Synthetic Biochemical Models

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To understand life is not only one of the highest aspirations of man, but also of vital importance to his well-being. Unfortunately many biological processes seem to involve so many relevant factors that the available experimental information is often insufficient for him to reach a definite conclusion on the detailed molecular mechanism. While experiments on biological systems continue their admirable progress, the study of biochemical mechanisms may meanwhile be approached from a new angle, *i.e.*, by synthesizing and studying model molecules or molecular systems of similar biochemical activity.

Because of their relative simplicity, relevant synthetic models sometimes enable us to identify the crucial factors among a large number of possible causes, shortcut years of laborious experimental work, and reach the core of the more complicated biological problem much sooner. To illustrate this approach, this Account summarizes some of the synthetic models developed in our laboratory during the last 15 years. As our chemical knowledge continues its splendid growth in the next few decades, it seems very likely that this type of approach will be even more effective in helping us solve exciting biochemical problems.

Catalysis through Strain

Because of their catalytic specificity and efficiency, enzymes play the central role of controlling the course of biological reactions. But how do enzymes perform their remarkable function? One theory of enzyme catalysis assumes that when a substrate molecule is bound at the active site in the enzyme–substrate complex, the susceptible bond is already distorted or under strain so that it is rendered more reactive.[1] It is by no means a simple task to design a catalyst molecule which binds its substrate not only in precisely the right way for the catalyzing groups to react but also under strain, let alone the actual synthesis of such a molecule. However in the fortuitous case where the binding group also serves as the catalyzing group, the construction of a synthetic test model may be feasible. For example, many transition metal ions are known to bind the hydroperoxide ion, OOH^-, and catalyze its decomposition. Since OOH^- is potentially a bidentate ligand, it may be possible to design a metal–ion complex in which OOH^- can be bound as a bidentate ligand under strain and hence rendered more reactive. We found[2] that the complex of ferric ion and triethylenetetramine (TETA), $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$, which may for convenience be represented by $(TETA)Fe(OH)_2^+$, could indeed satisfy these specifications.

A Synthetic Model. Steric considerations show that it is energetically unfavorable to have the four N atoms and the Fe(III) ion of the complex in one plane, but that the structure represented by I in Figure 1 should be relatively stable in alkaline aqueous solution. In the presence of H_2O_2, OH^- in this complex may be replaced by OOH^- to form compounds II and III. But since the O–O bond has a normal length of only about 1.3 Å, it should be under strain in compound III. Consequently III should be very reactive and could change to the reactive complex IV. Direct splitting of the O–O bond in H_2O_2 requires an activation energy of about 33 kcal/mol. However, in the change III → IV this can be partially compensated for by the formation of stronger Fe–O bonds. Compound IV can then extract a hydride ion rapidly from a second OOH^- to produce O_2 and regenerate I.

This $(TETA)Fe(OH)_2^+$ complex was indeed found to catalyze the decomposition of H_2O_2 with remarkable efficiency.[2] The observed second-order rate constant in dilute solutions of H_2O_2 at pH 10 is

$$k_2 = (1/[(TETA)Fe(OH)_2^+])(-\mathrm{d}\ln[H_2O_2]/\mathrm{d}t) = 1.2 \times 10^3\ M^{-1}\ \mathrm{sec}^{-1}$$

This is 10^4 times higher than the corresponding value for methemoglobin or hematin, although it is still lower by a factor of 10^4 than the corresponding value for catalase, *i.e.*, $1 \times 10^7\ \mathrm{sec}^{-1}$ per mole of hematin group.[3] Among the TETA complexes of Cr(III), Mn(II), Fe(III), Co(III), Ni(II), Cu(II), Zn(II), Ag(I), Cd(II), and Pb(II), only the Fe(III) and Mn(II) complexes catalyze the decomposition of H_2O_2 efficiently. Inasmuch as Fe(III) and Mn(II) ions are isoelectronic, these results show that it is possible for small molecules to have enzyme-like catalytic activity with the highest degree of specificity for the electronic configuration of the metal ion.

It is interesting to note that the catalytic mechanism in Figure 1 requires the availability of two adjacent octahedral hybrid atomic orbitals of the Fe(III) for combination with the same HOO^-. Therefore if tetraethylenepentamine (TEPA), $H_2NCH_2CH_2NHCH_2CH_2NHCH_2CH_2NHCH_2CH_2NH_2$, is used as the ligand instead of TETA, the resulting complex, $(TEPA)Fe(OH)^{2+}$, should not be able to catalyze according to the strain mechanism in Figure 1. In other words, the fifth N atom in $(TEPA)Fe(OH)^{2+}$ will act as an intra-

(1) J. B. S. Haldane, "Enzymes," Longmans, London, 1930.
(2) J. H. Wang, *J. Am. Chem. Soc.*, 77, 822, 4715 (1955).
(3) B. Chance, D. S. Greenstein, and F. J. W. Roughton, *Arch. Biochem. Biophys.*, 37, 301 (1952).

