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Energy coupling: an introduction

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Energy coupling in biology is associated with distinctly different processes and it is necessary to see the differences and similarities between them if one is to appreciate how they can be coupled. The overall connection is between energy store in chemical bonds, for example ATP relative to ADP + P, or in the NADH/O_2 couple relative to the NAD^+/H_2 couple, or in radiative energy, light. There are cross couplings between light, NADH/O_2, and ATP/ADP + P, and also with parallel energy rich redox systems such as NADH/S_n or couples utilising such oxidising chemicals as NO_3^- or even SO_4^{2-} with reducing systems of lower redox potential. In fact, we can often restate the coupling problem as that between redox potential differences and condensation (acid, base, e.g., ATP) reactions. It is condensation reactions which drive biological synthesis once there is available carbon at the oxidation state level of HCHO, nitrogen at the level of NH_3, and ATP. This simplification implies that we need to think out how redox and condensation reactions can be driven by common intermediates in either direction according to where most energy lies. I believe that I solved this problem in 1959 [1] and in detail in 1961 [2]. The parallel contribution of Mitchell in 1961 [3] and my correspondence with him in that year is available so that today the origin of ideas is open to examination [4]. I accept that Mitchell developed the basic ideas considerably.

The common intermediate between redox and condensation is a proton gradient or in more general terms a charge gradient of any ion, as stressed by Skulachev in particular [5]. A way of looking at the problem is as follows, starting from light.

The absorption of light can be converted into a charge potential initially as in a photo-cell

$$h\nu \rightarrow e + \oplus$$

where $\oplus$ represents a positive hole and e represents an electron. In an inorganic material, e.g., a selenium photo-cell, this separation is possible, as is well recognised. Biological systems are restricted to organic materials. Thus light absorption generates charge separation in free radicals which are not permitted to re-combine. Electrons flow away from the source in one direction and electrons flow to the $\oplus$ hole from another direction. Diffusion control increases charge separation in the matrix, a membrane. This is the creation of a redox energy gradient from light equivalent to a simple chemical redox energy between O_2 and NADH. In fact, light can be used to make these two chemicals. At some stage, to which I return, the $\oplus$ hole becomes a proton by oxidising an organic molecule which may or may not be in bulk water. Equally, at some stage, the e charge on a reduced organic molecule is neutralized by a proton which may or may not be in water. Thus $e + \oplus$ becomes R^- $(OH^-) + RH^+$ (H_3O^+) (or as we have mentioned O_2 + NADH).

Before proceeding, we see that specific diffusion paths are required here for electrons and could well be required for protons [2,6]. Once the gradient is in the form of ionic charges, there is no reason why it should not switch to any other ion gradient – Na^+, K^+, Ca^{2+} Mg^{2+}, Cl^- and so on – since the switch from redox potential electron gradients to Lewis acid gradients has already been made in the chemistry of organic molecules, e.g., quinones. As stated above, the O_2/NADH gradient of redox power can also be used to make the charge gradient and the proton gradient. Associated mechanical pumping will be described later following Wikström's work [7].

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The above is not the only way in which to achieve energy capture. The alternative is well known and involves mechanical stress rather than redox energy to generate a gradient of ions which can be of the proton. The simple well-known case is the following

trans-organic molecule + $h\nu$

→ *cis*-organic molecule + stress

The requirement is for the stress to act on a matrix so as to separate charge before relaxing. This is known in the piezo-electric effect even in bone. Once again, the charge separation must flow subsequently in a diffusion-controlled way in an organic matrix and the major Lewis acid that can diffuse and is readily adjusted in energy by stress is the proton, but this does not exclude other ions. We know in the cases of bacteriorhodopsin and halorhodopsin that other gradients can be generated in this way.

Our next problem is to discuss diffusion of the different charge carriers. The movement of the electron through a uniform dielectric medium, protein, has been thoroughly analysed and an adequate theory by Marcus has been well supported by the work of Dutton [8]. I do not dismiss through-bond or specific group influences on the path, but evidence for such a route, except at high potential, is slight. Thus, electrons move within a protein matrix using redox centres up to some 15Å apart, but this movement does not always extend across membranes. It has now been observed, as postulated in 1961, that the electron path switches to a proton path inside the matrix or membrane [1]. This is known in the cytochrome chain at the oxidases at least, and in the photosystems. The proton path is also known in the bacteriorhodopsins, where proton migration is the only charge migration in the membrane.

