$$O_2 + Zn(II) + 2e^- \xrightarrow[Me_2SO]{-0.5\ V} ZnO_2 \quad (2)$$

Likewise, reduction of dioxygen in the presence of activating substrates (transition metal complexes, esters, and methyl viologen (MV^{2+})) results in a concerted two-electron process to yield reactive oxygenating agents and reaction mimics for oxygenases [6].

$$MV^{2+} + O_2 + 2e^- \xrightarrow[DMF]{-0.5\ V} MV^+(O_2^-) \quad (3)$$

The interactions of O_2 and its reduction products with iron-porphyrin,

$$Fe^{III}TPP^+ + O_2 + e^- \rightarrow Fe^{II}TPP(O_2) \xrightarrow{e^-} Fe^{III}TPP(O_2^{2-})^- \quad (4)$$

copper-bipyridine,

$$Cu^{II}(Bipy)_2^{2+} + O_2 + 2e^- \rightarrow Cu^{II}(Bipy)(O_2^{2-}) \quad (5)$$

and manganese-catechol complexes [9]

$$Mn^{IV}(DTBC)_3^{2-} + O_2 + OH^- \rightleftharpoons Mn^{III}(DTBSQ)_2\ (DTBC)(O_2^-)(OH)^{3-} \rightarrow Mn^{III}(DTBC)_2(O_2^-)\ (OH)^{3-} + DTBQ \quad (6)$$

yield a variety of reactive intermediates; the O_2 adducts are further activated by reduction. Several examples will be discussed of the *oxidation* of organic and inorganic substrates *via reductive* activation of O_2.

1 J. Wilshire and D. T. Sawyer, *Accounts Chem. Res., 12,* 105 (1979).
2 D. T. Sawyer and J. S. Valentine, *Accounts Chem. Res., 14,* 393 (1981).
3 J. L. Roberts, Jr. and D. T. Sawyer, *J. Am. Chem. Soc., 103,* 712 (1981).
4 E. J. Nanni, Jr. and D. T. Sawyer, *J. Am. Chem. Soc., 102,* 7591 (1980).
5 D. T. Sawyer, G. Chiericato, Jr. and T. Tsuchiya, *J. Am. Chem. Soc., 104,* 6273 (1982).
6 E. J. Nanni, Jr., C. T. Angelis, J. Dickson and D. T. Sawyer, *J. Am. Chem. Soc., 103,* 4268 (1981).
7 D.-H. Chin, G. Chiericato, Jr., E. J. Nanni, Jr. and D. T. Sawyer, *J. Am. Chem. Soc., 104,* 1296 (1982).
8 D. T. Sawyer, G. Chiericato, Jr., C. T. Angelis, E. J. Nanni, Jr. and T. Tsuchiya, *Anal. Chem., 54,* 1720 (1982).
9 D.-H. Chin and D. T. Sawyer, *Inorg. Chem., 21,* 0000 (1982).

E7

Cyanide and Methylisocyanide: Probes for Nitrogenase Reactivity

BARBARA K. BURGESS*, JUDITH F. RUBINSON and JAMES L. CORBIN

C. F. Kettering Research Lab, 150 E.S. College St., Yellow Springs, Ohio 45387, U.S.A.

Nitrogenase (N_2ase) is composed of two separately purified proteins, the molybdenum–iron (MoFe) protein and the iron (Fe) protein. Nitrogen fixation requires both proteins, a reductant, protons and MgATP. The Fe protein is generally accepted as a specific one-electron donor for the MoFe protein, which is believed to contain the substrate-reduction site. Besides N_2, N_2ase catalyzes the reduction of protons and a number of alternative substrates [*e.g.* 1], including the two six-electron substrates, cyanide and methylisocyanide, we have recently studied.

The rate-limiting step for N_2ase turnover occurs prior to substrate reduction. Thus, total electron flow through the enzyme should be essentially independent of the substrate being reduced. Although this appears true for N_2 fixation, H_2 evolution and C_2H_2 reduction [2], both CN^- [3] and CH_3NC dramatically inhibit the rate of total electron flow through N_2ase. Inhibition by both substrates is completely reversed by CO. Not only do CN^- and CH_3NC inhibit nitrogenase turnover, they also reduce the enzyme's efficiency by increasing the amount of MgATP hydrolyzed for each electron pair used to reduce substrate. These data are interpreted in terms of CN^- or CH_3NC binding to the MoFe protein in such a way as to prevent electron transfer to substrate. With nowhere to go, the electrons fall back to the Fe protein to complete a futile cycle.

Are the substrates N_2, HCN and CH_3NC reduced in one six-electron step or via a series of lesser reduced intermediates? Previously, we proposed that N_2 is reduced to ammonia via the two-electron intermediates N_2H_2 and N_2H_4 [4]. For the six-electron reductions of HCN to $CH_4 + NH_3$ and CH_3NC to $CH_4 + CH_3NH_2$, we have definitively identified the four-electron products, CH_3NH_2 (for HCN) and CH_3NHCH_3 (for CH_3NC), and suggest them as intermediates. The formation of two-electron reduced intermediates for both HCN and CH_3NC is suggested by the product ratio of NH_3-to-CH_4 (for HCN) and CH_3NH_2-to-CH_4 (for CH_3NC) being greater than one.

