

## A HYPOTHESIS ON MEMBRANOUS PROTEINS SPECIALIZED IN LATERAL TRANSPORT

Alexander I. ARCHAKOV, Alexander V. KARYAKIN and Vladimir P. SKULACHEV

*Central Research Laboratory of the N. I. Pirogov Moscow Second Medical Institute, Moscow II9435, and Department of Bioenergetics of the Laboratory of Bioorganic Chemistry of M. V. Lomonosov Moscow State University, Moscow II7234, USSR*

Received 3 October 1975

The majority of membranous proteins represents enzymes catalyzing the metabolic steps that are localized inside membranes or on their surface. Besides, in membranes there are found proteins functioning as transmembrane carriers of different components, such as ions, metabolites, electrons, protons, hydrogen atoms etc.

Apart from these two types of proteins some membranous enzymes catalyzing reactions that look like 'dead ends' on the metabolic map have been described. The proteins in question seem to be involved neither in metabolic chains (cycles) nor in transmembrane transport processes. Such 'a way to nowhere' may be exemplified by the NADH-cytochrome  $b_5$  oxidoreductase ( $fp_5$ )—cytochrome  $b_5$  system. This redox chain, found in a number of biological membranes [1,2], comes to an end, failing to reach oxygen.

It was shown that cytochrome  $b_5$  in aerobic liver cells is completely reduced [3,4]. Despite the absence of an active oxidant, the reducing capacity of  $fp_5$ —cytochrome  $b_5$  system is very high. Among the redox enzymes,  $fp_5$  belongs to those that have the highest values of the turnover number [1]. In some tissues (e.g. liver) there is more cytochrome  $b_5$  than any other inner mitochondrial membrane cytochrome.

Recently it was found by our group [5–7] that the  $fp_5$ —cytochrome  $b_5$  system can carry out electron transport between microsomal vesicles. We mixed two portions of microsomes, one of which was treated with mersalyl to inhibit  $fp_5$ . Addition of NADH to such a mixture was shown to result in cytochrome  $b_5$  being reduced not only in the intact but also in the mersalyl-treated portion of the preparation. In the same way, intermembranous electron transport between

microsomes and outer mitochondrial membrane has been demonstrated.

The above observations lead to the conclusion that  $fp_5$  and/or cytochrome  $b_5$  can diffuse along the microsomal or outer mitochondrial membrane. Such lateral diffusion can result in intermembrane electron transfer when two membranous vesicles come into contact in an occasional collision.

An independent piece of evidence for lateral diffusion of  $fp_5$  and cytochrome  $b_5$  was furnished by Strittmatter et al. [8–10]. According to these authors, microsomal membrane can bind large amounts of added cytochrome  $b_5$ , so that cytochrome  $b_5$ :  $fp_5$  ratio becomes as high as 100. The rate of cytochrome  $b_5$  reduction by  $fp_5$  per molecule of cytochrome  $b_5$  was found to be higher in the cytochrome  $b_5$ -loaded microsomal membranes than in those containing a normal amount of this cytochrome. Similar relationships were shown in experiments with microsomes binding an excess of  $fp_5$ .

Molecules of  $fp_5$  and cytochrome  $b_5$  seem to function as carriers specially adapted for lateral diffusion along the lipid/water interphase. They are composed of two unequal parts: the larger one (three fourths of the enzyme molecule), hydrophilic, containing a flavin or a heme group responsible for the catalytic function, and the smaller one, hydrophobic, requiring the protein to be anchored to membrane [10,11]. Being organized like a float,  $fp_5$  and cytochrome  $b_5$  should move along the membrane, meeting with relative ease.

The ability to diffuse quickly along the membrane is not a common feature of membranous proteins. For example, mitochondrial cytochrome oxidase, according

to Junge [12], does not move by means of lateral diffusion. In our experiments [7], fragments of inner membrane of beef heart mitochondria containing the complete set of the phosphorylating respiratory chain enzymes proved unable to catalyze intermembrane electron transfer, this fact indicating that this chain includes no electron carriers arranged like  $fp_5$  or cytochrome  $b_5$ .

Taking into account all these facts we would like to suggest that the unknown function of  $fp_5$  and cytochrome  $b_5$  might consist in transport of reducing equivalents along the membrane.

