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Inhibition of photosystem II in *Chlorogloeopsis fritschii* with shikonin acetate

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Shikonin acetate extracted from the roots of the desert plant, *Arnebia decumbens*, was tested for its effect on the photosynthetic electron transport system of *C. fritschii*. Photosystem (PS) II-Hill reaction with water and diphenylcarbazide (DPC) as electron donors and ferricyanide as electron acceptor was inhibited completely. Shikonin acetate has little effect on 2,6-dichlorophenolindophenol (DCPIP)-Hill reaction and no effect on PSI with durohydroquinone as electron donor. Inhibition of Mehler reaction from water to methylviologen with whole cells was studied using 3-(3,4-dichlorophenyl)-1,1-dimethyl urea (DCMU) and shikonin acetate. About 70% inhibition of cyclic and non-cyclic photophosphorylation was found with 10^{-7} M shikonin acetate. These results suggest that shikonin acetate inhibits photosynthetic electron flow at the plastoquinone pool.

Chlorogloeopsis fritschii; Photosystem I; Photosystem II; Photophosphorylation; Shikonin acetate

1. INTRODUCTION

Shikonin (5,8-dihydroxy-2-(11-hydroxy-14-methyl-13-pentenyl)-1,4-naphthoquinone), its enantiomer alkanin and their derivatives have been shown to exhibit antimicrobial activity [1,2], antitumor activity [3,4] and anti-amoebic activity [5]. Various shikonin compounds have been extracted, purified and characterized from a desert plant by Afzal and Al-Oriquat [6]. One of these compounds, shikonin isovalerate was reported for its effect on photosynthesis [7]. DCMU, the most widely used inhibitor of photosystem II in photosynthetic eukaryotes [8] was studied for its site of effect on oxygen evolution and photosystem II in cyanobacteria [9–11]. The effect of 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone (DBMIB) on photosystem II, cyclic and non-cyclic electron flow in chloroplasts have been reported [12]. This report describes the effect of another shikonin compound, shikonin acetate, on photosystem II, photosystem I, dark and light phosphorylation in *Chlorogloeopsis fritschii*.

2. MATERIALS AND METHODS

2.1. *Organism and growth conditions*

Chlorogloeopsis fritschii (strain CU 1411/1) was obtained from the Culture Centre of Algae and Protozoa, Cambridge, England and was grown axenically in BG-11 medium [13] in 250 ml conical flasks. Cultures were incubated at 25°C in an orbital shaker under constant illumination of 60 μmol·m^{-2}·s^{-1}.

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2.2. *Electron transport reactions*

Cell-free extracts were prepared by centrifuging the cells at 5000 × *g* for 20 min. Cell pellets were suspended in 75 mM tricine-HCl buffer, pH 7.5, containing 10 mM NaCl. Cells were then disrupted by ultrasonication for 4 × 15 s, punctuated by 15 s rest periods in an ice bath. Disrupted cells were centrifuged at 2500 × *g* for 15 min and the supernatant was used to study the photosynthetic electron transport system.

2.3. *Oxygen evolution*

Oxygen evolution was measured using a Rank Pt–Ag oxygen electrode at 25°C. 3.0 ml of cells was incubated in the dark for 10 min and then exposed to light provided by a Leitz Pradovit tungsten filament projector which gave 120 μmol·m^{-2}·s^{-1} for another 10 min. The oxygen electrode was calibrated according to Lessler [14].

2.4. *Ferricyanide-Hill reaction*

The ferricyanide-Hill reaction was used to measure the transfer of electrons from water to ferricyanide via photosystem II, according to Nishimura et al. [15], with minor modifications. The reaction mixture contained in a final volume of 3.0 ml: 2.3 ml Hill buffer (50 mM tricine, 0.4 M sucrose and 10 mM NaCl, pH 7.5), 1 μmol $MgCl_2$, 1 μmol potassium ferricyanide and 0.5 ml cell-free extract. The reaction mixture in cuvettes was illuminated by a Leitz Pradovit tungsten filament lamp projector. The net photoreduction of ferricyanide was measured at 420 nm spectrophotometrically. In some assays the ferricyanide was replaced by 8×10^{-5} M DCPIP as electron acceptor and DCPIP reduction was monitored at 600 nm.

