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Phosphohistidine

Its isolation from mitochondrial protein sheds new light on the process of oxidative phosphorylation.

P. D. Boyer

Principal deterrents to experimental progress on the mechanism of oxidative phosphorylation have been the extreme lability of the phosphorylation reactions in contrast to the oxidation reactions, and the lack of definitive chemical information about any possible intermediates in the process. The recent discovery of a protein-bound phosphohistidine by my laboratory group at Minnesota gives promise of increasing considerably our insight into how oxidative phosphorylation occurs. This article reviews current information about bound phosphohistidine and its metabolic role, and advances the hypothesis that all phosphorylations of oxidative phosphorylation funnel through the formation of bound phosphohistidine.

The formation of ATP from ADP and P_i (*1*) serves as the principal means of utilization of energy from oxidation of foodstuffs. In aerobic organisms, some 90 percent or more of the ATP formation occurs through oxidative phosphorylation, in which reduced cofactors are oxidized by molecular oxygen. A minor portion of the ATP arises from oxidation of substrates by cofactors. The mechanisms of these substrate phosphorylations, in contrast to oxidative phosphorylation, are relatively well understood. Most evidence about the mechanism of oxidative phosphorylation has been indirect, so that formulation of reaction schemes based on relevant chemical information has been unfortunately limited.

Heretofore the phosphorylation reactions of oxidative phosphorylation have been observed only in intact mitochondria from cells, or in highly organized and insoluble subunits therefrom. Bound phosphohistidine appears to be an intermediate in oxidative phosphorylation by intact mitochondria as shown by its rapid labeling from either P_i^{32} or ATP^{32} and other factors governing its formation and disappearance. Our excitement was heightened when a remarkable soluble enzyme system was obtained which would catalyze the formation of bound phosphohistidine. Study of the phosphorylations unencumbered by the diffusion barriers and complexity of the mitochondria thus seemed possible, including delineation of discrete steps and reaction components. Explorations with this system are still in their early stages, but already the results are considerable. Interpretations and projections are made at this time because of present interest in the field, even though they must remain tenuous until our studies are more fully developed.

Our interest in possible intermediates formed from P_i in oxidative phosphorylation was an outgrowth of experiments demonstrating that P_i and not ADP lost an oxygen to water in the formation of ATP (*2*). Several years ago, we instituted a search for intermediates by incubating mitochondria for short periods with P_i^{32} and attempting to find any substances other than P_i or ATP which contained radioactivity. A readily soluble substance with expected properties of an intermediate was detected. This only resulted, however, in a rediscovery of carbamyl phosphate without shedding any light on oxidative phosphorylation (*3, 4*). We then directed attention to possible intermediates not readily extracted from the mitochondrial protein or lipid. This led to the detection of a trace of a phosphorylated component, which appeared to be an intermediate in oxidative phosphorylation, as reported at scientific meetings (*4, 5*) and in additional detail by Suelter *et al.* (*6*) in 1961. An isolation and fractionation program culminated in identification of the phosphorylated component as a protein-bound phosphohistidine, in which the phosphoryl moiety is attached to an imidazole nitrogen (*7*). Other concomitant studies showed formation of bound phosphohistidine directly from ATP^{32} by mitochondria, which added to the evidence that this phosphorylated imidazole structure was an intermediate in oxidative phosphorylation (*8*).

More recent experiments have led to the demonstration (*9*) of phosphorylation reactions that give rise to bound phosphohistidine from both P_i^{32} and ATP^{32} by soluble enzyme preparations from mitochondria. Relationships between precursor and product are difficult to establish unequivocally in structures as complex as the mitochondrion in which compartmentation of intramitochondrial P_i and ATP is possible. Thus, the convincing demonstration that bound phosphohistidine is formed directly from P_i or directly from ATP by a soluble enzyme system (*9*) strengthens the deduction that bound phosphohistidine is likewise formed directly from P_i or directly from ATP by intact mitochondria.

The soluble enzyme preparations retain the capacity for a dynamic equilibrium interchange between P_i and the phosphorylated imidazole group, as well as for net formation of the phosphorylated imidazole group from P_i upon an increase in *p*H or an increase in the

