Biochimica et Biophysica Acta, 546 (1979) 77—83

BBA 47644

CATECHOLS STIMULATE FERRICYANIDE REDUCTION IN CHLOROPLAST PHOTOSYSTEM II

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(Received August 16th, 1978)

Key words: Catechol stimulation; Ferricyanide reduction; O_2 evolution; Photosystem II; (Chloroplast)

Summary

In isolated chloroplasts (*Spinacia oleracea*), where electron transport to Photosystem I is blocked by the plastoquinone antagonist, dibromothymoquinone, lipophilic catechols in concentrations of 50—150 μM stimulate ferricyanide reduction in Photosystem II and associated O_2 evolution.

Non-permeating catechols, such as Tiron, are unable to stimulate this reaction. Those quinones, such as 2,5-dimethylbenzoquinone, which act as class III electron acceptors, do not lead to stimulation of ferricyanide reduction in Photosystem II or stimulation of associated O_2 evolution, when electron transport to Photosystem I is blocked by dibromoquinone.

Stimulation of ferricyanide reduction is not observed in Tris-treated chloroplasts, implying that electron donation to Photosystem II by catechols is not responsible for the stimulation.

Various mechanisms for this stimulation in class II chloroplasts are discussed.

Introduction

Izawa [1] has reported low photophosphorylation rates in EDTA-hydroxylamine-treated chloroplasts, using catechol as electron donor to Photosystem II, with electron transport to Photosystem I blocked by dibromothymoquinone (DBMIB). Other investigators [2,3] have used catechol as an electron donor to

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Abbreviations: DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; DMBQ, 2,5-dimethylbenzoquinone; Tiron, 4,5-dihydroxy-1,3-benzenedisulfonic acid (sodium salt).

Photosystem II in chloroplasts in which the O_2 evoluation system has been destroyed by different treatments. In this study, we have tested various catechols as stimulators of ferricyanide reduction in Photosystem II in presence of dibromothymoquinone or associated O_2 evolution in class II chloroplasts with an intact O_2 evolution system. Treatments are described, which differentiate this reaction from previously reported electron donor functions for catechol in isolated chloroplasts. Mechanisms for the stimulation of ferricyanide reduction and associated O_2 evolution in presence of catechols are discussed in class II chloroplasts in which electron transport has been blocked by dibromothymoquinone [4,5] or KCN treatment [6].

Materials and Methods

Chloroplasts were prepared from market spinach (*Spinacea oleracea*) in 0.4 M sucrose/0.05 M NaCl buffer as previously described [7]. Chlorophyll was determined according to Arnon [8]. The final chloroplast suspension in the sucrose/NaCl buffer contained mainly class II chloroplasts.

O_2 evolution was measured with a Clark-type oxygen electrode. Chloroplasts were illuminated with white light ($2.5 \cdot 10^6$ ergs $\cdot$ cm$^{-2} \cdot$ s^{-1}). Reaction mixtures are given in the various figure legends.

Tris-treated chloroplasts for the inactivation of the O_2 evolution system were prepared according to the method of Yamashita and Butler [9]. KCN-treated chloroplasts were prepared according to Ouitrakul and Izawa [6].

Results and Discussion

Ferricyanide is a nonpenetrating electron acceptor, which can be used to test chloroplasts for fragmentation [10]. According to Böhme et al. [5] broken chloroplasts are insensitive to dibromothymoquinone inhibition. Ferricyanide normally accepts electrons in Photosystem I [11]. However, Trebst and Reimer [12] and Heathcote and Hall [13] have shown that in chloroplasts, in which electron transport to Photosystem I has been inhibited by dibromothymoquinone, ferricyanide acts as a Photosystem II electron acceptor. In lettuce chloroplasts at low pH, ferricyanide also accepts electrons exclusively from Photosystem II, as shown by Ben-Hayyim et al. [14].

In this study, we provide evidence for a different type of ferricyanide acceptor site in Photosystem II, which occurs only in presence of lipophilic catechols, such as 4-*tert*-octylcatechol (Fig. 1). The pH optimum for this reaction is shown in Fig. 2. Only lipophilic, membrane-penetrating pyrocatechol (Fig. 3) or quercetin (Fig. 4) stimulate ferricyanide reduction or associated O_2 evolution in Photosystem II in presence of dibromothymoquinone. Coumarin (Fig. 5) is also a stimulator like 4-*tert*-octylcatechol, but a nonpenetrating phenol like Tiron, 2,3-dihydroxybenzoic acid or those quinones, which act as class III electron acceptors according to Saha et al. [15], are unable to stimulate ferricyanide reduction in Photosystem II or associated O_2 evolution.

