Biochimica et Biophysica Acta, 635 (1981) 434—439

BBA Report

BA 41344

THE IONIC STRENGTH DEPENDENCE OF THE RATE OF A REACTION BETWEEN A SMALL ION AND A LARGE ION WITH A DIPOLE MOMENT

JOHAN W. VAN LEEUWEN, FRANS J.M. MOFERS and ENNO C.I. VEERMAN

Department of Molecular Biophysics, Physics Laboratory, University of Utrecht, P.O. Box 80 000, 3508 TA Utrecht (The Netherlands)

(Received September 22nd, 1980)

Key words: Ionic strength dependence; Dipole moment; Electrostatic interaction; Cytochrome c; Cytochrome b

Summary

The ionic strength dependence of the rate constant of a reaction between a small ion and a large ion with a dipole moment (e.g. a protein) is described. This description takes into account only the electrostatic interactions between the two ions. This approach agrees with the Marcus theory treatment of the electrostatic interactions and also with the Debye-Hückel theory which is based on changes in the activity coefficients of the reactants. The contribution of the dipole moment of the protein to the ionic strength dependence of the rate constant has been calculated. A method is described whereby one can calculate the charge of the protein without knowing the precise ionic strength dependence of the rate constant. Two applications are mentioned to illustrate the usefulness of the method.

Generally the ionic strength dependence of the rate of a reaction between charged particles is given by the following Debye-Hückel expression

$$\ln k_I = \ln k_0 - \frac{e^2\kappa}{4\pi\epsilon_0\epsilon 2kT}\left\{\frac{Z_1^2}{1+\kappa R_1} + \frac{Z_2^2}{1+\kappa R_2} - \frac{(Z_1+Z_2)^2}{1+\kappa R}\right\} \quad (1)$$

where Z_i and R_i are the charge and the radius of the ions respectively, R is the radius of the activated complex and $\kappa = 0.33\sqrt{I}$ Å^{-1} where I is the ionic strength. Each of the last three terms on the right hand side of Eqn. 1 repres-

Abbreviation: Hipip, high-potential iron protein.

ents the difference between electrostatic energy at zero ionic strength and at ionic strength I. In this equation however the contribution from the activated complex is underestimated. Consider for example the reaction between O_2^- and ferricytochrome c. According to Eqn. 1 the O_2^- is incorporated in the activated complex and the radius of the ionic cloud around O_2^- increases from R_1 to R. It is more likely that in the activated complex the O_2^- remains in the water environment, and will not penetrate into the protein because the latter has a low dielectric constant. During the formation of an activated complex the ionic clouds around the reacting ions will be disturbed. For cytochrome c this disturbance can be neglected because the O_2^- can be considered as being an ion of the ionic cloud. The ionic cloud around the O_2^- is considerable disturbed when the O_2^- is in the vicinity of the cytochrome c molecule. However, when a charge is located in a medium with a high dielectric constant and is close to another medium which has a low dielectric constant it induces at the boundary surface between the mediums a polarisation charge which has the same sign as the first charge. We now assume that the disturbance of the ionic cloud around the O_2^- can be compensated by the ionic cloud caused by the polarisation charge on the surface of the protein.

Using the above arguments we can state that the difference between the solvation energy of the reacting ions at zero ionic strength and at ionic strength I in the activated complex is approximately the same as the difference between the solvation energy of the separate reacting ions at these ionic strengths. This is equivalent to replacing the term $(Z_1 + Z_2)^2/(1 + \kappa R)$ in Eqn. 1 by the following expression:

$$\frac{Z_1^2}{(1 + \kappa R_1)} + \frac{Z_2^2}{(1 + \kappa R_2)} + \frac{2Z_1Z_2}{(1 + \kappa(R_1 + R_2))} . \tag{2}$$

This assumption reduces Eqn. 1 to the following expression which in many cases gives a correct fit at low ionic strength [1,2].

$$\ln k_I = \ln k_0 + \frac{Z_1Z_2e^2}{4\pi\epsilon_0\epsilon kT} \cdot \frac{\kappa}{1 + \kappa(R_1 + R_2)} . \tag{3}$$

Using the above-mentioned assumption we can then describe the ionic strength dependence of the rate constant in a more general way as

$$k_I = k_\infty \exp(-V(R)/kT) \tag{4}$$

where k_∞ is the rate constant at infinite ionic strength and $V(R)$ represents only the electrostatic energy of the interaction between the two ions at closest approach ($R = R_1 + R_2$) at ionic strength I. Eqn. 4 and all expressions derived therefrom are valid only for reactions which are not diffusion controlled [3].