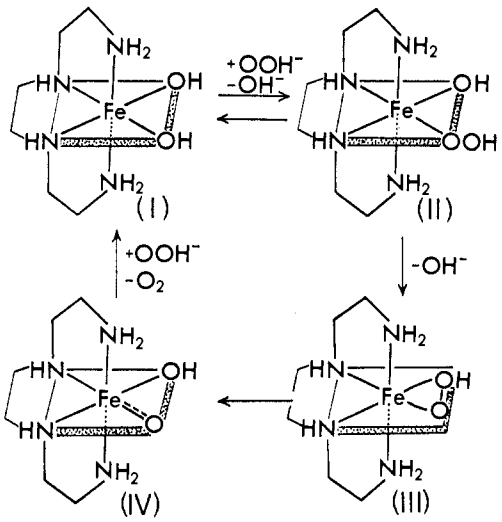


Figure 1. Catalytic decomposition of H_2O_2 by (TETA)Fe-$(OH)_2^+$.

molecular inhibitor for this mechanism. This expectation was subsequently confirmed by the observation that (TEPA)Fe$(OH)^{2+}$ is practically inert as compared to (TETA)Fe$(OH)_2^+$. Inhibition experiments with CN^- also showed that as soon as one of the two bound OH^- ions in (TETA)Fe$(OH)_2^+$ is replaced by CN^-, the complex becomes inactive.[4]

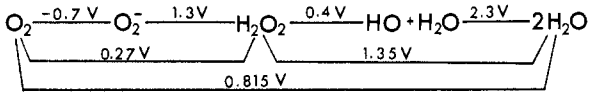
Other Reaction Paths. Since it is well known that Fe(III) and Fe(II) ions catalyze the decomposition of H_2O_2 through a free-radical mechanism,[5] we should also consider that reaction path for the present system. The fact that (TEPA)Fe$(OH)^{2+}$, which may still catalyze by the free-radical mechanism through its exchangeable OH^-, is practically inert compared to (TETA)Fe-$(OH)_2^+$ shows that the strain mechanism in Figure 1 is much more efficient than the free-radical mechanism, even though the latter might also slowly take place simultaneously.

One may also wonder whether the catalytic efficiency of (TETA)Fe$(OH)_2^+$ is mainly due to an activation entropy effect.[6] For example, if both OH^- in the complex are replaced by OOH^- to form (TETA)Fe$(OOH)_2^+$, then decomposition of the substrate could take place in two ways: (a) combination of two O atoms, one from each OOH^-, to form O_2 and leave two OH^-; (b) transfer of two electrons from one OOH^- to the other through the Fe(III) ion. The activation entropy effect is definitely an important factor. However, mechanism a is ruled out, since experiments with doubly ^{18}O-labeled H_2O_2 showed that both O atoms in the product O_2 came from the same H_2O_2 molecule.[7] According to Figure 1, O_2 is produced from the second HO_2^- *via* a two-electron oxidation by IV. Consequently both O atoms in the product O_2 are expected to come from the same H_2O_2 molecule as was observed experimentally. Mechanism b cannot account for the observed reaction rate, because hematin (or Fe^{III} heme), which can catalyze by mechanism b but cannot catalyze by the mechanism of Figure 1, has a catalytic efficiency lower than that of (TETA)Fe$(OH)_2^+$ by a factor of 10^4. Furthermore, mechanism b would predict the rate to be second order with respect to $[H_2O_2]$ in dilute solutions, whereas the mechanism of Figure 1 would predict it to be first order with respect to $[H_2O_2]$ if either II → III or III → IV is rate-limiting, and second-order if IV → I is rate-limiting. The observed reaction rate for (TETA)Fe-$(OH)_2^+$ catalysis in dilute H_2O_2 solutions is proportional to the first power of $[H_2O_2]$. Therefore, of the mechanisms discussed above, only that of Figure 1 agrees with the experimental data.

Hemoglobin and Myoglobin

All vertebrates depend on the rapidly reversible combination of molecular oxygen with hemoglobin and myoglobin for their oxygen supply. Although an aqueous solution of Fe^{II}heme is immediately oxidized to Fe^{III}heme upon exposure to air, in the presence of nitrogenous bases and the strict absence of water or other acid, Fe^{II}heme derivatives are quite resistant to oxidation by molecular oxygen.[8-10] These observations suggest that the globin of hemoglobin or myoglobin may protect Fe^{II}heme in a similar way, and that by taking all the relevant factors into consideration it may be possible to construct a synthetic model for these biological oxygen carriers which is stable in an aqueous environment.

Relevant Factors. It has often been jokingly said that the only chemical reactions with simple mechanisms are those which have not been studied thoroughly. If this statement is still true for many elementary reactions, it must be even more so for biochemical reactions. Let us consider the oxidation of ferroheme in solution by molecular oxygen. At pH 7 the midpoint reduction potentials of O_2 are given as[11]



These values show that O_2 is a rather poor one-electron oxidant, but quite good two-electron oxidant.

The oxidation of Fe^{II}heme (or its derivatives) may proceed along the five possible paths summarized in Figure 2. For simplicity, fast reaction steps following the rate-limiting step of each path are omitted from Figure 2. Since the Fe(III) ion in Fe^{III}heme bears a net formal charge of 1+, mechanisms 2 and 4 of Figure 2 involve charge separation at the rate-limiting step and consequently should be hindered by a hydrophobic

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(6) C. G. Swain and J. F. Brown, Jr., *J. Am. Chem. Soc.*, **74**, 2538 (1952).
(7) R. C. Jarnagin and J. H. Wang, *ibid.*, **80**, 786 (1958).
(8) A. H. Corwin and J. G. Erdman, *ibid.*, **68**, 2473 (1946).
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(10) J. H. Wang, A. Nakahara, and E. B. Fleischer, *ibid.*, **80**, 1109 (1958).
(11) W. M. Latimer, "Oxidation Potentials," 2nd ed, Prentice-Hall, New York, N. Y., 1952, p 50. The values have been recomputed for pH 7.

