BRIEF COMMUNICATION

ACTIVATION OF ELECTRON TRANSFER REACTIONS OF THE BLUE PROTEINS

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ABSTRACT Thermal activation of electron transfer reactions of the blue proteins is considered in terms of vibronic coupling. Use of the electron paramagnetic resonance spectral distribution to obtain an estimate of the force constant for the relevant protein mode is proposed and demonstrated. This analysis leads to a model in which the configurations of the cupric and cuprous sites are displaced only a few degrees from each other, both being close to the configuration midway between planar and tetrahedral.

In recent papers on biological electron transfer by thermally activated tunneling, Hopfield (1, 2) quantitatively treats the activation energy in terms of vibronic coupling in the donor and acceptor molecules. (See also ref. 3.) A model of minimal complexity is employed wherein the force constants for the vibrational modes of the molecules with and without the electron are taken to be equal, k_d for the donor and k_a for the acceotor. The parameters $\Delta_d \equiv \frac{1}{2} k_d x_d^2$ and $\Delta_a \equiv \frac{1}{2} k_a x_a^2$ play important roles in the temperature dependence of electron transfer, x_d being the displacement of the relevant donor harmonic potential in going from the state with the electron present to that with it absent, x_a being the corresponding displacement for the acceptor. At high temperatures ($T \gg T_a/2$)[1] Hopfield finds that the rate of electron transfer W_{da} can be expressed

$$W_{da} = \frac{2\pi}{h} \mid T_{da} \mid^2 \frac{1}{\sqrt{4\pi\Delta kT}} \exp - (E_d - E_a - \Delta)^2/4\Delta kT \tag{1}$$

where T_{da} is the matrix element of the Hamiltonian between the initial (on donor) and final (on acceptor) states of the electron, E_d and E_a are the reduction potentials of the donor and acceptor redox couples, $\Delta \equiv \Delta_d + \Delta_a$, k is the Boltzmann constant, and T, the absolute temperature. In this formulation the donor and acceptor sites are spatially fixed as they would be in solids. With modifications the formulation also applies to nonadiabatic outer sphere electron transfer reactions in solution, the greatest

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[1] T_a is the temperature equivalent of a quantum of vibrational energy.

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contribution to a positionally-averaged rate being produced during the lifetime of the donor-acceptor complex. Apart from a possible additional term arising from complex formation in solution, these electron transfer processes will exhibit an enthalpy of activation given by[2]

$$\Delta H\ddagger = (E_d - E_a - \Delta)^2/4\Delta. \tag{2}$$

In the context of the above model we wish to consider some properties of azurin, one of the copper-containing proteins which engage in electron transfer reactions and are deep blue when oxidized. Tetrahedral distortion of the usually planar complexes of the cupric ion has been recognized for some time as a structural factor of prime importance in producing the unusual magnetic and optical properties of the blue proteins (4–6). It was suggested that this displacement of coordination configuration toward that characteristic of the cuprous ion was in part responsible for the high positive values of redox potential (4, 7) and would also facilitate activation of redox reactions (7). Magnetic resonance and fluorescence measurements on azurin and other blue proteins containing a single copper show that the metal atom is not accessible from the aqueous phase (e.g. 8–11), a situation which requires a tunneling or outer sphere mechanism of electron transfer. To minimize the influence of factors other than vibrational facilitation, we wish to consider the smallest activation enthalpies observed for electron transfer to these proteins. In this category are the values (12, 13) 0.089, 0.093, and 0.13 eV for the reduction of *Pseudomonas aeruginosa* azurin ($E_a = -0.30$ eV), French bean plastocyanin ($E_a = -0.37$ eV), and stellacyanin ($E_a = -0.18$ eV) by Fe(EDTA)$^{2-}$($E_d = -0.12$ eV). (See ref. 14 for a tabulation of these and related redox potentials.) Solving the quadratic Eq. 2, one finds that the larger set of roots is self-consistent: 0.67 (azurin), 0.79 (plastocyanin), and 0.64 eV (stellacyanin). In the following considerations we take the average value, $\Delta = 0.70$ eV, to be the upper limit for the vibronic coupling factor of the copper site, i.e. $\Delta_a \equiv \frac{1}{2} k_a x_a^2 \leq 0.70$ eV.