We shall restrict our study to reactions between a large ion (charge Z_1, radius R_1) with a dipole moment P_1 and a small ion (Z_2, R_2) without a dipole moment (e.g. oxidation or reduction reactions of cytochrome c, Hipip, haem proteins).

Solving the Poisson equation with the Debye-Hückel approximation ($V(R) < kT$) the following expression can be derived for $V(R)$ which contains only the

ion-ion interaction [4]

$$V(R) = \frac{Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon R} \frac{e^{-\kappa R_2}}{1 + \kappa R_1} . \tag{5}$$

Eqn. 5 represents the potential energy of ion 2 in the field of ion 1 when the ions are separated by a distance $R = R_1 + R_2$. The ion-dipole interaction is given by Eqn. 6 which represents the electrostatic energy of a small ion in the potential field of the a dipole P_1 (radius R_1).

$$V_P(R) = \frac{Z_2 e P_1 \cos\theta}{4\pi\epsilon_0\epsilon R^2} \frac{(1 + \kappa R)\, e^{-\kappa R_2}}{(1 + \kappa R_1)} \tag{6}$$

θ representing the angle between the dipole moment and the interaction site on the large ion. The potential field around a dipole at ionic strength I has been calculated by adding together the potential fields of two oppositely charged ions (radius R_1) separated by a distance d. Eqn. 6 was obtained by applying the following limits, $d \to 0$, $Z \to \infty$ and by keeping $P_1 = Zd$ constant. In this way the dipole is formed by two oppositely charged penetrable ions, each of radius R_1 and ultimately merging.

In these calculations it is important to use the potential energy of the small ion in the electric field of the large ion because the small ion will hardly disturb the ionic cloud around the large ion. Theoretically the following relation should be valid: $V_{12} = V_{21}$, where V_{ij} is the potential energy for ion i in the field of ion j. This is not what we obtain if we use Eqn. 5 because Eqn. 5 does not include the large disturbance of the ionic cloud around the small ion which occurs when the large ion approaches the small ion. The formula derived by Wherland and Gray (see Eqn. 7) [5] can also be obtained by substituting the average potential energy, $V(R) = \frac{1}{2}(V_{12} + V_{21})$ in Eqn. 4.

$$\ln k_I = \ln k_\infty - \frac{Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon k T R} \cdot \frac{1}{2}\left\{\frac{e^{-\kappa R_2}}{1 + \kappa R_1} + \frac{e^{-\kappa R_1}}{1 + \kappa R_2}\right\} \tag{7}$$

Eqn. 7, however, underestimates the contribution of $V(R)$, especially when R_1 is much larger than R_2.

By substituting Eqns. 5 and 6 in Eqn. 4 we obtain the following ionic strength dependence of the rate constant which should be valid for the reaction between a small ion and a large ion with a dipole moment ($R_1 > R_2$).

$$\ln k_I = \ln k_\infty - \frac{Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon k T R} \cdot \frac{e^{-\kappa R_2}}{1 + \kappa R_1} - \frac{Z_2 e P_1 \cos\theta}{4\pi\epsilon_0\epsilon k T R^2} \cdot \frac{(1 + \kappa R)\, e^{-\kappa R_2}}{1 + \kappa R_1} \tag{8}$$

which, after substitution of Eqn. 4 (for $I = 0$) gives

$$\ln k_I = \ln k_0 + \frac{Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon k T R}\left(1 - \frac{e^{-\kappa R_2}}{1 + \kappa R_1}\right) + \frac{Z_2 e P_1 \cos\theta}{4\pi\epsilon_0\epsilon k T R^2}\left(1 - \frac{(1 + \kappa R)\, e^{-\kappa R_2}}{1 + \kappa R_1}\right) \tag{9}$$

and simplifies to the following Eqn. if $\kappa R_2 < 1$.

