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A Dynamic Stereochemical Reaction Mechanism for the ATP Synthesis Reaction of Mitochondrial Oxidative Phosphorylation

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Abstract. A mechanism is presented for the ATP synthesis reaction of mitochondrial oxidative phosphorylation. This mechanism is a dynamic, stereospecific, microscopically reversible mechanism in which enzyme-bound substrates, ADP, and inorganic phosphate directly combine in a S_N2 type of process which proceeds with retention of configuration at the phosphate phosphorus center. The mechanism involves unstable, pentacovalent trigonal bipyramidal reaction intermediates which are pseudorotationally related. The mechanism accounts for all of the observed net and equilibrium exchange reactions and their relative rates. All of the exchange reactions are directly referrable to the ATP synthesis mechanism, and are circumstantial traits of that mechanism.

This paper presents the formulation of a reaction mechanism for ATP synthesis in mitochondrial oxidative phosphorylation (Eq. 1).

$$ADP^{-3} + HPO_4^{-2} + H^+ \rightleftharpoons ATP^{-4} + H_2O \qquad (1)$$

Up to this time no mechanism has been available which, to our knowledge, satisfactorily accounts for the catalytic synthesis of ATP, and fully explains the various equilibrium exchange reactions that are known to attend the net synthesis. Together with Boyer,[1] we view the exchange reactions as directly referrable to the synthesis mechanism. The formulation of the mechanism described herein is explicitly directed towards the primary process, namely ATP synthesis.

Mechanistic Assumptions. We assume that: ADP and inorganic phosphate unite directly to form ATP and H_2O. The reaction is comprised of nucleophilic attack by ADP on inorganic phosphate with displacement of water from the phosphate center.[1] The displacement proceeds by way of reaction intermediates having classical trigonal bipyramidal geometry. Groups entering and leaving such trigonal bipyramidal intermediates do so apically with respect to that geometry.

Nature of the Trigonal Bipyramidal Reaction Intermediates. The trigonal bipyramidal reaction intermediates are conceived of as comparable to the unstable pentacovalent reaction intermediates deduced to occur for reactions of other phosphorus compounds.[2] The two apical bonds are relatively long and weak, while the three equatorial bonds are relatively short and strong. The apical bonds are considered to be labile, reactive bonds (i.e., bearing entering or leaving

groups), while the equatorial bonds are considered to be stable, nonreactive bonds.

According to current theory,[2] such trigonal bipyramidal pentacovalent phosphorus reaction intermediates are capable of pseudorotation governed by certain electronic stability factors, namely that electron-withdrawing groups are most stable in apical positions and electron-releasing groups are most stable in equatorial positions. The direction of pseudorotation will be predicted by the

FIG. 1.—1. In *a*, ADP-O$^-$ and the tetrahedral HPO_4^{-2} are depicted as associated with an active center on the enzyme. Four histidine imidazole groups, represented by the letter I, facilitate proton transfers. Three of the imidazole groups have been arbitrarily designated as the tautomer protonated at nitrogen 1 in their rings, and are represented $I_{-1}H$, while one imidazole group is designated as the tautomer protonated at nitrogen 3 in its ring, and is represented $I_{-3}H$.

At physiological pH, phosphate exists in solution primarily in the monoprotonated form, HPO_4^{-2}, possessing a neutral hydroxyl group, two oxygen atoms, each of which is singly negatively charged, and one neutral oxygen atom doubly bonded to phosphorous. We have chosen to represent the phosphorous-oxygen double bond in its heterovalent resonant form where the π electrons of the double bond localize on the oxygen atom resulting in a full negative charge on the oxygen atom and a full formal positive charge on the central phosphorous atom.