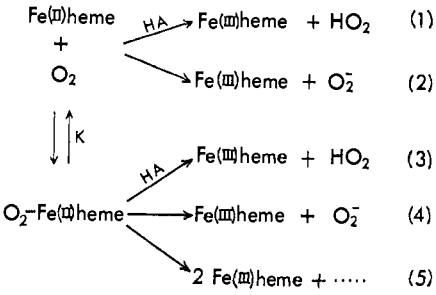

Figure 2. Possible reaction paths for the oxidation of Fe^{II}heme by O_2.

medium of low dielectric constant. Mechanism 4 involves the formation of an "oxyheme," O_2–Fe^{II}heme, prior to the rate-limiting step, and hence is generally faster than mechanism 2 which involves the extraction of an electron by O_2 during a chance collision of the latter with the porphyrin or a ligand of the Fe(II) ion. The relatives rates of (2) and (4) can be determined by measurements in the presence of a ligand which competes with O_2 for the binding site.[12] Mechanisms 3 and 1 do not involve charge separation but require a general acid, HA. Consequently they should be less affected by the dielectric property than by the acidity of the medium. Since O_2 is a poor one-electron oxidant, all these one-electron oxidation mechanisms can be retarded considerably by raising the reduction potential of the Fe^{III}heme, for example, by coordination with nitrogen bases which have a higher affinity for the Fe(II) than for the Fe(III) state.[13]

To illustrate the last effect, let us estimate the maximum forward rates of the following one-electron oxidation mechanism for different midpoint reduction potentials of Fe^{III}heme. The diffusion-limited bimolec-

$$Fe^{II}\text{heme} + O_2 \underset{k_b}{\overset{k_f}{\rightleftharpoons}} Fe^{III}\text{heme} + O_2^-$$

ular rate constant has been estimated[14,15] as $k \leqslant 10^{11}$ M^{-1} sec^{-1}. For Fe^{II}heme with a midpoint reduction potential of -0.2 V we obtain

$$k_f = k_b \exp[-\Delta G^\circ/(RT)] \leqslant 10^{11} \times \exp[(-0.7 + 0.2)(23.06)/(0.6)] \approx 10^3\ M^{-1}\ \text{sec}^{-1}$$

which is not a very severe upper limit. However, if we complex the Fe^{II}heme with a suitable nitrogen base to raise the midpoint reduction potential to 0.18 V (the value for hemoglobin at pH 7),[13] this upper limit becomes $k_f \leqslant 2 \times 10^{-4}\ M^{-1}$ sec^{-1}, which corresponds to a minimum half-life of 1 hr at unit O_2 concentration.

Finally since O_2 is a good two-electron oxidant, the "oxyheme" may rapidly react with another Fe^{II}heme molecule according to mechanism 5 of Figure 2 to produce two Fe^{III}heme molecules.

The Synthetic Model. If a synthetic model can be constructed in which O_2 and Fe^{II}heme can combine and dissociate freely, but for which all the five oxidation paths of Figure 2 are blocked, it should be able to combine reversibly with O_2 like hemoglobin or myoglobin. Such a synthetic model was made by embedding molecules of the diethyl ester of heme in a hydrophobic matrix of polystyrene and 1-(2-phenylethyl)-imidazole. The Fe(II) ion of each embedded heme group was firmly coordinated to a substituted imidazole on one side and, because of the constraint of the matrix, only partially coordinated to a second imidazole group on the other side, as illustrated in Figure 3. These embedded heme groups were indeed found to combine reversibly with O_2 even in the presence of water.[16,17]

In the synthetic model of Figure 3 the proximal substituted imidazole group not only assists the binding of O_2 or CO on the other side of the heme plane through back π bonding[18] but also raises the midpoint reduction potential of Fe^{III}heme and consequently retards the one-electron oxidation of Fe^{II}heme by O_2. The distal substituted imidazole group may also have some influence in stabilizing the Fe(II) state and facilitating the reversible dissociation of the bound O_2. The low dielectric constant of the hydrophobic matrix, represented by the shaded area, retards the charge-separation mechanisms 2 and 4; the nonacidic nature of the hydrophobic matrix prevents mechanisms 1 and 3. Finally, since the embedded Fe^{II}heme groups are isolated from each other by the matrix, oxidation by mechanism 5 is also prevented. The observed reversible oxygenation of this synthetic model suggests that hemoglobin and myoglobin may function in a similar way.

This hypothesis was later confirmed by the important X-ray diffraction studies of Kendrew, Perutz, and coworkers.[19,20] In ferrimyoglobin it was found that aside from the two propionic acid side chains which form salt linkages and the Fe(III) ion which is coordinated to the proximal imidazole group on one side and a water molecule on the other, the rest of the heme group is surrounded almost completely by nonpolar groups of the protein.[21] The data of ferromyoglobin[22] showed that the sixth coordinating position of the Fe(II) ion is not even coordinated to a water molecule but left vacant, as in the model of Figure 3, in a hydrophobic environment with a distal imidazole group. Recent X-ray data also showed that each of the four subunits in oxyhemoglobin molecule has a very similar structure.[23]

(12) O. H. W. Kao and J. H. Wang, *Biochemistry*, **4**, 342 (1965).
(13) J. H. Wang in "Oxygenases," O. Hayaishi, Ed., Academic Press, New York, N. Y., 1962, p 505.
(14) P. Debye, *Trans. Electrochem. Soc.*, **82**, 265 (1942).
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(22) C. L. Nobbs, H. C. Watson, and J. C. Kendrew, *Nature*, **209**, 339 (1966).
(23) M. F. Perutz, *et al.*, *ibid.*, **219**, 29, 131 (1968).