The properties of the new 4-*tert*-octylcatechol reaction are summarized in Table I. The 4-*tert*-octylcatechol pathway does not work in Tris-washed chloroplasts, in which the O_2 evolution mechanism has been inactivated [9], but it

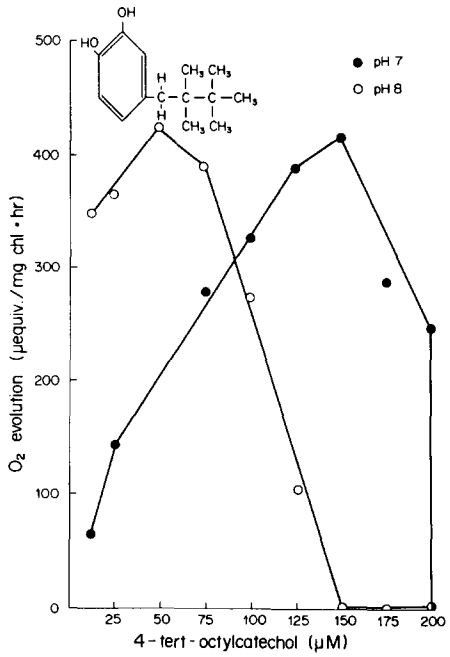

Fig. 1. Stimulation of Photosystem II ferricyanide (FeCy) reduction and associated O_2 evolution in presence of dibromothyoquinone by various concentrations of 4-*tert*-octylcatechol (TOC). Reaction mixtures contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes (pH 7 or 8), 2 mM NH_4Cl, 3 mM $MgCl_2$, 0.5 mM FeCN, 2 μM DBMIB and TOC at various concentrations as indicated.

works in KCN-treated chloroplasts, in which the pathway to Photosystem I has been blocked by removal of plastocyanin [6]. Whitmarsh and Cramer [16] have recently found that in KCN-treated chloroplasts cyclic electron flow around Photosystem II could occur, involving cytochrome *b*-559. Since the

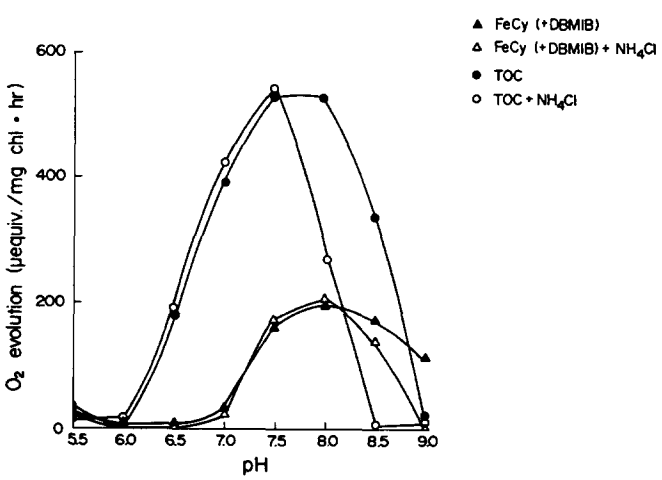

Fig. 2. Variations in 4-*tert*-octylcatechol-induced stimulation of ferricyanide (FeCy) reduction and associated O_2 evolution in Photosystem II of spinach chloroplasts according to pH. Reaction conditions are as in Fig. 1, except that the pH was varied from 5.5 to 9.0.

