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CARDIOLIPIN OF *CHLAMYDOMONAS REINHARDTII* 137$^+$

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Abstract—The fatty acids of cardiolipin from the phototrophic green alga *Chlamydomonas reinhardtii* 137$^+$ have been quantitatively analysed. Comparison is made at the molecular level between the cardiolipin of *Chlamydomonas* and that of higher plant tissue.

INTRODUCTION

Cardiolipin (diphosphatidylglycerol) has been analysed at the molecular level in only a few higher plant tissues [1–2]. Recently, the phospholipid was localized exclusively in the inner membrane of the plant mitochondrion and the fatty acids associated with the mitochondrial cardiolipin were quantitated [3]. The technical difficulty of obtaining purified mitochondria from green algae [4] and the relatively minor contribution that cardiolipin makes to plant tissue lipid [1–3] has hindered comparable direct study of cardiolipin from lower green plants. We have obviated these limitations by isolating cardiolipin from cellular lipid extracts of the green alga *Chlamydomonas reinhardtii* 137$^+$ grown phototrophically. Reported here is a quantitative analysis of the acyl groups associated with the cardiolipin of this typical chlorophyte.

RESULTS AND DISCUSSION

Purified *Chlamydomonas* cardiolipin was transesterified with sodium methoxide [5] and the fatty acid methyl ester derivatives recovered. The methyl ester fraction was analysed by GC either as recovered or after separation into classes of unsaturation by silver nitrate-TLC [6] with comparable results. In phototrophic *Chlamydomonas*, cardiolipin has an ester group unsaturated–saturated ratio of 2.41 ± 0.12 (s.d.; $n = 4$). Monoenes constitute 36.1% of the unsaturates; dienes 32.3%; trienes 19.9% and tetraenes 11.7%. Cardiolipin is one of the most highly unsaturated phospholipids of the alga; only phosphatidylglycerol is more unsaturated [7].

The fatty acid profile of *Chlamydomonas* cardiolipin is detailed in Table 1. Together, 16-carbon and 18-carbon acyl chains constitute over 70% of the major fatty acids, with lesser contributions from 14-, 20- and 22-carbon acyl groups. Prominent fatty acids are 16:0, 18:0 and 18:3. The prevalence of 16- and 18-carbon fatty acids is qualitatively reminiscent of the cell as a whole [7, 8] and of its major membrane, the chloroplast thylakoid [7].

Cauliflower (*Brassica oleracea* [1]) buds, mung bean (*Vigna radiata* [2, 3]) hypocotyls and sycamore (*Acer pseudoplatanus* [3]) cells are as yet the only plants from which cardiolipin has been analysed at the molecular level. In these higher plants at least 93% of the total cardiolipin fatty acids are C_{18}. The unsaturated–saturated ratio for higher plant cardiolipin ranges from *ca* 13 in mung bean [2, 3] to *ca* 32 in sycamore cells [3]. Comparison of these characteristics with the properties of *Chlamydomonas* cardiolipin demonstrates that the algal cardiolipin has both a wider variety of acyl groups and greater fatty acid saturation than do the higher plant cardiolipins.

Since the number of detail analyses on cardiolipin

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Table 1. Major* fatty acids of *C. reinhardtii* cardiolipin

Fatty acid	Mol % ± 2.d.†
14:0	1.11 ± 0.07
14:1	5.12 ± 0.26
16:0	12.06 ± 0.83
16:1	8.40 ± 0.12
16:2	5.01 ± 0.24
18:0	13.02 ± 0.76
18:1	5.50 ± 0.17
18:2	8.25 ± 0.23
18:3	13.40 ± 0.98
18:4	5.20 ± 0.31
20:1	2.82 ± 0.13
20:2	8.45 ± 0.36
20:4	2.72 ± 0.19
22:0	1.81 ± 0.11
22:1	2.44 ± 0.15
Total:	95.31 mol%

*Fatty acids representing ≥1.0 mol % of total.
†$n = 4$.

from plant tissue is restricted, the scope of such differences between algal and higher plant cardiolipins must await, for example, definition of positional unsaturated fatty acid isomers and study on a wider variety of green plants. Unfortunately, the trace levels at which cardiolipin is found in green plant tissue complicate such studies. For the moment, our report, the first molecular analysis of an algal cardiolipin, provides an initial step in broadening the details of plant cardiolipin biochemistry.

EXPERIMENTAL

Cell culture. Chlamydomonas reinhardtii 137$^+$ (wild-type) was grown phototrophically in axenic, log-phase culture in minimal medium [9] with constant stirring and aeration [10]. Cultures were harvested by low-speed centrifugation.

Cardiolipin isolation and identification. Algal lipid was quantitatively (recovery >98%) extracted by a modified Bligh–Dyer procedure [11]. Cardiolipin was resolved out of the extract by TLC [12] on Merck Type 60 Si gel with fluorescent indicator and visualized under UV. The spot, $R_f(x, y)$ (0.60, 0.57), identified as cardiolipin gave a positive reaction to phosphate detection spray [13] and contained phosphate [14], glycerol [15], and lipid ester [16] in the ratio 2:2.94:3.96, which approximates to the theoretical 2:3:4. The cardiolipin spot does not contain sugar [17] or sulphur [18]. The eluted [12] *Chlamydomonas* cardiolipin, intact, and the H_2O-soluble product of its mild alkaline hydrolysis [19] co-migrate on PC [20] and on TLC [21] with, respectively, cardiolipin standard and the deacylated standard [i.e. bis(glycerophosphoryl)glycerol].

Fatty acid ester preparation and analysis. Lipids were transesterified with 0.5 N NaOMe [5], and the methyl esters recovered quantitatively (recovery >98%) and fractionated into subclasses based on unsaturation by $AgNO_3$-TLC [6]. The methyl esters were separated by GC on a 10% stabilized diethylene glycol–succinate glass column, 2 m × 2 mm, under isothermal (200°) conditions and with carrier N_2 flow at 25 ml/min. Dual FID response was calibrated with standard methyl ester mixtures; all analyses were carried out well within the linear response range. Identification of fatty acids was the result of combined information from several sources, principally R_fs of known methyl ester standards and mathematical analyses of R_f–chain length relationships under the conditions employed [22]. Quantitation of peak areas on resulting chromatograms was by computer integration and conversion of relative ester areas to mol% composition was based on response factors obtained with the quantitative standards [22].

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