CHELATOR-SENSITIVE SITES IN CHLOROPLAST ELECTRON TRANSPORT[1,2]

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Received July 26, 1974

Summary: The effect of various chelators (orthophenanthroline, bathophenanthroline, bathophenanthroline sulfonate and bathocuproine) on electron transport of spinach chloroplasts has been studied by means of various photosystem I and II reactions. It was found that photosystem II has at least 3 chelator-sensitive sites, photosystem I from 3-4. An uncoupler-affected site was found in each photosystem. In addition, photosystem I had a stimulator site and a soak site. The soak site was sensitive to chelators only after a period of incubation with the chelator.

Introduction

Orthophenanthroline has been known to inhibit a site between photosystems I and II in spinach chloroplasts before the introduction of DCMU (1,2). Arnon, Tsujimoto and McSwain (3) found that orthophenanthroline strongly inhibited non-cyclic photophosphorylation. Satoh (4) studied the effect of various orthophenanthroline derivatives on chloroplast electron transport. He found that 4,7-dimethyl-1,10-phenanthroline was the most potent inhibitor of indophenol reduction and bathophenanthroline was a poor inhibitor.

In this study it was decided to find out if photophosphorylation and the reduction of indophenol were the only sites in chloroplasts affected by chelators. We, therefore, studied the effect of orthophenanthroline, bathophenanthroline and bathophenanthroline sulfonate on total electron transport and various partial reactions. We found several other chelator-sensitive sites and wish to characterize them in more detail in this communication.

[1]Supported by NSF Grant GB 27501 AI

[2]Abbreviations used: CCCP-carbonylcyanide-3-chlorophenyl hydrazone; DCMU-3-(3,4-dichlorophenyl)-1,1-dimethyurea; DAD-diaminodurene; MV-methyl viologen; PS I-photosystem I; PS II photosystem II; TMPD-N,N,N',N' - tetramethyl-p-phenylenediamine.

Materials and Methods

Spinach leaves were obtained from the local market. Chloroplasts were prepared according to the method of Jagendorf and Avron (5). Most PS I and II assays were performed as reported previously (6). Silicomolybdic acid reduction by water in PS II is described in greater detail by Giaquinta, Dilley, Crane and Barr (7). Non-enzymatic reduction of cytochrome c by silicomolybdic acid has been reported by Glenn and Crane (8). Reduction of silicomolybdic acid by diphenylcarbazide was carried out by following the reduction of cytochrome c by reduced silicomolybdic acid at 550 nm. The reaction mixture contained in 3 ml total volume: chloroplasts (50 µg chlorophyll), 150 µmoles Trizma-Mes, pH 7.0, 30 µmoles $MgCl_2$, 12 µmoles NH_4Cl, 0.2 mg silicomolybdic acid, and 1.5 µmoles diphenylcarbazide. DCMU was added where indicated.

Orthophenanthroline, bathophenanthroline, bathocuproine and bathophenanthroline sulfonate were purchased from Sigma or K and K Biochemicals. Solutions containing 2 mg/ml of the various chelators were dissolved in hot ethanol except bathophenanthroline sulfonate which is water-soluble. Appropriate ethanol addition was made to controls. The uncoupler, CCCP, was added in the required amounts from a 1 mM solution in methanol. The degree of prevention of chelator inhibition by the uncoupler was derived from a comparison between control versus chelator-inhibited rate against uncoupler versus uncoupler plus chelator rate.

Results and Discussion

Table 1 shows that a chelator-sensitive site close to the water-oxidation end of the electron transport chain occurs in the water→silicomolybdate pathway. This site is sensitive to both ortho- and bathophenanthroline and may involve the Mn of PS II. This site is different from the traditional orthophenanthroline and DCMU-sensitive sites because silicomolybdate accepts electrons before the DCMU block, possibly at Q (7).

The diphenylcarbazide→silicomolybdate pathway also shows ortho- and

Table 1

Chelator Effects on Chloroplast Photosystem II Reactions

Reaction	Rate[1]	Inhibition (%)
H_2O→silicomolybdate (Mn site)	313	
plus DCMU	299	5
plus 0.07 mg OP/ml[2]	134	58
plus 0.2 mg BP/ml[3]	164	48
Diphenylcarbazide→silicomolybdate	222	
plus DCMU	189	15
plus 0.27 mg OP/ml	137	38
plus 0.27 mg BP/ml	103	54
H_2O→ferricyanide (OP and BP sites)	306	
plus DCMU	0	100
plus 0.07 mg OP/ml	85	72
plus 0.07 mg BP/ml	163	47

[1] μequivalents O_2 evolved/mg chlorophyll/hr

[2] OP-orthophenanthroline

[3] BP-bathophenanthroline

bathophenanthroline inhibition but higher concentrations of chelators are necessary. This site is also located before the DCMU block because the diphenylcarbazide→silicomolybdic acid pathway is insensitive to DCMU inhibition.

