Lipid Composition of Tissues from *Electrophorus electricus* (35017)

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Our understanding of the molecular basis of cellular excitability and bioelectrogenesis requires basic information concerning the composition and structure of cell membranes which show electrical excitability, ion transport mechanisms, receptor function, and bioelectrogenic capability. Our studies are directed in part towards the elucidation of the composition of the nerve cell membrane. We also have used the electric organ of *Electrophorus electricus* in order to investigate the nature of the protein and peptide fractions thought to participate in bioelectrogenesis (1–3). In a collaborative study with P. Mueller and D. O. Rudin, a number of purified electroplax proteins were tested on reconstituted membranes and were found to have weak EIM (excitability inducing molecule) activity. From the investigations reported by Mueller *et al.* (4–7) it appeared likely that some complementarity was required for the lipid–protein interaction. Consequently, we have directed our attention to the electroplax lipids as an obvious extension of these studies. This communication deals with initial investigations on the lipid composition of whole electric organ of *Electrophorus*, of a microsomal ATPase preparation from the electroplax, and a comparison with the lipid composition of muscle from the same animal. We have tested some of these lipid fractions for their capability to form lipid bilayer membranes.

Materials and Methods. Four- to 5-ft long eels (*Electrophorus electricus*) were killed by immersion into ice water; the tissues were excised immediately and either frozen in liquid nitrogen or homogenized.

Preparation of the microsomal fraction. The electric organ of the eel was homogenized in a blendor with 4 vol of 0.05 *M* Tris buffer, pH 7.5, and stirred in the cold for 90 min. The homogenate was centrifuged at 10,000g_{av} for 45 min. The supernatant was decanted and again centrifuged for 1 hr at 44,300g_{av} in a no. 21 rotor in a Model L Spinco centrifuge. The sedimented microsomal fraction was washed once with buffer. This microsomal fraction is representative of the ATPase preparation described by Albers *et al.* (8).

Extraction of the lipids: Electric organ, its microsomal fraction or the dorsal muscles of the animal were homogenized with an equal volume of 0.05 *M* Tris buffer, pH 7.5. Ten vol of chloroform–methanol (CM) (2:1) were added under nitrogen, and the mixture was allowed to stand with stirring for 24–48 hr at + 4°. After filtration, the insoluble residues were extracted with acetone and ether, dried under a vacuum, and weighed. The combined lipid extracts were washed twice with water and evaporated to dryness in a rotary flash evaporator and the residue was resuspended in CM 2:1. This procedure was repeated until the CM phase showed no insoluble residues. After drying for several days under vacuum, the amounts of lipid extracted were determined gravimetrically.

Chromatographic separation of lipids. 200 mg of lipids in petroleum either were placed on a column 20 × 100 mm, consisting of 10 g of activated silicic acid mixed with 5 g of hyflo supercel. The lipids were eluted according to a scheme reported by Hirsch and Ahrens (9) and Biran and Bartley (10). The lipid fractions were collected under nitrogen and evaporated to dryness. The total amount of lipid in each fraction was determined gravimetrically after thoroughly drying under vacuum.

The following reactions were employed in the analysis of the lipid fractions obtained by chromatographic procedure: (a) acyl esters

by the method of Rapport and Alonzo (11), (b) aldehydogenic lipids by the method of Gray and MacFarlane (12), (c) total phosphorus according to Fiske and Subbarow (13), (d) total cholesterol after saponification by the method of Zlatkis *et al.* (14), (e) glycolipids by the anthrone reaction (15), and (f) sphingosine according to Lauter and Trams (16).

The testing of the lipid fractions for their ability to form bimolecular leaflets was carried out according to Mueller *et al.* (6, 7). The lipids were dissolved in *n*-tetradecane, containing 0.1% α-tocopherol, to a final concentration of 10 mg/ml. NaCl–histidine buffer at pH 7.3 was used in the aqueous compartments. The time to formation of secondary black (thickness of 60–80 Å) was observed optically and checked by applying square pulse potentials of from 100 to 300 mV across the membrane. Resistance of the membrane at that time was of the order of 10^6 to 10^8 ohms cm^2.

