REVIEW

Chemical Models of Oxidative Phosphorylation*

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Abstract

Chemical models for coupling oxidation to phosphorylation are summarized and examined both from the standpoint of organic reaction mechanisms and with respect to their relevance to mitochondria and chloroplasts. In order to accelerate the progress of our research in bioenergetics, it is suggested to focus at least as much attention on structural biochemistry as on phenomenological observations of energy-transducing membranes.

Introduction

The well-known results of two-stage photophosphorylation experiments by Shen and Shen [1], Hind and Jagendorf [2], Izawa [3], and others [4] show that the primary energy transduction step in chloroplasts does not involve either ADP and P_i or ATP. But does electron transport first generate a covalent precursor which subsequently gives rise to ATP as well as concentration gradients, electric fields, and conformation changes or is one of the latter generated first, which causes the formation of the covalent precursor? A universally acceptable answer to this question has yet to be found.

Discussion of coupling mechanisms without adequate structural information is unlikely to be very fruitful. For example, in studying the chemical intermediates of oxidative phosphorylation, it is not enough just to discuss in terms of $X \sim I$ or $X \sim P$. We have to know what their structures are, how they are formed through redox reactions, and by what mechanisms they are utilized. Likewise, it is not enough to discuss concentration gradients, electric fields, and conformation changes—we must identify the responsible molecules, their functional groups, and the molecular mechanisms which trigger the observed phenomenological changes. Unfortunately the complexity of mitochondria and chloroplasts has so far prevented us from identifying the structures and functional groups responsible for energy transduction. In most studies, the best one can do is to conclude that a certain postulated mechanism is consistent with experimental data. But since in bioenergetics there are often alternative mechanisms which also fit the data, such a conclusion is not very enlightening.

Model oxidative phosphorylation systems are advantageous, because they are simpler and hence can be more easily understood. However, no matter how interesting and successfully elucidated is their chemistry, these model systems are at best only suggestive of what might happen *in vivo*. While a thorough study of the successful model systems will probably yield some valuable information relevant to the oxidative phosphorylation problem, direct extrapolation of molecular mechanisms of simple model systems to mitochondria and chloroplasts is perilous.

In anaerobic glycolysis and citric acid cycle (substrate level) phosphorylation, the covalent precursors, i.e., D-1,3-diphosphoglycerate, phosphoenolpyruvate and succinyl-CoA, are permanently transformed in the energy transduction process. On the other hand, the electron carriers and coupling factors of respiratory chain oxidative phosphorylation are used repeatedly through many cycles without resynthesis. Therefore, in the following discussion of model oxidative phosphorylation systems, we shall consider not only the formation of covalent bonds, but also the regeneration of the reactants through simple oxidation-reduction reactions.

I. Redox Formation of C—O Bond

In 1961, Clark et al. [5] reported that the oxidation of quinol phosphate by bromine in N,N-dimethylformamide (DMF) solution containing mono-(tetra-n-butylammonium) salt of AMP produces ADP as indicated below.

$$+ \text{AMP} + \text{Br}_2 \longrightarrow \quad + \text{ADP} + 2\text{Br}^- \tag{1}$$
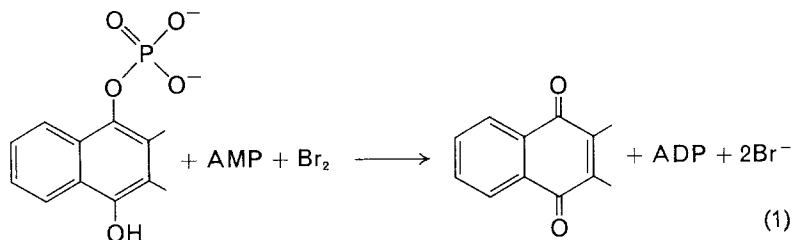
In light of this model reaction, Vilkas and Lederer [6] suggested the following mechanism for coupling phosphorylation to the oxidation of quinol phosphate in mitochondria:

$$\tag{2}$$
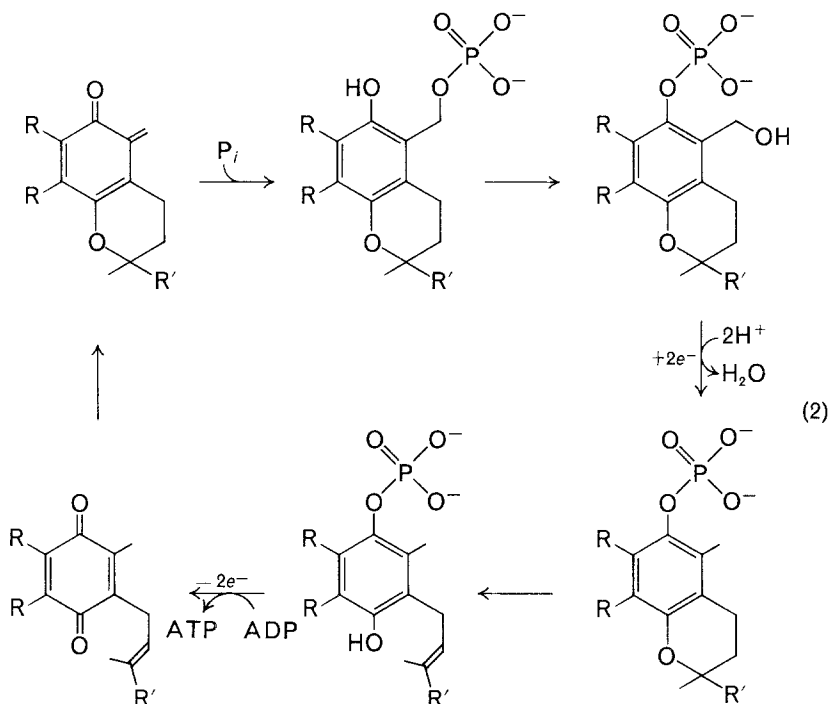
This mechanism predicts hydrogen exchange between the substituted quinone and water.

Examinations by Snyder et al. [7] of possible tritium incorporation from labeled water to the quinone in the cell-free extract from *Mycobacterium phlei* during oxidative phosphorylation gave unambiguously negative results. Similar experimental studies by Gutnick and Brodie [8] using mammalian mitochondria and native ubiquinone, and by Parson and Rudney [9] using endogenous ubiquinone in *R. rubrum* also failed to detect significant tritium incorporation.

An alternative quinol phosphate mechanism which involves redox formation of C—O bond as illustrated below was proposed by Brodie [10]:

$$\text{(3)}$$
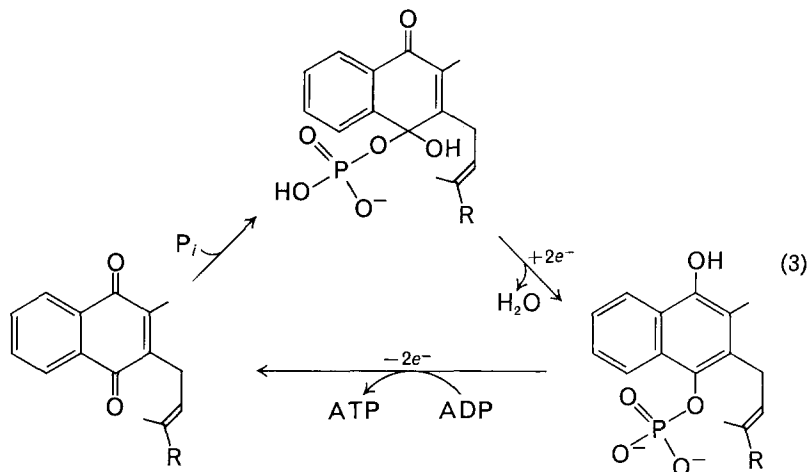
A similar mechanism may be written with nucleophilic attack of P_i at the C^1-atom. To test this mechanism, which predicts oxygen exchange between the substituted quinone and water, Snyder and Rapoport [11] reconstituted oxidative phosphorylation in cell-free, light-inactivated extracts from *Mycobacterium phlei* with both uniformly and specifically ^{18}O-labeled vitamin K_1 and determined the retention of the ^{18}O-label in the quinone after the oxidative phosphorylation experiments. The recovered vitamin K_1 showed complete retention of ^{18}O except for a slow loss roughly proportional to the exposure time of the quinone to the medium, which probably occurred via simple exchange between the carbonyl group with water. That such an exchange is not related to oxidative phosphorylation is established by the parallel exchange observed for cyanide ion-treated system, where no consumption of molecular oxygen occurred. These observations indicate that quinone involvement in oxidative phosphorylation must proceed with the original C—O bond remaining intact.