The relevant vibrational motion of these copper sites is somewhat similar to the symmetric bending mode of a tetrahedral complex such as Ni(CO)$_4$, but less symmetric, with one length of polypeptide attached to one pair of the tetrahedral bonds and joined to another length attached to the other pair. It has been shown that the unusual shape of the low-field quartet in the X-band electron paramagnetic resonance (EPR) spectrum of azurin can be explained on the basis of a Gaussian distribution in tetrahedral (out-of-planarity) angle Fig. 1, (15). The root mean square amplitude of the angular deviations $\Delta\eta$ required to simulate the observed shape is 0.7° or 0.024 radian for the angle subtended by a pair of bonds, to which corresponds a displacement of 0.048 Å at a ligand distance of 2 Å. The EPR measurement was taken at a cryogenic temperature, and this distribution could reflect the displacements of an ensemble of conformations fixed at approximately 210°K (16). Application of the principle of equipartition then gives a force constant $k_a = 1.3 \times 10^5$ dyn cm^{-1}. In small molecules the force constants for bending are smaller than this, and those for stretching bonds, greater.

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[2] It can be shown that *ln* (rate) is nearly linear in $1/T$ in the region $(E_d - E_a - \Delta)^2/2\Delta k \gg T \gg T_a/2$.

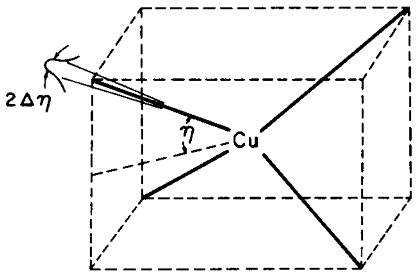

FIGURE 1 The lobes of the ground state (hole) orbital are maximally concentrated at angles η above and below the mean plane and are directed, approximately, at the four ligand atoms.

Because of the constraining influence of the polypeptide, some stretch should accompany the bending activity around the cupric site in the blue proteins and give rise to a force constant of intermediate stiffness such as the above value for k_a.[3]

An upper limit to the configuration coordinate displacement x_a can now be obtained, $x_a < \sqrt{2\Delta/k_a} = 0.4$ Å, to which corresponds a change of η of 6°. In the cupric states of azurin and stellacyanin the out-of-planarity angle η is approximately 16° (15). It appears that the configurations of the cuprous and cupric states of the blue proteins are only a few degrees apart, both being close to the configuration midway (η = 17.7°) between true planar and true tetrahedral.

In support of this model are the measurements on several blue proteins of light absorption and circular dichroism in the ultraviolet (5, 17), and ^{13}C nuclear magnetic resonance (18); the results are consistent with there being only minor changes in protein conformation accompanying change in the valence of the copper. X-ray absorption edge spectra of reduced and oxidized plastocyanin exhibit differences (19), which possibly can be used to quantitate the extent of rearrangement of ligand electronic structure. X-ray diffraction studies of azurin and plastocyanin are under way. The angles associated with the model are related to angles in the three-dimensional structures of the proteins. Within the resolution of the electron density maps, comparison of the oxidized and reduced structures will provide a test of the model.

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REFERENCES

1. HOPFIELD, J. J. 1974. Electron transfer between biological molecules by thermally activated tunneling. *Proc. Natl. Acad. Sci. U.S.A.* **71**:3640.
2. HOPFIELD, J. J. 1977. Photo-induced charge transfer. A critical test of the mechanism and range of biological electron transfer processes. *Biophys. J.* **18**:311.
3. JORTNER, J. 1976. Temperature dependent activation energy for electron transfer between biological molecules. *J. Chem. Phys.* **64**:4860.