Results. Average values for the protein dry weight in percentage of wet weight were for: Electric organ, 4.36; muscle, 15.6; and for microsomes, 6.6. The yield of microsomal fraction obtained by sedimentation at $40.3 \times 10^3 g$ was about 13% of the wet weight of the electric organ. In Table I the lipid composition of whole electric organ is compared to that of its microsomal fraction and to muscle. Recovery of the lipids from the column after silicic acid chromatography generally was satisfactory. It was found, however, that only about 65% of the cholesterol measured in the total lipid extract was recovered in Fractions 1–3. The lipid composition of whole electric organ was essentially identical to that of its microsomes, but was remarkably different from that of muscle. This difference is expressed principally in the contents of cholesterol and triglyceride which together amounted to about 70% of the total muscle lipids. It is assumed that a considerable portion of this difference is due to storage of neutral lipids in fish muscle. The significance of the differences in the steroid and polyglycerophosphatide fractions between whole electroplax and electroplax microsomes is uncertain.

The cholesterol is reported as total cholesterol in Table I, but the percentage of esterified cholesterol was small. The glycolipid content, as assayed by the anthrone reaction for bound hexoses was minute. In some fractions, the total hexose content was also analyzed on samples which had been hydrolyzed with hydrochloric acid. Determination of the carbohydrates as reducing materials yielded essentially the same results.

The phospholipids of Fraction 5 were fur-

TABLE I. Lipid Composition of *E. electricus* Tissues (mg of lipids/100 g of wet wt).

Fraction and composition	Muscle		Whole electric organ		Electroplax[a] Microsomal fraction	
		(% of total)		(% of total)		(% of total)
Total lipids	2932.0		555.0		31.8	
1. Steroid esters	26.4	0.94	7.6	1.37	0.8	3.0
2. Cholesterol and triglycerides	1935.0	68.22	91.4	16.5	5.8	18.2
3. Mono- and diglycerides	79.3	2.81	22.2	6.29	1.6	5.0
4. Polyglycerophosphatides and unknowns	36.7	1.31	12.9	2.33	1.3	4.18
5. Ethanolamine and serine phosphatides	203.3	7.22	124.5	22.40	7.7	24.15
6. Phosphoinositides	139.5	4.93	74.3	13.38	4.6	14.4
7. Phosphatidylcholine	381.0	13.50	139.5	26.50	8.7	27.48
8. Sphingolipids	62.4	2.20	19.7	3.53	1.1	3.45
9. Unknowns	70.2	2.54	17.8	3.20	Traces	—
10. Lipids recovered		100%		92%		94%

[a] Values per 100 g of electric organ.

TABLE II. Bilayer Formation from Lipid Fractions.

Fraction tested	No. of tests	Time (min) to (a) secondary black, and (b) breakdown									
		20°		25°		30°		35°		40°	
		a	b	a	b	a	b	a	b	a	b
Muscle: total lipids	26	—[a]	6.2	—[a]	12	—[a]	6.3	—[a]	3.2	—[a]	4.8
M-2	23	—[a]	2.1	—[a]	9.3	—[a]	—	—[a]	9.2	—[a]	10.8
M-7	28	—[a]	4.0	—[a]	7.4	—[a]	12.3	—[a]	1.5	6.7	5.0
Electroplax[b]: total lipids	20	—[a]	3.9	39	56	17	17	—[a]	15	—[a]	8.0
E-2	27	—[a]	3.0	—[a]	15.6	—[a]	21.5	—[a]	8.4	—[a]	6.8
E-3	23	—[a]	3.7	—[a]	4.9	—[a]	0.8	—[a]	0.4	—[c]	—[c]
E-7	23	5.8	7.4	6.8	10.0	8.6	8.6	6.2	7.0	2.4	4.0
E-8	20	—[a]	6.2	—[a]	7.5	18	18	9.3	9.3	—[a]	1.8

[a] No secondary black formed.