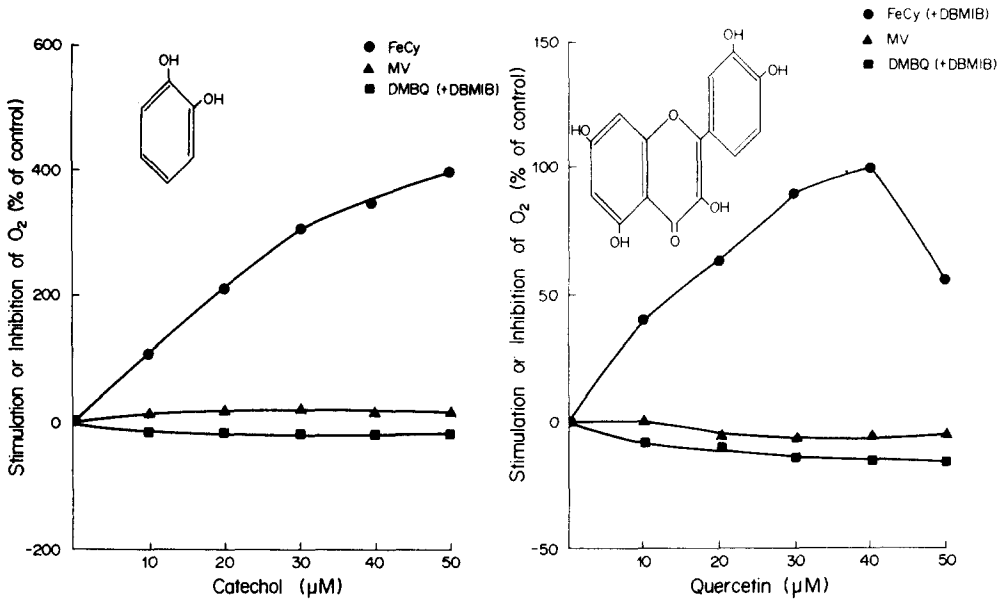

Fig. 3. Effect of catechol on ferricyanide reduction and associated O_2 evolution in Photosystem II of spinach chlorplasts. Reaction mixtures contained chloroplasts (50 μg chlorophyll). Reactions were run in 25 mM Tris-Mes (pH 7) in the presence of 2 mM NH_4Cl, 3 mM $MgCl_2$, 2 μM DBMIB and 0.5 mM ferricyanide, with catechol added in the indicated concentrations. In other assays 0.5 mM methyl viologen (MV) or 0.75 mM DMBQ was used instead of ferricyanide. +, stimulation; —, inhibition. Control rate: 45 μequiv./mg chlorophyll per h.

Fig. 4. Effect of quercetin of ferricyanide reduction and associated O_2 evolution in Photosystem II of spinach chloroplasts. Reaction conditions are the same as in Fig. 3, except that quercetin was added instead of catechol at the indicated concentrations. +, stimulation, —, inhibition. Control rate: 40 μequiv./mg chlorphyll per h. MV, methyl viologen.

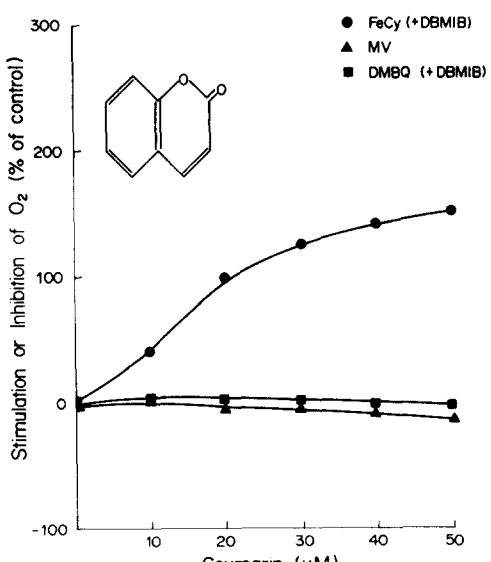

Fig. 5. Effect of coumarin on fericyanide reduction and associated O_2 evolution in Photosystem II of spinach chloroplasts. Reaction conditions as in Fig. 3, except that coumarin was added at the indicated concentrations instead of catechol. +, stimulation; —, inhibition. Control rate: 73 μequiv./mg chlorophyll per h. MV, methyl viologen

TABLE I

INHIBITION OF 4-*tert*-OCTYLCATECHOL-INDUCED STIMULATION OF FERRICYANIDE REDUCTION AND ASSOCIATED O_2 EVOLUTION IN PHOTOSYSTEM II OF SPINACH CHLOROPLASTS

Reaction mixture for $H_2O \rightarrow$ FeCN (+DBMIB and TOC) contained chloroplasts (50 μg chlorophyll), 25 mM Tris-Mes (pH 7), 2 mM NH_4Cl, 3 mM $MgCl_2$, 0.5 mM FeCN, 2 μM DBMIB and 0.1 mM TOC. Reaction mixtures for $H_2O \rightarrow$ DMBQ (+DBMIB) were the same except 0.75 mM DMBQ was used instead of FeCN and there was no TOC present. TOC = 4-*tert*-octylcatechol.

Inhibitor	Concentration (μM)	$H_2O \rightarrow$ FeCN (+DBMIB and TOC) (μequiv. O_2/mg chlorophyll per h)	Inhibition (%)	$H_2O \rightarrow$ DMBQ (+DBMIB) (μequiv. O_2/mg chlorophyll per h)	Inhibition (%)
None	—	520	0	660	0
DCMU	6	0	100	0	100
Dibromothymoquinone	2	520	0	660	0
o-Phenanthroline	7.5	0	100	0	100
8-Hydroxyquinoline	15	0	100	528	20
Picryl hydrazyl	500	208	40	65	75