More recently, Bechara and Cilento [12] reported that in the oxidation of 1-*n*-propyl-6-hydroxy-1,4,5,6-tetrahydronicotinamide to the 1-*n*-propyl-3-carboxamide-pyridinium cation by O_2 in pyridine-water mixture (49 : 1, v/v) containing N,N,N',N'-tetramethyl-*p*-phenylene-diamine (TMPD), P_i, and ADP, phosphorylation takes place with quite high yields of ATP. The coupling mechanism these investigators proposed, which similarly involves C—O bond formation, may be summarized as follows:

$$(4)$$
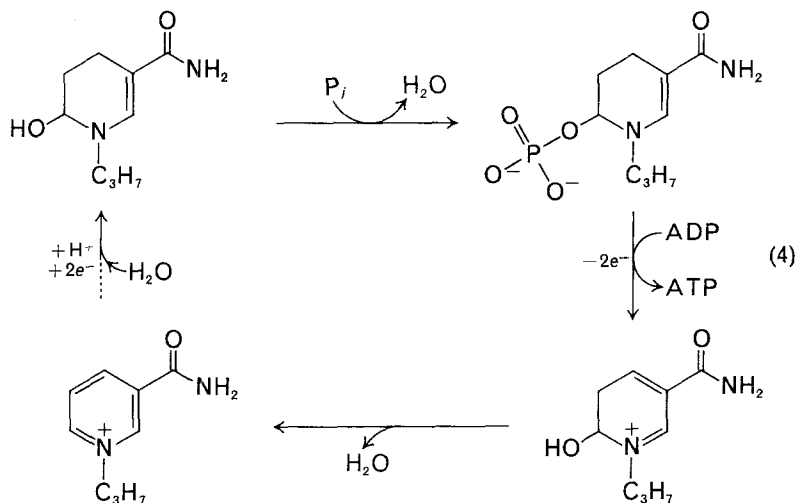
Kinetic studies suggest that the oxidized-TMPD radical (Wurster's Blue semiquinone) plays an important role in this model system [12, 13] and that the detailed coupling mechanism may be much more complex than the above diagram may seem to suggest.

II. Redox Formation of P—O Bond

An alternative scheme for the quinol phosphate mechanism with the original C—O bond remaining intact is formulated as follows:

$$(5)$$
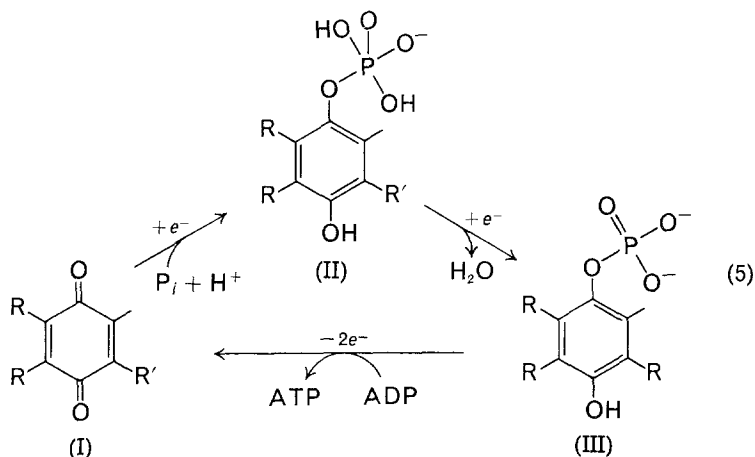
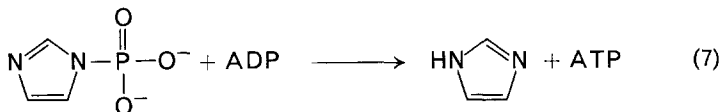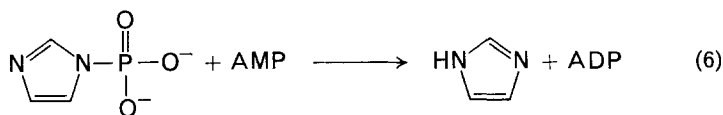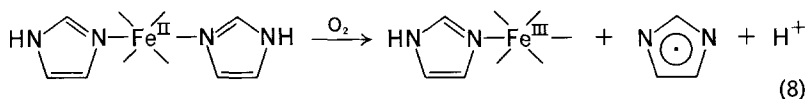By analogy to the phosphoimidazole mechanism to be discussed in the next section, the radical intermediate (II) is supposed to be formed through the reaction of the semiquinone radical with P_i. Such a mechanism would be consistent with the isotope-tracer experiments described above, since it does not require either ^3H exchange or ^{18}O exchange between the quinone and water.

III. Redox Formation of P–N Bond

Brinigar and co-workers showed in 1967 that when diimidazole-ferro-hemochrome was oxidized by air in N,N-dimethylacetamide (DMAC) solution containing P_i and AMP, generation of ADP and ATP was observed [14]. Further studies show that 1-phosphoimidazole was first generated through the redox formation of P–N bond which subsequently phosphorylate AMP or ADP to form ADP and ATP, respectively [15].

$$\text{N} \diagdown \text{N} - \overset{\overset{\text{O}}{\|}}{\underset{\underset{\text{O}^-}{|}}{\text{P}}} - \text{O}^- + \text{AMP} \longrightarrow \text{HN} \diagdown \text{N} + \text{ADP} \qquad (6)$$

$$\text{N} \diagdown \text{N} - \overset{\overset{\text{O}}{\|}}{\underset{\underset{\text{O}^-}{|}}{\text{P}}} - \text{O}^- + \text{ADP} \longrightarrow \text{HN} \diagdown \text{N} + \text{ATP} \qquad (7)$$

But how is 1-phosphoimidazole itself formed through the oxidation of heme by O_2? Studies with substituted imidazoles [15] suggest that molecular oxygen first extracts two electrons from the ferrohemochrome to produce a complex of ferriheme and the reactive imidazolyl radical ($C_3H_3N_2$):

$$\text{HN} \diagdown \text{N} - \overset{\text{II}}{\text{Fe}} - \text{N} \diagdown \text{NH} \xrightarrow{\text{O}_2} \text{HN} \diagdown \text{N} - \overset{\text{III}}{\text{Fe}} - \; + \; \text{N} \odot \text{N} \; + \; \text{H}^+ \qquad (8)$$

This radical can then react with P_i to form an unstable phosphoimidazolyl radical ($C_3H_5N_2PO_4^-$), which is subsequently reduced by another ferrohemochrome molecule to produce 1-phosphoimidazole and water:

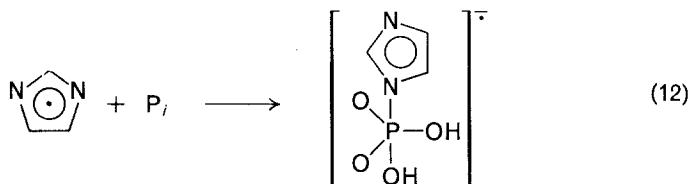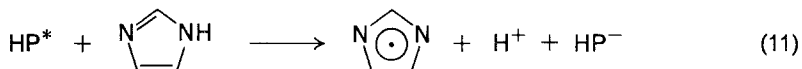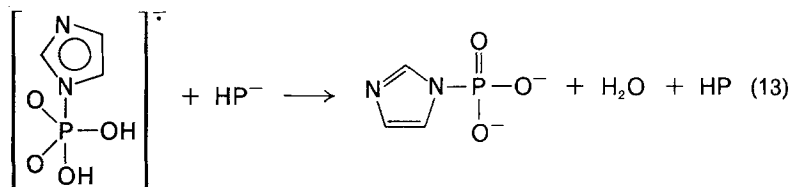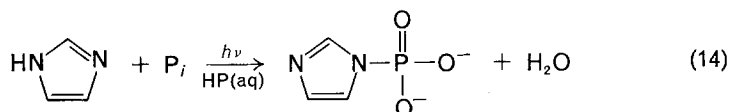$$N\!\!\bigodot\!\!N \;+\; H_2PO_4^- \;\rightarrow\; \left[\begin{array}{c} \text{imidazolyl-P} \\ \text{P-OH} \\ \text{OH} \end{array} \right]^{\overline{\cdot}} \quad \xrightarrow{+e^-} \quad \left[\begin{array}{c} \text{imidazolyl-P} \\ \text{P-OH} \\ \text{OH} \end{array} \right]^{2-}$$

$$(C_3H_5N_2PO_4^-) \qquad\qquad\qquad (C_3H_5N_2PO_4^{2-}) \tag{9}$$

$$\downarrow H_2O$$

$$N\!\!\bigwedge\!\!N-\overset{\overset{\displaystyle O}{\|}}{\underset{\underset{\displaystyle O^-}{|}}{P}}-O^-$$
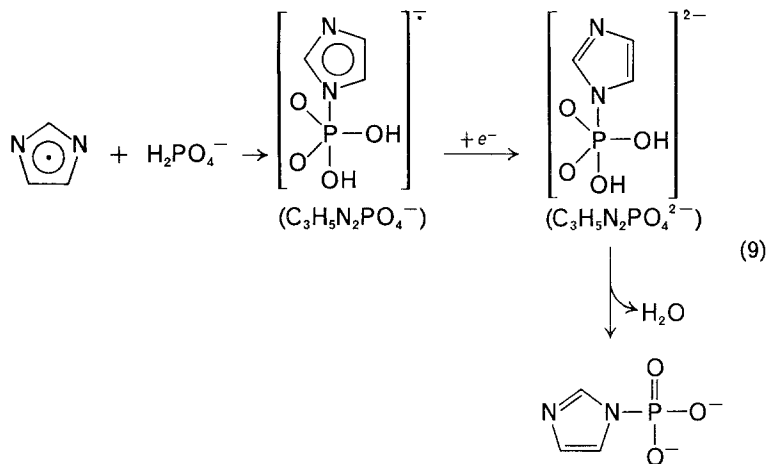
The formation of the trigonal–bipyramidal intermediate compound 1-orthophosphoimidazole $(C_3H_5N_2PO_4^{2-})$ through the direct nucleophilic attack at the P atom by imidazole is not only slow but thermodynamically unfavorable. However, since radical reactions generally require a much lower activation free energy, the trigonal–bipyramidal phosphoimidazolyl radical can be formed much more readily through radical addition to the P=O double bond as illustrated above. In the subsequent step driven by the oxidation-reduction free energy change, 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 [16].