[b] No membranes formed with fractions E-4, 5, 6, and 8; E-1 not tested.

[c] No membranes formed.

ther resolved by TLC on silica gel G (17) and it was found that in the microsomal fraction, phosphatidylserine accounted for up to 70% of phospholipid phosphorus.

In the testing of the lipid fractions for their ability to form membranes, the methods of Mueller *et al.* (6) were followed. Each lipid fraction was tested from four to eight times at each temperature and the time of the formation of "secondary black" (60–80 Å thick bilayers) was determined. Formation of a secondary black, or bilayer film, was verified optically and also electrically by recording the ohmic resistance of the membrane. Membranes of less than 10^7 ohm/cm^2 resistance were not included in the data shown in Table II. The data recorded in Table II show that the best membranes were formed by using unfractionated tissue lipids. The term "best membranes" here refers to their ability to form reasonably stable bilayers of high ohmic resistance and with dielectric breakdown strengths of from 100 to 300 mV. Stability of the membranes was optimal between 25 and 30°. Only Fractions 2 and 7 of the muscle lipids were tested. Fractions 4, 5, 6, and 9 of the electroplax lipids failed to make stable membranes either in tetradecane or in chloroform–methanol.

Occasionally some of the membranes which remained stable for prolonged periods of time were tested for interaction with EIM derived from ovalbumin and *Aerobacter cloacae* (6). A marked decrease in resistance was observed in most cases but data on the production of induced excitability were inconsistent. After several weeks of testing and repeated exposures to air–oxygen, the ability of the lipid solutions to form bilayer membranes decreased as judged by a marked decrease in the time to breakdown.

Discussion. The electric organ of *Electrophorus* embryologically develops from the mesoderm and ontogenetically it is derived from muscle. Thus it was conceivable that a specialization towards bioelectrogenic capability had been accompanied by a shift in lipid composition. Our data support this contention in a general way though the exact nature of the relationship remains obscure.

Lipid content in percentage of dry weight was 16 for muscle, for whole electric organ 12, and 29 for the microsomal fraction. If the preparative procedure for the latter was modified for maximum Na^+, K^+ ATPase activity, as much as 36% of the ATPase was constituted of lipid. This value does not include a proteolipid fraction amounting to about 12%.

Two thirds of the muscle lipid was found in the form of triglycerides and cholesterol. Triglyceride storage in muscle has been observed in a variety of teleosts and our data on *Electrophorus* are consistent with these observations. The cholesterol found in any of the tissues occurred to over 90% as free

cholesterol. The amounts of spingolipids and glycolipids recovered from the tissues of *Electrophorus* were comparatively small and gangliosides appear to be totally absent from the electroplax.

The most remarkable observation was that phosphatidyl serine occurred in comparatively large amounts in the microsomal ATPase preparation. Whether or not this is significant, especially in view of the observations that ATPases may require a phospholipid (18) remains to be demonstrated.

Bilayer formation in our experiments was best achieved with lipid fractions containing the phosphoglycerides. Our observations that unfractionated lipid extracts give optimal results in the Rudin–Mueller system indicate that at least the mechanical and electrical stability of the excitable membrane may depend on a complex lipid matrix.

Summary. The lipid composition of the electric organ of *Electrophorus electricus* was compared to that of its microsomal fraction and to that of muscle of the electric eel. Using column chromatography on silicic acid to separate the lipid extracts into nine lipid classes, it was found that the lipid composition of whole electric organ was identical to that of its microsomal fraction.

Muscle tissue differed from electric tissue mainly in its high content of triglycerides and cholesterol; less than 10% of the latter was esterified. The amounts of glycolipids and sphingolipids found in either electric organ or in muscle were small.

Some lipid fractions of electric organ and unfractionated lipid extracts formed bilayer membranes in the Rudin–Mueller system. Stability of the bilayers was optimal at 25–30°.

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