Discussion Letter

ENERGY TRANSDUCTION AND PROTON TRANSLOCATION BY ADENOSINE TRIPHOSPHATASES

Paul D. BOYER

Department of Chemistry and Molecular Biology Institute, University of California, Los Angeles, California 90024 USA

Received 30 October 1974

1. Introduction

In a recent letter to this journal, Peter Mitchell [1] suggested a mechanism for ATP synthesis coupled to a proton gradient across a membrane. This essentially represents an amplification of the component of his chemiosmotic hypothesis concerning how a potential gradient is used, the other components of the hypothesis being how oxidative energy may generate a proton or potential gradient and the function of such gradients to transmit energy from oxidation to phosphorylation sites.

My letter has three principal purposes. One is to point out incompatibilities between Mitchell's suggestions [1] and available chemical and thermodynamic information about cleavage and synthesis of phosphate esters. A second is to clarify aspects of ^{18}O exchanges associated with energy-transducing ATPases. A third objective is to direct attention to the alternate possibility of conformational coupling of phosphate bond synthesis to proton or potential gradients.

2. Evaluation of Mitchell's mechanism

Mitchell considers ATPases that can use energy from oxidations, ion gradients, or ATP cleavage for synthesis of ATP as reversible ATPases. For such ATPases, he suggests in the text and in fig.1 of his letter [1] a mechanism for cleavage and synthesis of ATP by such ATPases. For cleavage, an OH^- is shown as adding to the terminal phosphoryl group of ATP, $-PO_3^{2-}$, to form a pentacovalent addition compound that cleaves to give ADP and P_i with 3 negative charges ($O{=}PO_3^{3-}$). For synthesis, and $O{=}PO_3^{3-}$ is depicted as undergoing successive addition of two protons, the second addition being accompanied by reaction with ADP to give a pentacovalent species containing an oxonium ion ($-OH_2^+$) group. This species is depicted as losing water to form ATP. The scheme thus accounts for use of two protons per ATP molecule synthesized, in accord with Mitchell's earlier proposals.

Besides suggesting different intermediates for coupled hydrolysis and synthesis, the scheme has other deficiencies, among which are the following:

1. Addition of an OH^- ion to the terminal phosphoryl group of ATP for hydrolysis is a quite unlikely step. Water, not OH^- has been established as the preferential reacting species for phosphate monoester hydrolysis (by a factor of about 10^{10}), with conspicuous absence of base catalysis even in presence of metals (see review by Benkovic and Schray [2]). Also, the activity of OH^-, particularly in an area purportedly with high proton activity, would be very low.

2. The formation of a trinegative $O{=}PO_3^{3-}$ species is an energetically very unfavorable step. The pK for the 3rd ionization of phosphate is over 5 pH units above neutrality, equivalent to an energy requirement for formation near neutral pH of about 7 kcals. To create and destroy a specific binding site for $O{=}PO_3^{3-}$ in a cyclic series of reactions would be energetically expensive.

3. Use of $O{=}PO_3^{3-}$ to start ATP synthesis (step III of his sequence) is highly questionable. The protonation of $O{=}PO_3^{3-}$ would merely give $HOPO_3^{2-}$, a prominent species at neutral pH. The free energy barrier for formation of ATP at neutral pH would still remain; moreover no use would be made of any proton gradient.

4. The addition of a second proton to an –OH group

on phosphorus to give an $-OH^2$ seems highly improbable and is perhaps the weakest part of his scheme. Such oxonium ion formation with carboxylic acids requires acidities equivalent to about 10 pH units below their pK. A similar energetic barrier likely exists for phosphate. Further, such protonation of an –OH would need to occur while an $-O^-$ group was still attached to the phosphorus atom. This would not be expected even if the $-O^-$ were adjacent to Mg^{2+}. Further, even if the P_i were fully protonated (H_3PO_4), the oxygen of O=P, not of HO–P would be the likely acceptor.

Modifications of the nature of the charged species participating in steps of Mitchell's scheme, as suggested in his concluding remarks [1], do not obviate the chemical difficulties, For example, the suggestion that in ATP synthesis the second protonation might effectively occur by the departure of OH^- contrasts with the well recognized properties of OH^- as a poor leaving group.

