This enzymatic procedure may facilitate the production of D-cysteine, but cheap availability of the substrate, \(\beta\)-chloro-D-alanine remains a problem.

References


KENJI SODA

Institute for Chemical Research, Kyoto University, Uji, Kyoto-Fu 611, Japan.

Coupling factor B and the bovine mitochondrial H\(^{+}\)-ATP synthase

How many polypeptide components are required for the proper functioning of the mitochondrial H\(^{+}\)-ATP synthase/hydrolase? Obviously an answer to this question is a prerequisite to a reasonable understanding of the process of oxidative phosphorylation, and many groups have approached this problem by the techniques of resolution and reconstitution. Such studies, pioneered in the laboratories of Green and of Racker, have led to the identification of a variety of coupling factors, which, by definition (see Ref. 1), when added to partially extracted submicroscopic particles, stimulate the rate of ATP synthesis or of another energy-requiring process.

Most workers now recognize the conceptual subdivision of H\(^{+}\)-ATP synthase into a structural, membrane-associated F\(_1\) (for oligomycin-sensitive) portion and a catalytic, peripheral F\(_0\) portion. The isolated F\(_0\) catalyses ATP hydrolysis, but the entire F\(_{1}\) complex ('complex V') is required for uncoupler-sensitive ATP\(^{-2}\)P\(^{3}\) exchange activity, the usual criterion of competence in energy coupling, i.e. the ability to carry out electron transport-driven phosphorylation with a normal turnover number in the native membrane.

In Escherichia coli, recent molecular genetic and nucleotide sequencing studies by a number of groups (see for example Refs 2-4) have clearly established that the unc [ap] operon codes for eight polypeptides in the F\(_{1}\)F\(_{0}\) complex, plus a ninth of uncertain function: the F\(_1\) contains five types of subunit \((a\) to \(e\)) and the F\(_0\) three types — values consistent with those found in the isolated F\(_{1}\)F\(_{0}\) complex\(^{2,4}\). Thus, the answer to the opening question seems to be at hand.

In bovine mitochondria the problem is much more complex, since only one of the polypeptides of the F\(_{1}\)F\(_{0}\) complex is encoded in mitochondrial DNA (Ref. 5) and there is thus no satisfying correspondence between a well-characterized operon and the components of the H\(^{+}\)-ATP synthase. It is, therefore, of great importance to establish exactly what the F\(_{1}\)F\(_{0}\) complex requires for its activity, and it is here that the 'coupling factor' approach offers the possibility of progress.

The bovine F\(_1\) is relatively easy to discuss; there is general consensus (e.g. Ref. 6) that it contains at least the five \(a\) to \(e\) subunits, although their roles are in some cases distinct from those of their E. coli counterparts. However, it seems likely that the whole bovine F\(_{1}\)F\(_{0}\) complex contains at least 11 polypeptides (cf. Refs 7 and 8). One of these is the oligomycin-sensitivity conferring protein (OSCP) which, with factor F\(_6\), helps to bind F\(_{1}\) to F\(_{0}\). In the membrane sector itself, only the dicyclohexylcarbodiimide (DCCD)-binding protolipid seems unequivocally assigned. The role of factor B (previously called F\(_6\)) has, until relatively recently, remained uncertain. Now however, a series of publications from Sanadi's laboratory, culminating in a review\(^{9}\), has greatly strengthened the status of F\(_6\) as a crucial component of the F\(_{1}\F\(_{0}\) complex in beef-heart mitochondria.

What do we know of F\(_6\)? (Ref. 9) When added to submicroscopic particles, which have been extracted with ammonia and EDTA at alkaline pH ['AE-particles'], it greatly stimulates the rates of ATP synthesis, ATP-P\(^{2}\) exchange and ATP-driven NAD\(^{-}\)P\(^{-}\) reduction. When purified to homogeneity, it has a mol. wt of 14 600, dimerizes in solution, lacks carbohydrate or lipid and stains poorly on polyacrylamide gels. Antiserum raised against purified F\(_6\) cross-reacts with F\(_{1}\)F\(_{0}\) preparations from E. coli, Paracoccus denitrificans, spinach thylakoids and from the thermophilic Bacillus, PS3 exploited in Kagawa's laboratory. Although F\(_6\) is required absolutely for ATP-P\(^{2}\) exchange activity, it is not required for oligomycin-sensitive ATP hydrolyse activity, and a variety of binding experiments indicate that F\(_6\) is found in a stoichiometry of approximately one F\(_6\) per F\(_{1}\), binds to F\(_{0}\) but not to F\(_{1}\). Studies with membrane-impermeant thiold reagents localize F\(_6\) on the same side of the membrane as F\(_{1}\), and data from fluorescent probe experiments are consistent with the view that the role of F\(_6\) lies in coupling the ATP-synthetic and -hydrolytic reactions of the complex to H\(^{+}\) movements, conceivably by inhibiting 'slip' reactions such as those proposed to occur\(^{10}\) within redox-linked H\(^{+}\) pumps.

Thus, coupling factor B seems to be well-established constituent of the energy-transducing ATP synthase complex of bovine mitochondria, it will be of great interest to see if it has a counterpart in the E. coli enzyme.

