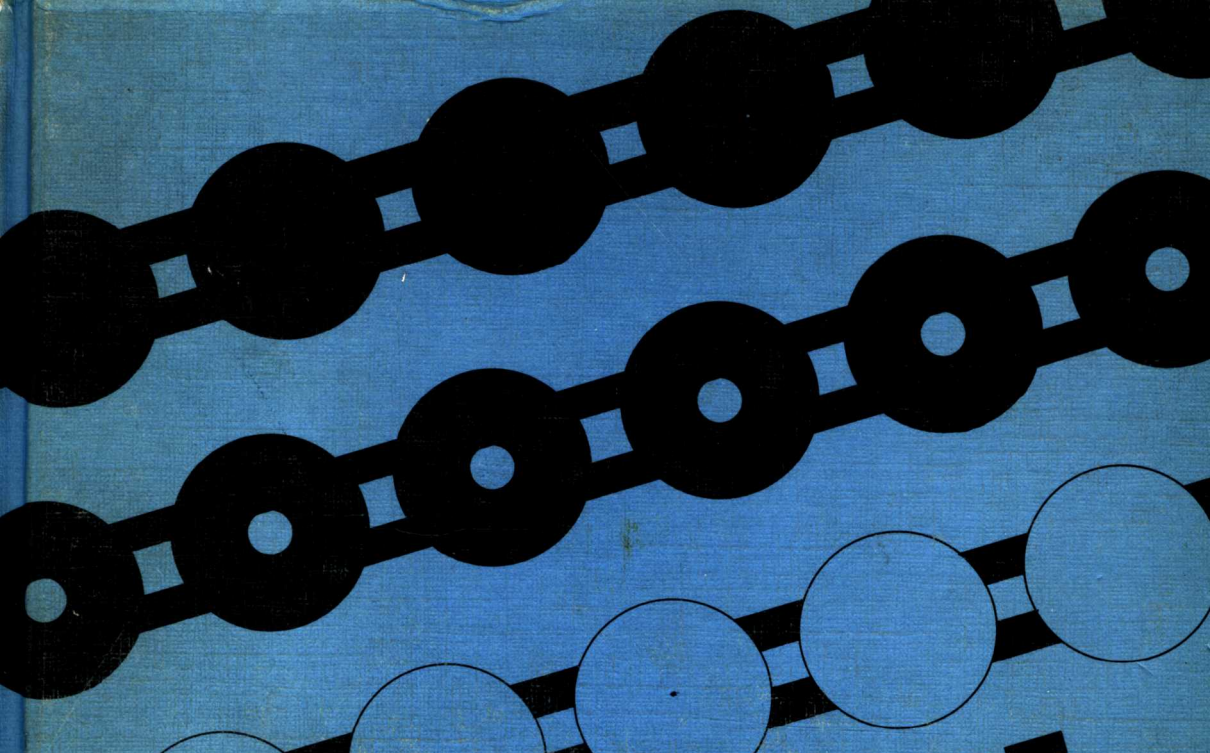

BIOENERGETICS OF MEMBRANES

Lester Packer
George C. Papageorgiou
Achim Trebst

Developments in Bioenergetics and Biomembranes Volume 1

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PREFACE

This volume contains contributions presented in the International Symposium on Membrane Bioenergetics, which was held on the Island of Spetsai, Greece, July 10-15, 1977.

This symposium was sponsored and supported by the Greek Ministry of Culture and Sciences, International Union of Biochemistry, International Union of Pure and Applied Biophysics, Federation of European Biochemical Societies, Nuclear Research Center at Demokritos and the National Hellenic Research Foundation. On this occasion it is worth adding a few words concerning the location of the meeting and the Spetsai tradition on international conferences. The Anargyrios and Korgialenios School of Spetsai (a boarding high school of the Island) where the symposium was convened, has established its international reputation in the field with a series of successful summer schools on molecular biology, molecular and cell biology and molecular and developmental biology which commenced in 1966. The symposium on membrane bioenergetics represent a departure from the Spetsai tradition and an enlargement of the scientific activities on the Island. Once again participants expressed their satisfaction for the arrangements of the scientific programme of the meeting. Senior scientists in the field have the opinion that they have participated in one of the most successful meetings in bioenergetics. Aside from the scientific merits of the place, Spetsai is the point where three Continents come close together (Africa, Asia and Europe) and indeed the conference was attended by persons from 16 countries. Since scientists and research workers are traditionally ranking among most suitable persons to act as spearheads for increasing understanding and international cooperation, it seems that in this global age of science, Spetsai scientific conferences may serve a dual purpose.

