Discussion Letter

The localized $\Delta\bar{\mu}H^+$ problem

The possible role of the local electric field in ATP synthesis

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Local electric field *$\Delta\bar{\mu}H^+$* *ATP synthesis* *H^+-ATP-synthetase* *Chemiosmosis* *Protonic coupling*

1. INTRODUCTION

After the Mitchellian chemiosmotic theory of energy coupling [1,2] won recognition, two extremist tendencies in bioenergetics have appeared: some follow blindfold 'The Oracle from Bodmin'; others try to ruin the 'intellectual cathedral' built by Mitchell and his adherents over the last two decades.

The latter may well be the great-great-grandsons of Herostratos. However, their opposition may arise from an unexpected, surprising result, not consistent with the accepted view and the scientific community will have to interpret the finding: what is it? An experimental mistake?, an artifact?, an exception to the rule?, disproof of the basic concept? To give a verdict, one must be sure that the alternative possibilities have been ruled out.

Over the long period of testing the chemiosmotic concept, different schemes have been considered as alternatives (table 1). The first was a chemical hypothesis of energy coupling which suggested that the chemical energy of a redox reaction is used directly to support ATP synthesis with no H^+ involved. Later, when it became obvious that H^+ plays a very important role, Williams' concept, that was advanced in parallel to the chemiosmotic scheme [3], was regarded by the majority of Mitchell's opponents as an alternative. In Williams' hypothesis, a proton is already present but it is directly translocated from a redox enzyme to ATP-synthetase without crossing the hydrophobic barrier of a membrane. This mechanism requires no topologically closed membrane vesicles. However, energy coupling was never demonstrated in systems as simple as enzyme complexes or open membranous sheets. On the other hand, direct evidence of the formation of $\Delta\bar{\mu}H^+$ across coupling membranes was obtained. Moreover, it was found that enzymes involved in energy coupling are com-

Table 1

Energy coupling concepts

1. *Chemical coupling*: direct utilization of chemical energy of a redox reaction for driving ATP synthesis.
2. *Conformational coupling*: direct exchange by conformational energy between a redox enzyme and ATP-synthetase.
3. *Protonic coupling*
   - 3.1. *Williams' scheme*: direct transfer of H^+ from a redox enzyme to ATP-synthetase; H^+ does not cross the hydrophobic barrier of the membrane.
   - 3.2. *Transmembrane mechanisms of protonic coupling*
     - 3.2.1. *Localized $\Delta\bar{\mu}H^+$ concept*: H^+ is transported through the hydrophobic barrier by a redox enzyme (or bacteriorhodopsin), moves in the interface *without entering the water phase* and is returned to the starting point by H^+-ATP-synthetase.
     - 3.2.2. *Delocalized $\Delta\bar{\mu}H^+$ concept*: as 3.2.1. but H^+ does enter the water phase.

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2.5. *Protein determination*

Protein was measured according to [14] using bovine serum albumin as a standard.

3. RESULTS

The Fab fragments of 3 monoclonal anti-apo B antibodies 5E11 (IgG1), 3A8 (IgG2a) and 4G3 (IgG2a) have been used in titration studies. An example of the separation of free Fab fragments (3A8) from LDL–Fab complexes by isoelectrofocusing in agarose is shown in fig.1. The stained gel (fig.1A) demonstrates that an excellent separation between LDL (lane 1) and Fab (lane 2) is obtained. An autoradiogram of the same gel (fig.1B) shows that when ^{125}I-Fab (3A8) is preincubated with excess LDL, LDL–Fab complexes have a pI similar to that of LDL and that almost all of the radioactivity is localized in this region (lane 3). The bound ^{125}I-Fab is displaced by excess unlabelled Fab fragments of 3A8 (lane 4) but not by Fab fragments of normal mouse IgG (lane 5). Similar results were obtained with Fab fragments of 5E11 and 4G3.