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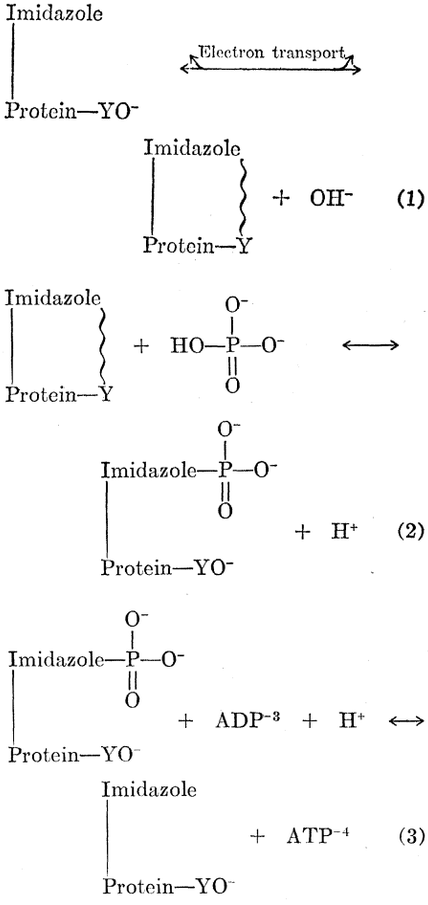
concentration of P_i or ATP (*9*). This suggested the presence in the mitochondrial extracts of a "high-energy" or activated imidazole structure capable of reacting with P_i. Such a possibility was substantiated by demonstrations that exposure of the extracts to alkali resulted in a loss of the capacity for formation of the phosphorylated imidazole from P_i but not from ATP, and that the capacity for formation from P_i could be at least partially restored by the presence of ATP.

The shift in the equilibrium with P_i toward formation of the phosphorylated imidazole group by an increase in *p*H from 7 to 9 suggests that the oxygen removed from P_i in the formation of the phosphoryl group is retained as a covalently bound and negatively charged oxygen. This and other results point to a reaction sequence as follows:



The mode of formation of the activated imidazole structure by coupling to oxidation-reduction reactions, as designated by over-all Eq. 1, is obviously an important problem and the intriguing chemistry of the imidazole group lends itself to feasible mechanisms. The nature of the suggested oxygen-containing group, designated YO^- in the above scheme, is also of keen importance. Carboxyl and phosphoryl groups carry a negatively charged oxygen at *p*H 8 to 9; of these, a carboxyl group appears more likely because of the alkali lability expected from the acyl imidazole structure which would be formed, and from an observed lability to hydroxylamine (*10*).

Possible Participation of Imidazole in Oxidative Phosphorylation

Our search for possible phosphorylated intermediates has included procedures expected to reveal the presence of low molecular weight substances which are freely soluble or else loosely bound to protein or lipid, substances covalently bound to protein or lipid, and fat-soluble substances. Acid, alkaline, and neutral extraction conditions have been used with the thought that labile substances which might have escaped earlier detection would at least survive the neutral extraction. No definitive evidence has been obtained for the presence of any phosphorylated intermediate other than the bound phosphohistidine. In addition, the rate of the labeling from P_i^{32} and ATP^{32} in intact mitochondria shows that no other alternate and more rapid pathway of interchange between P_i^{32} and ATP^{32} is operative in mitochondria. These results do not, of course, rule out the existence of undetected intermediates. Likewise, the possibility that in one or more of the steps of oxidative phosphorylation no covalent intermediate occurs (*2*) cannot be conclusively eliminated.

Our data do not as yet suffice to show if only one protein species contains bound phosphohistidine in mitochondria, although preliminary fractionation studies are consistent with this possibility. Occurrence of a single bound phosphohistidine, formed in all steps of oxidative phosphorylation, would follow if only one activated imidazole structure is present. That only one activated imidazole structure is present is consistent with demonstration of a "high-energy" substance which can drive various energy-linked reductions or participate in readily reversible reactions with P_i and ADP to form ATP. For example, NAD^+ reduction by succinate coupled to energy derived from succinate oxidation or from cleavage of ATP (*11*) points to the participation of a nonphosphorylated "high-energy" intermediate. Moreover, all electron transfer steps associated with oxidative phosphorylation can be reversed through coupling with energy derived either from oxidation reactions independent of phosphorylations or from cleavage of ATP (*12*). The activated imidazole structure is an attractive candidate for a "high-energy" intermediate common to the various electron transfer steps. The alternative possibility of different activated imidazole structures which can be interconverted must also be considered, but this is considerably more complicated and thus less appealing.

Participation of bound phosphohistidine as the only phosphorylated intermediate of oxidative phosphorylation is also in harmony with the views developed by Wadkins and Lehninger (*13*) that an ADP-ATP exchange activity represents the terminal reaction of the various steps of oxidative phosphorylation. Also, the ability of inhibitors, particularly 2,4-dinitrophenol and related compounds, to uncouple all oxidative phosphorylation steps with a relatively smooth mass-action dependence upon inhibitor concentration, as well as the ability of inhibitors like oligomycin (*14*) to block all phosphorylation, point to common mechanisms of action or to common intermediates (*15*). A role for the relatively small amount of bound phosphohistidine in the synthesis of the large amount of ATP made by the mitochondria would mean that the turnover rate of the phosphoryl group likely far exceeds that of any other phosphoryl compound in the cell.