A much cleaner method of generating imidazolyl radical and 1-phosphoimidazolyl radical found by Tu and Wang [17] is to illuminate an aqueous phosphate buffer at pH 7 containing imidazole, hematoporphyrin (HP), and a catalytic amount of O_2 with orange or yellow light. The detailed reaction steps are

$$HP \quad \underset{\longleftarrow}{\overset{h\nu}{\longrightarrow}} \quad HP^* \tag{10}$$

$$HP^* \;+\; N\!\!\bigwedge\!\!NH \quad\longrightarrow\quad N\!\!\bigodot\!\!N \;+\; H^+ \;+\; HP^- \tag{11}$$

$$N\!\!\bigodot\!\!N \;+\; P_i \quad\longrightarrow\quad \left[\begin{array}{c} \text{imidazolyl-P} \\ \text{P-OH} \\ \text{OH} \end{array} \right]^{\overline{\cdot}} \tag{12}$$

$$\left[\begin{array}{c} \text{imidazolyl-P structure} \end{array} \right]^{\cdot -} + \ HP^- \ \longrightarrow \ \text{imidazolyl-N-P(=O)(O^-)-O^-} + \ H_2O \ + \ HP \quad (13)$$

Summarizing reactions (10)–(13), we obtain the following overall reaction:

$$HN{\diagup}{\diagdown}N \ + \ P_i \ \xrightarrow[\text{HP(aq)}]{h\nu} \ N{\diagup}{\diagdown}N-\overset{\displaystyle O}{\underset{\displaystyle O^-}{\overset{\parallel}{P}}}-O^- \ + \ H_2O \qquad (14)$$

In some experiments it was found that as much as 60% of the imidazole was phosphorylated in 24 hr in aqueous phosphate buffer at pH 7 and $4°C$ [18]. Separation by paper chromatography showed that about one-third of the phosphorylated imidazole was in the form of 1-phosphoimidazole and two-thirds was in the form of 1,1'-diphosphoimidazole.

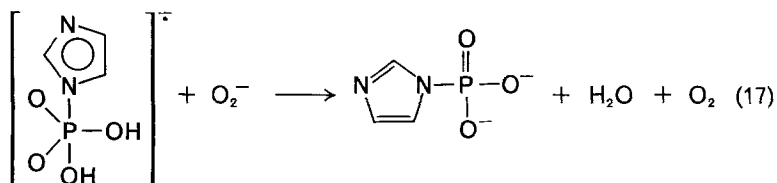It was observed that a catalytic amount of O_2 is required in the above photophosphorylation reaction, although there was no net reduction of molecular oxygen. Presumably the rate of overall net reaction [14] is proportional to the steady-state concentration of the imidazolyl radical, which is severely limited by the dissipative reaction

$$N{\diagup}{\overset{\cdot}{\bigcirc}}{\diagdown}N \ + \ HP^- \ + \ H^+ \ \to \ HN{\diagup}{\diagdown}N \ + \ HP \qquad (15)$$
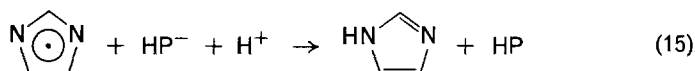
Through removing HP^- as indicated by reaction (16)

$$HP^- \ + \ O_2 \ \underset{\longleftarrow}{\overset{\longrightarrow}{}} \ HP \ + \ O_2{}^- \qquad (16)$$

molecular oxygen could retard reaction (15) and increase the steady-state concentration of the imidazolyl and phosphoimidazolyl radicals. Being a weaker reductant itself than HP^-, the superoxide radical O_2^- may allow reaction (12) to proceed to a greater extent before reducing the phosphoimidazolyl radical to the final products as indicated below:

$$\left[\begin{array}{c} \text{imidazolyl-P structure} \end{array} \right]^{\cdot -} + \ O_2^- \ \longrightarrow \ \text{imidazolyl-N-P(=O)(O^-)-O^-} + \ H_2O \ + \ O_2 \quad (17)$$
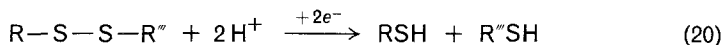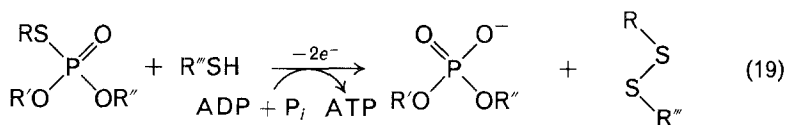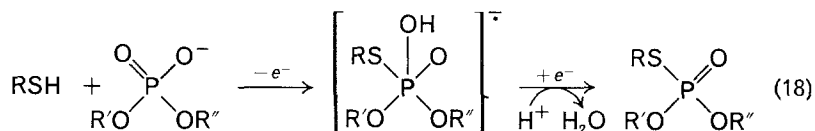
By freezing the illuminated mixture at different stages of reaction in liquid nitrogen and examining the ESR spectra, it was found that imidazolyl radical was formed first, which subsequently gave rise to phosphoimidazolyl radicals [17, 18].

Although both heme and imidazole groups are present in mitochondria, there is presently no direct experimental evidence to support such a coupling mechanism *in vivo*.

IV. Redox Formation of P–S Bond

An extension of the above coupling mechanism, with the sulfhydryl radical replacing the imidazolyl radical, may be formulated as follows:

$$\text{RSH} + \overset{O}{\underset{R'O}{\overset{|}{P}}}\overset{O^-}{\underset{OR''}{}} \xrightarrow{-e^-} \left[\begin{array}{c} OH \\ RS-\overset{|}{P}-O \\ R'O \quad OR'' \end{array}\right]^{\cdot-} \xrightarrow[H^+ \ H_2O]{+e^-} \overset{RS}{\underset{R'O}{\overset{O}{P}}}\overset{O}{\underset{OR''}{}} \qquad (18)$$

$$\overset{RS}{\underset{R'O}{\overset{O}{P}}}\overset{O}{\underset{OR''}{}} + R''SH \xrightarrow[ADP + P_i \quad ATP]{-2e^-} \overset{O}{\underset{R'O}{\overset{O^-}{P}}}\overset{O^-}{\underset{OR''}{}} + \overset{R}{\underset{S}{\overset{S}{\underset{R''}{}}}} \qquad (19)$$

$$R-S-S-R'' + 2H^+ \xrightarrow{+2e^-} RSH + R''SH \qquad (20)$$

Because of the strong tendency of sulfhydryl radicals to recombine through the formation of a disulfide linkage, such a coupling mechanism is practical only when the sulfhydryl radical groups are covalently attached to a macramolecule or membrane.

Since there is abundant evidence in the literature to support the view that specific sulfhydryl groups in the inner membrane of mitochondria play a crucial role in coupling oxidation to phosphorylation [19–25], this possible coupling mechanism deserves further study.

V. Redox Formation of C–S Bond

The oxidation of thioesters by iodine or bromine has been found by Wieland and Bäuerlein [26] to lead to the formation of the pyrophosphate bond. However, since the standard free energy of hydrolysis of a thioester linkage, 7.5 kcal/mole, is comparable to that for the hydrolysis
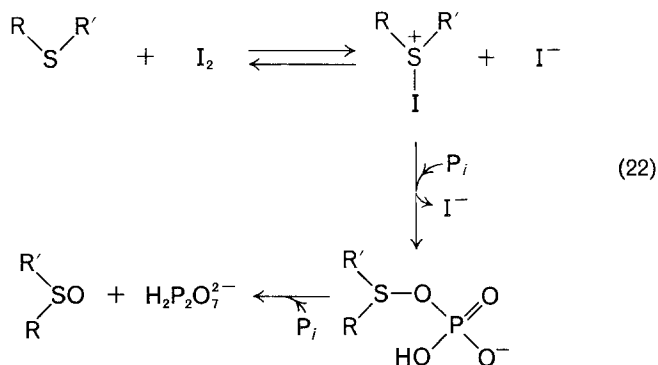
of a pyrophosphate bond, there is no net storage of redox free energy in
this model reaction. To be complete, a coupling mechanism based on this
model reaction must also provide an efficient route for generating the
thioester linkage through an oxidation-reduction reaction. In his earlier
proposal, Falcone [27] suggested that the thioester is formed by
oxidizing a hemithioacetal and that after phosphorylation the resulting
carboxyl group was enzymatically esterified and reduced back to the
aldehyde state. Alternative mechanicsms for thioester formation were
proposed by Boyer [28] and by Storey [29]. One of the simplest
mechanisms for the formation of a thioester through a redox reaction is
that involving the oxidation of a thiol to a radical intermediate as
illustrated below:

