Isolation of 2-Demethylphytylplastoquinone from *Euglena gracilis*

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White (1971), while investigating the synthesis of plastoquinones, tocopherols, tocopherolquinones and ubiquinones by a streptomycin-bleached strain of *Euglena gracilis* strain Z, isolated from this organism a quinone with the chromatographic and u.v.-spectral properties expected for a phytyltoluquinone. The quinone was also found to be present in autotrophically grown *E. gracilis* strain Z; however, the amount formed by the normal strain (3 µg/g dry wt.) is less than that produced by the bleached strain (7 µg/g dry wt.).

In the present communication we report on the characterization of this quinone as 2-demethylphytylplastoquinone (3-phytyltoluquinone).

The lipid was extracted from freshly harvested cells (10g dry wt.) of the bleached strain by the method of Threlfall & Goodwin (1967), and then chromatographed on a column of Brockmann grade III acid-washed alumina developed by stepwise elution with light petroleum containing increasing amounts of diethyl ether (Whistance, Threlfall & Goodwin, 1967). The 2-demethylphytylplastoquinone (70 µg), present in the fraction eluted from the column of 0.25% diethyl ether in light petroleum, was purified by t.l.c. on (i) silica gel G developed with benzene–light petroleum (2:3, v/v) (R_F 0.3), (ii) paraffin-impregnated silica gel G developed with aq. 90% (v/v) acetone (R_F 0.5) and (iii) silica gel G developed with benzene (R_F 0.45).

The identity of the 2-demethylphytylplastoquinone was established as follows. (1) Its u.v. spectra in cyclohexane (λ_{max} 251.5nm) and ethanol (λ_{max} 252nm, changing to λ_{max} 289nm after sodium borohydride treatment) were those expected for a phytyltoluquinone. (2) Its mass spectrum showed peaks at m/e 400 (M) and 175 (base peak). (The latter proves that the quinone contains a monoprenyltoluquinone unit.) (3) Its monomethyltocol, synthesized by the same sequence of reactions as those used to form γ -tocopherol from phytylplastoquinone (Whistance & Threlfall, 1970), was found to have the same t.l.c. properties as δ -tocopherol. [This establishes that the quinone is 2-demethylphytylplastoquinone and not 3-demethylphytylplastoquinone (4-phytyltoluquinone) or 6-phytyltoluquinone.]

In biosynthetic terms the occurrence of 2-demethylphytylplastoquinone is of obvious interest, since its reduced form (2-demethylphytylplastoquinol) can be envisaged as an intermediate on the

pathways leading from homogentisic acid to phytylplastoquinone and phytylplastohydroquinone monomethyl ether and to the tocopherols and their oxidation products, the tocopherolquinones (Threlfall & Whistance, 1971).

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Propionate Resistance in Blue-Green Algae

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Sodium propionate has an inhibitory effect on the growth of a variety of bacteria and moulds (Wolford, 1945). Recent investigations of the ability of blue-green algae to assimilate organic compounds have revealed that the growth of these organisms is also sensitive to sodium propionate (Hoare, Hoare & Moore, 1968; A. J. Smith, J. London & R. Y. Stanier, unpublished work) and that acetate specifically reverses the effect of propionate (Hoare *et al.* 1968).

Anacystis nidulans and *Gloeocapsa alpicola*, two unicellular blue-green algae, are sensitive to low concentrations of propionate; concentrations above 0.5mM completely inhibit growth. In both cases the inhibitory effect of 5mM-propionate is reversed by an equimolar amount of acetate. The growth-inhibitory effect of sodium propionate is not permanent; with both organisms growth in media containing propionate (5mM) recommences after a lag of 50–60 h. The organisms in a culture that has recovered from the inhibitory effect of propionate are propionate-tolerant, since on transfer to salts media with or without propionate (5mM) they grow without a lag and at about the same rate as do propionate-sensitive organisms. Propionate-tolerant strains of both organisms have been isolated by plating sensitive organisms on salts-agar containing sodium propionate (5mM). These tolerant strains grow in a salts medium with or without

propionate (5mM) without a lag and at about the same rate as do propionate-sensitive (wild-type) organisms. Propionate tolerance is a stable character, since prolonged subculture of tolerant organisms in the absence of propionate does not result in the loss of resistance to propionate.