The greatest problem with proton migration is the nature of the carrier groups. These must be Lowry-Bronsted acid/base centres and because the proton moves in short hops (0.5 Å) and by group rotation (3.0 Å), many centres are required. The best units are -OH (H_2O) or NH chains. Proteins can provide such chains of H-bonds, as can water. The difficulty is greater for long distance movements of ions such as Na^+ and Cl^-, since the Grötthus mechanism is not available. The nature of possible ion channels was first described at the Bari meetings [9,10] and has been thoroughly studied, as has the question of how they can be made selective [6].

A feature of the movement of charge is that it is of lowest energy when the relaxation required around the charge at each step is smallest. We know that relaxation energies are controlled by the internal mobility of proteins. A very rigid protein is useful for electron migration and can be 'designed' for such a simple function: see the description of the entatic state of the β-sheet blue copper proteins. It is more difficult to achieve migration of other charged particles without relaxation and in fact the inflexible interior of β-sheet proteins prohibits such motions. Helical constructs are more valuable. Relaxation of conformation has to be used also as a gating mechanism to prevent flow to recombination after ion separation. The stress is on creation of a diffusion controlled path; see Williams 1961 [8]. A direct way of seeing this is to look upon redox reactions in the same way as ATP/ADP + P reactions, both driving gated proton movements through membranes at long range, see below. Helical proteins are of the essence of the machinery of biology.

The difficulty with following proton migration as opposed to electron migration is that few spectroscopic methods will detect it. Possibly FTIR will be useful one day. We have used NMR to study redox linked pK_a shifts in proteins, for many years [11]. Thus redox and proton energy states can be shown to be coupled over long distances.

Just as for an electron, the flow we require to generate is unidirectional. Back reactions, recombination, is easily prevented in the case of electrons by some energy loss immediately after charge separation. It would appear that for protons the best mode of creating a unidirectional vector path in a matrix is by using conformational changes. Here, many general methods of study exist, but NMR gives specific structural data. We refer to cytochrome *c* [11]. In this protein there is a network of H-bonds which runs past the heme and virtually through the protein. This network is broken, so that access toward the haem does not extend to both sides at any one time. On oxidation of Fe^{2+} to Fe^{3+}, the network of H-bonds switches and there is now a new network. It takes little imagination to see that the pathway for protons in the protein has been gated by the redox reaction. A simple test is the measurement of H/D exchange on the tryptophan-59 ring NH deep in cytochrome *c*. The exchange rate is 100-times faster in the Fe(III) state whence the proton path energy is altered by redox state change. We can imagine, too, that if a protonated group had been positioned near the Fe(II) ion in the centre of the protein with access from one side, change of conformation to the Fe(III) state could close this access, open access to the opposite side and, by lowering the pK_a, drive protons out. Recovery would come about through a reduction followed by the H-bond rearrangement and change of pK_a when protons would be gained by the ionised group, say an aspartate. Thus, a cyclic gating of proton (or ion) movement is achieved.

An attractive feature of this conformational gating mechanism is that it requires considerable conformational flexibility within the tertiary structure. This is a feature of α-helical proteins which we have stressed. It is notable that most membrane proteins and all known haem proteins like cytochrome *c* are α-helical and

open to conformational changes of the twist and slide kind. Haemoglobin is a further example of this allosteric connection which can be used in gating.

Now the problem of coupling requires two more steps. The first to be examined is the condensation reaction ADP + P → ATP + H_2O which requires energy. The final connection is between the gradients developed above and this condensation and involves ion diffusion. The relationship between proton gradients and ATP/ADP + P has to be solved first. The ideas put forward by Mitchell in 1961, [4] namely long-range field effects, have no foundation in physical chemistry. Direct coupling by passing protons through the ATP/ADP + P site is feasible (Williams (1961) [1]), but is not the method used. The method preferred by biology is long-range energy transfer by conformational switching as proposed by Boyer [12] and myself [9,10] in the 1970's at the Bari conferences. The ideas were further advanced by Boyer, as will be described below. It has taken my own group some 20 years to produce substantial evidence concerning the coupling related to that of ATP-synthetase [13].

