

Allowance for effects of electrostatic repulsion on protein dimerization

Paul-Michael Agapow and Donald J. Winzor

Department of Biochemistry, University of Queensland, St. Lucia, Queensland (Australia)

(Received 8 September 1987)

(Revised manuscript received 25 November 1987)

Key words: α -Chymotrypsin; Electrostatic effect; Protein dimerization

A simple procedure for assessing the extent of electrostatic effects on protein dimerization is described and illustrated by application to published results on the ionic strength dependence of the dimerization constant for α -chymotrypsin at pH 4 (Aune, K.C., Goldsmith, L.C. and Timasheff, S.N. (1971) *Biochemistry* 10, 1617-1622). From the analysis it is concluded that the inverse dependence of α -chymotrypsin dimerization upon ionic strength is predominantly a general electrostatic effect, rather than a consequence of repulsion between two specific charged residues on the adjacent monomers comprising dimer.

The formation of dimer from two charged monomers requires two such monomers to be placed in direct contact, a situation that is clearly opposed by an electrostatic force. Despite the existence of deficiencies in regard to its inherent presumption that the charge is uniformly distributed over a spherical monomer, and also to its neglect of factors such as possible solvent-ordering on monomer surfaces and dielectric saturation of the solvent [1], the Verwey-Overbeek theory [2] still provides the best quantitative description, albeit approximate, of the electrostatic contribution to the free energy of protein dimerization [3]. This theory has, however, been little used for evaluating the extent of electrostatic effects in the energetics of protein dimerization. In that regard, a contributing factor to the apparent reluctance of biochemists to take advantage of the quantitative information available from the Verwey-Overbeek theory has undoubtedly been the complicated nature of the expressions describing the electrostatic effect. The purpose of this communication is to present a rearranged form of the Verwey-Overbeek expression that greatly facilitates the quantitative assessment of experimental results. Pub-

lished data on the effect of ionic strength on the dimerization of α -chymotrypsin [4] are then used to illustrate its application, and to comment on the likely value of the Verwey-Overbeek theory for the quantification of electrostatic effects in protein dimerization.

Provided that dimer formation is presumed to entail the placement of two spherical monomers such that the distance between their centres is twice the monomeric radius ($s = 2$ in Verwey-Overbeek terminology), the electrostatic contribution to the molar free-energy change for dimerization follows from Eqn. 82 of Ref. [2] by noting that:

$$\Delta G_e = N\psi_0^2 Da\gamma/2 \quad (1)$$

where N is Avogadro's number, and D the dielectric constant. ψ_0 , the surface potential on monomer molecule with radius a , and γ are both functions of ionic strength by virtue of their dependence on κ , the Debye-Huckel inverse screening length. The inherent requirement for κa to be small is unlikely to impose any great restriction on application of Eqn. 1 to the dimerization of globu-

lar proteins in buffers of low to moderate ionic strength. The quantitative expressions for ψ_0 and γ are Eqns. 79 and 83 of Verwey and Overbeek [2], which on substitution of a value 2 for s and reinstatement of the product κa for τ , become in present terminology:

$$\psi_0 = \frac{Ze[1 + \{(1 - \exp(-2\kappa a))/4\kappa a\}(1 + \alpha)]}{aD(1 + \kappa a)(1 - \delta(1 + \alpha))} \quad (2a)$$

$$\gamma = (1 + \alpha)/(1 - \delta(1 + \alpha)) \quad (2b)$$

where Z is the net charge (valence) of monomer, and ϵ is the electronic charge in esu; δ and α are subsidiary parameters defined (Verwey-Overbeek Eqn. 78) by the relationships:

$$\delta = (1/4\kappa a)[\{(\kappa a - 1)/(\kappa a + 1)\} + \exp(-2\kappa a)] \quad (3a)$$

$$\alpha = \lambda_1(1 + (1/2\kappa a)) + \lambda_2\{1 + (3/2\kappa a) + [3/(2\kappa a)^2]\} \quad (3b)$$

