BBA 71056

Involvement of hydrophobic and hydrophilic groups of phospholipids in membrane formation

LENARD AND SINGER[1] reported that treatment with phospholipase C to remove 68–74 % of ionic groups from phospholipids of red blood cell did not affect the membrane structure, as evidenced by phase microscopy and circular dichroism measurement. We wish to report results which show the involvement of hydrophobic groups of phospholipids in membrane formation, and in addition, that the ionic group may play a role in the membrane vesicle formation.

Purified lipid-depleted cytochrome oxidase from beef heart mitochondria was prepared as described previously[2,3] by employing Triton detergents and KCl in Tris–HCl buffer. Purified phospholipids, *i.e.* phosphatidyl choline, phosphatidyl ethanolamine and cardiolipin, were prepared by thin-layer chromatography from total mitochondrial phospholipid extract. Micellar sols of total or purified phospholipids were made according to the sonication method of FLEISCHER AND FLEISCHER[4].

Mixed mitochondrial phospholipids or purified phospholipids are able to reconstitute membrane structure when combined with lipid-depleted cytochrome oxidase[5]. Phospholipid micelles were added to lipid-depleted cytochrome oxidase to 30 μg P per mg of protein, followed by sonication and centrifugation washes to remove any unbound phospholipids or free cytochrome oxidase. Fig. 1 shows a thin section of the phosphatidyl choline–cytochrome oxidase complex. It appears exclusively as a membrane vesicle similar to reconstituted mixed phospholipid cytochrome oxidase membrane[6]. Digestion of this membrane phosphatidyl choline by *Clostridium welchii* phospholipase C results in release of phosphoryl choline, the ionic group of the phospholipid, into solution. Fig. 2 shows the membrane structure after removal of 80 % of the phosphorus. In spite of the drastic composition change, the complex still retains

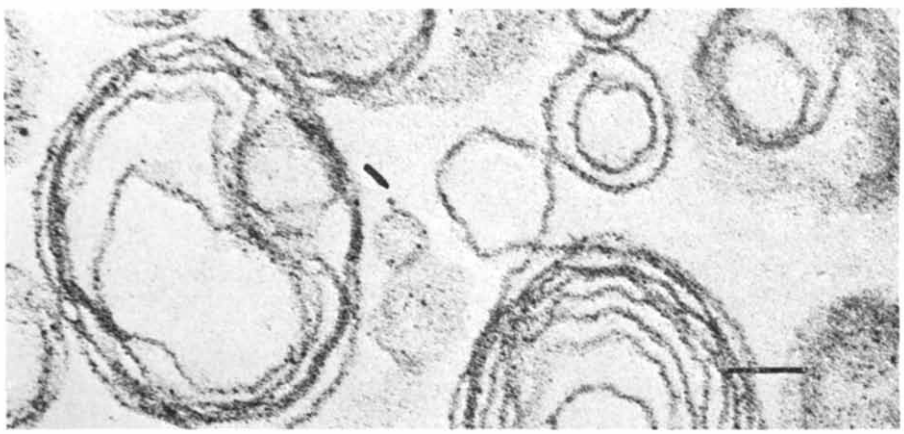

Fig. 1. Reconstituted phosphatidyl choline–cytochrome oxidase membrane. Specimen was prefixed in 4 % glutaldehyde solution buffered in 0.1 M phosphate, pH 7.4, post-fixed in 1 % OsO_4 buffered in veronal acetate, pH 7.4. Sample was dehydrated in an acetone series and embedded in Epon 812 based on a procedure by LUFT[8]. Sectioned specimen was post-stained with 2 % aqueous uranyl acetate and followed by lead citrate according to REYNOLDS[9], and examined in a Philips EM 300 microscope. × 104000. Marker 1000 Å.

its unit membrane structure. However, the membrane-bound vesicles are elongated, enlarged and show cross-fusion or "Y" shaped junctions. Fig. 3 shows that membranes can be formed between the same enzyme protein and diolein, which has the hydrophobic part of phosphatidyl choline but no ionic group. No membranous vesicles are seen but the membranes still show unit membrane structure.

Unsaturation of the hydrocarbon chain of phospholipids is essential for the membrane formation. Hydrogenated mixed phospholipids or phosphatidyl choline, prepared by exposure of lipid to H_2 in the presence of platinum oxide, do not form membrane structures with cytochrome oxidase at all, as seen under electron microscope. This is consistent with BENSON's[7] hypothesis that unsaturation of phospholipids plays a role in membrane structure.

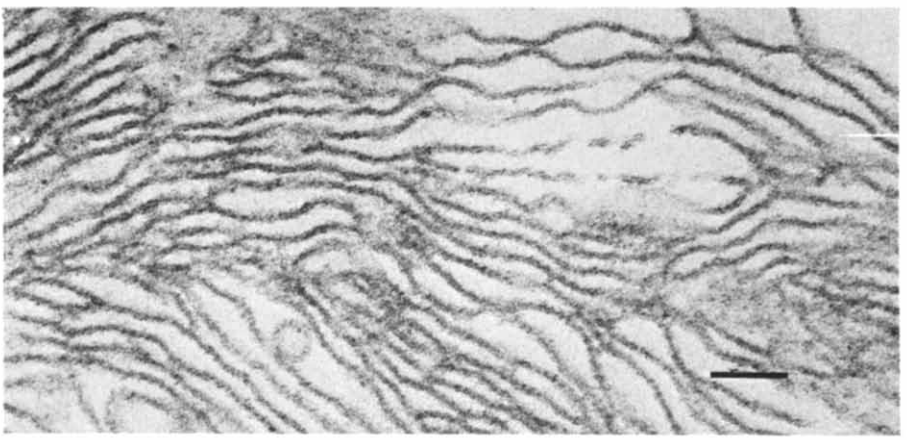

Fig. 2. Phospholipase C digested phosphatidyl choline–cytochrome oxidase membrane. Reconstituted membrane phospholipids were digested at 30° by 200 μg of *Cl. welchii* phospholipase C per mg of cytochrome oxidase membrane for 80 min in the presence of 2 mM Ca^{2+}. × 104000. Marker 1000 Å.