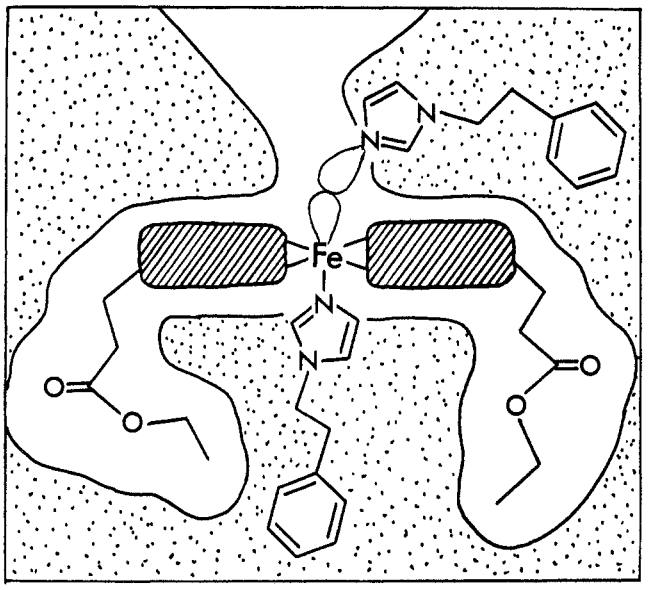


Figure 3. A synthetic model for hemoglobin or myoglobin.

The striking similarity between the actual structure of the active site in hemoglobin or myoglobin and the hypothetical structure based on the synthetic model gives us a deeper insight into the structural basis of biological function.

During the last decade, a large number of pathological hemoglobins were discovered. In every case, the observed change in biochemical property due to the replacement of certain amino acid residues is consistent with the above hypothesis.[24] For example, in hemoglobin Boston the distal imidazole group of the α chain is replaced by a phenolic group. Because of its acidic property this phenolic group is expected to accelerate the oxidation of the Fe(II) ion by O_2 through mechanism 3 of Figure 2. This was indeed found to be the case.

Aerobic Oxidation of Cytochrome *c*

Ferrocytochrome *c* does not combine with molecular oxygen because the fifth and sixth coordinating positions of the Fe(II) ion in its heme group are already fully occupied by an imidazole and thioether groups, respectively.[25] Consequently ferrocytochrome *c* cannot be oxidized by O_2 through the more efficient mechanisms 3, 4, and 5 of Figure 2. A computation similar to that given above shows that the half-lives of the oxidation reaction by mechanisms 1 and 2 of Figure 2 for ferrocytochrome *c*, with a midpoint reduction potential of 0.26 V for its oxidized form, must be longer than 30 hr at unit O_2 concentration. Therefore, even without protective hydrophobic groups, ferrocytochrome *c* is not oxidized by O_2 at an appreciable rate.

It is important to note that the slow oxidation rates discussed above originate from the very negative potential (−0.7 V) of the one-electron reduction of O_2. If we use a more powerful one-electron oxidant, *e.g.*, the ferricyanide ion (midpoint reduction potential +0.36 V), both hemoglobin and cytochrome *c* should be and actually are rapidly oxidized.

Because ferrocytochrome *c* is not directly oxidized by O_2, aerobes employ an efficient catalyst called cytochrome oxidase to capture an electron from ferrocytochrome *c* and pass it on to O_2. In order to oxidize ferrocytochrome *c* rapidly, the oxidized catalyst must have a higher midpoint reduction potential and consequently it must also be a very slow one-electron reductant of O_2. Therefore, to transfer electron efficiently from cytochrome *c* to O_2, the catalyst must reduce O_2 through a two- or four-electron reduction mechanism, *i.e.*, it must transfer two or four electrons to O_2 before the reduction product diffuses away. Cytochrome oxidase could function in this way if there is strong electronic interaction between its heme a group which binds O_2 and the other heme a group as well as the copper ions in the same molecule.

The Synthetic Catalyst. Although the mechanism of electron transfer between the heme a groups and copper atoms of cytochrome oxidase is yet to be elucidated, the above considerations suggest that a model catalyst can perhaps be designed and synthesized to illustrate this operating principle. Such a synthetic model was made by allowing heme to react with 4,4′-dipyridyl and poly-L-lysine.[26] The structure of an active site in this synthetic catalyst is illustrated in Figure 4. In its Fe(III) state the binary copolymer of heme and dipyridyl is unstable and tends to disintegrate. This drawback was partially alleviated by combining the binary copolymer in its Fe(II) state with poly-L-lysine which forms salt linkages with the negatively charged heme units and retards the disintegration upon exposure to air.

Since the successive heme units in the synthetic copolymer of Figure 4 are linked together through a conjugated double-bond system, the energy barrier for electron transfer between the successive heme units should be relatively low. Therefore, we would expect the O_2 molecule bound to each terminal heme to be reduced by more than one Fe^{II}heme unit. Moreover, since both the replacement of water by 4,4′-dipyridyl at the fifth and sixth coordinating positions of heme and the delocalization of d electrons of the Fe(II) ions along the direction of the connecting ligand tend to raise the reduction potential of the oxidized copolymer, the latter is expected to oxidize cytochrome *c* rapidly. This synthetic copolymer was indeed found to catalyze the oxidation of ferrocytochrome *c* by air.[26] Aside from the natural cytochrome oxidase, it is the only artificial catalyst reported in the literature for the aerobic oxidation of cytochrome *c*.