We can consider now the extreme of the reactions of ATP. Boyer showed that energy capture in the ATPase from the energised proton gradient is due to the reaction of release of ATP and not its formation in a bound state [12]. Once again, we need to see that in a reaction

ADP + P → ATP bound → ATP free

there is required conformation changes of a *cyclic nature*, since ATP bound has to be formed *catalytically* in a way different from the uncatalysed ATP release after energisation. To analyse this reaction, which has to be free from interference from water, which could make an ATPase from the ATP synthetase, we have studied the conformation changes of phosphoglycerate kinase [13]. This turned out to be a typical kinase (ATP synthetase is also one) with a hinge-bending mode, see Fig. 1. Using NMR and site-specific mutagenesis, we showed that in the reaction

ADP + 1,3diPG → ATP + 3PG

compare

ADP + P → ATP + H_2O

we could follow the conformational changes. (The enzyme rate like that of ATP synthetase is controlled by the rate of product leaving.) In fact, the required conformational change was not just a hinge-bending but spread through the enzyme along the helices while the more rigid β-sheet structures bound the substrates. Other kinases, many of which have now been studied, show how to protect ATP energy in reactions in a very similar way.

The next step to complete energy transduction uses the mechanical coupling of helical rods to transfer

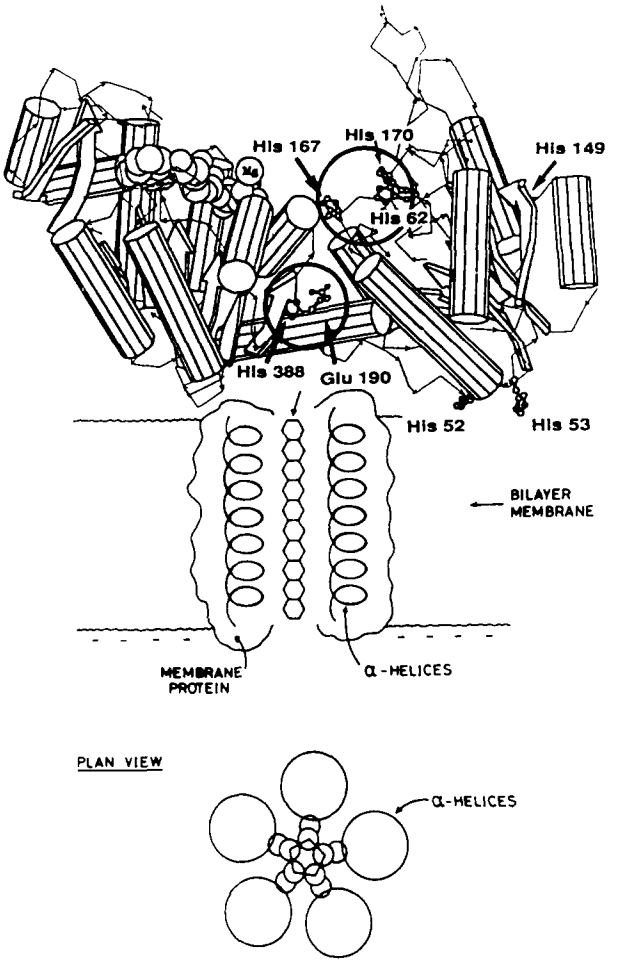

Fig. 1. The figure is an attempt to model the ATP-synthetase using the ideas expressed in Ref. [10] and detailed analysis of helical proteins, e.g., alamethicin, calmodulin and colicins, and a kinase, phosphoglycerate kinase as detailed in the references. The data would indicate that the passage of protons through a proton wire (made of helical proteins) [6] would, on binding to membrane carboxylate groups, twist the helices so that mechanical energy was transmitted to the hinge-region helices of the ATP synthetase, causing ATP to be released. A more detailed description is given elsewhere [4]. Today, there is no suggestion that the chemiosmotic field mechanism has any standing.