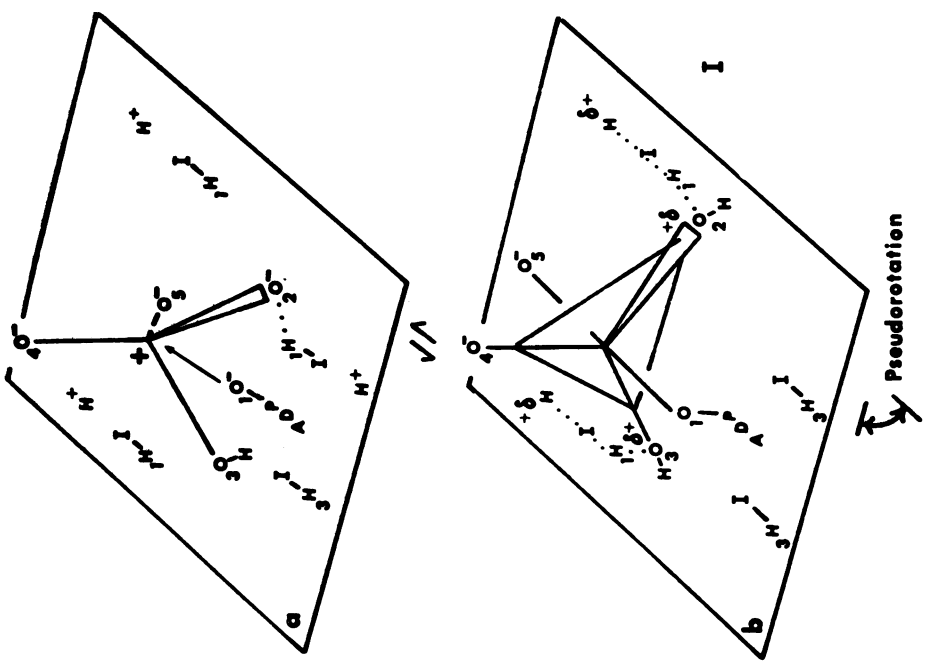
HPO_4^{-2} associates with the enzyme via three of its oxygen atoms to give a three point attachment, with its protonated oxygen atom, $_3$OH, adjacent to the $I_{-3}H$ group. Another oxygen atom, $_2O^-$, associates with a site close to a hydrogen atom of an $I_{-1}H$ group, to which it can hydrogen-bond. Formation of a full $_2$O—H bond reduces the charge on the phosphate ion by one full negative charge; the remaining two negative charges reside on the two oxygen atoms $_4O^-$ and $_5O^-$. ADP-O$^-$ associates with a site adjacent and close to the $HPO^-{}_4$, with one of the unprotonated oxygen atoms of its β-phosphate moiety positioned so that a pair of electrons in one of its orbitals can now advantageously interact with the positively charged phosphorous atom of $HPO^-{}_4$ (see arrow).

2. ADP-O$^-$ attacks HPO_4^{-2}. This nucleophilic attack is perpendicular to, and in the center of, the essentially equilateral triangular $_2$O-$_3$O-$_4$O face of the tetrahedron of HPO_4^{-2}, i.e., the attack is apical. As the $_1O^-$ oxygen atom of ADP-O$^-$ moves toward the phosphorous atom in its nucleophilic attack, $_2$O, $_3$O, and $_4$O are symmetrically displaced away from the attacking oxygen atom, i.e., "backward" toward the $_5O^-$ oxygen atom, to eventually transform the original tetrahedron of HPO_4^{-2} to a pentacovalent trigonal bipyramid, I, depicted in *b*. As $_2$O and $_3$O are displaced backward toward $_5O^-$, $_3$O carries the hydrogen atom it brought into the active center, while $_2$O carries the hydrogen atom it acquired from the adjacent imidazole group. That imidazole concomitantly acquires a proton, H^+, to form the $I_{-3}H$ tautomer. As $_2$OH and $_3$OH move backward, they each come into the vicinity of a hydrogen atom of an $I_{-1}H$ group. Hydrogen-bonding to these hydrogen atoms imparts to $_2$O and $_3$O partial oxonium character, $_2O\delta^+H$ and $_3O\delta^+H$. These two imidazoles each hydrogen-bond to a proton, H^+, at the nitrogen atom number 3 in their rings.

3. In the pentacovalent trigonal bipyramid, I, the apically oriented $_1$O oxygen atom of the ADP-O-moiety is relatively electrically neutral, $_4$O and $_5$O are both singly negatively charged, and $_2$O and $_3$O are both partially positively charged. This nonequivalence of electric charge on the various oxygen atoms in the trigonal bipyramid, I, will result in a pseudorotation to the trigonal bipyramid, II, shown in *c*. Concomitant with this pseudorotational process, the $_2$O and $_3$O groups develop essentially full oxonium character by virtue of full protonation by their imidazole neighbors. Note that the ADP-O- moiety, which had been apically oriented in the trigonal bipyramid, I, is equatorially oriented in the trigonal bipyramid, II.