Only a small portion of the participating imidazole group may be in the phosphorylated form within the mitochondria at a particular time. Thus, at *p*H 7 the equilibrium of the reaction between bound phosphohistidine and the activated imidazole structure favors the latter. Additional dynamic equilibria may exist between the activated imidazole structure and complexes of electron carriers and imidazole. The accumulation of the activated structure and of such bound complexes, in addition to bound phosphohistidine, could be responsible for the considerable "jump" in ATP formation observed by Schachinger, Eisenhardt, and Chance (*16*) when ADP was added to mitochondria in which respiration was inhibited by a lack of ADP.

The ease of extraction of the soluble

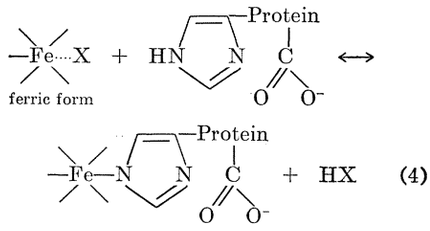
enzyme system also supports the concept of a mobile, bound imidazole structure existing in different forms and reacting at different loci in the mitochondrion. Merely freezing and thawing bovine liver mitochondria in isotonic sucrose followed by high speed centrifugation yields the system which catalyzes interconversions of the activated imidazole structure, the phosphorylated imidazole, and the bound imidazole group with unsubstituted nitrogen atoms.

A fundamental role for bound phosphohistidine may also exist in photophosphorylation. A rapid formation of a trace of a protein-bound phosphorylated substance occurs in chloroplasts upon exposure to light (*17*). The bound substance, like bound phosphohistidine, is acid-labile and alkali-stable.

If the imidazole group is closely associated with an electron carrier during an oxidation-reduction reaction, proton loss from the neutral imidazole ring may result in the formation of an imidazolide ion (*18*) or an addition compound of an imidazolide ion and an electron carrier. A nucleophilic imidazolide ion might react with a carboxyl group with the formation of an acyl imidazole and the departure of an OH^-. An O^{--} makes an unlikely leaving group, but the formation of an OH^- through participation of a proton-donating group becomes plausible. The presence of a metal to associate with the OH^- could further facilitate formation of the acyl imidazole. For simplicity in presentation, such participation is not schematically indicated in the equations given below. The over-all energetics of formation of an acyl imidazole are satisfactory. The dissociation of imidazole to the imidazolide ion occurs with a pK of about 14.5 (*19*). Thus to effect proton removal near *p*H 7 would require a free energy input of about 10 to 11 kcal. This is a reasonable amount to be derived from one oxidation-reduction step, and it is consistent with the approximate energy requirements for acyl imidazole formation.

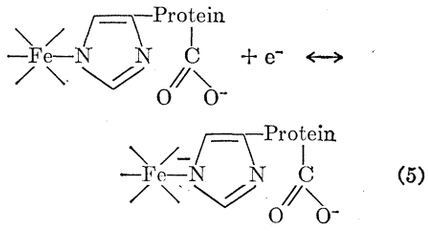
The following hypothetical considerations are based on the participation of a carboxyl group bound to the same protein as the imidazole group, but this is obviously not a requisite. Also, our present data are indicative but do not give proof that the activated imidazole structure is an acyl imidazole.

For reduction of cytochromes, or possibly of non-heme iron or other metal, association of the bound imidazole and the oxidized form could occur with replacement of a ligand, *X*, on one of the six coordination valencies of iron, as follows:



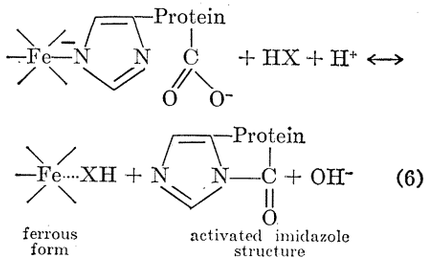
(4)

The ferric imidazolide, upon accepting an electron would form a ferrous imidazolide:



(5)

which could then form the acyl imidazole with group HX association with the ferrous form:



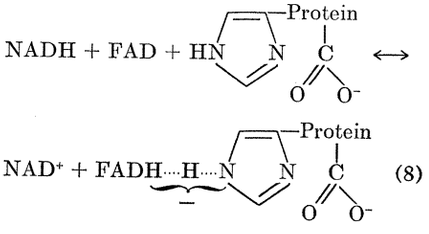
(6)

Upon oxidation, the ferrous form as indicated could lose a proton to give the ferric form for repetition of the sequence. These changes in coordination and ionization could be associated with a structural change in the cytochrome *c* upon oxidation and reduction.