$$(21)$$
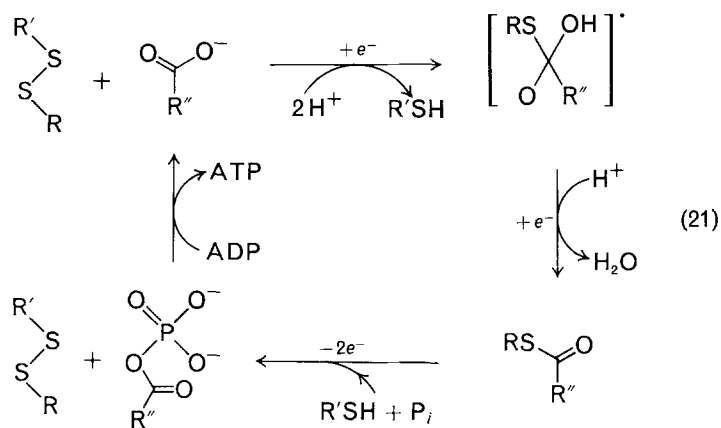
However, there is no convincing experimental evidence in the published
literature to support the acylphosphate intermediate in oxidative
phosphorylation.

VI. Redox Formation of S–O Bond

Higuchi and Gensch [30] reported in 1966 that the oxidation of
thioethers by iodine is accelerated by dicarboxylic acids and leads to the
formation of acid anhydrides. When the dicarboxylic acids were replaced
by P_i, the reaction produced pyrophosphate and sulfoxide as indicated
below:

$$R_{\diagdown}{}^{R'}\diagdown{}_{S} \quad + \quad I_2 \quad \rightleftharpoons \quad R_{\diagdown}{}^{+}{}^{R'}\diagdown{}_{S}{}_{|}{}_{I} \quad + \quad I^{-}$$

$$\downarrow \! \nwarrow^{P_i} \quad\quad\quad\quad (22)$$
$$\searrow I^{-}$$

$$R'_{\diagdown}{}_{SO} + H_2P_2O_7^{2-} \xleftarrow[P_i]{} R'_{\diagdown}{}_{S-O}{}_{\diagdown}{}_{P}{}^{\diagup O}_{HO}{}^{\diagdown}{}_{O^-}$$

This interesting model reaction has been studied by Wieland and Bäuerlein and their co-workers [31–33] and by Lambeth and Lardy [34]. Related studies of phosphorylation coupled to sulfoxide or sulfinic acid formation have been made by Allison and Benitez [35] and by Glass and co-workers [36]. However, in order for this model mechanism to be functional, the resulting sulfoxide must be reduced back to the thioether in an efficient way. Direct experimental support has yet to be found for such a mechanism to operate in mitochondria and chloroplasts.

In the hall of bioenergetics there are many authorities holding opposing points of view. That an incisively direct experiment is often more powerful than many authorities is convincingly illustrated by the effect of Lavoisier's experiment on the fate of the highly sophisticated phlogiston theory of combustion and by the effect of Büchner's experiment on Pasteur's widely applauded vitalistic theory of fermentation. What is most urgently needed in bioenergetics research today is definitive information on the structures and functional groups of those molecules which play a crucial role in the primary energy transduction step. Without knowing the chemical identity of the functional groups which participate in the coupling of electron transport to phosphorylation, we can never understand the biology of energy transduction at the truly molecular level no matter which point of view we may take in formulating our working hypothesis.

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