THE LIPID METABOLISM OF BLUE-GREEN ALGAE

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Although blue-green algae resemble the photosynthetic bacteria in their cellular structure (Echlin and Morris, 1965) one of them (Anabaena variabilis) has been shown (Levin, Lennarz and Bloch, 1964) to have some similarities to both green algae and the photosynthetic tissue of higher plants. For example, it evolves oxygen during photosynthesis (the Hill reaction) and contains α-linolenic acid . This supports a previous suggestion that α-linolenic acid is required for the operation of one or more of the steps leading to the photosynthetic production of oxygen (Erwin and Bloch, 1963). We have demonstrated (Nichols, Wood and James 1965) the presence of trans-3-hexadecenoic acid in the green tissue of a variety of higher plants and in green algae, and the absence of this acid both from the corresponding tissues when grown in the dark and from photosynthetic bacteria grown in the light. Consequently we suggested a possible connection between this fatty acid and the Hill reaction.

On the other hand, Holton and co-workers (1964) found no polyenoic acids in the blue-green alga Anacystis nidulans, although it can perform the Hill reaction (van Baalen 1957), but it was not clear whether or not the trans-3-hexadecenoic acid was present. Also, little is known of the nature of the lipid classes present in blue-green algae. We hoped that a more detailed study of the lipid content and lipid metabolism of the two blue-green algae Anabaena variabilis and Anacystis nidulans might throw light on the possible relationship between lipids and the Hill

reaction, and also on the biochemical relationships between photosynthetic bacteria, blue-green algae, green algae and higher plants.

MATERIALS AND METHODS

Anacystis nidulans (strain number 140 S/1) was obtained from the Cambridge collection of algae and protozoa and cultured according to the method of Kratz and Myers (1954).

Anabaena variabilis (Hecker stock No. 24) was kindly supplied by Professor G. E. Fogg and was cultured on the media described by Kratz and Myers (1954).

Lipid extracts Algal cells were harvested by centrifugation and the lipids extracted with chloroform-methanol (2:1 v/v) at room temperature for about 3 hours. Water-soluble compounds were removed from the concentrated extracts by the method of Folch, Lees and Sloane-Stanley (1957).

Identification and separation of lipids Lipids were fractionated and identified by a combination of thin layer chromatography on silicic acid and DEAE-cellulose column chromatography (Nichols and James 1964). Fatty acid methyl esters were prepared by refluxing each fraction for 90 minutes with a mixture of methanol, benzene and sulphuric acid (150:75:5 v/v). The esters were analysed by gas-liquid chromatography using polyethylene glycol adipate (EGA) and Apiezon L grease as stationary phases; ^{14}C analyses were carried out on EGA columns in the gas-liquid radiochemical chromatogram described by Hitchcock and James (1965). Fatty acids were identified on the basis of relative retention volumes on the two columns and of migration behaviour on thin layers of silicic acid impregnated with silver nitrate.

Incorporation of ^{14}C-labelled metabolites After harvesting, algal cells were resuspended in 10 ml aliquots of culture media and 10 μc of 2-^{14}C-acetate was added. The cell suspension was shaken in bright (artificial) light for various periods after which the cells were harvested and extracted as described above. Similar studies with 1-^{14}C-palmitate,

1-^{14}C-stearate or 1-^{14}C-oleate were also carried out.

RESULTS AND DISCUSSION

Lipid composition Both of the blue-green algae studied resemble the green algae and the photosynthetic tissue of higher plants in containing the four major fatty acid containing lipids found in chloroplasts, namely the two galactosyl diglycerides, sulphoquinovosyl diglyceride and phosphatidyl glycerol (Figure 1). However, they differ from these tissues in containing neither lecithin, phosphatidyl-ethanolamine nor phosphatidyl-inositol. In this respect, and in their lack of sterols (Levin and Bloch 1964), they resemble photosynthetic bacteria. The unknown lipid present in Anabaena variabilis and in rather smaller quantities in Anacystis nidulans has chromatographic properties on silica gel and DEAE cellulose columns different from any known lipid, but is as yet unidentified.

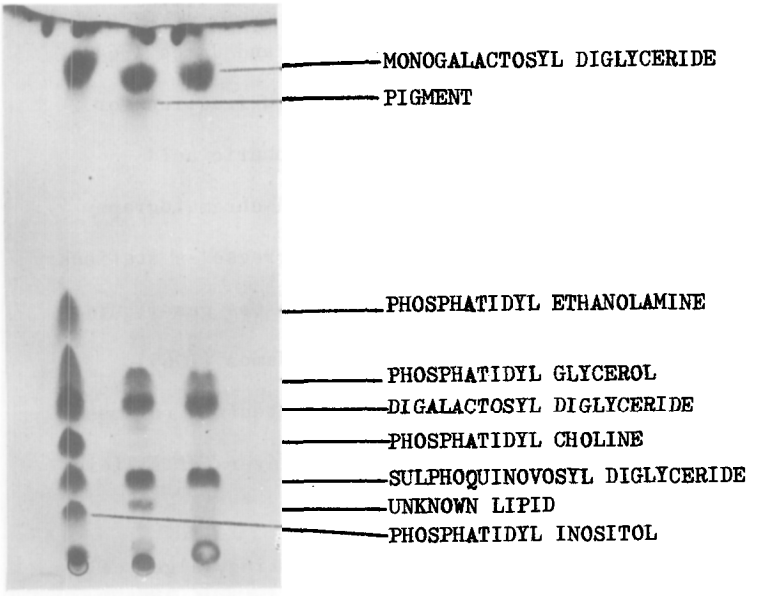

Figure 1

Comparative chromatogram of the polar lipids in (R to L) Anacystis nidulans, Anabaena variabilis and Chlorella vulgaris. Adsorbent : Silica gel G : Mobile phase : $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (170:30:20:7 v/v) Visualisation : H_2SO_4.