4. Oxonium groups are inherently good leaving groups; their being apically oriented vastly favors their departure as neutral water. Negatively charged -O$^-$ groups are poor leaving groups; equatorial orientation further disfavors their departure. ADP-O-, although an excellent leaving group in the form ADP-O$^-$, is constrained from departure by virtue of its equatorial orientation. The trigonal bipyramid, II, is structured, therefore, for loss of water and for the bonding of the ADP-O- moiety to the phosphorous atom.

The trigonal bipyramid, II, collapses by loss of one of the oxonium groups as a molecule of water. This is accompanied by the transformation of the trigonal bipyramid, II, to a tetrahedral phosphate with a bond between the phosphrous atom and the $_1$O oxygen atom of ADP. A molecule of ATP has been formed, as shown in *d* and *d'*.


I
Pseudorotation
a
b

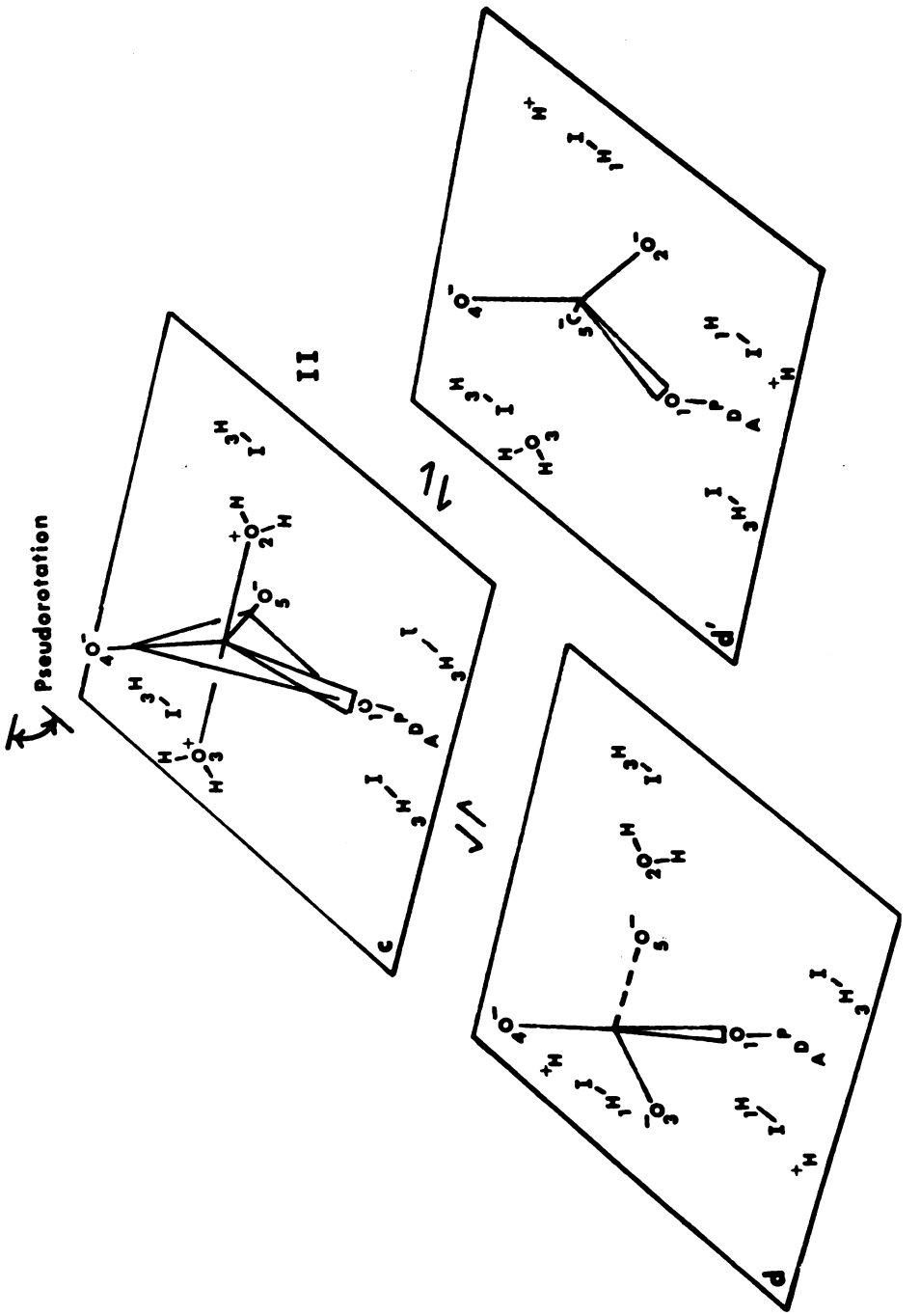


FIG. 1.