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[3] Taking a reduced mass of one-eighth the molecular weight of the protein, one finds an undamped vibrational frequency of 1×10^{12} Hz (33 cm^{-1}) and an equivalent temperature, T_a, of 48°K (0.0041 eV).

4. **Brill**, A. S., R. B. **Martin**, and R. J. P. **Williams**. 1964. Copper in biological systems. *Electron. Aspects Biochem. Proc. Int. Symp.* Academic Press, Inc., New York. 519–557.
5. **Maria**, H. J. 1966. Colour, magnetic resonance, and symmetry of the cupric site in blue protein. *Nature (Lond.).* **209**:1023.
6. **Brill**, A. S., and G. F. **Bryce**. 1968. Cupric ion in blue proteins. *J. Chem. Phys.* **48**:4398.
7. **Vallee**, B. L., and R. J. P. **Williams**. 1968. Metalloenzymes: the entatic nature of their active sites. *Proc. Natl. Acad. Sci. U.S.A.* **59**:498.
8. **Blumberg**, W. E., and J. **Peisach**. 1966. The optical and magnetic properties of copper in *Chenopodium album* plastocyanin. *Biochim. Biophys. Acta.* **126**:269.
9. **Brill**, A. S., G. F. **Bryce**, and H. J. **Maria**. 1968. Optical and magnetic properties of *Pseudomonas* azurins. *Biochim. Biophys. Acta.* **154**:342.
10. **Rist**, G. H., J. S. **Hyde**, and T. **Vänngård**. 1970. Electron-nuclear double resonance of a protein that contains copper: evidence for nitrogen coordination to Cu(II) in stellacyanin. *Proc. Natl. Acad. Sci. U.S.A.* **67**:79.
11. **Avigliano**, L., A. **Finazzi-Agrò**, and B. **Mondovi**. 1974. Perturbation studies on some blue proteins. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **38**:205.
12. **Wherland**, S., R. A. **Holwerda**, R. C. **Rosenberg**, and H. B. **Gray**. 1975. Kinetic studies of the reduction of blue copper proteins by Fe(EDTA)$^{2-}$. *J. Am. Chem. Soc.* **97**:5260.
13. **Holwerda**, R. A., S. **Wherland**, and H. B. **Gray**. 1976. Electron transfer reactions of copper proteins. *Annu. Rev. Biophys. Bioeng.* **5**:363.
14. **Moore**, G. R., and R. J. P. **Williams**. 1976. Electron-transfer proteins. *Coord. Chem. Rev.* **18**:125.
15. **Brill**, A. S. 1977. Transition Metals in Biochemistry. Springer-Verlag KG, Berlin, W. Germany. Chapter 3, 47–77.
16. **Austin**, R. H., K. W. **Beeson**, L. **Eisenstein**, H. **Frauenfelder**, and I. C. **Gunsalus**. 1975. Dynamics of ligand binding to myoglobin. *Biochemistry.* **14**:5355.
17. **Pecht**, I., O. **Farver**, and M. **Goldberg**. 1977. Electron transfer pathways in blue copper proteins. *In* Bioinorganic Chemistry. II. K. N. Raymond, editor. American Chemical Society, Washington, D. C. 179–206.
18. **Ugurbil**, K., R. S. **Norton**, A. **Allerhand**, and R. **Bersohn**. 1977. Studies of individual carbon sites of azurin from *Pseudomonas aeruginosa* by natural-abundance carbon-13 nuclear magnetic resonance spectroscopy. *Biochemistry.* **16**:886.
19. **Hu**, V. W., S. I. **Chan**, and G. S. **Brown**. 1977. X-ray absorption edge studies on oxidized and reduced cytochrome c oxidase. *Proc. Natl. Acad. Sci. U.S.A.* **74**:3821.