Published records of scientific symposia lose much of their value if publication is unduly delayed. In the present instance, we have chosen to proceed with publication at the expense of omitting the interesting discussion which occurred during the meeting and some of the articles which could not be ready by the time the volume went to press. This symposium volume should be of great value to scholars and educators in the fields of :

Structure and biogenesis of the three membrane systems: mitochondria, chloroplasts and sarcoplasmic reticulum.

Mechanisms of biological oxidation; iron sulphur proteins, interaction of iron sulphur proteins with quinones, photosynthetic electron transport.

Concepts of bioenergetics, ionophores, ionophoric proteins and transport mechanisms. Mechanism of action of bacteriorhodopsin (and rhodopsin) and ATP-

synthetase-ATPase complex and their role in energy transduction or as ion pumps.

Some of the most recent advances in these fields are presented, with emphasis on membrane organization with respect to bioenergetic functions. These advances include the role of biological oxidations, electrical forces and ion gradients in mechanisms of energy coupling. In these areas there has been exceedingly rapid progress some of which is recorded here. The past few years almost as many redox components of the respiratory chain, as were known to exist before, have been identified by low-temperature EPR as iron sulphur proteins. These are now being characterized in terms of how they interact in flavoprotein dehydrogenases and with ubiquinone and the rest of the respiratory chain. Advanced technologies being used to exploit the understanding of membranes and bioenergetics as high resolution ^{31}P-NMR, ESR-spectroscopy, nano- and pico-second spectroscopy and the use of selective chemical probes for labelling, are well represented in this volume.

Most importantly, we thank each of the authors and other participants whose contributions made the Spetsai Symposium on Membrane Bioenergetics an intellectually stimulating and profitable occasion, finally, we thank one of the authors, Dr. J. Isaakidou for her invaluable endeavours for the success of the symposium.

A. E. Evangelopoulos
J. G. Georgatsos
L. Packer
G. C. Papageorgiou
A. Trebst

CONTENTS

MEMBRANE STRUCTURE AND BIOGENESIS

Bioenergetics of Membranes. L. Packer et al. ed.

THE MOLECULAR ORGANISATION OF CELL MEMBRANES

D.Chapman,
Chemistry Department,
Chelsea College,
University of London,
England.

INTRODUCTION

Considerable scientific work over the last ten years has increased our knowledge of the molecular organisation of cell membrane structure. The original idea suggested some 50 years ago that cell membranes are built upon a matrix of a bilayer of lipid has been preserved. This idea has now been strengthened and enlarged to include knowledge of the mobility and asymmetry of the lipid components. Considerable information has also been obtained concerning the protein arrangements associated with the lipid bilayer. In some cases details of the interior structure of the proteins themselves is now available.

It has been clear for some time that to understand the many functions associated with cell membranes that it would be necessary for us to understand their detailed structure. We now seem to be close to this important goal thereby linking membrane structure with function. Furthermore these studies are also revealing the possibilities and the potential for simulating these functions *e.g. the simulation of energy* transducer processes for technological exploitation.

In this paper we attempt to briefly summarise the situation regarding membrane structure so as to form an introduction and basis for the subsequent discussions on Membrane Bioenergetics at this Conference.

CELL MEMBRANES AND FUNCTION

Biochemical and electron microscope studies by many scientists have revealed the existence of many cell membranes including not only the outer plasma cell membrane but also membranes associated with the various organelles within the cell. Associated with the membranes are various functions including the control of permeability to organic molecules, water and ions. Certain membranes are also associated with the organisation of enzymes, visual pigments, chlorophyll and other energy transducing molecules.

THE LIPID BILAYER MATRIX

We know that cell membranes consist of various lipid and protein components of varying ratios dependent upon the particular membrane chosen. Other molecules such as cholesterol or chlorophyll occur in some membranes e.g. the former in appreciable quantities in the plasma membranes and chlorophyll in chloroplast membranes. Carbohydrate groups also are found associated either with the lipid or protein structures.