implicit relative "preference rules" of Westheimer[2,3] for reaction intermediates in which phosphorus is pentacovalently bonded to oxygen groups. These rules are: (1) $-OH_2^+$ (oxonium) groups will prefer apical positioning, (2) $-O^-$ groups will prefer equatorial positioning, and (3) neutral $-OH$ and $-OR$ groups will have relatively no positional preference.

Presentation and Discussion of the Mechanism. Because of its low basicity, $ADPO^-$ is a poor nucleophile; for the very same reason it is an excellent leaving group. The lower basicity of $ADPO^-$ as compared to H_2O definitely favors ATP hydrolysis and disfavors ATP formation. The disfavor of ATP formation is, however, overcome by the enzyme-catalyzed synthesis mechanism.

The dynamic stereochemistry of this mechanism is depicted in Fig. 1. Histidine imidazole groups have been invoked as reasonable groups for facilitated proton transfer in the active site.

Positioning of $ADPO^-$ and HPO_4^{-2} in the active site provides geometry favorable for nucleophilic attack by the negative oxygen of $ADPO^-$ on the phosphorus center of HPO_4^{-2} (Fig. 1*a*). This otherwise weak attack is probably assisted by the positive character of the phosphorus center. Such apical attack on tetrahedral phosphate, together with protonation of oxygen $_2O$ (see Fig. 1*a* and its legend) gives rise to unstable trigonal bipyramidal reaction intermediate I, depicted in Fig. 1*b*. This structure has the attacking $ADPO^-$ oxygen ($_1O$) and a formal negatively charged oxygen ($_5O$)$^-$ occupying the apical positions, while it has a second formal negatively charged phosphate oxygen ($_4O^-$) and two $-OH$ groups occupying the equatorial positions. Note that designation of full negative charges on $_4O$ and $_5O$ is done so only in a formal sense. The actual charge on one or both of these oxygens need not be full. This aspect of partial charge is important to the mechanism and is further commented on below.

The two equatorial $-OH$ groups, by reason of the tetrahedral to trigonal bipyramidal geometry transition within the active site design, each find themselves in proximity to an imidazole hydrogen. The imidazole hydrogens may form hydrogen bonds to the pair of $-OH$ groups and thereby impress partial oxonium character on those oxygens (Fig. 1*b*).

The charge state of this initial reaction intermediate I renders it unstable with respect to pseudorotation to intermediate II, because structure I involves a formal negative oxygen ($_5O^-$) in an apical position and a pair of partial positive oxonium groups in equatorial positions. Pseudorotation to form II disposes the formal negative oxygen ($_5O^-$) and the ADPO group (together with the formal negative pivot oxygen $_4O^-$) in *equatorial* positions, while it disposes the two partial oxonium groups in *apical* positions (Fig. 1*c*). The pair of partial oxonium groups can fully acquire protons from the juxtaposed imidazoles and assume full oxonium character. Development of oxonium groups in apical positions is energetically favored.[3] Either one or the other of these oxonium groups ($-OH_2^+$) may now proceed to leave as a water molecule, with simultaneous collapse of structure II to give product ATP (Fig. 1*d* and *d'*).

The pseudorotation of intermediate I to intermediate II is crucial for ATP formation. Without it, intermediate I would merely collapse back to starting materials. This is because the only other reaction path involves displacement

of the apical ${}_5O^-$ group (as O^{-2}) by a Walden inversion. This path is vastly disfavored because of the high basicity of O^{-2}. It is important to note that the formation of intermediate I when written with a full negative charge on the apical oxygen ${}_5O$ would be somewhat unfavored. However, ${}_5O$ is likely to participate in chelation of Mg^{2+} (ref. 4), attenuating its negative charge. The formation of reaction intermediate I is probably the slow, rate-determining step in the overall reaction mechanism. We consider the collapse of reaction intermediate I back to starting materials, the pseudorotational event, and the collapse of reaction intermediate II to products to be rapid relative to the formation of reaction intermediate I.