In the reaction of NADH (*1*) with flavin, the uncoupled reaction is

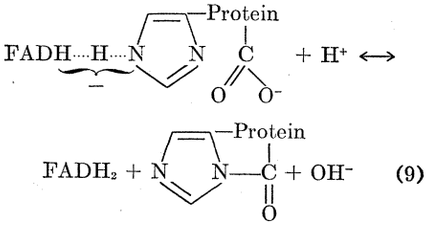
$$NADH + H^+ + FAD \longleftrightarrow NAD^+ + FADH_2 \quad (7)$$

In the coupled reaction, an imidazole group near the FAD could serve as a proton donor to the FAD, with resultant formation of an enzyme-bound $FADH_2$-imidazolide complex:



(8)

In a concomitant or subsequent step, the acyl imidazole structure may form:



(9)

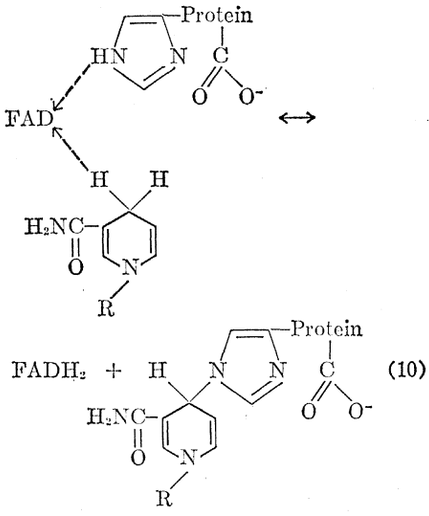
Similar reaction schemes can be formulated for other oxidation-reduction carriers. For example, in reduction of coenzyme Q by $FADH_2$, a hydride ion could be donated directly to the quinone from $FADH_2$ and a proton furnished by imidazole. This might occur concomitantly with the formation of an acyl imidazole structure, or result in an addition compound between the imidazolide ion and coenzyme Q. The latter could conceivably represent the type of compound suggested by Smith and Hansen (*20*) as a precursor to the formation of ATP.

In the preceding formulations a reduced form of the carrier is depicted as associated with the imidazole group. Such association receives some support from the observations of Chance and Williams (*21*) indicative of a combination of reduced carriers with an "inhibitor" when net phosphorylation is blocked by lack of an acceptor. Indeed, their postulates may be correlated with the preceding suggestions if one considers the symbol "*I*" in their schemes to represent "imidazole" and not "inhibitor," and that "*I*," and not the unknown substance "*X*," of their symbols, becomes phosphorylated.

As an alternate to these suggestions, association of an imidazolide ion with oxidized forms of the carriers is conceivable. In the sequence depicted by Eqs. 4, 5, and 6, the group XH could be an imidazole group associated with the ferrous form. Oxidation to the ferric form accompanied by proton loss would form the ferric imidazolide. The imidazole group could then form bound phosphohistidine, or be replaced by the bound imidazole as indicated in Eq. 4.

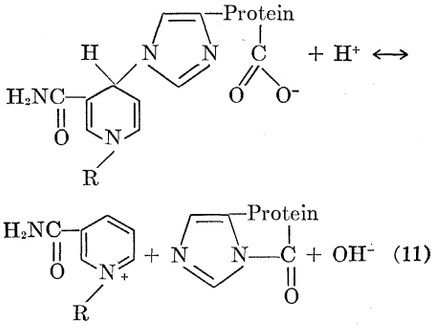
Association with oxidized forms merits consideration since Pinchot suggests a bound NAD^+ as a participant in oxidative phosphorylation (*22*). NADH might donate a hydride ion directly and imidazole a proton (either directly or through solvent water) to FAD, with formation of $FADH_2$ and an imidazo-

lide-NAD^+ addition compound as follows:



(10)

An NAD^+ addition compound with imidazole has been described by van Eys (*23*), with an equilibrium constant for the reaction $NAD^+ + \text{imidazole} \rightleftharpoons \text{NAD-imidazolide} + H^+$ of about 10^{-12}. Thus if such a compound had access to a proton donor at neutral *p*H, dissociation would be greatly favored. In the coupled reaction, the acyl imidazole may form as follows:



(11)

The imidazole ring of histidine, with its two nearly equivalent nitrogens, forms an excellent chemical structure for reactions such as these. Increased electron density at one nitrogen can impart increased nucleophilicity to the other nitrogen. The availability of a second nitrogen in the ring also could be important spatially in allowing approach of another reactant, for example, a carboxyl group, while the potential imidazolide structure is still stabilized by interaction between the first nitrogen and the electron carrier. Which of the two nitrogens of the imidazole might form an acyl imidazole is unknown, and the choices indicated above are arbitrary.

