Reprinted from Nature, Vol. 301, No. 5896, pp. 169-171, 13 January 1983

Electrostatic orientation during electron transfer between flavodoxin and cytochrome *c*

James B. Matthew*, P. C. Weber*, F. R. Salemme* & F. M. Richards

Department of Molecular Biophysics and Biochemistry, Yale University, PO Box 6666, New Haven, Connecticut 06511, USA

Various studies have shown that reaction rates between reversibly binding electron transfer proteins depend strongly on solution ionic strength[1-7]. These observations suggest that intermolecular electrostatic interactions are important in facilitating the formation of a productive reaction complex. A recently examined system involves the reduction of vertebrate cytochrome *c* by bacterial flavodoxin[8,9]. Although this is a non-physiological reaction, it proceeds with rates typical for natural partners and is similarly inhibited at high ionic strengths. Here we describe computational studies which examine the role of electrostatics in the formation of a putative reaction complex between flavodoxin and cytochrome *c*. The results suggest that electrostatic interactions preorient the molecules before they make physical contact, facilitating the formation of an optimal reaction complex.

* Present addresses: Department of Biochemistry, University of New Hampshire, Durham, New Hampshire 03824, USA (J.B.M.); Department of Biochemistry, University of Arizona, Tucson, Arizona 85721, USA (P.C.W., F.R.S.).

Figure 1*a*, *b* shows the $2kT$ electrostatic potential fields around tuna ferricytochrome *c*[10] at two ionic strengths. The fields were computed from the crystallographic coordinates[11] using a modified Tanford–Kirkwood theory which scales the local dielectric constant of the charged groups according to their solvent-accessible surface areas[12-14]. As first inferred from comparative structural studies[15,16], and subsequently verified by Koppenol *et al.*[17], the presence of a 'necklace' of positively charged lysine residues around the perimeter of the haem crevice leads to a pronounced asymmetry in the electrostatic field of the molecule. Figure 1*c*, *d* shows similar representations for *Clostridium* MP flavodoxin[18]. Here it is again apparent that the molecule possesses a highly asymmetric potential field, in this case due to a concentration of negatively charged amino acids in the vicinity of the flavin mononucleotide (FMN) prosthetic group. Therefore, it is clear that the complementary electrostatic fields on these proteins can mutually orient them to facilitate a direct approach between the flavin and haem prosthetic groups.

Figure 2 shows stereoscopic views of a putative reaction complex of flavodoxin and cytochrome *c* generated by computer graphics[8,19,20]. This arrangement minimizes the prosthetic group separation subject to intermolecular steric constraints at the interface in the complex, and additionally incorporates four specific ionic interactions between lysine residues on cytochrome *c* and acidic residues of flavodoxin[8]. (These are the same charged groups whose close proximity on the isolated molecules makes a predominant contribution to their local electrostatic fields[8,19].)