According to Adam and Delbrück [13], a mechanism involving lateral diffusion along membranes permits a much faster transfer of a component from cytoplasm to a small target on the cell membrane than free diffusion in the three-dimensional cytosol. Sumper and Träuble [14] calculated that there is quite a wide range of parameters (the sizes and density of targets, cell radius) for which involvement of lateral diffusion proves favourable for carrying out intracellular transport processes.

Keith and Snipes [15] reported recently that a spin-labelled synthetic compound of low molecular weight, which has similar solubilities in water and lipids, moves preferably in the lipid phase of various types of the cells. The authors concluded that the viscosity of cytosol is higher than that of membranes.

Lateral diffusion in its simplest form should occur when a transported component is of high affinity to the membrane. In this case no special carrier can be involved. Transport of steroids [16,17] and fatty acyl CoA [14] along the membranes seem to be examples of this kind.

Energy transfer along the membrane, requiring no carriers, can take place in mitochondria, chloroplasts and bacteria that generate transmembrane electric potential differences [3]. Such a transfer should occur due to high electric resistance of the membranes of the above organelles and bacteria and high conductance of cytosol.

In halophilic bacteria, migration of electric potential along the membrane seems to be the obligate stage of the photophosphorylation process. In these microorganisms the light energy is transduced into electric potential by bacteriorhodopsin which is localized in special membrane areas ('bacteriorhodopsin sheets') containing no proteins other than bacterio-

rhodopsin and, hence, lacking ATP-synthetase [18,19]. The latter is localized, apparently, in other areas of the same bacterial membrane.

Migration of electric potential along membranes of giant mitochondria described recently in liver [20] and yeast [21] may be a way of accomplishing a very fast energy exchange between different parts of the cell.

In chlorophyll-containing photosynthetic membranes, it is well established that excitation energy migrates along the membrane between chlorophylls of the light-collecting antennae to reach the chlorophyll molecule of the reaction center (for review see [22]). The chlorophyll antenna may exemplify a structure specialized in lateral transport. Another type of mechanisms of lateral transport may include Brownian movement of proteins functioning as carriers of a lipid-insoluble component in the membrane plane. The role of lateral carriers might be performed by protein floats, such as  $fp_5$  and cytochrome  $b_5$ .

$Fp_5$ , a hydrogen atom carrier of  $-0.3$  V redox potential, may serve as a mechanism to equilibrate different, spacially separated cytosol pools of NADH ( $NAD^+$ ). According to calculations carried out by Dr V. J. Chernyak, lateral diffusion of  $fp_5$  and  $fp_5H_2$  along intracellular membranes between two intracellular regions differing, e.g. in concentration of NAD-linked substrates, may be faster than diffusion of these substrated or NADH via cytosol.

Assuming that  $fp_5$  functions as a lateral hydrogen atom carrier shuttling between different  $NAD^+$  pools we would like to use the same approach to cytochrome  $b_5$ . This cytochrome might operate as a lateral electron carrier of redox potential of about 0.

It seems to be of importance that the system  $fp_5$ -cytochrome  $b_5$  can carry reducing equivalents not only along a membrane but also between two membranes as was mentioned above. Combination of these two types of processes can allow the limitation of lateral transport of reducing equivalents by a single cistern of endoplasmic reticulum or a single mitochondrion to be overcome. Interplay of lateral and intermembrane transport may result in the overall intracellular system of extramitochondrial redox reactions being formed.

It seems reasonable to consider the possibility that such a system, united by means of intracellular membranes and lateral carriers, may be used for transport

of components other than reducing equivalents (for example  $\sim\text{P}$ ;  $\sim\text{COCH}_3$  etc.).

Considering the possible mechanisms of high-energy acetyl transport, we believe that attention should be paid to acetyl carnitine whose formation is an example of a 'metabolic dead end'. It is known that esters of carnitine and fatty acids represent transport forms of fatty acyls moving across the inner mitochondrial membrane (for review, see [23]). Palmitoyl carnitine, as was shown by our group [24], is a penetrating cation for artificial and mitochondrial membranes. The same experiments showed that both types of membrane are impermeable to acetyl carnitine. This very fact is in agreement with the finding that citrate, rather than acetyl carnitine, is a transport form of acetyl, shuffling between cytosol and mitochondrial matrix [25]. It is not excluded, however, that the role of acetyl carnitine consists in acetyl transport along the membranes. If this is the case, an acetyl carnitine-binding 'protein float' should exist. Maybe, this function is performed by acetyl carnitine transferase, the enzyme catalyzing reversible formation of acetyl carnitine from carnitine and acetyl CoA. It should be noted that microsomes contain large amounts of this enzyme.