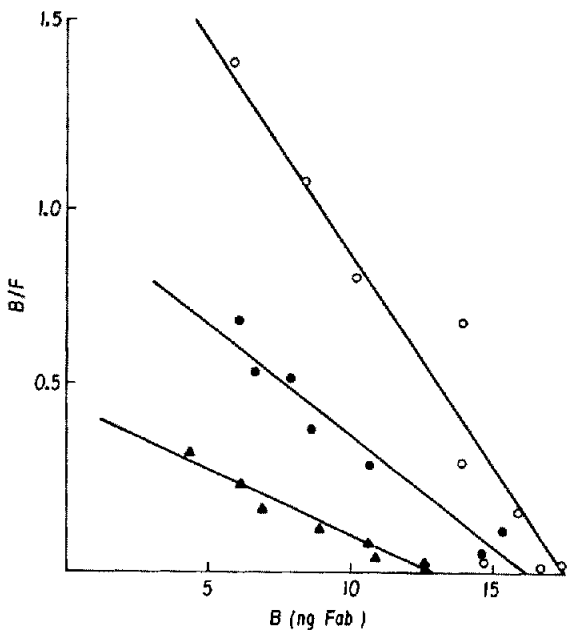

Fig.2. Fab fragments (4 ng to 2 μg) of 3A8, 4G3 and 5E11 were incubated with 15 ng of the corresponding ^{125}I-Fab fragments and 200 ng LDL. Radioactivity associated with bound (B) and free (F) Fab was determined after isoelectrofocusing and the results expressed as Scatchard plots.

With fresh LDL preparations, ~90% of applied radioactivity was recovered, with virtually all associated with either the Fab or LDL bands. In older LDL preparations (> 14 days), a distinct band of radioactivity was observed between the bands of Fab and LDL and which was not associated with the point of application. The radioactivity of this band (as high as 20% total recovered radioactivity) could be displaced with unlabelled anti-apo B Fab and may thus represent degradation products of LDL. The phenomenon was not prevented by inclusion of the protease inhibitor PMSF. Degradation of apo B in the presence of protease inhibitors has been described in [15].

Varying dilutions of unlabelled Fab fragments of 3A8, 4G3 and 5E11 containing 15 ng of the corresponding ^{125}I-Fab fragment were incubated with a fixed quantity (200 ng) of LDL as in section 2. The radioactivity representing free Fab and Fab complexed with LDL was determined, and the concentration of specifically bound Fab calculated. Results of typical experiments for the 3 antibodies are illustrated in fig.2 in the form of Scatchard plots [16]. As expected, each of the antibodies gave a linear binding plot. The maximum amount of Fab which could be bound by 200 ng LDL, determined from the intercept on the abcissa, was for 3A8, 14.6 ± 2.0 ng; for 4G3, 14.6 ± 2.8 ng; and for 5E11, 18.7 ± 3.1 ng (mean and standard deviation, n = 3). The maximum amount of Fab fragments of the antibody 3A8 which could be bound by 200 ng LDL prepared from 3 different individuals was also determined. The 3 preparations of LDL (200 ng), respectively bound 14.1 ± 2 ng, 15.0 ± 1.4 ng, and 14.7 ± 2.0 ng of Fab proteins (n = 3).

4. DISCUSSION

A maximum of 16 ng Fab (mean value for the 3 antibodies) was bound by 200 ng LDL protein. Knowing a Fab fragment to be 5 × 10^4 M_r and the LDL protein concentration, it can be calculated that 1 molecule of Fab reacts with 6 × 10^5 M_r of LDL protein. Since, estimates of 5.1 × 10^5 [17] and 6.3 × 10^5 [18] M_r of apo B protein per LDL particle have been reported, it follows that a

For example, in the reaction centers of chromatophores, charge separation between bacteriochlorophyll (BChl) and Fe–Q results in the former being charged positively and the latter negatively [12]. This process is a partial reaction of $\Delta\bar{\mu}H^+$ generation by the reaction center complex. It takes about 200 ps. The separated charges exist until $BChl^+$ is reduced by cytochrome *c*, and Fe–Q$^-$ is oxidized by a secondary quinone ($\psi_{1/2}$ of the overall process is 60–300 μs) [12]. According to our data on the direct measurement of $\Delta\psi$ formation in chromatophores, BChl–FeQ oxidoreduction is responsible for the major portion of photoelectrogenesis [14]. The hydrophobicity of the region where this oxidoreduction takes place [12] should prevent the fast dissipation of the local field. If there is an H^+-ATP-synthetase near the photosynthetic reaction center complex, a $\Delta\bar{\mu}H^+$-linked stage of ATP synthesis may be triggered by the local field existing between $BChl^+$ and Fe–Q^-.