Evaluation of α requires magnitudes of λ_1 and λ_2 , which must first be obtained by solving the following two simultaneous equations (Verwey-Overbeek Eqns. 71 and 72):

$$\frac{1}{2}\lambda_1 + \delta \left(1 + \frac{1}{(2\kappa a)} + \lambda_1 \left\{ 1 + \frac{2}{(2\kappa a)} + \frac{2}{(2\kappa a)^2} \right\} + \lambda_2 \left\{ 1 + \frac{4}{(2\kappa a)} + \frac{9}{(2\kappa a)^2} + \frac{9}{(2\kappa a)^3} \right\} \right) = 0 \quad (4a)$$

$$\frac{1}{2}\lambda_2 + \frac{1}{(4\kappa a)} \left(\frac{(\kappa a)^2 - 3\kappa a + 3}{(\kappa a)^2 + 3\kappa a + 3} - \exp(-2\kappa a) \right) \times \left(\left\{ 1 + \frac{3}{(2\kappa a)} + \frac{3}{(2\kappa a)^2} \right\} + \lambda_1 \left\{ 1 + \frac{4}{(2\kappa a)} + \frac{9}{(2\kappa a)^2} + \frac{9}{(2\kappa a)^3} \right\} + \lambda_2 \left\{ 1 + \frac{6}{(2\kappa a)} + \frac{24}{(2\kappa a)^2} + \frac{54}{(2\kappa a)^3} + \frac{54}{(2\kappa a)^4} \right\} \right) = 0 \quad (4b)$$

For routine application of Eqn. 1, it is clearly more convenient to reformulate Eqns. 1-4 in terms of monomer radius, a , its valence, Z , and a single parameter, $\xi(\kappa a)$, solely dependent on the product κa .

Solution of Eqns. 4a and 4b for a specified value of κa yields values of λ_1 and λ_2 , which in turn allow calculation of the corresponding magnitudes of γ and $(aD\psi_0/Z\epsilon)$. Eqn. 1 thus becomes:

$$\Delta G_e = N\epsilon^2 Z^2 (\psi_0 a D / Z\epsilon)^2 \gamma / 2aD \quad (5)$$

Further simplification follows on noting that $N\epsilon^2/D = (7.154 \cdot 10^{-8})RT$, a substitution obtained by combing the expression for the Debye-Huckel screening parameter, $\kappa = (8\pi N^2 \epsilon^2 / 1000DRT)^{1/2} \sqrt{I}$, with its value for aqueous solutions of univalent electrolytes at 25°C, $\kappa = (3.29 \cdot 10^7) \sqrt{I}$. The electrostatic component of the molar free energy change for dimerization may therefore be written as:

$$\Delta G_e = (3.577 \cdot 10^{-8}) RT \xi(\kappa a) Z^2 / a \quad (6)$$

where $\xi(\kappa a) = (\psi_0 a D / Z\epsilon)^2 \gamma$ is a quantity which may be evaluated for any given κa . Results of such calculations, performed at intervals of 0.1 in κa , are summarized graphically in Fig. 1. Empirically, it has been found that the relationship $\xi(\kappa a) = (0.709 + 1.267\kappa a)^{-1.87}$ is an essentially linear transform of this dependence, the maximum

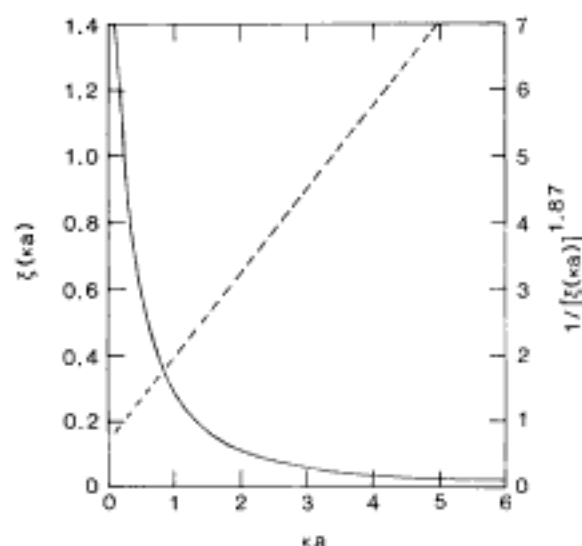


Fig. 1. Graphical representation of the dependence upon κa of the function $\xi(\kappa a)$, a quantity required for assessment of electrostatic effects in protein dimerization (Eqn. 6). The broken line, referring to the right-hand ordinate, is an empirical linear transform of the dependence.

difference between actual and predicted values being 1% in the range $1 \leq \kappa a \leq 6$: for lower but less relevant values of κa , the discrepancy is still less than 2%.

From an experimental viewpoint, the measured standard free energy change for dimerization, ΔG^0 , is given by:

$$\Delta G^0 = \Delta G_{int}^0 + \Delta G_e \quad (7a)$$

where ΔG_{int}^0 is the intrinsic standard free energy change in the absence of an electrostatic effect. In terms of association equilibrium constants, the corresponding relationship may therefore be expressed as:

$$\ln K = \ln K_{int} - (3.577 \cdot 10^{-8}) Z^2 \xi(\kappa a) / a \quad (7b)$$

A plot of $\ln K$ vs. $(3.577 \cdot 10^{-8}) \xi(\kappa a) / a$ should thus be linear, with a slope of $-Z^2$, if the dominant effect of ionic strength is the change in general electrostatic repulsion.