Ordinary ferrohemochromes are bright red, with well-defined α, β, and Soret absorption maxima. The Fe(II) copolymer of Figure 4 is purplish black and exhibits an additional, very broad absorption band which extends from 630 nm to the infrared. Replacement of

(24) M. F. Perutz and H. Lehmann, *Nature*, **219**, 902 (1968).
(25) H. A. Harbury, *et al.*, *Proc. Natl. Acad. Sci. U. S.*, **54**, 1658 (1965).
(26) J. H. Wang and W. S. Brinigar, *ibid.*, **46**, 958 (1960).

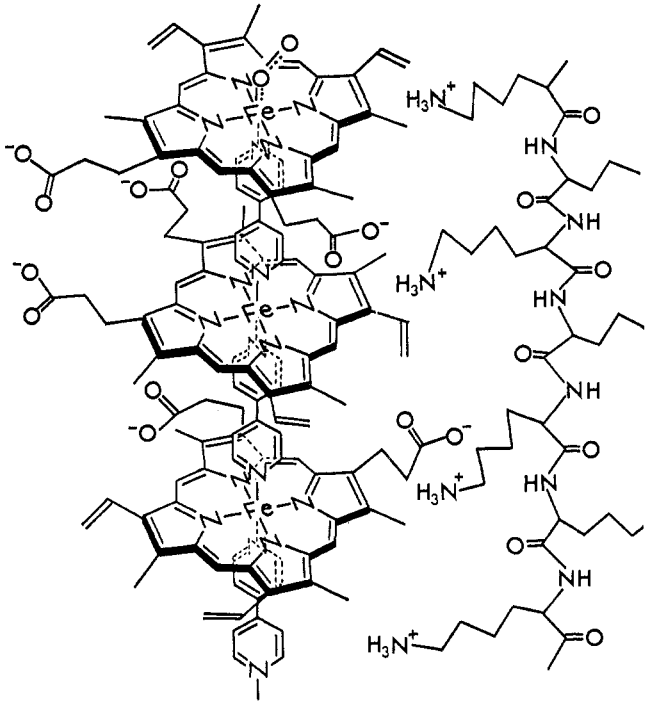


Figure 4. An active center in the synthetic catalyst for the aerobic oxidation of cytochrome *c*.

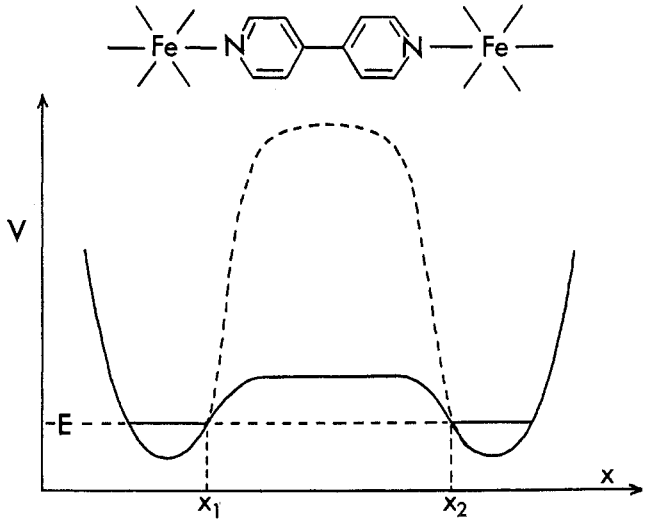


Figure 5. Electron tunneling *via* 4,4′-dipyridyl bridge.

4,4′-dipyridyl by 1,2-di(4-pyridyl)ethylene as the bridging ligand yielded another purplish black copolymer which is catalytically about 50% as active as the copolymer of Figure 4.[26] On the other hand, similar copolymers made with bidentate ligands without completely delocalized π-electron systems, *e.g.*, 1,2-di(4-pyridyl)ethane or 1,4-diazabicyclo[2.2.2]octane, are catalytically inert and look bright red in the Fe(II) state. Measurements in the absence of ferrocytochrome *c* showed that the Fe^{II}heme units in the synthetic model of Figure 4 cooperate in the reduction of O_2, and that the bound O_2 was slowly reduced to water without releasing any appreciable amount of radical or hydroperoxide intermediate.[27]

(27) W. S. Brinigar, in preparation.

Electron Tunneling. The observed optical and kinetic properties of the synthetic copolymer of Figure 4 may also be regarded as a demonstration of electron transfer through bridging organic molecules with delocalized π electrons. Additional insight into this problem may be gained by considering the tunneling process illustrated in Figure 5. In Figure 5, the potential energy V of an electron of mass m and total energy E traveling between two Fe^{III}heme units connected by a bridging 4,4′-dipyridyl molecule is qualitatively represented by the solid curve as a function of the coordinate x. If a saturated bidentate molecule of the same length were used as the bridging group, the potential energy of the electron traveling between x_1 and x_2 would be several volts above E, as represented by the broken curve in Figure 5; consequently if this electron is originally in one of the two potential wells, the probability

$$\tau = \exp\left\{-(2/\hbar)\int_{x_1}^{x_2}[2m(V-E)]^{1/2}\,dx\right\}$$

for it to tunnel through the high potential barrier and arrive at the other side would be relatively small.[28] However, the doubly coordinated 4,4′-dipyridyl in Figure 5 must have fairly high affinity for an additional electron, because it is well known that doubly quaternized 4,4′-dipyridines can be reduced to intensely colored stable free radicals.[29] For example, the observed midpoint reduction potentials of N,N′-dimethyl-4,4′-dipyridyl ion (methyl-viologen) and N,N′-dibenzyl-4,4′-dipyridyl ion (benzyl-viologen) are −0.44 and −0.36 V, respectively. Assuming the midpoint reduction potentials of the bridging dipyridyl group and the coordinated Fe^{III}hemes in Figure 5 are approximately −0.4 and +0.3 V, respectively,[26] we may obtain an order-of-magnitude estimate of the minimum tunneling frequency as follows.