4-*tert*-octylcatechol reaction is sensitive to DCMU, but not to dibromothymoquinone, and since it was not affected by the KCN treatment of chloroplasts, it is possible that the 4-*tert*-octylcatechol reaction also involves cyclic electron flow around Photosystem II. Other alternatives to interpret the mechanism of the 4-*tert*-octylcatechol reaction, such as electron donation to Photosystem II by 4-*tert*-octylcatechol or by other catechols, as described by Izawa in EDTA-hydroxylamine-inactivated chloroplasts [1] appears inlikely in this case, because O_2 evolution, not O_2 uptake as in Izawa's case, is measured in the 4-*tert*-octylcatechol reaction. Another alternative, such as bypassing the DBMIB block on the inside of the thylakoid membrane, as in the case of phenylenediamine described by Selman [7], can be excluded on the basis of the 4-*tert*-octylcatechol reaction remaining unaffected by washing chloroplasts with KCN.

4-*tert*-Octylcatechol can act as a chelator. It has previously been shown by Crane and Barr [18] that it inhibits the DCMU-insensitive silicomolybdate reduction in Photosystem II, while stimulating ferricyanide reduction in the presence of dibromothymoquinone. The mechanism proposed by Crane and Barr [18] for stimulation of forward electron transport, when silicomolybdate reduction is inhibited, probably does not apply to the 4-*tert*octylcatechol action observed in the present study, because coumarin, a non-chelatig agent, also stimulates ferricyanide reduction in Photosystem II, when forward electron transport is inhibited by dibromothymoquinone. Inhibition of 4-*tert*-octylcatechol stimulation of ferricyanide reduction (Table I) by 8-hydroxyquinoline, a Cu^{2+} chelator, may indicate that a copper-containing Photosystem II protein is involved in this patthway. Since dimethylbenzoquinone reduction by Photosystem II is only inhibited 20%, while ferricyanide reduction is totally inhibited by 8-hydroxyquinoline, it is possible that there is a divergence of electron transport pathways before the class III acceptor site. Cytochrome *b*-559 may be involved in the 4-*tert*-octylcatechol stimulated ferricyanide reduction or associated O_2 evolution in Photosystem II as outlined in Fig. 6. Whitmarsh and

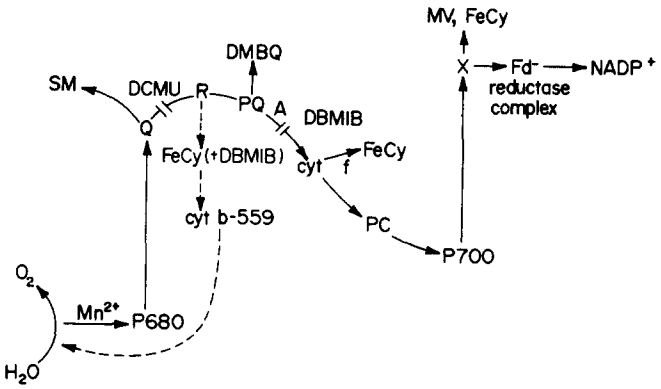

Fig. 6. Scheme of the electron transport in spinach chloroplasts, showing a cyclic pathway around Photosystem II and including a site for ferricyanide reduction. 4-*tert*-Octylcatechol stimulates ferricyanide reduction in the presnce of DBMIB in Photosystem II, possibly involving cytochrome *b*-559. MV, methyl viologen; PQ, plastoquinone; PC, plastocyanin; SM, silicomolybdic acid; R, an unknown electron acceptor, presumed to be a special type of PQ A.

Cramer [16] have shown a similar pathway in Photosystem II.

The fact that coumarin (Fig. 5) can also stimulate the dibromothymoquinone-insensitive ferricyanide reduction in Photosystem II indicates that the stimulation is not dependent on the redox function of the stimulating compound. The effectiveness of quercetin (Fig. 4), an energy transfer inhibitor [19], which binds to coupling factor 1, but acts as a stimulator of ferricyanide reduction in Photosystem II , may point to a control mechanism of electron transport in Photosystem II.

The stimulation of ferricyanide reduction or associated O_2 evolution in Photosystem II by catechols cannot be explained entirely on the basis of chemical oxidation of catechols to the corresponding quinones by ferricyanide in the reaction mixture. When these quinones are used in the catechol-stimulated reaction in place of catechols, no stimulation of ferricyanide reduction in presence of dibromothymoquinone is observed, although these quinones act as class III electron acceptors and give good Photosystem II electron transport rates. Therefore, the interaction between catechols and ferricyanide, leading to stimulation of ferricyanide reduction by Photosystem II must involve a chloroplast component other than the class III electron acceptor site.

Acknowledgements

This study was supported by N.S.F. grant 7713047. F.L. Crane is recipient of an NIH research career award GM K6-21,839.

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