$$\ln k_I = \ln k_0 + \frac{Z_2 e^2}{4\pi\epsilon_0 \epsilon kT} \cdot \frac{\kappa}{1 + \kappa R_1} \left(Z_1 + \frac{P_1 \cos\theta}{eR} \cdot \kappa R_2 \right) . \tag{10}$$

Upon comparing Eqn. 8 (if $P_1 = 0$) with Eqn. 7 for reactions between a small ion ($R_2 = 2$—5 Å) and a large ion ($R_1 \simeq 17$ Å) we found that both equations could fit the data equally well for $I < 0.3$ M and that $Z_1 Z_2$ as derived from Eqn. 7 was only about 5 to 10% less than the value obtained from Eqn. 8. This means that the Marcus theory treatment of electrostatic interactions [5] gives the same results as the direct application of Eqn. 4. This is easily verified by substituting $\kappa = 0$ in Eqn. 7. Another consequence of using Eqn. 4 is that the derived Eqn. 10 with $P_1 = 0$ gives nearly the same result as the often applied Eqn. 3, which originates from changes in the activity coefficients of the reactants. These facts support the earlier assumption.

There are several factors which can cause deviations in the normal type of ionic strength plots: (1) contribution of an ion-dipole interaction term (see Eqn. 10); (2) ion binding to the studied protein; (3) ionic strength dependence of the pK_a of the amino acid residues; (4) the condition $V(R) < kT$ may not be fulfilled. These factors can make it difficult to correlate the charge of the protein with the slope of the ionic strength plots. An alternative way of obtaining the charge of a protein is to determine k_0 and k_∞ through extrapolation of the measurements at low ($I < 0.1$ M) and high ($I \geqq 0.5$ M) ionic strength. Then by substituting the values found for k_0 and k_∞ in Eqn. 11, which is a direct consequence of Eqn. 4, the product of charges ($Z'_1 Z_2$) at zero ionic strength can be calculated.

$$\ln(k_\infty / k_0) = \frac{Z'_1 Z_2 e^2}{4\pi\epsilon_0 \epsilon kTR} . \tag{11}$$

In Eqn. 11

$$Z'_1 = Z_1 + \frac{P_1 \cos\theta}{eR} : \tag{12}$$

Normally rate constants are measured at low ionic strength ($I < 0.1$ M) [1,6]. We would stress that measurements at high ionic strength ($I \sim 1$ M) also yield valuable information about the charge and the dipole moment of the reactants. In fact one need only measure the rate constant at zero ionic strength and at high ionic strength to obtain the product of charges of the reactant (see Eqn. 11).

Applications

The dipole moment of cytochrome *c* has been calculated to be approx. 300 Debye [7] and, therefore, depending on the value of $\cos\theta$, the maximum charge correction (P_1/eR) will be ±3 (if $R = 20$ Å). If we assume that the charge on cytochrome *c* is $Z_1 = +8$ then it can be calculated (see Eqn. 10) that for $I <$ 0.1 M the dipole moment will hardly make any contribution to the ionic strength dependence and that for $I > 0.4$ M the ionic strength dependence is

mainly determined by the dipole interaction (see Eqn. 9).

The presence of ions might lead to deviations in the net charge of the protein under study (ion binding [2,8] and changes in pK_a of the amino acid residues [9]). These deviations will hardly affect k_∞ and hence using the method described above (see Eqn. 11) we are able to calculate the charge of the protein without knowing the precise ionic strength dependence of the rate constant.

For Eqn. 4 there is no restriction that $V(R) < kT$ [3]. Eqn. 5 however was derived under the condition that $V(R) < kT$ but recently it has been shown that the validity of Eqn. 5 can go far beyond the kT value [10]. For example if $\kappa R_1 = 0.1$ then Eqn. 5 is valid as long as $V(R) < 8\,kT$ and if $\kappa R_1 = 1.0$ then $V(R) < 4\,kT$. This means that the reaction of cytochrome c ($R_1 = 17$ Å, $Z_1 = 8$) with single- or double-charged small ions can be described with the Debye-Hückel approximation, whereas if this approximation is used for the reaction with small ions having a valency of three or more, deviations can be expected.