Let us assume that for the present system the terms due to entropy and volume changes are small so that the difference in the above midpoint potentials, −0.4 and +0.3 V, is mainly due to the difference in total energies E and E' of an additional electron bound to the coordinated Fe^{III}heme and the bridging dipyridyl group, respectively, *i.e.*, $E' - E \approx 0.3 - (-0.4) = 0.7$ V. Let us further assume that the potential energy of an electron in the region between x_1 and x_2 of Figure 5 may be regarded as constant at some average value $\bar{V}$. Since $\bar{V} \leqslant E'$, we have $\bar{V} - E \leqslant E' - E \approx 0.7$ V. Therefore $\tau \geqslant \exp(-I)$, where $I = (2/\hbar)[2m(E' - E)]^{1/2}(x_2 - x_1)$. Estimating $x_2 - x_1 = 9 \times 10^{-8}$ cm and substituting in the values of $\hbar$ and m, we obtain $\tau \geqslant 5 \times 10^{-4}$.

From the uncertainty principle, the average velocity $\bar{v}$ of an additional electron bound to the Fe(III) ion of 0.7 Å radius may be estimated as $\bar{v} \geqslant \hbar/[(0.7 \times 10^{-8}) \cdot (9.1 \times 10^{-28})] = 2 \times 10^{8}$ cm sec^{-1}. Estimating the

(28) G. Gamow, *Z. Physik*, **51**, 204 (1928); R. W. Gurney and E. U. Condon, *Phys. Rev.*, **33**, 127 (1929).
(29) L. Michaelis, *Chem. Rev.*, **16**, 243 (1935).

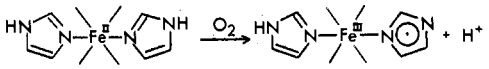
fraction of a 4π solid angle subtended by the second Fe(III) ion relative to the first Fe(III) ion as $\pi(0.7)^2/[4\pi(9 + 2 \times 0.7)^2] \approx 1 \times 10^{-3}$, we obtain the collision frequency of the electron with the barrier as $f \approx [\bar{v}/(2 \times 0.7 \times 10^{-8})](1 \times 10^{-3}) \geq 1.2 \times 10^{13}$ sec^{-1}. Therefore the tunneling frequency becomes $k_1 = fr \geq 6 \times 10^9$ sec^{-1}. Since the observed initial oxidation rate of the fully reduced copolymer in Figure 5 corresponds to a pseudo-first-order rate constant of 5×10^{-3} sec^{-1}, we concluded that the Fe^{II}heme units of this copolymer cooperate in the reduction of O_2.[27]

Oxidation Phosphorylation

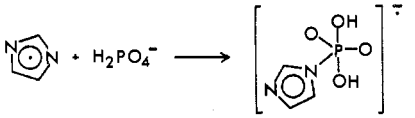
During respiration, the free energy stored in the substrate or food is released by the controlled aerobic oxidation in mitochondria. Part of this free energy is utilized to synthesize adenosine 5′-triphosphate (ATP) for the maintenance of life;[30] the remainder is expended so that these vital energy conversion reactions can take place with satisfactory speed. The biosynthesis of ATP from adenosine 5′-diphosphate (ADP) and inorganic orthophosphate (P_i) coupled to the oxidation of substrates is known as oxidative phosphorylation and has been shown to take place mainly in the respiratory chain embedded in the inner membrane of mitochondria.[31,32] But by what molecular mechanism is the free energy liberated in an oxidation process utilized to drive a phosphorylation reaction in which P_i and ADP condense to form ATP and water? There is not yet a universally accepted answer.

Model Reactions. Believing in the ultimate simplicity of nature but frustrated by the complexity of mitochondria, we started in 1961 to search for relevant model oxidative phosphorylation reactions to guide our thinking on the biological system. It was found that when ferrohemochrome was oxidized by air in N,N-dimethylacetamide solution containing AMP, P_i, and imidazole, ATP was formed.[33] Additional experiments showed that in this model reaction 1-phosphoimidazole was first produced and subsequently transferred its phosphoryl group to AMP or ADP to form ADP or ATP, respectively. But how was 1-phosphoimidazole formed?

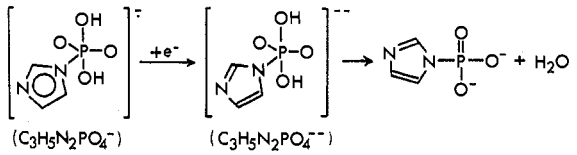
Studies with substituted imidazoles[34] suggest that O_2 first extracts two electrons from the ferrohemochrome to produce a complex of Fe^{III}heme and the reactive imidazole radical ($C_3H_3N_2$), *i.e.*



This radical can then react with P_i to form an unstable



phosphoimidazolyl radical, which is subsequently reduced by another ferrohemochrome molecule to produce 1-phosphoimidazole and water. The formation of



the trigonal-bipyramidal intermediate compound 1-orthophosphoimidazole ($C_3H_5N_2PO_4^{2-}$) by the usual nucleophilic attack at the P atom is a very slow process. However, since radical reactions generally require a much lower activation free energy, the trigonal-bipyramidal phosphoimidazolyl radical ($C_3H_5N_2PO_4^{-}$) can be formed much more rapidly. In the subsequent step driven by the oxidation–reduction free energy, this phosphoimidazolyl radical is reduced to the unstable 1-orthophosphoimidazole which then spontaneously eliminates H_2O to form 1-phosphoimidazole. In this way, oxidation can be coupled to phosphorylation.