2.5. *Mehler reaction*

Oxygen consumption of the methylviologen-catalyzed Mehler reaction in whole cell suspension was followed with the addition of 2.3 ml fresh BG-11 medium, 0.1 μmol methylviologen, 2.5 μmol KCN and 0.5 ml fresh cyanobacterial culture as described by Whitelam and Codd [16]. In cell-free extracts with DCPIP/ascorbate couple as electron donor, methylviologen-Mehler reaction was done according to Schmid et al. [17]. For some assays durohydroquinone was used as electron donor. The reaction mixture contained in a final volume of 3.0 ml: 2.3 ml Mehler buffer (75 mM tricine, 0.2 M KCl), 2.5 μmol DCPIP, 60 μmol ascorbate, 2.5 μmol KCN, 0.1 μmol DCMU,

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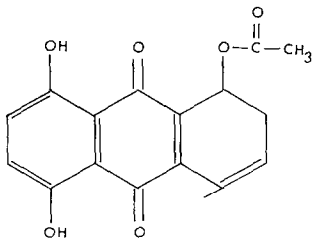

Scheme 1. Shikonin acetate structure

0.1 μmol methylviologen and 0.5 ml cell-free extract. The reaction mixture was equilibrated in the oxygen electrode in the dark for 5 min and light-dependent oxygen consumption was measured.

2.6. *Photophosphorylation*

Log phase cultures of *Chlorogloeopsis fritschii* were incubated in light and dark in the presence of DCMU (1×10^{-5} M) and shikonin acetate (1×10^{-7} M) for 24 h under the same growth conditions mentioned. Duplicate 6 ml samples of cultures were removed from the culture flasks and injected into 1.5 ml of 3 M perchloric acid, then extracted and assayed for ATP by the luciferin-luciferase technique according to Bottomley and Stewart [18,19].

2.7. *Chlorophyll measurement*

Chlorophyll *a* was measured according to the method of Kirk [20].

3. RESULTS AND DISCUSSION

Shikonin acetate extracted from dried roots of a desert plant, *Arnebia decumbens*, was a gift from Professor M. Afzal. Various concentrations of shikonin acetate were tested for their effects on photosystems II and I as shown in Table I. 10^{-5} M shikonin acetate was found to inhibit the ferricyanide-Hill reaction completely (Table I). Photosystem I was not affected by the usual concentrations which inhibited photosystem II; however, higher concentrations, i.e. 10^{-4} M and 10^{-5} M, caused slight inhibition of PSI using DCPIP/ascorbate couple as electron donor (Table I). Photosystem II, with water and DPC as electron donors and potassium ferricyanide as electron acceptor was inhibited using 10^{-7} M shikonin acetate (Table II). The photoreduction of DCPIP by cell-free extract of *C. fritschii* was slightly inhibited with shikonin acetate using DPC as electron donor (Table II). The effects of DCMU and DBMIB on photosystem II were reported in a similar study using the same cyanobacterium [7]. DBMIB was found to inhibit PSII-Hill reaction with water and DPC as electron donors, while DCMU inhibited the reaction with water but not DPC as electron donor [7]. These results agree with other reports on the site of inhibitions by DCMU and DBMIB [11,12]. The permeability of *C. fritschii* to shikonin acetate was tested by inhibiting Mehler reaction with water as electron donor using intact cells of *C. fritschii* as shown in Table III. This is also supported by the inhibition of light-grown cells of *C. fritschii* with shikonin acetate (unpublished data).

Table I

The effect of different concentrations of shikonin acetate on Hill reaction and Mehler reaction of *C. fritschii*

Shikonin acetate (M)	Photosystem II-Hill reaction		Photosystem I-Mehler reaction	
	Reaction rate[a]	Relative rate	Reaction rate[b]	Relative rate
0	360.0	100.00	40.1	100.00
10^{-10}	358.0	99.50	40.0	99.75
10^{-9}	337.5	93.75	40.0	99.75
10^{-8}	168.5	46.80	39.5	98.50
10^{-7}	56.2	15.60	39.0	97.26
10^{-6}	22.5	6.25	38.0	94.80
10^{-5}	0	0	34.3	85.50
10^{-4}	0	0	31.1	77.60