References


The semantics of 'chemiosmosis'

Giovanni Felice Azzone

The fact that e⁻ transfer or ATP hydrolysis (chemical scalar reactions) generate Δμ, an osmotic force, and vice versa⁴ and that Δμ can transfer energy between two H⁺ pumps, has led to widespread use of the term 'chemiosmosis'. But what is chemiosmosis? According to one view, the term chemiosmosis indicates the hypothesis where the e⁻ transfer chains . . . are coupled to ATP synthesis by Δμ (Ref. 2). A similar view has recently been taken with respect to the scheme 'microchemiosmosis' or 'localized Δμ' (Refs 3–6) proposed to explain some of the discrepancies with the prediction of the chemiosmotic hypothesis. According to Skulachev⁴, 'this scheme is in fact so close to Mitchell's original hypothesis that it is rather difficult for a bioenergeticist to explain to an outsider why the localized Δμ concept contradicts the chemiosmotic theory'. I do not share this view and, as stated elsewhere, I believe that the concept of microcircuits connecting redox and ATPase H⁺ pumps leads directly to that of the molecular energy machine⁵. The purpose of my writing, however, is not that of analysing the differences between macrochemiosmosis, microchemiosmosis and molecular energy machine but rather to show that, in the current bioenergetic literature, the term chemiosmosis has different meanings. This can easily be appreciated by comparing the above statements with those of the proponent of the chemiosmotic concept⁶.

Consider, first, chemiosmosis during the operation of single pumps. The loose meaning of chemiosmosis is that of an enzyme complex connecting metabolism to transport and thus converting chemical into vectorial energy. All pumps perform this role and thus they are intrinsically 'chemiosmotic'. However, denoting the Ca²⁺ pump of the sarcoplasmic reticulum or the Na⁺/K⁺ pump of the plasma membrane as a 'cation–motive chemiosmotic pump'⁷ is not only a matter of language but has precise implications regarding the molecular mechanism of the pumps.

The strict use of the term chemiosmosis is bound to the concept of group translocation, described by Mitchell as follows⁸: 'I suggested that for enzymes and catalytic carriers that have spatially separated binding sites for donor and accepting species, one should recognize the vectorial conductivity of transport of the catalytic domain by describing the group transfer as a group translocation process . . .'. I introduced the term chemiosmotic to describe this anisotropically catalysed chemical-cum-osmotic type of group translocation process. Thus, not all pumps necessarily operate according to chemiosmotic reaction mechanisms; pumps that do are those where the coupling of osmotic and chemical steps complies with the specific requirements of the group-translocation concept. 'I considered the chemiosmotic reaction mechanism to depend on very tightly controlled diffusion of electronic chemical group and molecular ligands through the intrinsically anisotropic chemiosmotic catalytic complex between two (or more) phases'. The loop arrangement of the redox carriers in the respiratory chain has hence, been strongly defended as the most classical expression of a chemiosmotic reaction mechanism and opposed in principle to the non-chemiosmotic chemical pumps where: 'The osmotic proton translocations are conceived as occurring through centers in protein or polypeptide subunits that are spatially separated from the chemical reaction centers, and coupling is attributed to physical conformational movements communicated through the intervening polypeptide system'. The question then arises as to whether enough knowledge has accumulated to conclude that the molecular mechanisms of the pump satisfy the requirements of chemiosmosis. If not, the notation of the pumps, redox and ATPase H⁺, or Na⁺/K⁺, as 'chemiosmotic', reflects a research strategy rather than an established concept.

When applied to the mechanism of energy coupling between the two redox and ATPase H⁺ pumps, the term chemiosmosis can also be used in loose or strict terms. The very strict meaning of chemiosmosis was derived from the group-translocation concept which 'also provided the general basis for describing macroscopic chemiosmotic coupling between different chemiosmotic catalytic units osmotically connected by being plugged through the same topologically closed osmotic barrier'. Again, as stated in the words of P. Mitchell⁹, 'the macroscopic chemiosmotic coupling can be seen to differ in a clear-cut and most important respect from the local interaction or localized protonic anhydride type of mechanism in that it has a function of power transmission over the whole area of the topologically closed membrane between the protonic conductors on either side. This power-transmission function of proticity in chemiosmotic systems is very important organizationally because it allows the proticity-producing and proticity-consuming units to interact freely while distributed over the membrane surface'.

In contrast, chemiosmosis can be used in loose terms to indicate any coupling between the redox and ATPase H⁺ pumps occurring by means of a proton current, i.e. by proticity. From this loose usage it would follow that, since most energy transfer mechanisms assign a coupling role to the proton current, they are all chemiosmotic in nature. A deeper insight is obtained by considering the topology of the proton circuits. In the case of classical chemiosmosis, 'all that is required is a thin, topologically closed insulating lipid membrane between two aqueous proton-conductor phases', for this reason 'the widespread habit of speaking of membrane energization in chemiosmotic systems is mistaken and confusing. Rather, one should speak of the energized aqueous media that are brought to different protonic potentials by the protonic power-generating systems plugged through the membrane'.

An alternative to classical chemiosmosis assumes, by contrast that the membrane of energy-transducing systems is not simply a thin insulating lipid layer. Rather, it contains polypeptides where the primary conformational changes associated with e⁻ transfer or illumination take place (cf. Ref. 10). These conformational changes are not

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DOUGLAS KELL
Department of Botany and Microbiology, School of Biological Science, The University College of Wales, Aberystwyth SY23 3DA, UK.