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THE SITE OF INHIBITION OF PHOTOSYSTEM II BY 3-(3,4-DICHLOROPHENYL)-N-N'-DIMETHYLUREA IN THYLAKOIDS OF THE CYANOBACTERIUM ANABAENA CYLINDRICA

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ABSTRACT: Thylakoids isolated from the cyanobacterium Anabaena cylindrica exhibit Photosystem II activity. Photosynthetic electron transfer from water to ferricyanide and to 2,6-dichlorophenolindophenol is inhibited by 3-(3,4-dichlorophenyl)-N-N'-dimethyl urea. Diphenylcarbazide stimulates ferricyanide and 2,6-dichlorophenolindophenol photoreduction, whilst inhibiting oxygen evolution. Diphenylcarbazide-supported Photosystem II activity is completely insensitive to 3-(3,4-dichlorophenyl)-N-N'-dimethyl urea, indicating that the site of action of this inhibitor lies on the donor side of Photosystem II in A. cylindrica, before the site of electron donation by diphenylcarbazide.

INTRODUCTION: DCMU has probably been the most widely used inhibitor of Photosystem II in eukaryotic photosynthesis research (1). Its site of action in chloroplasts is generally believed to lie between the primary acceptor and secondary acceptor (plastoquinone) of Photosystem II (1, 2). DCMU has also been effectively used as an inhibitor of Photosystem II in physiological and nutritional in vivo studies of cyanobacteria (3-6). The present communication presents data on photosynthetic reactions by thylakoids isolated from the cyanobacterium Anabaena cylindrica, which indicate that the site of inhibition

Abbreviations: DCMU; 3-(3,4-dichlorophenyl)-N-N'-dimethyl urea. DCPIP; 2-6-dichlorophenolindophenol.

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by DCMU differs from that in chloroplasts and lies on the donor side of Photosystem II. These results concur with a recent report on the effect of DCMU on thylakoids of the cyanobacterium Oscillatoria chalybea (7) and the implications of these findings obtained with prokaryotic phototrophs are discussed.

MATERIALS AND METHODS

Organism: Anabaena cylindrica Lemm. (strain CU 1403/2a) was obtained from the Culture Centre of Algae and Protozoa, Cambridge, U.K. Cells were grown axenically in 10 L batches as detailed previously (8), and log-phase cultures harvested by centrifugation at 5000 x g for 20 min.

Preparation of thylakoids: 4 L of culture were used. The disruption of cells into 0.75 M tricine buffer pH 7.5, containing 0.01 M NaCl and 0.2 M sucrose and subsequent differential centrifugation were performed according to Sallal and Codd (9). The resulting thylakoid pellet was gently resuspended, washed twice by repeated spins at 35,000 x g for 30 min and finally resuspended to 4 ml with tricine NaCl buffer.

Electron transport reactions: The ferricyanide-Hill reaction was carried out as reported previously (10). In some assays, the ferricyanide was replaced by DCPIP (8×10^{-5} M) as electron acceptor, and DCPIP reduction was monitored at 600 nm. Oxygen evolution in the Hill reaction was measured using at Pt-Ag electrode (Rank Bros., Bottisham, Cambridge, U.K.). DCMU and diphenylcarbazide were used at a range of concentrations (see results) and chlorophyll measured according to Kirk (11).

RESULTS AND DISCUSSION

As shown in Table 1, thylakoid preparations from Anabaena cylindrica carried out the Hill reaction from water to ferricyanide. The addition of increasing amounts of diphenylcarbazide, an electron donor to Photosystem II in chloroplasts (12-14), resulted in an increase in the rate of ferricyanide reduction, suggesting, in part, that components which function between the sites of photolysis of water and electron donation by diphenylcarbazide may have been lost during membrane isolation (see 12). However, as found with isolated Oscillatoria chalybea thylakoids (7), the presence of diphenylcarbazide also caused a decrease in the rate of oxygen evolution (Table 1). This indicates that

Table 1

The effect of diphenylcarbazide on the Hill reaction by thylakoids of Anabaena cylindrica