In addition to the chemical difficulties mentioned above, by presenting more specific chemical suggestions, Mitchell opens his chemiosmotic hypothesis to earlier thermodynamic criticisms (see [3–8]). When the probable absence of any appreciable proton gradient in mitochondria was recognized, Mitchell stressed that the chemiosmotic coupling as envisaged by him would not require a pH difference [9], but might use a potential difference arising from other ion gradients as part of a total 'protonmotive' force. However, the scheme given in fig.1 of Mitchell's letter [1] provides for use only of protons and not of a potential gradient. The activity of protons for additions to phosphate oxygens at the active site in a membrane would not be expected to be increased by presence of a potential gradient across the membrane. Thus each ATP molecule made would require use of two protons at an activity sufficient to yield the free energy necessary for the ATP synthesis. The mitochondrial inner membrane may achieve an (ATP)/(ADP)(P_i) ratio equivalent to input of up to 13–14 kcals per mole of ATP synthesized [10,11], which would demand a membrane pH gradient of nearly 5 pH units for use of two protons per ATP molecule synthesized.

3. Displacements on phosphorus and ^{18}O exchanges

As one basis for development of his mechanism Mitchell states, 'Nucleophilic substitution reactions at phosphate phosphorus centers are generally recognized as proceeding by way of pentavalent transitional intermediates, having trigonal bipyramidal geometry.' It is pertinent to note, however, that phosphate ester monoanion hydrolysis, including that of ATP and ADP, very likely proceeds by a metaphosphate mechanism not involving pentacovalency. Phosphate ester dianion hydrolysis, which also appears to involve a metaphosphate formation, has a transition state in which P–O bond cleavage is well advanced, i.e., a pentacovalent intermediate is not formed (see [2]). In the ribonuclease catalysis of phosphate diester hydrolysis, where the cyclic diester itself incorporates a neighboring group that might favor a pentacovalent hydrolysis mechanism, Usher using stereochemical probes found no evidence for such an intermediate [12]. Further, even if pentacovalent intermediate did occur in ATP cleavage, such occurrence would not support the chemistry proposed by Mitchell.

The above considerations are related to Mitchell's adoption of the suggestion of Korman and McClick [13] that the ^{18}O exchanges associated with the oxidative phosphorylation might involve a 'pseudo-rotation mechanism' with a pentacovalent intermediate. This is in contrast to the possibility that the exchanges result from reversible formation and cleavage of ATP at the catalytic site (see [14,15]). Indeed, for all known enzymically-catalyzed exchanges of phosphate oxygens, reversible hydrolytic cleavage of a P–O bond in a $R-O-PO_3^{2-}$ substrate has been established as at least one and possibly the only mechanism for the exchange of water oxygens with phosphate oxygens. This includes the ^{18}O exchange catalyzed by alkaline phosphatase [16], myosin [17,18], sarcoplasmic reticulum ATPase [15,19], Na^+,K^+-ATPase [15,20], pyrophosphatase [15], and submitochondrial particles [15].

4. Energy transduction through protein conformation changes

There is abundant evidence, the obtaining of which has been usefully stimulated by the concepts of Mitchell, that cation and proton gradients can be in reversible equilibrium with ATP hydrolysis or synthesis. At this stage a chemically-satisfying hypothesis is that such an

energy transduction occurs through changes in protein conformation. That such conformation changes might be coupled to covalent bond formation in muscle or mitochondria was suggested a decade ago [21]. More recently, the concept has been sharpened to suggest that ATP synthesis is driven by conformationally-linked changes in the affinity for reactants at the catalytic site [14,15,22,23]. These affinity changes could readily allow conformational energy to be used for covalent bond formation.

With membrane systems having a proton gradient coupled to ATP cleavage of synthesis, changes in protein conformation might be induced by the proton gradients. Such a possibility is in harmony with known properties of proteins. It is implicit in suggestions of Azzone (see [7]) and of Chance [24], and has been clearly stated by Papa et al. [8]. A conformational coupling mechanism of this type could involve participation of more than two protons per ATP synthesized, although the use of progressively smaller pH gradients would necessitate interlinking of an increasing and perhaps cumbersomely large number of protonatable groups to changes in affinities at the catalytic site. With hemoglobin protonation of only one surface imidazole group is involved in change in affinity of oxygen at one binding site.

Conformationally-linked energy transductions could also involve potential gradients caused by asymmetric distributions of ions other than protons or even of uncharged solutes, singly or in combination. In this regard Azzone and Massari [7] have reviewed evidence that the combined gradient of K^+ and of H^+ observed with mitochondria under some circumstances is insufficient for the observed ATP synthesis according to stoichiometries required by Mitchell's mechanisms. More analyses of this type might be helpful.