energy from a gated proton gradient in the membrane (not as Mitchell proposed) to gated ATP release. The figure illustrates the idea. It is assumed that the c-peptides of the ATP-synthetase, F_o portion, provide the gated channel using central aspartates to gather protons. Its helices are then connected to those of the ATP synthetase and once loading is sufficient driving reactions through a cycle of steps of lower energy. There is no difficulty in principle with a replacement of the proton channel with any other ion channel with similar properties. Skulachev has drawn attention to Na^+, but the description is general [5]. In fact, any gradient will suffice, even of a sugar. The system is reversible, so that ATP can be used to drive transport as shown by Crane in 1960 [14]. The use of charged

particles has the advantage observed in mitochondria that instead of a dependence on concentration terms for intermediate energy storage, an electric field can be used. This was put forward by Mitchell and Rottenberg.

Curiously, in the discussion of the last step in coupling, the transfer of energy between the energy-generating sites of redox and condensation reactions, the discussion between localised and delocalised protons became wilfully muddled [4]. My original idea of localised proton migration was movement of protons in and around two large particles, redox and ATP sites, in a diffusion path which did not allow collapse of the ion gradient. As pointed out in 1961 in letters to Mitchell before his publications, this did not exclude limitation by a membrane system, delocalised. However, the smaller the volume involved the smaller the buffer capacity which has to be overcome. No matter what biology does, localised connections are better. There is now considerable evidence that localised circuits exist. Of course, in steady state the bulk has to be considered, but the steady state does not reveal the pathway. You must analyse the pre-steady state. A somewhat different analysis of the internal reactions of the membrane electron carriers, proton carriers and ATP generating centres involves a diffusion in the membrane of the reaction sites. Such diffusion can short-circuit and/or come in balance in the steady state with bulk gradients [15].

I conclude that in essence we understand energy coupling, but as with all other biological reaction sequences, feedback controls are essential. Today the stress is on control, which includes conformational changes of whole sections of membranes as well as chemical steps. I leave the topic to the following lectures.

References

[1] Williams, R.J.P. (1959) in The Enzymes, Vol.1, (Boyer, P., Lardy, H. and Myrbäck, H., eds.), pp. 391–441, Academic Press, New York.
[2] Williams, R.J.P. (1961) J. Theoret. Biol. 1, 1–17.
[3] Mitchell, P. (1961) Biochem. J. 79, 23P.
[4] Williams, R.J.P. (1993) Biosci. Rep. 13, 191–212.
[5] Skulachev, V.P. (1991) Biosci. Rep. 11, 387–444.
[6] Williams, R.J.P. (1985) in The Enzymes of Biological Membranes, 2nd Edn., Vol. 4 (Martonosi, A.N., ed.), pp. 71–110, Plenum, New York.
[7] Krab, K. and Wikström, M. (1987) Biochim. Biophys. Acta 895, 25–39.
[8] Moser, C.C., Keske, J.M., Warncke, K., Farid, R.S. and Dutton, P.L. (1992) Nature 355, 786–802.
[9] Williams, R.J.P. (1970) in Electron Transport and Energy Conservation (Tager, J.M., Papa, S., Quagliariello, E. and Slater, E.C., eds.), pp. 7–23, Adriatica Edirice, Bari.
[10] Williams, R.J.P. (1975) in Electron Trasfer Chains and Oxidative Phosphorylation (Quagliariello, E., Papa, S., Palmieri, F., Slater, E.C. and Siliprandi, N., eds.), pp. 417–422, North-Holland, Amsterdam.
[11] Gao, Y., McLendon, G., Pielak, G.J. and Williams, R.J.P. (1992) Eur. J. Biochem. 204, 337–352.
[12] Boyer, P.D. (1993) Biochim. Biophys. Acta 1140, 215–250.
[13] João, H.C. and Williams, R.J.P. (1993) Eur. J. Biochem. 216, 1–18.
[14] Crane, P.K. (1983) in Comprehensive Biochemistry (Florkin, M. and Stotz, E.H., eds.), Vol. 35, pp. 43–69, Elsevier, Amsterdam (see Fig. 4 of this paper).
[15] Slater, E.C., Berden, J.A. and Herweijer, M.A. (1985) Biochim. Biophys. Acta 811, 217–231.