Mitochondrial Oxidative Phosphorylation. With the above model reactions as a guide, a molecular mechanism for mitochondrial oxidative phosphorylation has recently been proposed.[35,36] This mechanism is summarized in Figure 6, where the direction of electron transport under normal conditions is represented by the bold-faced arrows between the respiratory electron carriers, *i.e.*, nicotinamide–adenine dinucleotide (NAD), flavin (Fl), quinones (Q), and the cytochromes (*b*, c_1, *c*, *a*, a_3).

The oxidative phosphorylation scheme in Figure 6 utilizes a coupling mechanism similar to that of the model reactions, with the important difference that in mitochondria the imidazolyl radical cannot diffuse away to cause biological damage because it is a part of the structure of the protein, *e.g.*, cytochromes. If in the mitochondrial system the cytochrome is adjacent to a suitable phospholipid, (RO)(R′O) PO_2^-, molecule, the imidazoly radical can rapidly react with the latter to form a phosphoimidazolyl radical which can subsequently be reduced to the "energy-rich" intermediates IV, V, or VI in Figure 6. Each of these intermediates can likewise react with P_i in the presence of a specific enzyme or coupling factor to give VII, which can subsequently transfer its phosphoryl group to ADP in the presence of another enzyme or coupling factor to give ATP and regenerate the phospholipid VIII for the next round of oxidative phosphorylation. Since the phosphoryl group in IV, V, or VI is not from inorganic phosphate but from endogenous phospholipid (or phosphoprotein), each of these "energy-rich" compounds should behave as a "nonphosphorylated intermediate" according to the conventional criterion.[37] A theory

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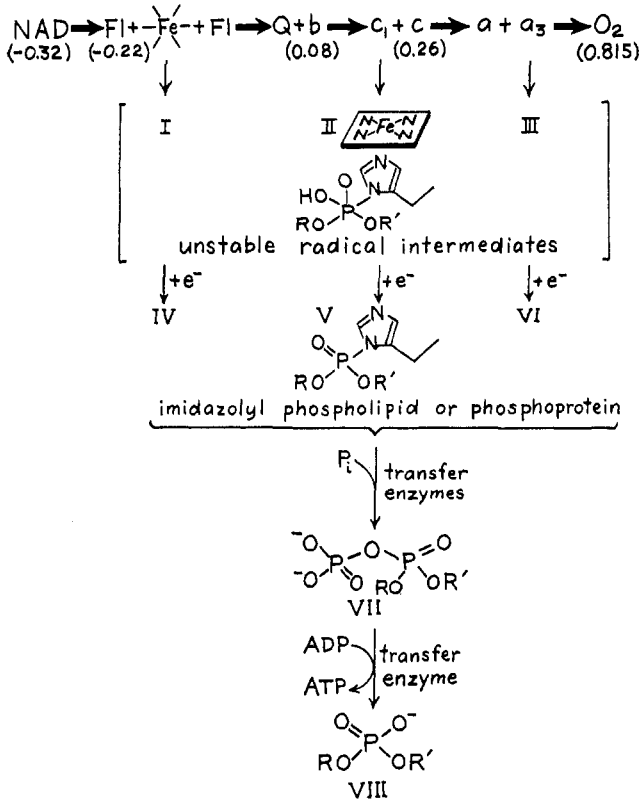


Figure 6. A proposed molecular mechanism of mitochondrial oxidative phosphorylation.

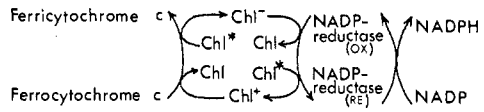
based on the scheme in Figure 6 has been developed to account for the observed respiratory control, the membrane morphological changes, and the effect of concentration gradients.[36]

Harvesting Light for Life

To maintain life, all living systems must feed on free energy from their surroundings either directly in the form of light or in the form of chemical free energy converted from light. For this reason, the molecular mechanism of the primary energy conversion processes in photosynthesis is of great interest. It is now generally accepted that in these primary energy conversion reactions of photosynthesis electrons are driven by light from molecules of higher reduction potential to molecules of lower reduction potential, and that in photosynthesis ATP is produced by essentially the same mechanism which couples phosphorylation to electron transport in the respiratory chain.

A Model Energy-Conversion System. In general an electronically excited pigment molecule can be a stronger reductant than the same molecule in its ground state because the removal of an electron from its antibonding molecular orbital requires less energy. It can also be a stronger oxidant since the positive hole left in the bonding or nonbonding orbital may be filled by abstracting an electron from a donor molecule. For this reason, certain pigment molecules can utilize the absorbed light energy to drive electrons up a free energy hill.