To assess the role of complementary electrostatics in the

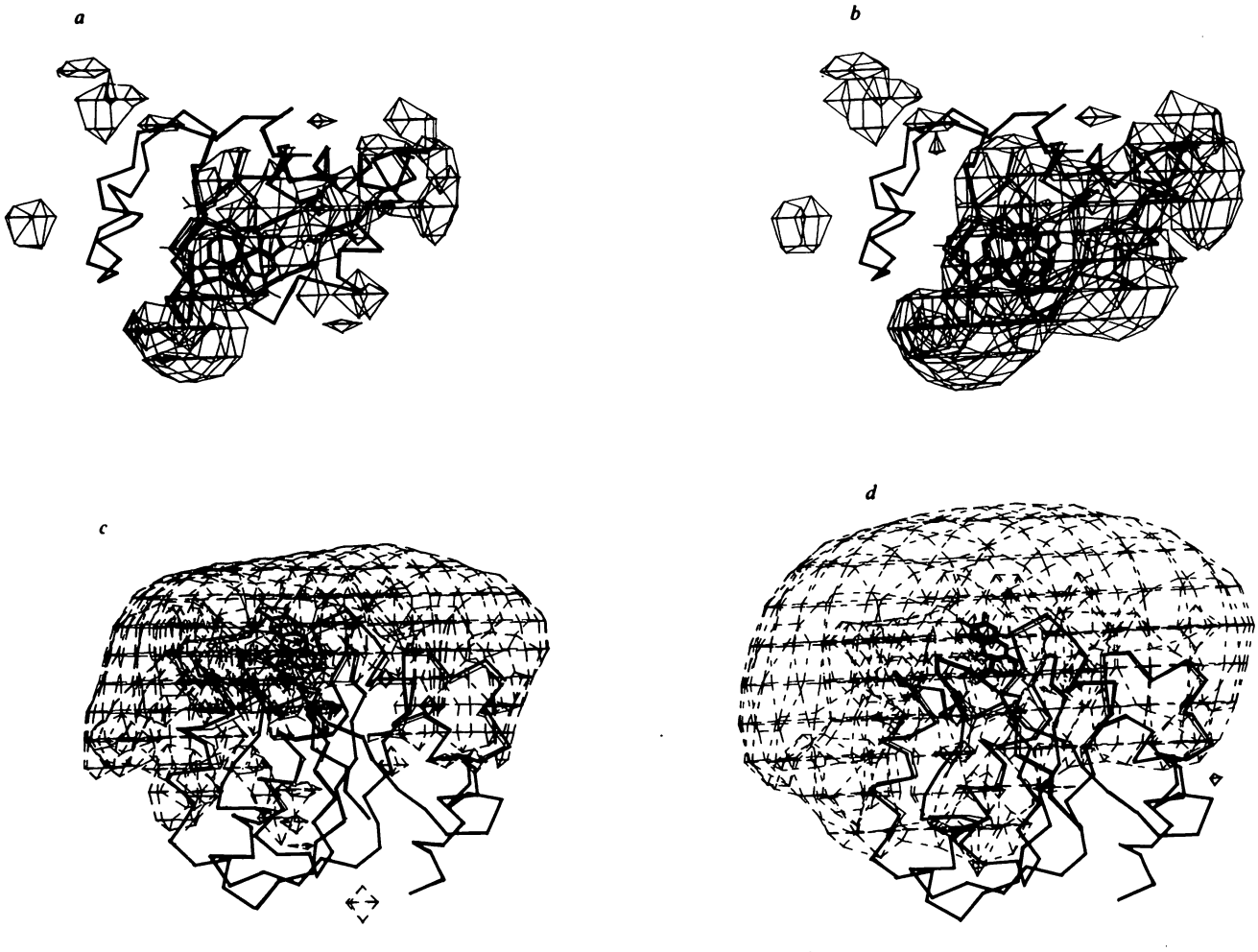

Fig. 1 Electrostatic fields, contoured at an energy level of + or − 2 kT (=1.2 kcal mol^{-1} = 0.052 eV) around tuna ferricytochrome *c* (*a*, *b*) and *Clostridium* MP flavodoxin (*c*, *d*) at pH 7 and solution ionic strengths of 0.08 M (*a*, *c*) and 0.04 M (*b*, *d*). The C^{α} chains of the two proteins are shown as heavy lines. Cytochrome *c* and flavodoxin have respective net charges of +7 and −18. The fields are asymmetrically distributed on the molecular surfaces near the prosthetic groups. The scale of these diagrams and those in Fig. 2 can be estimated from the C^{α}–C^{α} distance in the backbone chains which is about 4 Å.

formation of the complex, we have computed the change in electrostatic free energy during both reactant association (ferricytochrome *c* and flavodoxin semiquinone) and product dissociation (ferrocytochrome *c* and oxidized flavodoxin) assuming a simplest case trajectory where there is no relative rotational reorientation of the molecules. It has been suggested previously that the driving force for complementary electrostatic association reflects the participation of two factors: (1) the stabilization which occurs on complex formation because the groups of each molecule have like charge, and (2) the reduction of local dielectric constant at the molecular interface that results from solvent exclusion as the molecules associate[16,19]. For molecules having asymmetric charge distributions, a complementary electrostatic complex is more stable than the isolated molecules. The interaction energy decreases smoothly on association and reaches a minimum when the reaction complex is formed (Fig. 3). The subsequent loss of a cytochrome *c* positive charge when it is reduced destabilizes the product complex by ~ 1 kcal mol^{-1}, a relative difference between reactants and products which is preserved as the molecules dissociate. The scaling factor for each charged atom, which is proportional to its solvent-accessible surface area[12,15], can be viewed as a quantitative measure of the extent to which the water (or ionic solvent) surrounding each group shields its charge[14]. We have estimated the extent to which solvent exclusion, or equivalently, the drop in local dielectric constant, enhances the electrostatic free energy of association. The association energy profiles were computed with the accessible areas left at their infinite separation values (dashed curve of Fig. 3) or with the areas recomputed at each separation distance to allow for solvent exclusion (solid curve of Fig. 3). The difference between these curves suggests that solvent exclusion enhances