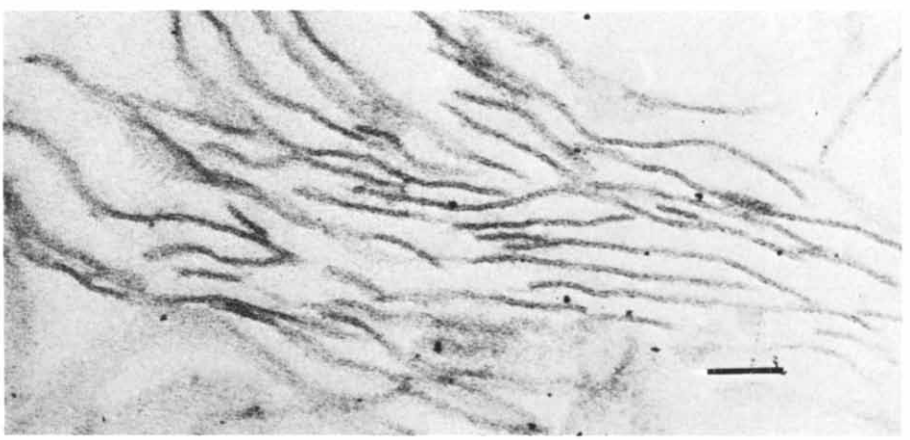

Fig. 3. Diolein–cytochrome oxidase membrane. Method of membrane formation is identical to reconstituted phospholipid–cytochrome oxidase membrane. × 104000. Marker 1000 Å.

Table I shows enzymatic activity of cytochrome oxidase after it is complexed with phosphatidyl choline, diolein or saturated phospholipids. 54 % of enzyme activity remains after hydrolysis of 80 % of the phosphatidyl choline using phospholipase C.

TABLE I

ENZYME ACTIVITY OF CYTOCHROME OXIDASE

Samples	*Activity μmoles O_2/min per mg*
Phosphatidyl choline–cytochrome oxidase	14.9
Phosphatidyl choline–cytochrome oxidase after phospholipase C digestion	8.1
Diolein–cytochrome oxidase	1.5
Hydrogenated phospholipid–cytochrome oxidase	4.0
Lipid-depleted cytochrome oxidase	2.8

Neither diolein nor saturated phospholipids can bring the enzyme to full activity, which may indicate that they cannot reach or interact with sites essential for activity.

This result indicates that (1) cleavage and release of the ionic heads of the phospholipids by the action of phospholipase C on reconstituted phosphatidyl choline–cytochrome oxidase membrane do not disrupt the unit membrane structure; (2) the presence of ionic groups in phospholipids encourages curvature of membranes and the formation of vesicular structure; (3) phospholipids are ineffective in membrane formation after saturation of the fatty acid chains; (4) hydrophobic interactions between phospholipid and membrane protein play an essential role in maintaining the integrity of the membranes; (5) to get the maximum stimulation of cytochrome oxidase activity with lecithin, both unsaturated fatty acids and ionic groups are necessary.

This work was supported by grant AM 04663 from the National Institute of Arthritis and Metabolic Diseases. F.L.C. is supported by a career grant K6-21839 from the National Institute for General Medical Research.

Department of Biological Sciences,
Purdue University,
Lafayette, Ind. (U.S.A.)

T. F. CHUANG
Y. C. AWASTHI
LINDA FUNK
F. L. CRANE

1 J. LENARD AND S. J. SINGER, *Science*, 159 (1968) 738.
2 T. F. CHUANG, F. F. SUN AND F. L. CRANE, *J. Bioenergetics*, 1 (1970) 227.
3 F. F. SUN, K. S. PREZBINDOWSKI, F. L. CRANE AND E. E. JACOBS, *Biochim. Biophys. Acta*, 153 (1968) 804.
4 S. FLEISCHER AND B. FLEISCHER, in R. W. ESTABROOK AND M. Z. PULLMAN, *Methods in Enzymology*, Vol. 10, Academic Press, New York, 1967, p. 407.
5 T. F. CHUANG, Y. C. AWASTHI AND F. L. CRANE, *Federation Proc.*, 29 (1970) 540 abs.
6 T. F. CHUANG, Y. C. AWASTHI AND F. L. CRANE, *Proc. Indiana Acad. Sci.*, 79 (1970) in the press.
7 A. A. BENSON, in L. BOLIS AND B. A. PETHICA, *Membrane Models and the Formation of Biological Membranes*, North Holland Publishing Co., Amsterdam, 1968, p. 190.
8 J. H. LUFT, *J. Biophys. Biochem. Cytol.*, 9 (1961) 409.
9 E. S. REYNOLDS, *J. Cell Biol.*, 17 (1963) 208.

Received May 22nd, 1970