The biochemical basis of propionate resistance was investigated in experiments with cell suspensions and cell-free extracts. Suspensions of wild-type organisms assimilate $[2-^{14}\text{C}]$ propionate and $[1-^{14}\text{C}]$ acetate in the light under an atmosphere of air + CO_2 (95:5); in contrast, suspensions of propionate-resistant organisms do not incorporate significant amounts of either organic compound into cell material under identical conditions. Dialysed extracts of wild-type *A. nidulans* contain readily detectable acetate and propionate thiokinase activity, whereas extracts of propionate-resistant organisms do not contain these activities.

It is proposed that the loss by spontaneous mutation of the enzyme acetate thiokinase is the biochemical basis of propionate resistance in the blue-green alga *A. nidulans*.

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Inhibition of Photosynthetic Electron Transport by 1,1,1-Trichloro-2,2-bis-(*p*-chlorophenyl)ethane (DDT) at a Site before Photosystem 2

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The inhibitory effect of DDT* on photosynthesis by susceptible barley varieties is shown by a decrease in the rate of O_2 evolution and by diminished Hill activity, paralleled by loss of cyclic and non-cyclic photophosphorylations (e.g. see Lawler & Rogers, 1967; Owen, Rogers & Hayes, 1970).

A site of inhibition of electron transport by DDT before photosystem 2 was identified from spectrophotometric studies on tris-washed chloroplasts in which electron donation from water, and O_2 evolution, have been eliminated (Yamashita & Butler, 1968). In this system artificial electron donors such as diphenylcarbazide were used to donate electrons beyond the site of tris inhibition (Yamashita & Butler, 1969), and electron flow through photosystem 2 was measured by use of 2,6-dichlorophenol

indophenol or ferricyanide as electron acceptor from the intermediate electron-transport chain.

The properties of chloroplasts from DDT-treated susceptible barley, where electron transport from water, as evidenced by photoreduction of dichlorophenol-indophenol, was inhibited some 50%, were compared with those of untreated controls. In both cases washing with tris almost completely inhibited electron donation from water, although electron transport could be restored to the control (untreated barley) value by diphenylcarbazide. Thus with electron transport from diphenylcarbazide no inhibition of electron transport to dichlorophenol-indophenol was observed in chloroplasts from DDT-treated barley. This indicates that a site blocked by DDT in the electron-transport chain before photosystem 2 is by-passed by electron donation from diphenylcarbazide. In contrast with work on spinach chloroplasts no electron donation beyond the tris block from benzidine or semicarbazide (Yamashita & Butler, 1969) was observed.

Studies of electron donation by Mn^{2+} or ascorbate to the electron-transport chain before photosystem 2 (Ben-Hayyim & Avron, 1970) were made by measuring O_2 consumption in the oxygen electrode with paraquat or diquat as electron acceptor from photosystem 1 (Izawa, Connolly, Winget & Good, 1966; Böhme & Trebst, 1969). There was no change in relative rates of electron donation from the artificial donors compared with donation from water in chloroplasts isolated from DDT-treated or untreated susceptible barley. This suggests that the sites of electron donation from Mn^{2+} or ascorbate are before the target site for DDT in the electron-transport chain before photosystem 2, since in chloroplasts from treated barley some alleviation of the observed inhibition of electron transfer from water might be expected if the DDT-sensitive site were by-passed.

The interpretation of this latter result was made in the knowledge that the inhibition observed in electron donation from Mn^{2+} or ascorbate is a function of inhibitions by DDT of both a site before photosystem 2 and a further site that preliminary results locate in the intermediate electron-transport chain (W. J. Owen & L. J. Rogers, unpublished work).

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* Abbreviation: DDT, 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl)ethane.