Diphenylcarbazide concentration	Oxygen evolution		Ferricyanide reduction	
	Reaction rate [a]	Relative rate	Reaction rate[b]	Relative rate
0	23	100	112	100
10^{-5} M	20.2	87.8	168	150
3×10^{-5} M	14.7	63.9	192.6	172
10^{-4} M	2.1	9.1	246.4	220
1.3×10^{-4} M	0	0	246.4	220

a, μ mol oxygen evolved/mg chlorophyll hr^{-1}

b, μ mol ferricyanide reduced/mg chlorophyll hr^{-1}

diphenylcarbazide can substitute for water as the electron donor to Photosystem II in isolated A. cylindrica thylakoids in which the photolysis of water can otherwise occur. The inhibition of the Hill reaction from water to ferricyanide by DCMU is shown in Fig. 1. 10^{-6} M DCMU caused a complete inhibition of this reaction when catalyzed by thylakoids containing 10 μg chlorophyll a per assay. However, ferricyanide photoreduction in the presence of 10^{-4} M diphenylcarbazide was completely unaffected by the presence of up to 10^{-5} M DCMU (Fig. 1). The photoreduction of DCPIP by A. cylindrica thylakoids was also measured from water as electron donor and rates of 20 to 34 μmoles DCPIP reduced/hr.mg chlorophyll^{-1} were recorded. This reaction was completely inhibited by DCMU and the inhibition was again prevented by the addition of 3×10^{-5} M diphenylcarbazide. By contrast, the addition of diphenylcarbazide

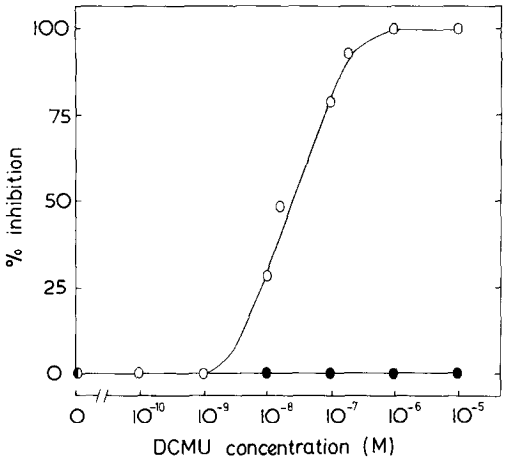

Fig. 1. The effect of increasing concentrations of DCMU on the photoreduction of ferricyanide by Anabaena cylindrica thylakoids. Assays contained 10 μg chlorophyll a. Reaction rates in the absence of DCMU were 112 and 246 μ moles ferricyanide reduced/ mg chlorophyll hr^{-1} in the absence and presence of diphenylcarbazide (10^{-4} M) respectively. (O—O , minus diphenylcarbazide; ●—● plus 10^{-4} M diphenylcarbazide).

does not relieve the inhibition of the Hill reaction in tobacco chloroplasts which is caused by DCMU acting on the donor side of Photosystem II (7, 12).

Assuming that diphenylcarbazide serves to donate electrons to Photosystem II in A. cylindrica, the data obtained indicate that the site of inhibition by DCMU lies on the electron donor side of Photosystem II in this prokaryote, between the sites of water photolysis and electron donation by diphenylcarbazide. Further resolution of the site of inhibition by DCMU in A. cylindrica cannot be ascertained at present. However, it is noteworthy that Etienne (15) has found an additional site of DCMU in chloroplasts. Here, DCMU acts on the donor side of Photosystem II, on the S_3 state of the complex.

Finally, whether the inhibition by DCMU on the donor side of Photosystem II occurs in other cyanobacteria is not apparent.

A. cylindrica and O. chalybea (7) are filamentous forms and represent an evolutionary divergence from the coccoid line (6). It is possible that the coccoid cyanobacteria are more closely related to chloroplasts through evolution (16) and hence may show a similar site of action of DCMU on the acceptor side of Photosystem II.

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