Another method of calculating the contribution of a dipole moment to the ionic strength dependence of the rate constant is to include the dipole moment in the activity coefficient of the reactant. This method has been used by Koppenol [7] who calculated the contribution that a dipole moment of 300 Debye makes to the reaction between a large ion (18 Å) and a small ion (4 Å) for $I <$ 0.1 M. Using the same parameters as Koppenol [7] for R_1, R_2, Z_1, Z_2 and P_1 we calculated that according to Eqn. 9 the dipole correction to the rate constant is about half the correction as calculated by Koppenol. Measurements, especially at high ionic strengths, should show whether our method is adequate to explain the obtained results.

Finally, we would like to mention two special applications of Eqn. 11.

(a) Goldkorn and Schejter [1] have determined the rate constant for reduction of native cytochrome c ($k = 335\ M^{-1} \cdot s^{-1}$) and of pyrixodal phosphate-1-cytochrome c ($k = 158\ M^{-1} \cdot s^{-1}$) by ascorbate at zero ionic strength. If we assume that this modication of the charge on cytochrome c will hardly change the dipole moment and that the modification is not at the site of interaction between cytochrome c and ascorbate (i.e. k_∞ remains the same upon modification) then Eqn. 11 gives

$$\ln\left(\frac{k_{mod}}{k_{nat}}\right)_{I=0} = \frac{\Delta Z_1 Z_2 e^2}{4\pi\epsilon_0\epsilon kTR} \tag{13}$$

where k_{mod} and k_{nat} are the rate constants for the modified and the native form of the protein and ΔZ_1 is the charge difference between the modified and the native protein. By using Eqn. 13 we found $\Delta Z_1 = 2.1$ (if $R = 20$ Å) which is in good agreement with the theoretical value of $\Delta Z_1 = 2$.

(b) The reaction between cytochrome c and cytochrome b_5 has been measured over a large ionic strength range [11]. Although this is a reaction between two large ions Eqn. 11 will still be valid if we include the dipole moment contribution in the effective charges (see Eqn. 12). Using the data of Stonehuerner et al. [11] we determine $k_0 = 40$ (a.u.) by making a plot of k_I versus κ. From a plot of k_I versus $(1 + \kappa R_1)^{-1}$ we obtained $k_\infty = 7 \cdot 10^{-5}$ (a.u.). Substituting k_∞ and k_0 in Enq. 11 and taking $R = 33$ Å one obtains $Z_1'Z_2' = -61$. This is in reasonable agreement with a value of +9 for cytochrome c and −6 for cyto-

chrome b_5 as reported by these authors. We therefore believe that it is not necessary to describe the interaction between the two proteins as the sum of six complementary charge interactions, as was done by the authors [11].

References

1 Goldkorn, T. and Schejter, A. (1979) J. Biol. Chem. 254, 12 562—12 566
2 Koppenol, W.H. (1976) Biochim. Biophys. Acta 449, 157—168
3 Weston, R. and Schwarz, H.A. (1972) Chemical Kinetics, pp. 151—179, Prentice Hall Inc., Englewood Cliffs, NJ
4 Volkenstein, M.V. (1977) Molecular Biophysics pp. 48—52, Academic Press, New York
5 Wherland, S. and Gray, H.B. (1976) Proc. Natl. Acad. Sci. U.S.A. 73, 2950—2954
6 Feinberg, B.A., Ryan, M.D. and Wei, J. (1977) Biochem. Biophys. Res. Commun. 79, 769—775
7 Koppenol, W.H. (1980) Biophys. J. 29, 493—508
8 Osheroff, N., Borden, D., Koppenol, W.H. and Margoliash, E. (1980) J. Biol. Chem. 255, 1689—1697
9 Tanford, C. and Kirkwood, J.G. (1957) J. Am. Chem. Soc. 79, 5333—5339
10 Lampert, M.A. and Crandall, R.S. (1979) Chem. Phys. Lett. 68, 473—476
11 Stonehuerner, J., Williams, J.B. and Millett, F. (1979) Biochemistry 18, 5422—5427