INHIBITION OF PHOTOSYNTHETIC ELECTRON TRANSFER BY AMPHOTERICIN B

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Summary. The polyene antibiotic amphotericin B inhibits photosynthetic electron transfer by Class II maize mesophyll chloroplasts, from water to FeCN, DCIP and diquat but not to plastocyanin. Photosystem 1 activity is also inhibited by amphotericin B, but ferredoxin-NADP reductase activity is not affected. The activity of all the photosynthetic electron transfer systems inhibited by amphotericin B can be restored by the addition of carrier amounts of plastocyanin. The results suggest that amphotericin B inhibits photosynthetic electron transfer by acting only at the plastocyanin site in the chain, and that the primary site of reduction of FeCN and DCIP from water by Class II chloroplasts lies on the reducing side of photosystem 1.

The photoreduction of NADP from water by chloroplasts is generally believed to occur by electron transfer through photosystem 2 and photosystem 1 acting in series (1,2). The role of the copper-containing protein, plastocyanin, is thought to be that of an electron carrier situated between the two photosystems (1,2). This communication describes the inhibitory effect of the polyene antibiotic amphotericin B on photosynthetic electron transfer, and provides evidence that the site of inhibition is located at plastocyanin.

Abbreviations: DCIP, 2,6-dichlorophenolindophenol: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea: Diquat, 1,1'-ethylene-2,2'-dipyridylium dibromide: DPCO, 1,5-diphenylcarbazone: FeCN, ferricyanide: MeV, methyl viologen: PC, plastocyanin.

MATERIALS AND METHODS

Class II mesophyll chloroplasts were isolated from maize plants (Zea mays var. GH128) as previously described (3). The photoreduction of FeCN, DCIP, and plastocyanin was measured with an Aminco-Chance dual wavelength spectrophotometer as previously described (3), except that each reaction mixture contained 3.0 µg chlorophyll and the substrates were potassium ferricyanide 333 µM, DCIP 17 µM, and oxidized plastocyanin 25 µM. The disproportionation of DPCO was measured in a similar fashion except that Tricine buffer, pH 8.0, replaced phosphate buffer, pH 7.5, and the substrate was DPCO, 270 µM. Photosystem 1 activity was also measured by the autoxidation of MeV in an oxygen electrode as previously described (3), and photosystem 1 + 2 by the autoxidation of diquat in a similar reaction mixture from which DCMU, DCIP, MeV, and sodium ascorbate were omitted and diquat 33 µM was added. Ferredoxin-NADP reductase activity was measured by the diaphorase assay (4).

Plastocyanin was isolated from bean leaves (Phaseolus vulgaris) by the method of Milne and Wells (5). Amphotericin B (Calbiochem) was dissolved in dimethylformamide:HCl (99:1) v/v, and aliquots of the antibiotic solution mixed with a chloroplast suspension to give a chloroplast concentration of 300 µg chlorophyll/ml, an amphotericin B concentration of 0.3 mM, and a dimethylformamide concentration of 5% (v/v). Controls contained 5% v/v dimethylformamide:HCl (99:1). The chloroplast suspensions were incubated at 25^{o} for 60 min before assay of photochemical activity.

RESULTS

The effect of amphotericin B on a number of photochemical reactions of maize mesophyll chloroplasts is shown in Table 1,

TABLE 1

INHIBITION OF PHOTOCHEMICAL ACTIVITY BY AMPHOTERICIN B[a]

	ACTIVITY μmoles substrate min^{-1} mg $chlorophyll^{-1}$			
	CONTROL		AMPHOTERICIN B	
	-PC	+PC 6μM	-PC	+PC 6μM
A. H_2O→Diquat	1.47	1.75	0.24	1.28
B. Ferredoxin Reductase	0.09	-	0.11	-
C. Ascorbate/DCIP→Methyl Viologen	5.94	6.66	1.80	7.02
D. Diphenylcarbazone	25.8	28.8	4.5	22.9
E. H_2O→Plastocyanin 25μM	4.24	-	4.87	-
F. H_2O→Plastocyanin 6μM	0.83	-	0.76	-
G. H_2O→Ferricyanide	8.83	9.38	2.94	9.20
H. H_2O→DCIP	1.63	1.45[b]	0.30	1.40[b]

[a]All values are corrected for dark activity.

[b]As plastocyanin and DCIP reduction are measured at the same wavelength, this value may not be the maximum. It was obtained by subtracting the maximum rate of plastocyanin reduction from the rate observed, but the relative rates of reduction of plastocyanin and DCIP in a reaction containing both substrates is not known.

and the sites at which these reactions are believed to occur are shown in Figure 1. The photoreduction of diquat from water (A, Table 1 and Fig. 1) occurs on the reducing side of photosystem 1 (6) and this reaction is therefore a measure of the coupled system, photosystem 2 + 1. No loss of activity occurred in the control sample after 60 min incubation at 25°, as previously demonstrated (3), but severe inhibition occurs in the presence of