In the preceding suggestions for formation of the activated imidazole, the imidazole group donating a proton is considered not to have access to water, but the carboxyl group or acyl group is accessible to a proton-donating group and possibly a metal for departure or addition of an OH^-. The uncharged imidazole group can be assumed to penetrate a water-free locus and become activated. The relatively stable, activated imidazole structure may move to an aqueous environment where interaction with P_i and ADP is facilitated.

As an alternate to the type of suggestions made above, one might consider reactions in which the $-COO^-$ group is activated with the loss of an oxygen. For example, a $-COO^-$ associated with a ferrous ion might leave an OH^- group with the ferric iron formed by electron transport, concomitant with formation of the acyl imidazole. Similar "dehydration" reactions are somewhat more difficult to visualize at other electron-transfer steps.

Inhibition

The relative ease with which phosphorylation can be uncoupled from oxidation and the inhibitory action on all phosphorylation by various agents can readily be visualized in systems catalyzing reactions such as depicted by Eqs. 1 to 11. Four plausible mechanisms for the inhibition of the phosphorylation of oxidative phosphorylation are as follows.

Uncoupling by spatial dislocation. In schemes such as those depicted here, in which proton donation by the bound imidazole occurs, the imidazole group is a compulsory participant in the oxidation-reduction reaction only when some other proton-donating group is not present. The frustrating ease with which the electron-transport system loses the capacity for phosphorylation could result from spatial dislocations allowing water to have access to electron carriers. This might represent the most common form of structural dislocation. Access of water to the imidazole nitrogen before it reacts with a $-COO^-$ group could likewise result in uncoupling. Some uncoupling agents could act by promoting one or both types of spatial dislocation.

Uncoupling by proton donors. The action of the widely used uncoupling agent, 2,4-dinitrophenol, and of related phenols might be explained in terms of their ability to donate a proton in a nonaqueous environment. The data of Hemker (*24*) give important evidence that the uncoupling results from the lipid-soluble undissociated phenol. Donation of a proton to either the carrier directly or to the imidazole nitrogen would result in uncoupling.

An earlier interpretation of the actions of dinitrophenol on the basis of its interference in oxygen exchange reactions (*2*) is consistent with the suggested mode of action. The lack of sensitivity of the soluble system catalyzing reactions of Eqs. 2 and 3 to 2,4-dinitrophenol rules out alternate modes of action of 2,4-dinitrophenol as considered by Drysdale and Cohn (*25*) if bound phosphohistidine is an intermediate in all oxidative phosphorylation steps.

Inhibition by prevention of interactions. Inhibition of both phosphorylation and oxidation would occur if an agent interfered with either the approach of the bound imidazole group to the oxidation-reduction site or its release from the site. Inhibition of release would be expected to decrease the $P_i \leftrightarrow ATP$ and P_i-oxygen exchange reactions in addition to preventing net phosphorylation. Alternatively, interchange of the bound imidazole structure from compartments associated with electron transport to compartments for phosphorylations might occur. Such action could explain the effect of oligomycin and related inhibitors.

Inhibition by destruction of catalytic activities. Catalytic activities associated with one or more proteins, which must exist for participation of a phosphorylated imidazole as suggested here, include a reaction to form the activated imidazole structure, phosphorylation of the activated imidazole by P_i, and phosphorylation of ADP by the phosphorylated imidazole. Any agent which specifically reacts with the site of one or more of these catalytic activities would obviously disrupt the net phosphorylation process.

Exchange Reactions, Adenosine Triphosphatase, "Coupling Factors"

The recognition of the phosphorylated imidazole as an intermediate in oxidative phosphorylation may provide much-needed experimental approaches by which various investigators can assess the nature and role of exchange reactions, "coupling factors," and adenosine triphosphatase activities that have been reported.

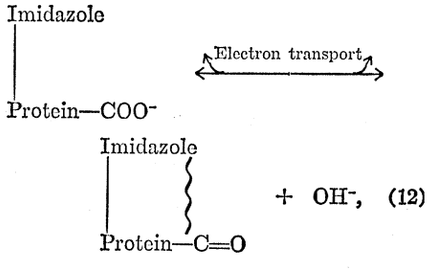
The equilibrium-reaction system described by Eqs. 2 and 3 may be written as follows,