The lipid component often consists of a variety of lipid classes e.g. lecithins, phosphatidyl-ethanolamines and associated with each of these lipid classes is a range of fatty acids of varying chain length and unsaturation[1]. Some lipids such as the lecithins are known to form spontaneously in water (above the lipid transition temperature) many bilayers each layer separated by water[2]. Various experiments e.g. spectroscopic and calorimetric studies have shown that the lipid bilayer is an important structural feature of many cell membrane structures.

(a) Molecular mobility and Bilayer fluidity

A range of physical techniques, calorimetry x-ray techniques and spectroscopic methods have given valuable information about the molecular mobility of the lipids within model lipid bilayer structures.[3] This information has been useful in providing insight into the molecular processes involved within the lipid bilayer matrix of natural cell membranes and to develop the concept of membrane fluidity[4].

Studies of these model systems have shown that the lipids exhibit a phase transition at a characteristic temperature[5]. Below this temperature the lipid chains are in an ordered or crystalline arrangement. Above this temperature the chains are "melted" and in a fluid condition. In this state rotational isomerism occurs about the C-C bonds of the chain. Spectroscopic studies including nmr, esr, ir and Raman spectroscopy have given information about the order parameter along the length of the chain. These studies are consistent in showing that greater disorder occurs at the methyl end of the chain[6].

As well as this type of mobility within the chain it has also been shown that the lipid molecules (above the transition temperature) can readily diffuse within the plane of the bilayer[7]. On the other hand the movement of lipid molecules from one side of the bilayer to the other (sometimes termed flip flop) is a slow process[8].

(b) Asymmetric arrangement

A variety of experiments including chemical and enzyme treatments have now confirmed a suggestion made by Bretscher[9] that in the erythrocyte membrane

that the lipid classes are asymmetrically arranged. The lecithin and sphingomyelins are arranged on the outside half of the membrane and the phosphatidyl ethanolamine and phosphatidyl serine molecules are arranged on the inner half of the membrane, Experiments with some other membranes show that lipid asymmetry occurs in other membrane systems. The biological significance of lipid asymmetry is at present still uncertain,

(c) Cholesterol organisation

Many experiments using a variety of techniques have shown that the presence of cholesterol within the bilayer matrix is to modulate the lipid fluidity[10]. When the lipid is above its transition temperature in the fluid condition the presence of the cholesterol is to inhibit the rotational isomerism of the methylene groups in the chain, causing the chain to become more rigid. The presence of cholesterol also prevents the lipid chains from crystallising. At high concentrations the cholesterol removes the lipid phase transition[11]. Essentially the presence of cholesterol is to cause the lipid to adopt an "intermediate fluid" structure[12].

The organisation of the cholesterol molecules within the plane of the bilayer has been discussed. Various types of complex have been postulated including 1:1 and 2:1 lipid to cholesterol complexes[13,14]. An alternative arrangement of <u>random</u> structure has also recently been discussed[15].

PROTEIN ARRANGEMENTS

For some time it had been considered that the proteins of cell membranes were arranged on the outside of the lipid bilayer - the Danielli-Davson model. In recent years it was realised that intrinsic protein may also occur within the lipid bilayer matrix[16]. A variety of arrangements of the proteins can occur even with a single membrane structure. A simple generalisation concerning the protein organisation is not possible.

Some of the proteins (intrinsic) have been shown to span the bilayer using chemical and electron microscope studies[17]. The latter using the techniques of freeze-fracture exhibit these spanning proteins in the form of particles seen on the fracture faces.[18] In many membranes these particles appear to be randomly distributed within the plane of the bilayer. A detailed structure of a membrane protein which spans the lipid bilayer has been revealed by the technique of electron diffraction with the proteins of the purple membrane of Halobacterium halobium[19]. This shows that the protein consists of seven helical

polypeptide chains folded back and forth across the membrane. These helical segments run approximately paralled to the plane of the membrane.