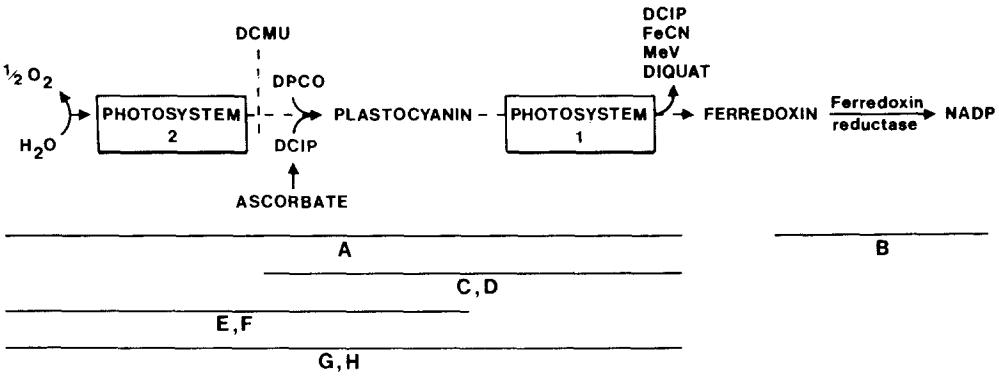

Fig. 1. Partial reactions of photosynthetic electron transfer.

amphotericin B. The addition of plastocyanin slightly stimulated the activity of the control but substantially relieved the inhibition caused by amphotericin B.

The activity of ferredoxin-NADP reductase (B) was not inhibited by amphotericin B, but photosystem 1 activity, measured by the autoxidation of MeV (C) (7) or by the disproportionation of DPCO (D) (8), was strongly inhibited. In both cases, however, activity was restored to the inhibited sample by the addition of plastocyanin.

The reduction of FeCN (G) or DCIP (H) from water, which are commonly used as assays of photosystem 2 activity (1), were both inhibited by amphotericin B. However, the reduction of plastocyanin from water was not affected by the antibiotic, regardless of whether the plastocyanin was present in saturating (E) or limiting (F) concentrations. Reduction of all three substrates was sensitive to DCMU. The addition of carrier amounts of plastocyanin overcame the inhibition of FeCN and DCIP reduction by amphotericin B.

Incubation of chloroplasts with amphotericin B under the

conditions described did not result in disintegration of the chloroplast. After 60 min incubation at 25°, only 10% of the total chlorophyll was present in the supernatant if the chloroplast suspension was centrifuged at 10,000 x g for 60 sec (Beckman Microfuge). A similar amount was present in the supernatant of the control sample. No differences between control and amphotericin B treated chloroplasts could be detected by electron microscopy of thin sections.

DISCUSSION

The results demonstrate five partial reactions of photosynthetic electron transfer from water to NADP which are sensitive to the polyene antibiotic amphotericin B. In each case, activity is substantially or completely overcome by addition of the photosynthetic electron transfer protein plastocyanin. The photoreduction of plastocyanin from water is not inhibited by amphotericin B, suggesting that the mode of action of the antibiotic is to remove plastocyanin from its site in the electron transfer chain. Plastocyanin could be detected in the 100,000 x g supernatant of chloroplasts treated with amphotericin B as has also been demonstrated for filipin (3), another polyene antibiotic.

The ability of plastocyanin to relieve the inhibition by amphotericin B of photoreduction of FeCN and DCIP from water, suggests that in Class II maize mesophyll chloroplasts the site of donation of electrons to FeCN and DCIP lies on the reducing side of photosystem 1. If FeCN is reduced in Class II chloroplasts by photosystem 2 alone, then its reduction should not be inhibited by amphotericin B, since the flow of electrons from water to plastocyanin is not inhibited by the antibiotic. FeCN can be chemically reduced by reduced plastocyanin. If this

reaction is occurring in chloroplasts treated with amphotericin B, then the observed rate of reduction of FeCN in the presence of plastocyanin should be the sum of the rate of photoreduction of ferricyanide alone plus the rate of photoreduction of plastocyanin. However, the actual rate of reduction of FeCN by amphotericin B-treated chloroplasts in the presence of 6 μM plastocyanin is more than twice the sum of the rates of reduction of the individual components. It is therefore concluded that in Class II chloroplasts the site of reduction of FeCN lies on the reducing side of photosystem 1. A similar conclusion can be drawn for the site of reduction of DCIP.

The involvement of the photosystem 1 reaction centre in the photoreduction of FeCN and DCIP from water has been suggested by other workers. Govindjee and Bazzaz (9) demonstrated the Emerson effect for the photoreduction of FeCN from water and concluded that at high light intensity, the reduction depended on the co-operation of both photosystems 1 and 2. Using the plastoquinone antagonist dibromothymoquinone, Böhme et al. (10) concluded that FeCN and DCIP are preferentially reduced by photosystem 1 in Class II chloroplasts, and Kimimura and Katoh (11) concluded that FeCN and DCIP accept electrons mainly at the reducing side of photosystem 1 in intact chloroplasts.

The most commonly used techniques for removal of plastocyanin from chloroplasts are sonication, passage through a French press, or detergent extraction, all of which result in fragmentation of the chloroplasts (12). While sonication and passage through a French press result in a lower capacity for photoreduction of FeCN, these treatments also greatly suppress the inhibitory effect on FeCN photoreduction of amphotericin B (unpublished results) and another polyene antibiotic, filipin (3). This observation would

support the proposal (13,14) that in sonicated chloroplasts the photoreduction of FeCN occurs by photosystem 2 alone.

The results indicate that amphotericin B may be a suitable reagent for the reversible inhibition of photosynthetic electron transfer under conditions where fragmentation of the chloroplasts is not desirable.

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