$$P_i + \sim I \longleftrightarrow PI$$
$$PI + ADP \longleftrightarrow ATP + I$$

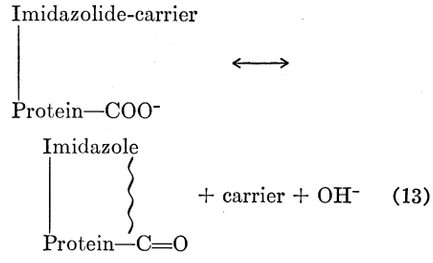
where I is imidazole. Such a system would be capable of catalyzing $P_i \longleftrightarrow$ ATP and ADP $\longleftrightarrow$ ATP exchange reactions. Indeed, it appears possible that the enzyme system (*26*) which catalyzes these exchange reactions may function through the reactions depicted by Eqs. 2 and 3, but that the significance of the system has escaped recognition because of lack of information about the participation of the phosphorylated bound imidazole. The very brief mention or the secondary role assigned the system in recent reviews (*27, 28*) indicates the difficulty in assessing its potential importance. Chiga and Plaut (*26*) noted a puzzling requirement of P_i for the ADP $\longleftrightarrow$ ATP exchange reaction. Such a requirement is readily explainable by the formation of the activated, not the free imidazole, group, upon release of P_i from the bound phosphohistidine. At low P_i levels, removal of P_i by equilibrium shift would decrease the rate of the ADP $\longleftrightarrow$ ATP exchange.

A capacity for an ADP $\longleftrightarrow$ ATP exchange in mitochondrial extracts independent of P_i concentration could arise from a system catalyzing the reactions of Eqs. 2 and 3 by the loss of the capacity for catalyzing reaction 2. This would obliterate the P_i dependence of the ADP $\longleftrightarrow$ ATP exchange as well as the catalysis of a $P_i \longleftrightarrow$ ATP exchange.

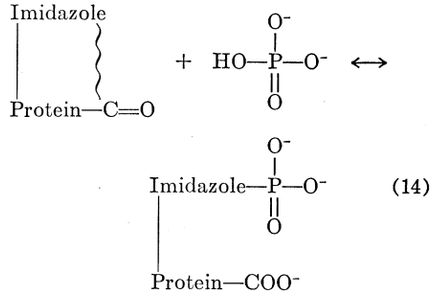
The oxygen exchange reaction catalyzed by mitochondria, as discovered by Mildred Cohn (*29*), poses a particularly interesting problem. If an acyl imidazole is formed, catalysis of an oxygen exchange between P_i and water oxygens could readily occur. Thus, exchange between water and the acyl group would result from dynamic reversal of either of the following reactions:



or



Exchange between $-COO^-$ and P_i oxygens would result from dynamic reversal of the reaction of Eq. 14, if the carboxyl oxygens have, as seems likely, equal possibility of donating an oxygen to P_i in the reverse of the reaction.



The schemes given here are in harmony with the data of Chan *et al.* showing occurrence of the oxygen exchange in digitonin particles independent of the participation of ATP, but dependent upon respiration (*30*).

The mechanism for the oxygen exchange as presented above would provide a satisfying explanation for the observed greater rate of the oxygen exchange than of $P_i \longleftrightarrow$ ATP exchange in intact mitochondria (*31*). A rapid flux of the reactions catalyzing energy-linked reductions is in accord with the suggested dynamic role of the activated imidazole, formed either by electron transport or ATP cleavage, in energy-linked reductions. In the mechanisms visualized above, the P_i-oxygen exchange and the $P_i \longleftrightarrow$ ATP exchange occur by distinct and separable reactions. Thus, the oxygen exchange may not, as suggested in the initial report on the $P_i \longleftrightarrow$ ATP exchange (*31*), represent a part of the $P_i \longleftrightarrow$ ATP exchange system. I previously directed attention to the possibility that the loss of P_i oxygen to water is indirect (*2*). Our soluble enzyme system catalyzes the oxygen exchange (*10*); this opens the way for clarification of the reaction.

The original suggestion of Lardy and Elvehjem (*32*) that the adenosine triphosphatase activity of mitochondria arises from enzymes which catalyze oxidative phosphorylation has received wide acceptance. The capacity for ATP cleavage might arise from the system catalyzing the reactions of Eqs. 2 and 3 by various ways. The alkaline lability of the activated imidazole structure would give rise to an adenosine triphosphatase operative at *p*H 8 to 10 and independent of electron carriers. Alternatively, an enzyme might be modified to catalyze cleavage of the activated imidazole by water as well as by P_i. Reaction of the activated imidazole with water would prevent reversal of the P_i formation from ATP and would release the bound imidazole group for rephosphorylation by ATP. In the presence of electron carriers, adenosine triphosphatase activity could result from formation of a carrier imidazole complex, with breakdown of the complex by addition of a water proton rather than reformation of the activated imidazole. Similarly, reversal of electron transport could occur with liberation of the unsubstituted bound imidazole, but access to water might prevent reformation of the activated imidazole. This latter concept of an adenosine triphosphatase action receives some support from the observations that reduction of electron carriers inhibits such activity of "digitonin particles" (*33*) or the dinitrophenol-induced adenosine triphosphatase of mitochondria (*34*). Not all such activities associated with mitochondria need arise from the systems catalyzing the reactions of Eqs. 2 and 3, even if these represent the primary or only path for oxidative phosphorylation. For example, enzymes catalyzing reversible phosphorylations of proteins of mitochondria might give rise to adenosine triphosphatase activity. In our laboratory, a considerable amount of acid-labile phosphoprotein of unknown nature has been found in mitochondria (*35*). This phosphoprotein is not rapidly labeled with P_i^{32}, in contrast to the bound phosphohistidine. The possibility of direct phosphorylation of acceptors by the bound phosphohistidine also merits consideration, particularly in view of phosphoryl transfers from free phosphohistidine as demonstrated by Fujimoto and Smith (*36*).