Even though Mitchell's recent suggestions [1] of how proton and/or potential gradients might be used for ATP synthesis are unsatisfactory, the possibility must remain open that energy is transmitted through such gradients, with appropriate numbers of protons, or the equivalent in potential gradient, used per ATP molecule synthesized. Indeed, the recent experiments of Racker and Stoeckenius [25] give evidence for such transmission from a bacterial rhodopsin-containing membrane to a crude mitochondrial preparation. In ATP synthesis by mitochrondia or chloroplasts, an alternate possibility is that conformational changes in the respiratory enzymes are transmitted through an interlinked protein network to the phosphorylation site [14,15].

Acknowledgments

I am indebted to present members of my research group for helpful discussions of points raised in this letter. Researches in my laboratory pertinent to the views presented have been supported by the Institute of General Medical Sciences, U. S. Public Health Service (GM 11904), the U. S. Atomic Energy Commission (Contract AT-(04-3)), and the National Science Foundation (Grant GB-36344X).

References

[1] Mitchell, P. (1974) FEBS Lett. 43, 189–194.
[2] Benkovic, S. J. and Schray, K. J. (1973) in: The Enzymes, 3rd Edn. (Boyer, P. D. ed.) Vol. 8, p. 201–239, Academic Press, New York.
[3] Chance, B. C. and Mela, L. (1966) Nature 212, 373–376.
[4] Slater, E. C. (1967) European J. Biochem. 1, 317–326.
[5] Harris, E. J., Catlin, G. and Pressman, B. C. (1967) Biochemistry 1360–1369.
[6] Wang, J. H., Yang, C.-S. and Tu, S.-I. (1971) 10, 4922–4930.
[7] Azzone, G. F. and Massari, S. (1973) Biochim. Biophys. Acta 301, 195–226.
[8] Papa, S., Guerrieri, F., Lorusso, M. and Simone, S. (1973) Biochimie 55, 703–716.
[9] Mitchell, P. (1967) Nature 214, 1327–1328.
[10] Heldt, H. W., Klingenberg, M. and Milovancev, M. (1962) European J. Biochem. 30, 434–440.
[11] Slater, E. C., Rosing, J. and Mol, A. (1973) Biochim. Biophys. Acta 292, 534–553.
[12] Usher, D. A., Erenrich, E. S. and Eckstein, F. (1972) Proc. Natl. Acad. Sci. U.S. 69, 115–118.
[13] Korman, E. F. and McClick, J. (1972) J. Bioenergetics 3, 147–158.
[14] Boyer, P. D. (1974) in: Dynamics of Energy-Transducing Membranes (Ernster, L., Estabrook, R. W. and Slater, E. C., eds.) B.B.A. Library No. 13, Amsterdam, Elsevier Press. p. 289–301.
[15] Boyer, P. D., Stokes, B. O., Wolcott, R. G. and Degani, C. (1974) Fed. Proc. (Symposia), in press.
[16] Reid, T. W. and Wilson, I. B. (1971) in: The Enzymes, 3rd Ed. (Boyer, P. D., ed.) Vol. 4, p. 373–449, Academic Press, New York.
[17] Bagshaw, C. R. and Trentham, D. R. (1973) Biochem. J. 133, 323–328.

[18] Wolcott, R. G. and Boyer, P. D. (1974) Biochem. Biophys. Res. Commun. 57, 709–716.
[19] Kanazawa, T. and Boyer, P. D. (1973) J. Biol. Chem. 248, 3163–3172.
[20] Dahms, A. S. and Boyer, P. D. (1973) J. Biol. Chem. 248, 3155–3162.
[21] Boyer, P. D. (1965) in: Oxidases and Related Redox Systems (King, T. E., Mason, H. S. and Morrison, M. eds.) 2, p. 994–1017, J. Wiley and Sons, Inc.
[22] Boyer, P. D., Cross, R. L. and Momsen, W. (1973) Proc. Natl. Acad. Sci., U.S. 70, 2837–2839.
[23] Slater, E. C. (1974) in: Dynamics of Energy Transducing Membranes (Ernster, L., Estabrook, R. W. and Slater, E. C., eds.) B.B.A. Library No. 13, p. 1–20, Amsterdam, Elsevier Press.
[24] Chance, B. (1972) FEBS Lett. 23, 3–20.
[25] Racker, E. and Stoeckenius, W. (1974) J. Biol. Chem. 249, 662–663.