Substituted *p*-Benzoquinones Having High Electron Affinity as Photosystem II Electron Acceptors

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Substituted Benzoquinones, Photosystem II Acceptors

Benzoquinones (BQ) substituted with alkyl or alkoxy groups 2,5-dimethoxy-3,6-dichloro-*p*-benzoquinone (DCDMQ), 2,3,5,6-tetramethoxy-*p*-benzoquinone (TMQ) have been tested for their electron affinity and site of action in the photosynthetic electron transport chain in whole cells and chloroplasts. Both the substituted compounds were found to be good electron acceptors of photosystem II. DCDMQ showed higher electron affinity than BQ as demonstrated by its two fold stimulation of O_2 evolution in chloroplasts and efficiency in quenching DCMU fluorescence. TMQ on the other hand showed low electron affinity.

Introduction

Quinones have attracted much interest not only by their presence in large quantities in chloroplasts and photosynthetic microorganisms [1,2] but also because of their role as intermediate in the photosynthetic electron transport system. It was shown that substituted naphthoquinones serve as inhibitors of Hill reaction [3]. A number of quinones also serve as oxidants in the Hill reaction [4, 5]. DBMIB, an antagonist of plastoquinone allows the study of the partial reactions of electron transport and photophosphorylation which are either connected to PS I or PS II [6]. Several other benzoquinones substituted by long alkyl side chains are also known to be inhibitors [7]. This paper introduces DCDMQ and TMQ as more efficient photosystem II acceptors.

Materials and Methods

The eucaryotic green alga *Scenedesmus obliquus* D_3 was used in all experiments. For growth and ageing conditions see ref. [8]. *Amaranthus gangeticus* L. was grown in botanical garden. Type II broken chloroplasts from fresh leaves were isolated in isotonic sorbitol-tricine medium pH 7.5 with 5 mM $MgCl_2$, 1 mM $MnCl_2$ and 10 mM KCl. Measurements of photochemical activities and fluorescence induction were made as reported previously [8]. DCDMQ and TMQ were prepared as described by Vorter and Rogers [9]. All compounds were first dissolved in ethanol and diluted with water. Final ethanol concentration in the reaction mixture was kept below 0.5%. Electron affinity was calculated from PPP-CI calculation using the relation of Kunii and Kuroda [10].

Abbreviations: BQ, *p*-benzoquinone; DBMIB, 2,5-dibromo-3-methyl-1,6-isopropyl-1,4-benzoquinone; DCDMQ, 2,5-dimethoxy-3,6-dichloro-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; DCPIP, 2,6-dichlorophenol-indophenol; PQ, plastoquinone; TMQ, 2,3,5,6-tetramethoxy-*p*-benzoquinone.

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Results and Discussion

The compound tetrachloro-*p*-benzoquinone is a powerful electron acceptor with a very high electron affinity of 2.16 eV. But the insolubility of this compound in aqueous solvent renders it unsuitable for its application in biological systems. However, the replacement of the two chloride atoms in the molecule by the $-OCH_3$ groups to form 2,5-dimethoxy-3,6-dichloro-*p*-benzoquinone (DCDMQ) makes it soluble in water considerably at the expense of its electron affinity which is brought down to 1.67 eV. Further replacement of the remaining chloride atoms by the OCH_3 groups to form 2,3,5,6-tetramethoxy-*p*-benzoquinone (TMQ) makes it highly soluble in water but its electron affinity has been reduced to 1.38 eV, very close to that of *p*-benzoquinone (1.35 eV). Unlike BQ all these compounds exhibit exceptionally good stability towards oxidation in light.

Addition of DCDMQ and TMQ to the cells of *Scenedesmus* decreased the overall rate of O_2 evolution to various extent (Table I). The decrease was more with DCDMQ and less with TMQ when com-

pared to BQ. Addition of PS II acceptors like BQ was reported to reduce the O_2 evolution rate to half [8]. This reduction in rate is not due to their inhibitory action but possibly due to the utilization of only PS II. This is evident from the half stoichiometry of coupled photophosphorylation with BQ and operation of only one energy conserving site [11].

Changes in the level of O_2 evolution in *Amaranthus* chloroplasts upon the addition of these compounds are given in Table II. DCDMQ brought about 50% higher rate of O_2 evolution than BQ indicating that it is a more powerful electron acceptor. TMQ was effective only by 21% as compared to DCDMQ. However, TMQ stimulates the O_2 evolution to a higher level in aged cells in which PQ is highly inactivated (see below), indicating that this collects only part of electrons flowing from Q in young active algal cells. This is clear from Table I. TMQ also shows 60% recovery of O_2 evolution in DCMU treated cells (results not shown here). It is therefore possible that the effect of TMQ would be high only in the absence of any other electron acceptor or alternative electron flow reaction.