In his valuable review, Racker (*27*) maintains that there is no convincing evidence for more than one protein with adenosine triphosphatase activity associated with oxidative phosphorylation. The suggestions presented would modify this view in that adenosine triphosphatase activity is considered to

arise from a single enzyme system. The number of discrete proteins would very likely depend upon which stage the reaction with water was facilitated. Thus, cleavage by water of an acyl imidazole generated by formation of P_i from ATP might prove to be a property of a single protein. Alternatively, cleavage involving reversal of electron transport would likely involve several proteins. All activities would be expressions of a common mechanism and enzyme system for all steps of oxidative phosphorylation.

Phosphorus-Oxygen Ratios

The concept of a phosphorus-oxygen ratio of 3 has become thoroughly ingrained in the literature, as may be noted in reviews on oxidative phosphorylation (*27*, *28*). In such considerations, transfer of two electrons is tacitly assumed necessary for each ATP formed coupled to reduction of cytochrome *c*, or to reduction of cytochrome *a* or a_3, without clear confrontation with the difficulties in mechanism that this implies. The difficulty is particularly apparent in phosphorylation accompanying the oxidation of reduced cytochrome *c*.

Mechanisms, as suggested in this article, would invoke formation of two ATP molecules with the passage of two electrons through two separate molecules of cytochrome *c* to oxygen. Thus, consideration of the possibility that phosphorus-oxygen ratios might actually be higher than 3, possibly as high as 4 or 5 under some circumstances, is in order, and feasible from considerations of energy exchanges. The span from the O-R potential of NADH-NAD^+ to oxygen theoretically could be coupled to formation of over 5 ATP molecules for each O consumed.

Ratios higher than 3 may not usually be observed because partial uncoupling exists in systems used for assay, and might even exist in mitochondria in the intact cell. Variability of access to water could represent a physiological control system. Alternatively, shunt mechanisms may exist whereby part of the electron transport occurs through phosphorylating and part through nonphosphorylating reactions. Good "respiratory control" demands only that one step of the respiratory chain be blocked, and not that all steps are necessarily fixed to a stoichiometric relation between electron transport and phosphorylation. Also, other mechanisms for respiratory control, such as change in structure or accessibility with increase in ATP-ADP ratio or concentration of bound phosphohistidine, might be operative.

A second possible explanation for not observing phosphorus-oxygen ratios higher than 3 is that part of the energy derived from oxidations is used for other purposes. This could include energy-linked reductions of endogenous or metabolically produced compounds in the mitochondria. Also, through internal phosphorylation reactions or other means, mitochondria need to meet energy requirements of active transport, osmotic work, and structural changes. This would obviously result in less ATP formation for each O atom consumed.

Assumption that phosphorus-oxygen ratios should be simple whole numbers has perhaps clouded the interpretation and reporting of experiments in which ratios greater than 3 were observed and appeared to be outside the range of experimental error. If the maximum possible ratio, without dissipation of energy to other mitochondrial processes, should prove to be only 3, additional consideration would need to be given to the possibility that only one phosphorylation site, coupled to a single electron transfer, exists for oxidation of reduced flavin by O_2.

Conclusion

The discovery of protein-bound phosphohistidine and its mode of formation from both P_i and ATP in intact mitochondria and in soluble enzyme preparations from mitochondria gives convincing evidence that the phosphorylated imidazole group of a bound histidine is an intermediate in oxidative phosphorylation. The suggestion is warranted on the basis of these findings together with other data that all phosphorylation of oxidative phosphorylation occurs in a mechanistically similar process in which electron transport is coupled to formation of an activated imidazole structure, possibly an acyl imidazole. The activated imidazole structure is cleaved by P_i to form bound phosphohistidine, which can react with ADP to give ATP and bound histidine, in readily reversible reactions. The activated imidazole structure is also considered to serve as a common reactant in various energy-linked reductions in a new means of energy transfer in cells. Many properties of oxidative phosphorylation and its inhibition correlate well with the suggestions made, except that the magnitude of phosphorus-oxygen ratios may need further consideration (*37*).