The data support mechanisms whereby the six-electron reduction of N_2, HCN and CH_3NC occur via a series of analogous two- and four-electron reduced intermediates. Thus, a common phenomenon is likely as an intimate part of the mechanisms of N_2, HCN and CH_3NC reduction. Although H_2 evolution is suggested as an obligatory part of the N_2-fixation mechanism, it is *not* required for either HCN or CH_3NC

reduction. This apparent anomaly might be explained if N_2, HCN or CH_3NC were either reduced at different sites or bound and reduced by different redox states of the MoFe protein. So, as increasing the ratio of Fe protein-to-MoFe protein increases electron flow, component protein ratio titration experiments in the presence of N_2, HCN and CH_3NC were used. They indicate that HCN and CH_3NC bind to and are reduced at a redox state of the MoFe protein more oxidized than that responsible for either N_2 fixation or H_2 evolution.

Do all substrates and inhibitors of nitrogenase bind to the same site on nitrogenase? Experiments with various combinations of substrates (N_2, HCN, CH_3NC, C_2H_2, N_2O, N_3^-) and inhibitors (H_2, CO, CN^-, CH_3NC) indicate that either C_2H_2 or N_2O stimulate HCN reduction and influence its product distribution, implying simultaneous binding and at least two interaction sites on N_2ase. CH_3NC appears to act as both substrate and inhibitor on binding to the same N_2ase site, implying productive and non-productive modes of binding.

Acknowledgement. This study was supported by Project No. 79-59-2394-0-1-383-1 from SEA/CGO of the USDA.

1 L. E. Mortenson and R. N. F. Thorneley, *Ann. Rev. Biochem., 48,* 387 (1979).
2 G. D. Watt and A. Burns, *Biochemistry, 16,* 265 (1977).
3 J.-G. Ki, B. K. Burgess and J. L. Corbin, *Biochemistry, 21,* 4393 (1982).
4 B. K. Burgess, S. Wherland, E. I. Stiefel and W. E. Newton, *Biochemistry, 20,* 5141 (1981).

E8

Thermal and Optical Electron Transfer Probabilities in Biological Systems

N. S. HUSH

Department of Theoretical Chemistry, University of Sydney, N.S.W. 2006, Australia

Electron-transfer reactions are perhaps the most important elementary chemical processes. In ionizing solution, the rates of the simplest of such exchanges (outer-sphere self-exchange between solvated or complex ions) vary enormously — in fact by a factor of $\sim 10^{19}$. The reasons for this very striking behaviour, and also for variation in the rates of cross-reactions, are now thought to be basically understood, as a result of work over the last two decades [1]. It is also known that there is an intimate connection between the probability of thermal exchange and of the corresponding optical or photon-assisted exchange (intervalence transfer) [2]; in principle, the thermal probability can be calculated from the optical transfer probability and *vice versa*. Intramolecular transfer in polynuclear complexes [3] and solid-state transfer (*e.g.* in organic or organometallic semiconductor/metals) can be interpreted within a very similar framework.

Electron transfer processes are very important in biological systems. In these, we are usually faced with the problem that the precise pathway is not known. However, it is known that the overall donor–acceptor distance is often much greater (*e.g.* 15–20 Å) than is usual in simpler systems. There has (mainly as a result of this) been much discussion of the role of tunnelling in biological electron transfer [4], often with the implication that this is of greater importance here than in simpler chemical processes. It is therefore of interest to ask whether one can, at this stage, make useful generalizations about this and other possible determinants of biological transfers. In order to attempt to do so, one must first focus attention on the basic parameters governing transfer probabilities. The most important of these are:

(1) the coupling of electronic to intramolecular vibrational and to phonon modes of the system, and the frequencies involved,

(2) the nature and magnitude of the transfer integral (J) coupling the reactant and product hypersurfaces,

(3) the overall free energy change,

(4) steric factors.

The interplay of factors (1) and (2) will firstly be illustrated by reference to optical transfer in iron–sulphur proteins. The (thermal) frequency factors for transfer rates over large distances will then be discussed with particular attention to the anticipated form of the distance-dependence of J. This is very important, as the electronic transmission coefficient multiplying the frequency factor is (for $J \ll k_B T$) proportional to J^2. It will be shown that there is good reason to suppose that the decrease of J with increasing donor–acceptor distance R in important systems is typically much less than exponential, and may be a fairly low inverse power of R. The magnitude of expected electron–phonon coupling energies and frequencies will also have an important effect in increasing the electronic transmission coefficients. It will be concluded that while nuclear tunnelling plays a significant but minor role at ordinary temperatures, there is at present no need to invoke electron tunnelling as a rate-determining step in typical large-distance biological electron-transfer processes in order to account for their moderate to rapid rates.

1 For recent summaries, see N. Sutin, *Acc. Chem. Res., 1982, 15,* 275; N. S. Hush, in 'Mechanistic Aspects of Inorganic Reactions',. (ACS Symposium Series 198), D. B. Rorabacher and J. F. Endicott (eds.), 1982, 301.
2 N. S. Hush, *Prog. Inorg. Chem., 1967, 8,* 391; *Electrochim. Acta, 1968, 13,* 1004.
3 C. Creutz and H. Taube, *J. Am. Chem. Soc., 95,* 1086 (1973).
4 For a recent discussion, see 'Tunnelling in Biological Systems', Britton Chance *et al.* (eds.), Academic Press, New York and London, 1979.