When the cells were treated with DCMU the level of fluorescence increased greatly and only com-

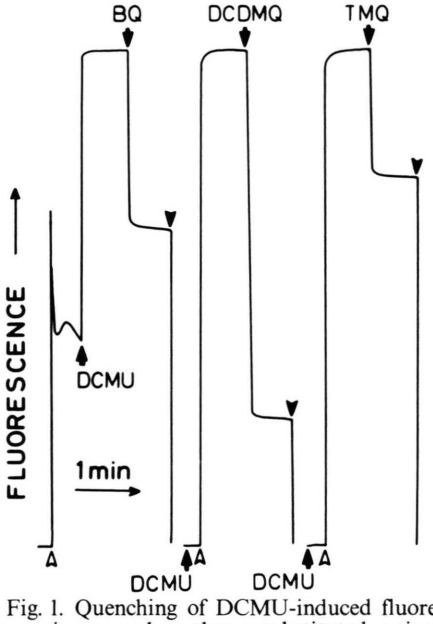

Fig. 1. Quenching of DCMU-induced fluorescence by benzoquinone and methoxy substituted quinones (DCDMQ and TMQ) in *Scenedesmus* cells. Final concentration of DCMU and quinones was 10 and 500 μM respectively.

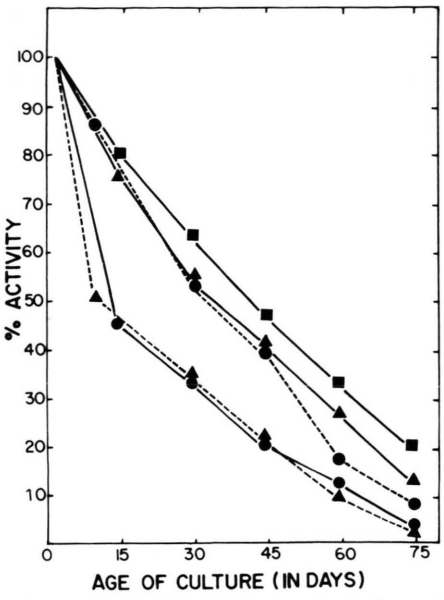

Fig. 2. Changes in the photosynthetic O_2 evolution and Hill reactions with DCPIP or quinones, in ageing cultures of *Scenedesmus*. O_2 evolution was measured polarographically in whole cells and DCPIP reduction spectrophotometrically in chloroplast particles at 578 nm. Maximum values for DCPIP Hill reaction in chloroplast particles were 476 and 426 μmol DCPIP reduced/mg Chl · h with and without PQ respectively. Maximum values of O_2 evolution in whole cells were: control, 252; + DCDMQ, 115 and + TMQ, 218 μmol O_2/mg Chl · h. ▲- - - -▲ Hill reaction with DCPIP; ●- - - -● Hill reaction with PQ and DCPIP; ●—● Control O_2 evolution; ■—■ Hill reaction with DCDMQ (0.5 mM); ▲—▲ Hill reaction with TMQ (0.5 mM).

Table I. Comparative effect of different quinones on the rate of O_2 evolution in 2-day-old *Scenedesmus* cells. The assay mixture contained 100 mM PO_4 buffer, pH 7.5, 5 mM $MgCl_2$, 2 mM$MnCl_2$, 2 mM EDTA, 35 mM NaCl. The final concentration of all quinones was 500 μM.

Addition	μmol of O_2/mg Chl, h	
		% activity
None	252.0	100.0
+DCDMQ	115.1	45.6
+BQ	142.5	56.5
+TMQ	217.6	86.4

Table II. Rate of O_2 evolution in *Amaranthus* chloroplasts mediated by different quinones. Details of measurement as in Table I.

Addition	μmol O_2/mg Chl, h	
		% activity
+DCDMQ	196.2	100.0
+BQ	115.4	58.8
+TMQ	42.0	21.4

pounds which accept electrons directly from the Q could reduce the fluorescence yield in the absence of direct quenching on pigment beds and alteration of membrane structure. The extent of quenching was found to be in the order of their electron accepting capacity (Fig. 1).

In ageing algal cultures the sharp decline in the photosynthetic activity during the initial stages was shown to be due to the block at PQ [8]. Overcoming the block at PQ by the addition of exogenous PQ, brought about a linear decay. DCDMQ and TMQ also behave similarly (Fig. 2). This is possible only if these compounds accept electrons at or before PQ since DCPIP alone which accepts near cytochrome fails to show a linear decay. Further work is in progress in washed thylakoid preparations to pin point their site of action in the photosynthetic electron transport chain.

Acknowledgements

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