(a) Protein mobility

Evidence that proteins can rotate about an axis perpendicular to the plane of the membrane was first obtained from studies of the visual pigment, rhodopsin. The highly regular parallel alignment of the disc membranes in retinal rod outer segments has enabled the performance of detailed studies of the arrangement of the major protein in these membranes[20]. Spectroscopic studies showed that the retinal rod is strongly dichroic in that light polarised perpendicular to the long axis of the rod is strongly absorbed compared with light polarised parallel to the rod. This indicates that the chromophore of rhodopsin, retinal, is preferentially oriented parallel to the plane of the disc membrane. Initial studies of partial bleaching with short flashes of polarised light lacked sufficient time resolution to demonstrate dichroism in the plane of the membrane[21]. The rotational relaxation time of rhodopsin was measured by Cone[22] using a flash photolysis apparatus capable of resolving events in the microsecond time range. He used lumi-rhodopsin as a tracer to measure the (polarised) flash induced linear dichroism. With the light polarised parallel and perpendicular in output to the laser pulse he determined the dichroic ratio as a function of time. The rotational relaxation time of the rhodopsin molecule was calculated and was of the order of 20 μsecs at room temperature.

From this result and making some assumption about the size of the protein he was able to calculate the viscosity of the surrounding lipid matrix (about 2 poise). This value is in good agreement with the value determined from translational diffusion measurements for the same system[23].

Similar studies have been reported for bacteriorhodopsin located in the purple membrane of Halobacterium halobium in which a transient spectroscopic chromophore centred at 410 nm was used to investigate rotational diffusion[24]. The absorbane has a lifetime of about 10 ms and was found to be strongly dichroic following flash illumination with plane polarised light but in contrast to rhodopsin this did not decay rapidly. The rotational relaxation time was found to be at least 10^3 times slower than rhodopsin consistent with the crystal-like hexagonal packing of bacteriorhodopsin in purple membranes inferred from X-ray analysis and unlike the liquid-like array of rhodopsin in the thylakoid membrane. Examination of the photolytic dissociation of cytochrome a_3-CO complex in the inner mitochondrial membrane[25] and photoinduced dichroism of chlorophyll a_1 in chloroplast membranes[26] has also suggested that these proteins do not rotate rapidly about an axis perpendicular to the membrane.

Fluorescence techniques have also been applied to study protein rotation in membranes. The fluorescence polarisation of analino naphthalene sulphonate and dansyl chloride bound to electroplax membrane fragments, for example, have been resolved into two components; a rapid but partial decay, believed to be rotation of the probe at its binding site and the residual decay due to rotation of the protein which set a lower limit for rotational relaxation time[27] of 0.7 us. Tryptophan fluorescence has been explored as an intrinsic probe to measure rotational kinetics of other membrane proteins and external probes such as eosin have been used successfully in model systems and erythrocyte membranes to study the rotation of proteins which have no natural chromophores[24,28]. In general, these studies indicate that rotational relaxation times for different membrane proteins vary considerably and probably reflect differences in the molecular environment surrounding each particular protein.

(b) Protein aggregation

Changes in the distribution of protein in the plane of the membrane was first noted with freeze-cleaved erythrocyte membrane preparations that had been quenched from acidic media. Membrane-associated particles are distributed randomly on complementary inner fracture of these membranes at pH 7.5 or pH 9.5 but they aggregate when the pH is reduced to 5.5 or less. Adjusting the pH again to 7.4 restores the original particle distribution so that the process is freely reversible[29].

Freeze-fracture techniques have been used to examine membrane-associated particle distribution of chloroplast membranes[30]. Particle distribution in these membranes appeared to be random in dark adapted chloroplasts but they became aggregated following illumination. In other experiments, temperature dependent membrane-associated particle aggregation has been observed with the inner mitochondrial membrane[31]. Protein induced aggregation has also been reported in mitochondrial membranes and lipid reconstituted Ca^{2+} -activated ATPase of sarcoplasmic reticulum.[33] Proteins of some bacterial membranes also remain dispersed even when the membranes are cooled to below the lipid phase transition temperature[34]. Erythrocyte glycophorin also appears to be dispersed both above and below the phase transition temperature[35]. Some interesting studies of reconstituted membranes containing rhodopsin have shown that dark adapted rhodopsin is aggregated below the lipid transition temperature but is dispersed randomly at higher temperatures. When the chromophore is bleached, however, the rhodopsin molecules remain dispersed at all temperatures[36].

