Hydrogenase in Chlorella: Qualitative Differences in Quinone Composition[1]

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Chlorella pyrenoidosa cultivated on inorganic medium supplemented with glucose develops an active hydrogenase (Stiller and Lee 1964) and is able to carry out the photoreduction of CO_2 with molecular hydrogen (Stiller 1965). Bishop and Gaffron (1962) have reported that hydrogenase adapted Scenedesmus can carry out photoreduction of CO_2 when illuminated by light at 705 mμ when normal photosynthesis does not occur. They have suggested that photoreduction can be mediated by the long wave length light reaction alone, and that the two light reactions are coupled together by plastoquinone during normal photosynthesis. This paper describes the quinone composition of glucose grown Chlorella, which can carry out both photosynthesis and photoreduction, and compares it with that of autotrophically grown cells which are not capable of photoreduction.

Extraction of quinones from Chlorella was carried out with the propanol-heptane-water mixture previously described (Henninger and Crane 1964) with extra steps of grinding and washing the cells. The combined extract is chromatographed on a 2 x 61 cm. column packed under nitrogen gas at a pressure of 4 lb/in^2 with a heptane suspension of 1:3 silicic acid and Hyflo Super-Cel. The first elution is carried out with about 1000 ml heptane, with samples collected in about 300 ml aliquots, depending upon the band movements on the column. This procedure affords a rough separation of cartenoids, PQ A, PQ B, non-polar naphthoquinones and coenzyme Q_{10}.

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The second elution with 500 ml of a mixture containing increasing amounts of chloroform (2-25%) in heptane removes PQ C, PQ D and polar naphthoquinones. The third elution with ethanol removes any remaining quinones and chlorophyll.

Quinones were identified by their chromatographic properties on thin layer silica gel GHR (table I) and by their ultraviolet spectra. Extensive purification by thin layer chromatography was necessary in order to separate the quinones from steroid type compounds which mask the spectral properties of the quinones in the ultraviolet region.

Chlorella cells grown on a medium supplemented with glucose contain only about 1% chlorophyll on a dry weight basis which is about 50% less than the chlorophyll content of normal Chlorella cells. These cells retain their

Table I. Chromatographic Properties of Quinones from Chlorella as Compared to Other Quinones on Silica gel GHR

Compound	Rf in Benzene	Rf in Chloroform
Chlorella Vitamin K_1	.71	.85
Chlorella Naphthoquinone	.48	.83
Vitamin K_1	.71	.85
Chlorella Plastoquinone A	.74	.90
Chlorella Plastoquinone B	.75	.90
Chlorella Plastoquinone C	.12	.67
Chlorella Plastoquinone D	.08	.60
Plastoquinone A	.74	.90
Plastoquinone B	.75	.90
Plastoquinone C	.12	.67
Plastoquinone D	.08	.61
Chlorella Coenzyme Q_{10}	.30	.76
Coenzyme Q_{10}	.30	.76
Chlorella α-tocopherylquinone	.05	.42
α-tocopherylquinone	.05	.43

ability to carry out low rates of photosynthesis after acquiring the ability to photoreduce CO_2 with molecular hydrogen. Quinone analysis of glucose grown cells shows a general decrease of at least 60% from the concentration of the quinones found in normal Chlorella cells including PQ A, PQ B, PQ C, PQ D, Vitamin K_1 and tocopherylquinones (table II). These quinones are also characteristic of higher plants. In contrast to the decrease of the above listed quinones about five times as much coenzyme Q_{10} was found in the glucose grown cells and a new naphthoquinone appears which is not found in Chlorella cells grown on inorganic media. The naphthoquinone is similar in thin layer chromatographic properties and ultraviolet spectra to a hydroxy naphthoquinone recently found in blue green algae, Anacystis nidulans (Henninger et al., 1965). The new naphthoquinone in glucose grown Chlorella gives a positive antimony pentachloride and Emmerie Engel test which are identical with the positive test colors for the hydroxy naphthoquinone in Anacystis nidulans.

Table II

Quinone Content of Chlorella Cells Grown on Different Media[1]

Compound	Normal Cells		Glucose Cells	
	μmoles/gram dry wt.	μmoles/mg. chlorophyll	μmoles/gram dry wt.	μmoles/mg. chlorophyll
Plastoquinone A	.2613	.0100	.045	.0040
Plastoquinone B	Trace	Trace	None	None
Vitamin K_1	.0185	.0005	Trace	Trace
Naphthoquinone (hydroxy)	None	None	.104	.0070
Plastoquinone C + D	.0443	.0017	Trace	Trace
Coenzyme Q	.0436	.0016	.089	.0060
α-tocopherylquinone	Trace	Trace	None	None

[1]Trace indicates a positive spot on thin layer chromatography which cannot be determined spectrophotometrically. It represents .0005 to .0002 μmoles quinone per mg. chlorophyll. α-tocopherylquinone levels in glucose grown cells is less than 0.001 μmoles mg. chlorophyll.

In the blue green algae Anabaena cylindrica an active hydrogenase and photoreduction has been reported (Hattori 1963). The fact that the hydroxylated naphthoquinone is found in two types of plants which have a hydrogen based photoreduction system but is not present in higher plants suggests an approach to the function of this quinone in the hydrogen dependent pathway.

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