[a] μmol ferricyanide reduced/h per mg chlorophyll
[b] μmol oxygen consumed/h per mg chlorophyll

Table II

Effects of shikonin acetate on oxygen evolution and photosystem II of *Chlorogloeopsis fritschii*

Reaction	Reaction rate	Relative rate
Oxygen evolution[a]	7.5	100.0
+ 10^{-7} M shikonin acetate	7.2	96.0
Photosystem II minus[b] DPC	310.0	100.0
+ 10^{-7} M shikonin acetate	43.5	14.0
Photosystem II plus[b] DPC	320.0	100.0
+ 10^{-7} M shikonin acetate	50.0	15.6
Photosystem II plus DPC[c] and DCPIP as electron acceptor	29.0	100.0
+ 10^{-7} M shikonin acetate	23.5	81.0

[a] Oxygen evolution was measured as μmol oxygen evolved/h per mg chlorophyll
[b] Photosystem II-Hill reaction was measured as μmol ferricyanide reduced/h per mg chlorophyll using water and 2.5 μmol DPC as electron donors. For details of the reaction, see section 2
[c] Photosystem II-Hill reaction was measured using 2.5 μmol DPC as electron donor and 8×10^{-5} M DCPIP as electron acceptor. Reaction rate was measured as μmol DCPIP reduced/h per mg chlorophyll

Table III

Effects of shikonin acetate and DCMU on methylviologen-Mehler reaction from water in whole cells of *C. fritschii*

Reaction	Reaction rate	Relative rate
Whole cell Mehler reaction	33.0[a]	100.0
Whole cell Mehler reaction		
+ 1×10^{-7} M shikonin acetate	19.6	59.4
+ 1×10^{-5} M DCMU	20.3	61.5

[a] Reaction rate was measured as μmol oxygen consumed/h per mg chlorophyll

Photosystem I-Mehler reaction from durohydroquinone to methylviologen was not affected with shikonin acetate as shown in Table IV. Durohydro-

Table IV

Effects of shikonin acetate on photosystem I of *C. fritschii*

Reaction	Reaction rate	Relative rate
PSI from durohydroquinone to methylviologen	40.1[a]	100.0
+ 1×10^{-7} M shikonin acetate	40.1	100.0

[a] Photosystem I was measured as μmol oxygen uptake/h per mg chlorophyll using 8×10^{-5} M durohydroquinone as electron donor

Table V

Effects of shikonin acetate and DCMU on photophosphorylation of light and dark incubated cells of *C. fritschii*

Reaction	Reaction rate	Relative rate
Dark incubated cells	35.0[a]	100.0
Dark incubated cells + 1×10^{-7} M shikonin acetate	32.5	91.4
Light incubated cells	287.5	100.0
Light incubated cells + 1×10^{-7} M shikonin acetate	87.4	30.4
Light incubated cells + 1×10^{-5} M DCMU	165.0	57.4
Light incubated cells + 1×10^{-7} M shikonin acetate + 1×10^{-5} M DCMU	80.8	28.1

[a] Photophosphorylation was measured as nmol ATP formed/mg chlorophyll

quinone was found to donate electrons at the cytochrome bf-FeS complex in chloroplasts which is not included in the electron carriers from DCPIP donation site to methylviologen [21]. The result on the effect of shikonin acetate on PSI with durohydroquinone as electron donor and the low percentage inhibition of DCPIP photoreduction through PSII, in addition to the inhibition of PSII with water and DPC as electron donors indicated that the site of inhibition of shikonin acetate lies on the plastoquinone pool which is almost similar to the site of inhibition of DBMIB [7,22].

The involvement of plastoquinone in both cyclic and non-cyclic electron flow in chloroplasts was reported [12]. For further evidence on the effect of shikonin acetate on plastoquinone pool, inhibition of cyclic and non-cyclic photophosphorylation was done (Table V). 69–71% inhibition of both photophosphorylation reactions was recorded. However, shikonin acetate exerted very little effect on dark phosphorylation (Table V).

Finally, we can conclude that shikonin acetate has its effect on plastoquinone pool in the electron transport system of *C. fritschii*.

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