That prediction has been tested by employing Eqn. 7b to assess the apparent valence of α -chymotrypsin at pH 4.1 from published results on the ionic strength dependence of the dimerization constant (Figs. 1 and 2 of Ref. 4). From the molecular weight of 25 000 and sedimentation coefficient ($s_{20,w}^0$) of 2.5 S for monomer under these conditions [4], its Stokes radius, a , is calculated to be 2.3 nm. This radius of hydrated monomer (in cm) is then combined with values of κ

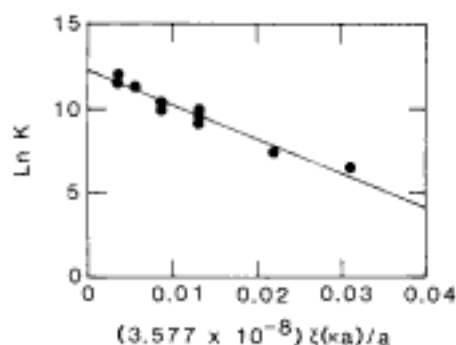


Fig. 2. Allowance for effects of electrostatic repulsion on the dimerization of α -chymotrypsin in 0.01 M acetate buffer (pH 4.1), of which the ionic strength was varied by including NaCl. The results, taken from Fig. 1 of Ref. 4, are plotted in accordance with Eqn. 7b; and the line corresponds to the relationship obtained by least-squares calculations.

derived from the ionic strengths to give the appropriate values of κa for estimating $\xi(\kappa a)$ from Fig. 1 (or from the above empirical relationship). Consideration of the resulting plot of $\ln K$ vs. $(3.577 \cdot 10^{-8}) \xi(\kappa a) / a$ (Fig. 2) to be linear leads to values (± 2 S.E.) of 12.2 (± 1.0) and -201 (± 38) for the ordinate intercept ($\ln K_{int}$) and slope ($-Z^2$), respectively. On this basis the apparent valence of α -chymotrypsin at pH 4.1 is 14.2 (± 1.4), an estimate to be compared with values of +10 to +11 derived from pH-titration data [5] and direct measurements of Z [6] under similar conditions.

Although the present analysis has given rise to a slight overestimate of α -chymotrypsin valence, the discrepancy must be viewed in the light that the evaluation of Z from Eqn. 7b is based on the premise [2] that the charge is uniformly distributed over spherical monomer — a condition that can only be fulfilled imperfectly. Moreover, it has been assumed that solvation effects [1] are taken into account adequately by the use of a hydrated radius for monomer. In view of these factors, the extent of agreement between present and previous estimates of Z is considered sufficient to justify consideration of the ionic strength dependence of the dimerization of α -chymotrypsin at pH 4.1 to be largely a general electrostatic effect, rather than a consequence of direct interaction between a specific pair of similarly charged groups on the two monomers comprising dimer.

In summary, a simple procedure based on Verwey-Overbeek theory [2] has been devised for assessing the extent of general electrostatic effects on protein dimerization. As is evident from the analysis of α -chymotrypsin data used to illustrate its application, there is likely to be sufficient deviation from the assumed uniformity of charge distribution to preclude use of the method for accurate evaluation of monomer valence. Nevertheless, by yielding an approximate estimate of the apparent valence, the procedure has potential to provide a means for distinction between general electrostatic effects and changes in intrinsic association constant as the dominant source of ionic strength dependence of protein dimerization.

This investigation was financed in part by the Australian Research Grants Scheme, to whom we express our gratitude.

References

- 1 Eagland, D. (1975) in *Water, a Comprehensive Treatise* (Franks, F., ed.), Vol. 5, pp. 1-74, Plenum Press, New York.
- 2 Verwey, E.J.W. and Overbeek, J.T.G. (1948) *Theory of the Stability of Lyophilic Colloids*, Elsevier, Amsterdam.
- 3 Aune, K.C. and Timasheff, S.N. (1971) *Biochemistry* 10, 1609-1617.
- 4 Aune, K.C., Goldsmith, L.C. and Timasheff, S.N. (1971) *Biochemistry* 10, 1617-1621.
- 5 Marini, M.A. and Wunsch, C. (1963) *Biochemistry* 2, 1454-1460.
- 6 Ford, C.L. and Winzor, D.J. (1983) *Biochim. Biophys. Acta* 756, 49-55.