Certainly, such a model requires a close proximity of H^+-ATP-synthetase and $\Delta\bar{\mu}H^+$-generator. Apparently, this is usually the case in natural membranes because of a very high concentration of H^+-ATP-synthetases in these membranes.

According to Kozlov [15–17], $\Delta\bar{\mu}H^+$ performs two functions in the ATP formation:

(i) Energetic, providing a positive overall balance of free energy for the ATP-synthetase reaction;
and
(ii) Kinetic, accelerating the slowest stage of this reaction.

The threshold of the ATP synthesis is due to the second function since below the threshold no formation of ATP, by, e.g., submitochondrial particles, takes place even in the presence of the hexokinase–glucose-6-phosphate dehydrogenase trap when the overall process including ATP synthesis is energy-releasing. The local field may first of all play an important role in the kinetics of H^+-ATP-synthetase. When a delocalized field is below the threshold, the sum of the local $\Delta\psi$ and delocalized $\Delta\bar{\mu}H^+$ may prove to exceed it, so that the rate of ATP formation will still be high.

4. PREDICTIONS

The direct way to test the above reasoning is to measure a local electric field across the membrane-linked H^+-ATP-synthetase molecule. This will be possible when one succeeds in the covalent binding of a field-sensitive probe to the portion of H^+-ATP-synthetase immersed into the membrane.

Another way is to probe into certain consequences of the concept. It predicts, e.g., that the molar ratio of $\Delta\bar{\mu}H^+$-generators and H^+-ATP-synthetases in the membrane must be critical for the threshold $\Delta\bar{\mu}H^+$-value of the phosphorylation process.

(A) Under conditions when $\Delta\bar{\mu}H^+$-generators are in great excess over H^+-ATP-synthetases, one can abolish the contribution of the local field by merely switching off most of the generators. In this case, the threshold $\Delta\bar{\mu}H^+$ estimated experimentally must be higher, since it is measured (i.e., delocalized) $\Delta\bar{\mu}H^+$ that is responsible for ATP formation.

(B) The local field which defies conventional methods should be involved when:

(i) The ratio $\Delta\bar{\mu}H^+$-generator/H^+-ATP-synthetase is low;
and
(ii) The concentration of the active forms of both enzymes is high.

Under these conditions, the measured $\Delta\bar{\mu}H^+$ threshold must shift to lower values.

For the same reason in case (B), but not (A), the threshold for artificially-imposed $\Delta\psi$ must be higher than that for enzymatically-generated $\Delta\psi$.

Apparently, case (B) is more typical of natural coupling membranes, whereas case (A) is an exclusion inherent in systems specializing in $\Delta\bar{\mu}H^+$-linked functions alternative to ATP synthesis, such as brown fat mitochondria. In proteoliposomes, both cases can be observed if one varies the concentrations of $\Delta\bar{\mu}H^+$-generator and H^+-ATP-synthetase in the reconstitution mixture.

In conclusion, the concept is being considered which assumes that the local electric field arising due to charge separation in the molecule of a $\Delta\bar{\mu}H^+$-generating enzyme affects the adjacent molecule of H^+-ATP-synthetase. This is postulated to be critical when the delocalized $\Delta\bar{\mu}H^+$ is somewhat below the phosphorylating threshold, so that the sum of local $\Delta\psi$ and delocalized $\Delta\bar{\mu}H^+$

proves to be above the threshold. This assumption takes care of the experimental data which are otherwise rationalized in terms of 'localized energy coupling', with no provision of mechanisms specialized in lateral H^+ transfer.

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