The relationship between membrane-associated particle aggregation and lipid crystallisation has been demonstrated convincingly in the micro-organism[37] Mycoplasma mycoides var. capri. This shows that in freeze-fracture replicas of a strain of the organism devoid of cholesterol two regions can be observed, one with a high density of particles, the other smooth and containing no particles. Similar replicas of the parent strain which contains cholesterol show that the proteins remain dispersed more or less randomly throughout the plane of the membrane. A simulation of the protein aggregation processes has been discussed[38].

Protein aggregation within the fluid lipid bilayer triggered by mechanisms other than lipid crystallisation has been related to various cell processes including pinocytosis, the stage of the cell growth cycle and to membrane interactions and fusion.

PROTEIN - LIPID INTERACTIONS

The fact that some proteins are embedded within the lipid bilayer has led to discussions of the perturbation which this produces on the neighbouring lipid[39,40]. Various terms have been used to describe this e.g. boundary layer lipid, halo lipid and annulus lipid. This has sometimes been associated with "residual lipid" i.e. the lipid which still appears to be tightly bound to the protein after extensive solvent extraction or the amount of lipid which is required to restore enzyme activity. The present situation is confused although it seems clear that proteins can affect the lipid chain gauche isomers. More experiments are required to clarify whether proteins in general have a tightly bound lipid annulus which they carry around with them within the fluid bilayer structure.

CATALYTIC HYDROGENATION AND MEMBRANE FLUIDITY

A new technique that we have been developing in our laboratory[41] may lead to the selective control of membrane fluidity. This is the technique of catalytic hydrogenation. Our recent experiments have shown that certain homogenous catalysts can be incorporated into phospholipid membrane systems and that indeed catalytic hydrogenation is observed. Thus the effect of hydrogenation of biological membranes on particular membrane functions will enable detailed and specific correlations to be established with saturation of particular double bonds of membrane phospholipids.

Genetic, nutritional and other methods of manipulating the fluidity of biological membranes will continue to make an important contribution to our understanding of how these membranes perform their various functions. Catalytic

hydrogenation may play an equally important role in many future studies. Various catalysts will be investigated leading to selectivity of hydrogenation and fine modulation of membrane fluidity. Studies of enzymic and transport processes, excitable membranes, retinal, chloroplast and mitochondrial function, may become possible as a function of this hydrogenation process.

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REFERENCES

1. van Deenen, L.L.M. (1965) in Progress in the Chemistry of Fats and other Lipids ed Holman, R.T. (Pergamon Press, Oxford) Vol. VIII Part 1 pp 1-127.
2. Chapman, D., Williams, R.M. and Ladbrooke, B.D. (1967) Chem. Phys. Lipids 1, 445 - 475.
3. Oldfield, E., and Chapman, D. (1972) FEBS Lett. 21, 303 - 306.
4. Chapman, D. Byrne, P. and Shipley, G.G. (1966) Proc.Roy.Soc.London Ser.A. 290, 115 - 142.
5. Chapman, D. (1975) Q.Rev.Biophys. 8, 185 - 235.
6. Seelig, A. and Seelig, J. (1974) Biochemistry 14, 2283.
7. Kornberg, R.D. and McConnell, H.M. (1971) Proc.Nat.Acad.Sci. USA, 68, 2564.
8. Rothman, J.E. and Lenard J. (1977) Science 195, 743 - 753.
9. Bretscher, M.S. (1972) Nature New Biol. 236, 11 - 12.
10. (a) Chapman, D. and Penkett, S.A. (1966) Nature Lond. 211, 1304 - 1305.
    (b) Gally, H.V. Seelig, A and Seelig, J. (1976) Hoppe-Seyler's Z.Physical Chem. 357 1447 - 1450.
11. Ladbrooke, B.D. Williams, R.M. and Chapman, D. (1968) Biochim. Biophys. Acta 150, 333 - 340.
12. Williams, R.M. and Chapman, D. (1970) in Progress in the Chemistry of Fats and other Lipids ed. Holman, R.T. (Pergamon Press Oxford)Vol 2, p 1 - 79.
13. Phillips, M.C. and Finer, E.G. (1974) Biochim.Biophys.Acta 356, 199 - 208.
14. Engleman, D.M. and Rothman, J.E. (1972) J.Biol Chem. 247, 3694 - 3697.
15. Cornell, B.A. Chapman, D. and Peel, W.E. (1977) Biochim.Biophys.Acta in press.