The water→ferricyanide pathway involves the traditional orthophenanthroline/DCMU-sensitive site (2). This site is also sensitive to bathophenanthroline, or 2 different sites may be involved. According to the water→ferricyanide reaction in presence of ammonia and dibromothymoquinone (Table 2), the orthophenanthroline inhibition site is less protected by the addition of the uncoupler, CCCP, than the bathophenanthroline site (53 versus 9% inhibition).

Table 2

Prevention of Chelator Inhibition by Uncouplers in Photosystem II

Reaction	Rate[1]	Inhibition (%)
$H_2O \rightarrow K_3Fe(CN)_6$ (plus NH_4 and DBMIB[2])	267	--
Control plus 0.07 mg OP/ml	85	68
" 0.005 µmoles CCCP	221	-
" CCCP and OP	104	53
" 0.07 mg BP/ml	163	39
" 0.005 µmoles CCCP	221	--
" CCCP and BP	202	9
$H_2O \rightarrow$ DMBQ[3] (plus DBMIB)	325	--
Control plus 0.07 mg BP/ml	195	40
" 0.005 µmoles CCCP	195	--
" CCCP and BP	254	24

[1] µequivalents O_2 evolved/mg chlorophyll/hr

[2] DBMIB - 2,5-dibromothymoquinone

[3] DMBQ-dimethyl benzoquinone

In the water→dimethyl benzoquinone pathway in presence of dibromothymoquinone, CCCP has no effect on the orthophenanthroline site but still prevents inhibition of the bathophenanthroline site although to a lesser degree than in the water→ferricyanide pathway. The protection against inhibition by chelators is remarkable because it is observed in the presence of ammonium ions which destroy proton gradients.

In the upper portion of PS I, with ascorbate plus DAD as electron donors and methyl viologen as the electron acceptor, there is another chelator-sensitive site (Table 3) which can be inhibited by a narrow range of

Table 3

The Effect of Bathophenanthroline on Chloroplast Photosystem I with DAD and TMPD as Electron Donors (PS I Coupler Site)

Reaction	Rate[1]	Change (%)
Asc. plus DAD → MV (plus DCMU and DBMIB[2])	234	--
Control plus 0.03 mg BP/ml	195	-17
" 0.01 µmoles CCCP	189	--
" CCCP and BP	234	+23
Asc. plus TMPD → MV (plus DCMU)	371	--
Control plus 0.03 mg BP/ml	585	+58
" 0.01 µmoles CCCP	559	--
" CCCP and BP	637	+14

[1] µmoles O_2 evolved/mg chlorophyll/hr

[2] DBMIB - 2,5 dibromothymoquinone

bathophenanthroline concentrations (0.02-0.03 mg/ml) in well coupled chloroplasts; lower concentrations don't inhibit while higher concentrations stimulate. In presence of CCCP, inhibition by bathophenanthroline is again prevented to a degree which makes this the uncoupler-sensitive site of PS I. Inhibition by orthophenanthroline of this site is hard to obtain, and prevention of inhibition by CCCP does not occur.

A similar concentration of bathophenanthroline used in the reaction ascorbate plus TMPD→methyl viologen in presence of DCMU gives no inhibition although in presence of CCCP a change in the rate can be observed (Table 3).

If higher concentrations of batho- or orthophenanthroline than shown in Table 3 are used, a chelator-stimulated site is seen in the ascorbate plus TMPD→methyl viologen pathway (Table 4). This stimulation is increased

Table 4

Uncoupler Effects on 2 Chelator Stimulator Sites of Photosystem I

Reaction	Rate[1]	Stimulation (%)
Ascorbate plus TMPD→MV (plus DCMU)	182	0
" " plus 0.2 mg BP	442	+170
" " plus 0.01 µmoles CCCP	98	-40
" " plus 0.01 µmoles CCCP plus 0.2 mg BP	501	+410
Ascorbate plus 0.3 M $K_4Fe(CN)_6$ (plus DBMIB[2])→MV		
Control	150	0
" plus 0.2 mg BP	309	+110
" plus 0.01 µmoles CCCP	153	0
" plus 0.01 µmoles CCCP plus 0.2 mg BP	358	+130

[1] µmoles O_2 evolved/mg chlorophyll/hr.

[2] DBMIB - 2,5-dibromo thymoquinone

more than two-fold in presence of CCCP. Under similar conditions but with ascorbate plus ferrocyanide as electron donors, a lesser degree of stimulation is seen which indicates that ferrocyanide feeds in electrons slightly past the main chelator stimulation site seen in the ascorbate plus TMPD→methyl viologen pathway. The presence of CCCP in conjunction with bathophenanthroline shows only a slight stimulation (Table 4).