References and Notes

1. Abbreviations are adenosine triphosphate, ATP; adenosine diphosphate, ADP; and inorganic orthophosphate, P_i; NAD and NADH, oxidized and reduced nicotinamide adenine dinucleotide, respectively; FAD and $FADH_2$, oxidized and reduced flavin adenine dinucleotide, respectively.
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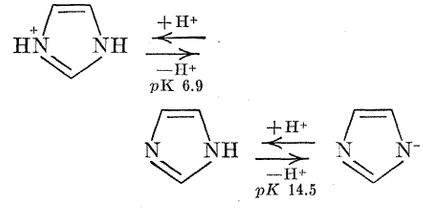
18. The forms participating in the equilibria



can be appropriately designated as imidazolium ion, imidazole, and imidazolide ion, respectively.

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37. Development of the concepts presented has been made possible only through the able participation of graduate students and postdoctorate fellows. At work now are J. B. Peter, M. DeLuca, Donald Hultquist, Jimmy Hinkson, Gunther Kreil, Larry Butler, and Richard Moyer. Past contributions were made by Clarence Suelter, Kurt Ebner, Arthur Schulz, and Mary Dempsey. The researches have been supported by grants from the U.S. Public Health Service, U.S. Atomic Energy Commission, and Hill Family Foundation.

Oriental Renaissance in Education and Medicine

A Canadian physician sees a sudden renaissance of Western learning on the Chinese mainland.

Wilder Penfield

This is a new China, after thousands of years as an empire—a new nation. Politically the Republic of 700 million people is young, but it is conscious of new power and great ambition. For a month in the autumn of 1962 my wife and I were guests of the Chinese Medical Association, traveling thousands of miles by rail and road to visit the colleges and hospitals of the larger cities and to see all that the time would allow in that vast country. Physicians welcomed us. Lectures, hospital rounds, university visits and discussions, and social events were arranged. I was made a member of the Chinese Medical Association.

The fact that we are not Communists was taken for granted, and we heard no political discussion except when we asked to have broadcasts or speeches translated. This article is made up of random observations which are as objective and critical as I can make them. It is in no sense a complete study, and I can only hope that my colleagues in China will forgive any possible errors or misconceptions. I know that my colleagues in the English-speaking world who may read the article will welcome an introduction, however inadequate, to the scientists, doctors, and educators of a very young country which is, at the same time, old and proud (*1*).

To understand what is happening in the People's Republic of China one must realize that there are practically no foreigners there now, except from the Orient. But, in spite of that, a remarkable renaissance of Western learning is going on. This is important to us as well as to the Chinese. There is a general expectation among them that science and higher education will solve the unsolved problems, that mechanization will banish hunger and bring plenty, the afforestation and the construction of more dams will control the floods and the droughts of the past. The people are temperate, frugal, puritanical, and remarkably law-abiding. It is the nature of these people, as I discovered in 1943 on a visit to western China, then under Chiang Kai-shek, to be fastidiously clean, to work hard, and to find something to laugh about.

An Interview

To give you the perspective of the Chinese on education and medicine, I can do no better than to report one interview literally. At my request, the more or less official atitude toward medical education was expressed to me by the Vice Minister of Health, Tsui Yi-tien (a physician who is in charge of medical education and is one of the vice presidents of the Chinese Medical Association). In such an interview note-taking is made easy by the delays during which the interpreter must listen to Chinese in one direction and speak Chinese in the other.

"The problem of medical education is inseparable," he began, "from that of education in general in China." Backwardness in China was due to "colonialism and to our feudalism and capitalism." For a hundred years after invasion by foreign powers, new culture, modern science, and modern industry made their start. But the work was not in the hands of Chinese. There was no overall plan for higher education, and there could not be, until independence came. "For medicine," Tsui said, "there were colleges run by the British, Americans, Germans, Japanese, and others. They trained personnel and research workers, but," he added, "the purpose was to serve their own interests.

"China has a long tradition of her own in culture and art. Now China has been liberated, thanks to the Communist Party under Mao Tse-tung. When we say 'liberation,' we refer to the feudalists inside, imperialists outside, and capitalists both sides." When he mentioned the capitalists both sides, the minister laughed. "Starting in 1949 to 1950 or 1952," he continued, "restoration of the economy was our major concern. But during this period, culture and education were also reorganized.

"Economically speaking, foreign capital was confiscated. The same was true in the field of culture and education. We took over the schools of the British, French, Japanese, the United States, and others. Personnel was used as long as the members of the staff would follow the law and the spirit. Since China is socialist in nature, there must be a unified plan.

"In the first five-year plan of 1953," he said, "education was included. In Old China, there were medical colleges

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