TABLE 1. Fatty acid composition of the lipids of Anabaena variabilis

Lipid	Fatty acid									
	12:0	14:0	16:0	16:1	16:2	17:0	18:0	18:1	18:2	18:3
Monogalactosyl diglyceride	0	0	27.1	27.5	1.0	0	t	12.0	18.7	14.7
Digalactosyl diglyceride	0	0	26.4	26.0	2.3	0	2.1	9.0	20.4	17.0
Phosphatidyl glycerol	5.7	7.5	31.0	8.9	1.2	1.4	5.9	21.1	11.5	6.3
Sulphoquinovosyl diglyceride	0	0	52.7	5.3	t	t	2.3	18.7	13.7	7.1
Unknown lipid	0	2.7	31.7	17.8	2.7	2.3	5.9	22.0	7.9	7.0

TABLE 2. Fatty acid composition of the lipids of Anacystis nidulans

Lipid	Fatty acid								
	12:0	14:0	14:1	16:0	16:1	17:0	17:1	18:0	18:1
Monogalactosyl diglyceride	0	1.2	t	42.6	33.6	t	t	4.0	20.4
Digalactosyl diglyceride	0	1.7	0	51.8	28.3	0	0	3.6	16.2
Phosphatidyl glycerol	4.3	8.0	2.2	35.2	17.3	t	t	7.5	22.0
Sulphoquinovosyl diglyceride	3.5	4.5	0	43.1	28.2	t	t	4.2	16.2
Unknown lipid	t	5.4	6.2	37.9	17.7	t	t	12.6	22.4

t = present but less than 0.1%

Tables 1 and 2 confirm the findings of Levin, Lennarz and Bloch (1964) that polyenoic acids are present in Anabaena variabilis and of Holton and co-workers (1964) that they are absent from the lipids of Anacystis nidulans. No trans-3-hexadecenoic acid could be found in the extracts of either culture, and therefore on present evidence the essential participation either α-linolenic acid or trans-3-hexadecenoic acid in the Hill reaction can be discounted. Thus in terms of fatty-acid containing lipids, the only factor common to all cells studied which are capable of performing the Hill reaction is the presence of the two galactosyl diglycerides, sulphoquinovosyl diglyceride and phosphatidyl glycerol. Consequently, as Holton et al (1964) have suggested, the function of these lipids would seem to be maintenance of a specific structure, with their fatty acid composition being less critical.

As suggested by Levin and Bloch (1964) the α-linolenic acid in Anabaena variabilis is primarily concentrated in the galactosyl diglycerides. A similar concentration of polyunsaturated acids in the galactosyl diglycerides is found in green algae and the leaves of higher plants (Nichols 1965).

When 2-^{14}C-acetate is used as precursor, the fatty acids of the phosphatidyl glycerol fraction are the most rapidly labelled, as is found with Chlorella vulgaris and plant leaves (unpublished results). However, the fatty acids of the monogalactosyl diglyceride fraction are labelled far more rapidly than in Chlorella vulgaris or plant leaves, possibly because in the blue-green algae this glycolipid contains a much higher proportion of the more saturated fatty acids than it does in the latter tissues.

As observed with the green alga Chlorella vulgaris (James, Harris and Harris 1965) both blue-green algae convert 1-^{14}C-palmitic acid into labelled palmitoleic, stearic and oleic acids. No label was found in the shorter chain acids, indicating little degradation to acetate followed by resynthesis. Anabaena variabilis was also, like Chlorella vulgaris,

capable of desaturating 1-^{14}C-stearic acid to give labelled oleic and linoleic acids, and of desaturating oleic acid to give labelled linoleic and linolenic acids. In both cases palmitic acid was unlabelled. Photosynthetic bacteria, on the other hand, cannot directly desaturate long chain fatty acids and rapidly breakdown added specifically labelled saturated fatty acids giving randomly labelled unsaturated fatty acids, (Harris, Wood and James 1965).

Thus the suggestion by Echlin and Morris (1965) that blue-green algae and photosynthetic bacteria should be classified together could only be supported biochemically by our finding that lecithin, phosphatidyl ethanolamine and phosphatidyl inositol are absent from the blue-green algae studied. In opposition to this suggestion we can quote the following:-

1) Unlike the photosynthetic bacteria, blue-green algae can directly dehydrogenate saturated long chain fatty acids.

2) They contain the polar lipids shown to be typical of green algae and plant leaves.

3) Some blue-green algae synthesise polyenoic acids which are absent from the photosynthetic bacteria.

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