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NOTES

Studies on Lipid Profile of *Chara Contraria*

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There are scanty reports on the analysis of lipods in algae. *Chara contraria,* an unworked fresh water alga (Class *Chlorophyceae, Phylum-Eucaryota*) has been studied using HPLC. The disadvantage with the most of the methods is that the main polar lipid—phospho and galacto lipids appear together. The situation becomes even more critical in the analysis of the algae lipids, which contain much more polar class of lipids than higher plants lipids. HPLC has been found to be a better method of resolution. The advantage over TLC and TLC/FID have been discussed.

Except for reports on the fatty acid pattern in marine weed lipids[1] and Chlorella[2], there is virtually no work on lipid profile in algae, may be due to difficulty in extraction and low yield. In the present work, *Chara contraria* was collected from local lake, identified at Botany Department of the University and analysed for its lipid profile, using HPLC.

The material was kept for 2–3 minutes in hot water and lipid content was extracted by a modification[3] of the Bligh and Dyer[4] method. Ground material was extracted in accordance with the basic procedure and filtered. The residue was reextracted three times with small portions of $CHCl_3$/MeOH (2 : 1 v/v). The extracts so obtained (yield 1.33%) was contaminated heavily with chlorophyll pigments. For removal of these pigments which co-extract with lipids, the extractive was passed through a column (length 4.5 cm, diameter 1.2 cm) containing 1.5 g mixture of activated charcoal and celite (2.1 w/w). Chloroform was used for elution. Chlorophyll pigments were mostly retained in the column, but there was still a broad pigment peak, masking other lipid classes, in the chromatogram. For removal of the pigment, as far as possible, and better resolution of polar lipid classes, the extractive was further passed through a column of silicic acid using $CHCl_3$: MeOH : H_2O : NH_4OH (80 : 35 : 3 : 1) as the eluting solvent. The eluent was concentrated after removal of the excess solvent and taken for analysis. Standards were supplied by Sigma Chemical Co. U.S.A. HPLC unit from Water Associates, Inc: Model 440, with UV detector, sensitive to UV aborbance at 254 nm with Bonda Pak C 18 column was used. 10% H_2O/MeOH was used as mobile phase (flow rate 1 ml/min, recoder speed 1 cm/min).

Fig. 1 shows the chromatogram of the extractive after filtration and charcoal treatment. There is a broad pigment peak, intermixing with the other peaks. Fig. 2 shows the same after silicic acid chromatography, with distinct peaks. Peak 1

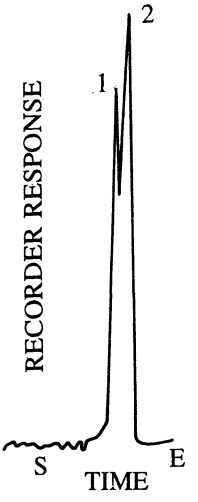

Fig. 1 HPLC Chomatogram of *Chara contraria* extractive after filtration and charcoal treatment.

HPLC Specification: HPLC (Water Associates)
Model 440, UV dector
Bondapak C 18 column
Solvent System: 10% H_2O/MeOH
Flow Rate—1 ml/min
Recorder Speed—2cm/min.
S = Start of Scan
E = End of Scan.

Peak assignment: Two intermixed peaks (1 and 2) with no good resolution.

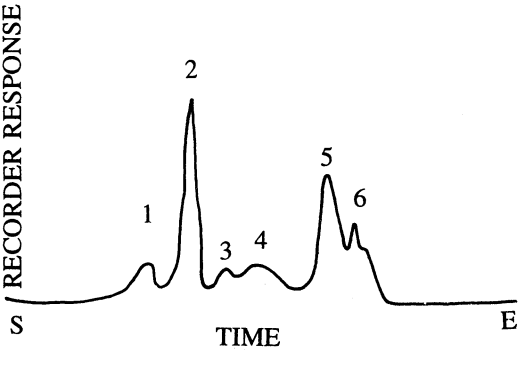

Fig. 2 HPLC Chromatiogram of *Chara contraria* extractive after silicic acid chromatography.

HPLC Specifications: Same as fig. 1.
Peak assignments: 1. NPL 2. MGDG 3. UN 4. PE 5. DGDG 6. PC

belongs to non polar lipids (NPL), 2 to monogalactosyldiglyceride (MGDG), 3 could not be identified (UN), 4 is phosphatidylethanolamine (PE), 5 is digalactosyldiglyceride (DGDG) and 6 is phosphatidycholine (PC). Identification of the peaks was done from the general pattern of elution and by comparing and spiking with authentic standards chromatographed under similar conditions.

Similarity in polarity brings phospho and glyco lipids close to each other. Algal lipids contain greater proportion of polar lipids. Presence of colouring matter makes the sepration more difficult. In TLC[5–12], PC and DGDG and PE and MGDP appear together. Same difficulty was noticed with TLC/FID where PC and DGDG appear as one peak using a particular solvent system. If PC and DGDG are separated, PE and MGDG get coincided. HPLC has advantage over TLC or TLC/FID. In TLC, the separation of algal lipids in the solvent mixtures depend on the properties of silica gel. In TLC or TLC/FID, the function of the mobile phase is to carry the samples through the system whereas in HPLC, the mobile phase is not inert and plays role in interacting with the sample molecule.[13–5] Furthermore, a single solvent or a mixture of solvents may be used as the mobile phase. A constant composition or a changed composition of the mobile phase can be maintained. In HPLC there is also a possibility of analysing a large number of polar compound by reverse phase. For HPLC of amphipathic molecules *e.g.* phospho and glyco lipids, photometric detection method is used, making use of the end abosorption in a specific region.

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