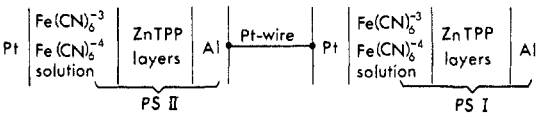
In one of the energy conversion systems developed in our laboratory,[38] electrons were driven by red light from cytochrome *c via* aggregated chlorophyllin a and the flavin group of NADP reductase to NADP against an electrochemical potential of 0.36 V. The experimental data show that the path of electron transfer in this model reaction can be represented by the following scheme, in which Chl represents chlorophyll a in its ground electronic state, Chl* chlorophyllin a in its excited electronic state, Chl^+ the oxidized chlorophyllin radical, Chl^- the reduced chlorophyllin radical, the curved arrows represent the directions of coupled oxidation–reduction change, and the symbols OX and RE designate the oxidized and reduced states, respectively.



Presumably resonant energy transfer[39] can readily take place between different monomer units of the same chlorophyllin aggregate, and either Chl^- or Chl^+ or both participate in the light-driven electron transfer. Although this model reaction involves three successive steps, it has a high overall quantum yield of 0.05.

A Possible Model for P700. In a different experimental system, it was observed that flavin and chlorophyll a form a molecular complex with a new absorption maximum near 700 nm which can be bleached reversibly by light.[40] The sum of these observations suggests that the energy converter of green plants, often called P700 because of its absorption maximum at 700 nm,[41] may be a molecular complex of a particular chlorophyll a molecular and a particular flavin group or its equivalent of similar structure (Fl). The latter could accept the photoelectron from the chlorophyll a and subsequently transfer it either to ferreodoxin (Fd) or to the flavin group of NADP reductase as illustrated in Figure 7, where PC represents plastocyanine.

A Two-Quanta Model System. Recently a model system has also been constructed for the conversion of light to chemical free energy through the cooperation of two light-harvesting subunits (PS I and PS II) connected electrically in series.[42] Each subunit was made of a layer of zinc tetraphenylprophyrin (ZnTPP) deposited on a clean aluminum surface and immersed in an aqueous solution of potassium ferri- and ferrocyanide as



indicated. Upon illumination by orange light, charge transfer took place across more than 70 molecular layers

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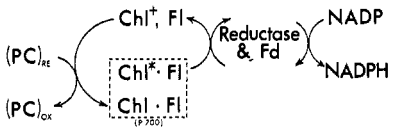


Figure 7. A possible model for the P700 of green plants.

of the pigment with a total electromotive force of 2.2 to 2.6 V. With NADP as the electron acceptor and NADP reductase as a mediator, this model system, like the photosynthetic apparatus of green plants, photooxidized water to oxygen gas.

Biological Systems. With the above model experiments as a guide, the mechanistic scheme shown in Figure 8 has recently been proposed for the primary energy conversion reactions of photosynthesis.[36] This scheme represents a synthesis of several earlier formulations[43] based on a wide range of experimental data reported in the literature.

For example, Arnon and coworkers made the important discovery that, when deprived of CO_2 and exogenous NADP, illuminated chloroplasts can sustain cyclic photophosphorylation, *i.e*, generation of ATP and H_2O from ADP and P_i with no other net chemical change.[44,45] According to the scheme in Figure 8, the light energy absorbed by the assembly of pigment molecules in photosystem I (PS I) is first transferred by a Förster mechanism to a particular chlorophyll a molecule which is complexed to a particular flavin group. This electronically excited chlorophyll molecule (Chl) can then transfer the electron to its acceptor flavin group (Fl), which can subsequently pass it on along the cyclic electron-transport path with coupled phosphorylation by the radical mechanism. Phosphorylation can take place at the coupling sites between ferredoxin (Fd) and plastoquinone (Q) and between Q and plastocyanine (PC), respectively. Since for a given number of electrons which this particular chlorophyll molecule supplies to its acceptor flavin group, exactly the same number is recovered from PC or its equivalent, cyclic

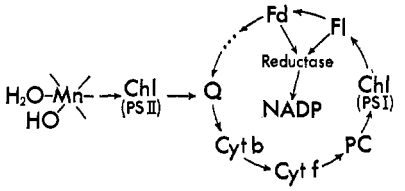
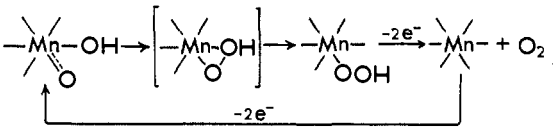


Figure 8. Primary energy conversion reactions in photosynthesis.

photophosphorylation does not produce a net redox change.

With CO_2 reduction taking place, the withdrawal of electrons at the NADP in Figure 8 by reactions of the Calvin–Bassham cycle creates a deficit of electrons at Q. This electron deficit makes Q a better acceptor, thereby activating the light-driven electron transfer from the Chl of photosystem II (PS II) to Q, and the Chl^+ radical thus produced in PS II can continue to raise the oxidation state of the manganese complex until the latter starts to produce O_2 by oxidizing its own water of hydration. The manganese complex could oxidize its water of hydration by a mechanism analogous to that of Figure 1, but in the reverse direction, *i.e.*



Finally, it is conceivable that as photosynthesis continued to produce organic matter and oxygen gas on earth during an early evolutionary era, heterotrophs could emerge by deleting the light-harvesting equipment from the scheme in Figure 8 and further developing the electron-transport chain to improve the yield of coupled phosphorylation. As an example, by substituting cytochrome oxidase for PC and replacing the NADP for CO_2 reduction by the NAD attached to the Krebs cycle, the sequence of electron carriers in Figure 8 is made formally identical with the respiratory chain of aerobes. Thus this scheme also seems consistent with the general assumption of the common origin of living systems and reflects a possible evolutionary pattern for the driving force of life at the molecular level.

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