) + V_P(R)$) < 0, then the rate constant decreases, and if ($V(R) + V_P(R)$) > 0, the rate constant increases upon increasing the ionic strength. For the reaction of O_2^- with SOD ($V(R) + V_P(R)$) < 0, even at low ionic strengths ($\varkappa \longrightarrow 0$) [3]. Using eq. (1) and (2) one then obtains:

$$Z_1 Z_2 + Z_2 \frac{P_1 \cos\theta}{eR} < 0 \quad (3)$$

If $Z_2 = -1$, $R_2 = 2$ Å, $R_1 = 23$ Å and $Z_1 = -3$ [6] it is calculated that $P_1\cos\theta > 346$ Debye (1 Debye = 3.3×10^{-30} c.m.). Because $0 < \cos\theta < 1$ it follows that the dipole moment of SOD is at least >364 Debye.

Upon neutralisation of the charge of arginine 141 (which means that $Z_1 = -4$) it was found that eq. (3) was still valid. This means that $P_1 > 485$ Debye. So the interaction of SOD with O_2^- is determined by the monopole–dipole interaction energy. Modification of only arginine 141 is not sufficient to reverse the effect of the ionic strength dependence. However, if > 8 lysines/subunit are neutralised, then the net charge becomes so low that $Z_1 Z_2 + Z_2 P_1 \cos\theta/(eR) > 0$. Furthermore, when the lysines are neutralised, the asymmetry in the charge distribution decreases and therefore the dipole moment P_1 decreases. This explains why the ionic strength dependence is reversed upon modification of the lysines. Because SOD is a dimer, the value of $P_1\cos\theta$ obtained from eq. (3) is some sort of average of the two components of the total dipole moment, each of which points in the direction of a catalytic site.

The lower limit for the magnitude of the dipole moment (485 Debye) seems reasonable because the estimated dipole moments of other proteins are of the same order of magnitude. For cytochrome *c*, P = 325 Debye [7], for cytochrome b_5, P = 570 Debye [5], for cytochrome *c* oxidase, P = 2130 Debye [5], for flavodoxin, P = 460 Debye [8] and for ferredoxin, P = 210 Debye [8].

This letter shows that an abnormal behaviour in the ionic strength dependence of a rate constant can be explained by taking into account the monopole–dipole interaction between the reactants. A method is indicated to estimate the value of the dipole moment.

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