The PS I chelator-stimulation site may be the same site described by Arnon, Tsujimoto and McSwain (9) who found that cyclic photophosphorylation was markedly stimulated by orthophenanthroline.

If chloroplasts are incubated with bathophenanthroline or bathocuproine and assayed for activity over a period of 4 hrs. (Fig. 1), different results

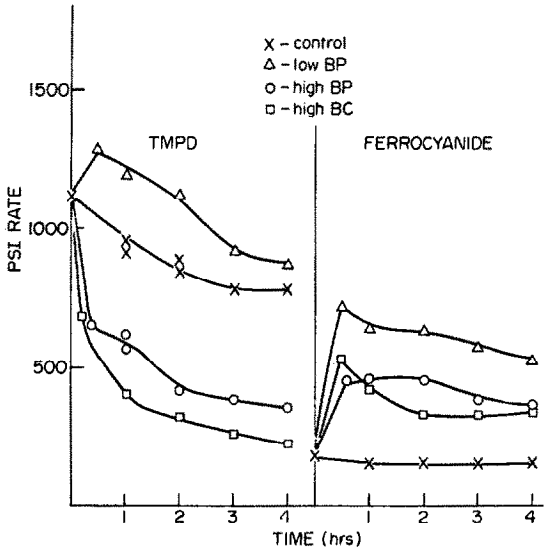

Figure 1. The effect of incubating chloroplasts with various chelators on the ascorbate plus TMPD→MV reaction and on the ascorbate plus ferrocyanide→MV reaction. Low BP denotes incubating chloroplasts with 0.01 mg BP/assay containing chloroplasts with 50 µg chlorophyll, high BP -0.04 mg BP, high BC -0.04 mg BC. The reaction rates were assayed over a period of 4 hrs. BP - bathophenanthroline, BC - bathocuproine

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are obtained by various PS I partial reactions. Low concentrations of bathophenanthroline (0.01 mg/assay) only show the stimulation site described earlier but high concentrations of bathophenanthroline or bathocuproine (0.04 mg/assay) inhibit the ascorbate plus TMPD→methyl viologen pathway within the first hour. In this case incubation with the chelator affects plastocyanin, as had been shown for incubation of chloroplasts with KCN by Izawa et al. (10) or with $HgCl_2$ by Kimimura and Katoh (11). Katoh and San Pietro (12) had shown before that the addition of chelators to chloroplasts did not affect plastocyanin instantaneously.

Figure 1 also shows that incubation with the chelator does not inhibit the ascorbate plus ferrocyanide→methyl viologen pathway. The stimulation effect seen in presence of all chelators diminishes with time.

Bathophenanthroline sulfonate inhibits electron transport from water→ NADP but not the water→methyl viologen reaction. This implies that a

chelator-sensitive site is located between methyl viologen and NADP. Added ferredoxin does not reverse this inhibition.

The application of chelators to partial reactions involving PS I thus shows the existence of several chelator-sensitive sites: an uncoupler site, a stimulation site, a soak site, and a bathophenanthroline sulfonate sensitive site. Inhibition of the soak site can be restored by exogenous plastocyanin. The variety of responses to chelators exhibited by the electron transport chain of spinach chloroplasts may be due to different modes of action on a variety of components in the chain, some still unknown.

References

1. Izawa, S. and Good, N.E. (1972) Methods in Enzymol., vol. XXIV, part B, pp. 355-377, Academic Press, New York.
2. Nishimura, M. (1966) Brookhaven Symp. Biol. 19, 132-142.
3. Arnon, D. I., Tsujimoto, H.Y. and McSwain, B.D. (1967) Nature 214, 562-566.
4. Satoh, K. (1974) Biochim. Biophys. Acta 333, 127-135.
5. Jagendorf, A.T. and Avron, M. (1958) J. Biol. Chem. 231, 277-290.
6. Brand, J., Baszynski, T., Crane, F.L. and Krogmann, D. W. (1972) J. Biol. Chem. 247, 2814-2819.
7. Giaquinta, R.T., Dilley, R.A., Crane, F.L. and Barr, R. (accepted for publication by BBRC).
8. Glenn, J.L. and Crane, F.L. (1956) Biochim. Biophys. Acta 22, 111-115.
9. Arnon, D.I., Tsujimoto, H.Y. and McSwain, B.D. (1967) Nature 214, 562-566.
10. Izawa, S., Kraayenhof, R., Ruuge, E.K. and DeVault, D. (1973) Biochim. Biophys. Acta 314, 328-339.
11. Kimimura, M. and Katoh, S. (1972) Biochim. Biophys. Acta 283, 279-292.
12. Katoh, S. and San Pietro, A. (1966) Biochem. Biophys. Res. Communs. 24, 903-908.