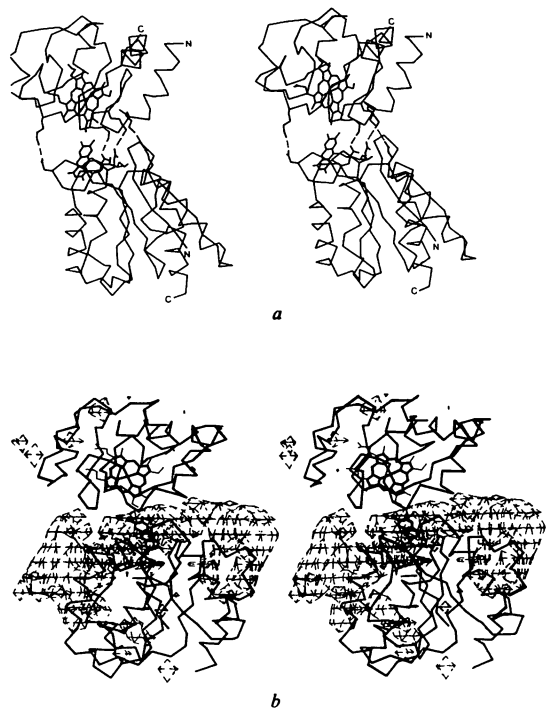

Fig. 2 Stereoscopic views of a computer-generated complex of *Clostridium* MP flavodoxin and tuna cytochrome *c*. In *a*, the C^{α} chains only are shown. Side-chain interactions constrain the relative orientations of the haem and FMN prosthetic groups and exclude solvent from the molecular interface. Four complementary ionic interactions (dotted lines) were optimized by slight reorientations of the crystallographically observed conformations of interacting charged residues. In *b* the complex is seen from a different aspect, together with its 2 *kT* electrostatic potential field at *p*H 7.0 and 0.08 M ionic strength. The field is wholly negative and reflects discharge of the positive field of cytochrome *c* on association with the more highly charged flavodoxin molecule.

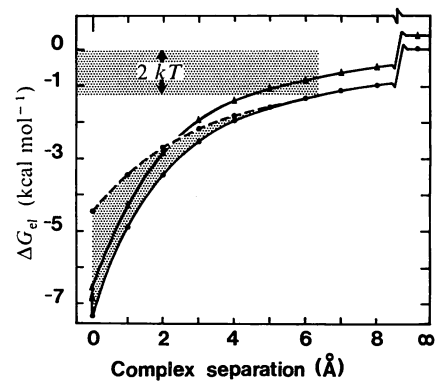

Fig. 3 The calculated change in electrostatic free energy (ΔG_{el}) on association of ferricytochrome *c* and flavodoxin to form the complex shown in Fig. 2 (*p*H 7.0, $I = 0.08$ M) is indicated by the dots and the solid curve, and by the triangles, the dissociation behaviour after cytochrome *c* reduction. The difference between the dashed and solid association curves (shaded) reflects the enhancement of ΔG_{el} due to solvent exclusion as the complex is formed. The molecules begin to orient when ΔG_{el} becomes more favourable relative to the isolated molecules by 2 *kT*, that is at ~6.5 Å separation.

the electrostatic interactions by an amount corresponding to about one-half of the total stabilization energy.

In Fig. 4, observed apparent and computed association constants[8] are plotted as a function of ionic strength. The close correspondence suggests that electrostatic interactions make the principal contributions to the free energy of complex association, and that entropic effects due to solvent release essentially compensate for those arising from reduction in independent molecular degrees of freedom[21–23].

The role of electrostatic interactions in enhancing the kinetics of the flavodoxin–cytochrome *c* reaction can be summarized in terms of two interrelated effects. First, the interaction of the electrostatic fields preorients the molecules along a trajectory leading to close approach of the prosthetic groups[7,15,16,19,20]. Thus, instead of random collisions, the fields interact preferentially so that only a single degree of rotational freedom (along an axis connecting prosthetic groups) remains to be defined in the formation of the structurally unique complex. This orientation effect becomes significant when the interaction free energy between the molecules is about 2 *kT* less than the sum of the free energies of the isolated states. In the physiological conditions simulated in Fig. 3, this occurs when the molecules are still physically separated by about 6.5 Å. Second, the fields could serve additionally to accelerate the actual association during complex formation[7,15,16,19,20]. This effect may account

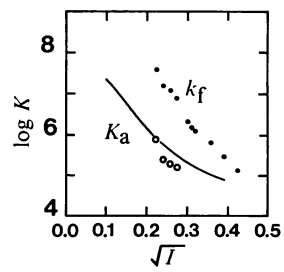

Fig. 4 The variation in the computed association constants ($\log K_a = -\Delta G_{el}/2.3RT$ from Fig. 3) for the complex as a function of solution ionic strength is shown by the solid line. ○, Observed values of K_a (apparent) derived from kinetic studies on electron transfer rates between cytochrome *c* and *Clostridium pasteruianum* flavodoxin semiquinone, a molecule homologous to the *Clostridium* MP species used for the computational studies. ●, Observed ionic strength dependence of the association rate constants for the latter system (referred to the same ordinate log scale). The observed rate accelerations may reflect progressively longer-range electrostatic orientation effects as the ionic strength is decreased (see Figs 1, 3).

for the observed increase in flavodoxin–cytochrome *c* association rates[8] at decreased ionic strengths (Fig. 4). Figure 1 shows that a decrease in solution ionic strength deshields the molecules so that they undergo attractive interactions at larger separation distances.