The pseudorotation is promoted by the charge state which the enzyme imposes on the initial intermediate. It yields an intermediate II that is structured for *equatorial capture* of ADPO and *apical expulsion* of H_2O.

The above mechanism comprises a type of S_N2 process which proceeds with *retention* of configuration at the reaction center. Retention of configuration results from a 90 degree geometrical relationship between entering and leaving groups in trigonal bipyramidal intermediates.[5]

It is of fundamental importance to compare this *retention* mechanism with the classical S_N2 Walden inversion mechanism. Were inversion to occur in ATP synthesis, it would involve a 180 degree, i.e. *co-apical*, relationship of ADPO and H_2O^+ in a trigonal bipyramidal intermediate. The fate of such an intermediate, in which ADPO and H_2O^+ are geometrically opposed in competition for full bonding to the phosphorus center, is collapse with expulsion of the $ADPO^-$ group as determined by the lower basicity, i.e., the greater leaving potential, of the $ADPO^-$ groups as compared to H_2O.

By comparison, in the S_N2 *retention* mechanism, no such direct, co-apical competition between ADPO and H_2O^+ is involved. Instead, a 90 degree relationship between these groups is involved. It is precisely this feature of the retention mechanism that allows ATP synthesis.

Important Mechanism Features. We summarize here a number of important features of the mechanism:

1. It is microscopically reversible.
2. It is of the S_N2 type and proceeds with *retention* stereochemistry.
3. It involves two unstable, pentacovalent trigonal bipyramidal reaction intermediates that are pseudorotationally related.
4. It involves a charge-state dependent pseudorotation of the first intermediate to form the second.
5. The second pseudorotational intermediate is structured for ADPO bonding and H_2O leaving.
6. The enzyme active center imposes the particular charge state on the first intermediate and pseudorotation proceeds according to established principles.
7. The enzyme active center, in its governing role on the charge state of the reaction intermediates, utilizes *two* water sites to achieve ATP synthesis.
8. The reaction intermediates of the mechanism are always stereospecifically bound to the enzyme.

Interpretation of Observed Exchange Reactions. We interpret the several observed equilibrium exchange reactions (i.e., ATP-$H_2{}^{18}O$, P_i-$H_2{}^{18}O$, ATP-$^{32}P_i$, and ADP-ATP exchange reactions)[1] as circumstantial traits of the ATP synthesis mechanism. Our formulation of the mechanism (Fig. 1) is abbreviated in Eq. 2 below:

$$ADP + P_i + H^+ \rightleftharpoons [I] \rightleftharpoons [II] \rightleftharpoons ATP + H_2O \qquad (2)$$

Our interpretation of each exchange reaction is:

1. *ATP-$H_2{}^{18}O$ exchange:* This exchange occurs *via* intermediate II (see Fig. 1*c*) by direct combination of ATP and $H_2{}^{18}O$ and proceeds by way of a simple Walden inversion in the double water site. It is rapid because at *no* point is ATP cleavage involved, i.e., it is a *true* exchange that does not involve overall reversal of catalysis.

2. *P_i-$H_2{}^{18}O$ exchange:* We believe that this exchange occurs in a fashion analogous to that of the ATP-$H_2{}^{18}O$ exchange above, except that the exchange occurs on P_i instead of ATP (either in the presence or the absence of ADP, but requiring energization of the enzyme). That is, P_i undergoes an $H_2{}^{18}O$ exchange in the double water site via a Walden inversion process.

3. *ATP-$^{32}P_i$ and ADP-ATP exchange:* Both of these exchanges require *full reversal* of the catalysis mechanism through both of the reaction intermediates I and II. They are therefore considerably slower than the true, ^{18}O exchanges described above.

Final Considerations. Our formulation of the ATP synthesis mechanism is, of necessity, simplified and idealized. Our structure and charge arguments possibly represent formalizations at one extreme. At the same time, we have omitted other important considerations such as active site regeneration and dynamic reversibility. We wish to defer these and other aspects of the mechanism to future papers.

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