The specific versions of lateral diffusion mechanisms presented above are certainly speculative. They were discussed with the purpose only to emphasize the possibility that intracellular membranes not only divide the cell into isolated compartments but also unite the cellular metabolism by means of transport systems operating along the membranes.

In conclusion, a hypothesis is advanced postulating a new type of membranous carriers specialized in transport of a certain component along the membrane by means of lateral diffusion. NADH-cytochrome  $b_5$  reductase and cytochrome  $b_5$  were suggested to function as lateral carriers of reducing equivalents of two different redox potentials.

### Acknowledgements

The authors thank Academician Yu. A. Ovchinnikov, Drs V. J. Chernyak, L. A. Drachev, A. M. Shkrob and L. S. Yaguzhinsky for useful discussion and advice, and Miss T. I. Kheifets for correcting the English version of the paper.

### References

- [1] Strittmatter, P. (1968) in: *Biological Oxidations* (Singer, T. ed.) pp. 171–191, Interscience Publ. New York–London.
- [2] Archakov, A. I. (1971) *Usp. Sovrem. Biol.*, 12, 136–163.
- [3] Skulachev, V. P. (1969) *Energy Accumulation in the Cell*, Nauka Press, Moscow.
- [4] Moldeus, P. W., Cho Y.-N., Cinti, D. L. and Schenkman, J. B. (1973) *J. Biol. Chem.* 248, 8574–8584.
- [5] Archakov, A. I., Karyakin, A. V. and Skulachev, V. P. (1973) *Dokl. Akad. Nauk SSR* 209, 221–223.
- [6] Archakov, A. I., Karyakin, A. V. and Skulachev, V. P. (1974) *FEBS Lett.* 39, 239–242.
- [7] Archakov, A. V., Karyakin, A. V. and Skulachev, V. P. (1974) *Dokl. Acad. Nauk SSSR* 217, 1423–1427.
- [8] Strittmatter, P., Rogers, M. J. and Spatz, L. (1972) *J. Biol. Chem.* 247, 7188–7194.
- [9] Rogers, M. J. and Strittmatter, P. (1974) *J. Biol. Chem.* 249, 895–900.
- [10] Rogers, M. J. and Strittmatter, P. (1974) *J. Biol. Chem.* 249, 5565–5569.
- [11] Spatz, L. and Strittmatter, P. (1971) *Proc. Natl. Acad. Sci. USA*, 68, 1042–1046.
- [12] Junge, W. (1972) *FEBS Lett.* 25, 109–112.
- [13] Adam, D. and Deibrück, M. (1968) in: *Structural chemistry and molecular biology*, (A. Rick and N. Davidson eds.) p. 198–215, W. H. Freeman, San Francisco, Calif.
- [14] Sumper, M. and Träuble, H. (1973) *FEBS Lett.* 30, 29–34.
- [15] Keith, A. D. and Snipes, W. (1974) *Science* 185, 666–668.
- [16] Sackmann, E. and Träuble, H. (1972) *J. Am. Chem. Soc.* 94, 4482–4491.
- [17] Sackmann, E. and Träuble, H. (1972) *J. Am. Chem. Soc.* 94, 4492–4498.
- [18] Oesterhelt, D. and Stoerkenius, W. (1973) *Proc. Natl. Acad. Sci. USA* 70, 2853–2857.
- [19] Skulachev, V. P. (1974) *Usp. Sovrem. Biol.* 77, 125–154.
- [20] Brandt, J. T., Martir, A. P., Lucas, F. V. and Vorbeck, M. L. (1974) *Biochem. Biophys. Res. Commun.* 59, 1097–1103.
- [21] Hoffmann, H. P. and Avers, C. I. (1973) *Science* 181, 749.
- [22] Borishov, A. Yu. and Godik, V. J. (1973) *Biochim. Biophys. Acta* 301, 227–248.
- [23] Skulachev, V. P. (1972) *Energy Transformation in Biomembranes*, Nauka Press, Moscow.
- [24] Levitsky, D. O. and Skulachev, V. P. (1972) *Biochim. Biophys. Acta* 275, 33–50.
- [25] Bressler, R. and Brendel, K. (1966) *J. Biol. Chem.* 241, 4092.