Some of the general properties considered here may apply to other biological systems where complementary electrostatic interactions are important, for example protein–DNA and protein–membrane interactions. It might be anticipated that field interactions similar to those described above could constrain protein diffusion on DNA[23] or membrane surfaces[16,25] to one or two dimensions, respectively. Interestingly, the present results (Fig. 3) suggest that when a proper (that is solvent-excluding) fit is achieved between the protein and a complementary structural feature of the DNA or membrane substrate, there is a dramatic increase in the binding free energy. The electrostatic forces can consequently act in concert with other specific steric or hydrogen-bonding interactions to immobilize the protein in a specific complex.

We thank Mark Handschumacher for assistance with the molecular graphics displays. This work was supported by NHI grants GM 21534 and 25664 to F.R.S., and by NCI fellowship F32 CA06633 to J.B.M.

Received 16 August; accepted 2 November 1982.

1. Smith, H. T., Ahmed, A. J. & Millett, F. *J. biol. Chem.* **256,** 4984–4990 (1981).
2. Geren, L. M. & Millett, F. *J. biol. Chem.* **256,** 4851–4855 (1981).
3. Foust, G. P., Mayhew, S. G. & Massey, V. *J. biol. Chem.* **244,** 964–970 (1969).
4. Batie, C. J. & Kamin, H. *J. biol. Chem.* **256,** 7756–7763 (1981).
5. Gacon, G., Lostanlen, D., Labie, D. & Kaplan, J. *Proc. natn. Acad. Sci. U.S.A.* **77,** 1917–1921 (1980).
6. Kuma, F. *J. biol. Chem.* **256,** 5518–5523 (1981).
7. Koppenol, W. H. & Margoliash, E. *J. biol. Chem.* **257,** 4426–4437 (1982).
8. Simondsen, R. P., Weber, P. C., Salemme, F. R. & Tollin, G. *Biochemistry* (in the press).
9. Simondsen, R. P. & Tollin, G. in *Flavins and Flavoproteins* (eds Massey, V. & Williams, C. H.) 719–724 (Elsevier, New York, 1982).
10. Swanson, R. *et al. J. biol. Chem.* **252,** 759–775 (1977).
11. Bernstein, F. G. *et al. J. molec. Biol.* **112,** 535–542 (1977).
12. Shire, S. J., Hanania, G. I. H. & Gurd, F. R. N. *Biochemistry* **13,** 2967–2974 (1974).
13. Matthew, J. B., Hanania, G. H. I. & Gurd, F. R. N. *Biochemistry* **18,** 1919–1928 (1979).
14. Matthew, J. B. & Richards, F. M. *Biochemistry* (in the press).
15. Salemme, F. R., Kraut, J. & Kamen, M. D. *J. biol. Chem.* **245,** 7701–7716 (1973).
16. Salemme, F. R. *A. Rev. Biochem.* **46,** 299–329 (1977).
17. Koppenol, W. H., Vroonland, C. A. J. & Braams, R. *Biochim. biophys. Acta* **503,** 499–508 (1978).
18. Smith, W. W., Burnett, R. M., Darling, G. D. & Ludwig, M. L. *J. molec. Biol.* **117,** 195–225 (1977).
19. Salemme, F. R. *J. molec. Biol.* **102,** 563–568 (1977).
20. Poulos, T. L. & Kraut, J. *J. biol. Chem.* 10322–10330 (1980).
21. Ross, P. D. & Subramanian, S. *Biochemistry* **20,** 3096–3102 (1981).
22. Chothia, C. & Janin, J. *Nature* **256,** 705–707 (1975).
23. Winter, R. B., Berg, O. G. & Von Hippel, P. H. *Biochemistry* **20,** 6861–6877 (1981).
24. Mauk, M. R., Reid, L. S. & Mauk, A. G. *Biochemistry* **21,** 1843–1846 (1982).
25. Overfield, R. & Wraight, C. A. *Biochemistry* **19,** 3322–3327, 3328–3334 (1980).