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EFFECTS OF ASCORBATE ON O_2-CONSUMPTION MEDIATED BY METHYL-VIOLOGEN IN CHLOROPLASTS[1]

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Abstract

Ascorbate stimulates the rate of electron transport (measured by the sum of O_2 consumptions in light and a transient O_2 evolution following the cessation of illumination) slightly in the presence of superoxide dismutase, the extent of rate increment is larger in the absence of gramicidin D than in the presence of the uncoupler. It is suggested that ascorbate may donate, bypassing partially a rate-limiting site, electron to Photosystem II in normal chloroplasts.

Introduction

Elstner *et al.* (1970) and Epel and Neumann (1972) predicted the involvement of superoxide radical ions in ascorbate photooxidation by chloroplasts. And the radical reaction mechanism proposed by Epel and Neumann (1972) was subsequently confirmed by the observations that superoxide dismutase reversed the ascorbate-stimulated rate of O_2 consumption (Allen and Hall, 1973; Ort and Izawa, 1974). Both Allen and Hall (1973) and Ort and Izawa (1974) groups concluded that in normal chloroplasts, ascorbate may not replace water as the electron donor.

However, the confirmation of the involvement of superoxide radical reaction in the photooxidation of ascorbate is one thing, and the disproving of the electron donation capability of ascorbate to Photosystem II, which has been suggested by workers of earlier studies of the photooxidation of ascorbate by isolated chloroplasts (Böhme and Trebst, 1969, and references quoted therein), is another matter. And whether ascorbate can replace H_2O as the electron donor is still another thing. The electron donation capability of ascorbate to Photosystem II is suggested by the observation that ascorbate stimulates chlorophyll a fluorescence of isolated chloroplasts (Li, 1978a).

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We have undertaken a study on the ascorbate effects on photosynthetic electron transport mediated by methylviologen, using chloroplasts prepared the same way as we did in the fluorescence studies (Li, 1978a), where ascorbate is suggested to donate electron to Photosystem II. And we have found that ascorbate, even in the presence of superoxide dismutase, stimulates slightly electron transport (see also Ort and Izawa, 1974), which, among other reasons (see later this report), corroborates the fluorescence study (Li, 1978a), suggesting that ascorbate may be able to donate electron to Photosystem II. The reason that its effect on electron transport is small may be explained by the existing of a rate limiting site of electron transport located after the primary electron acceptor (Q)—the molecule its redox states dictates the intensity of fluorescence yield—of Photosystem II.

Materials and Methods

Lettuce or oat chloroplasts were prepared according to the methods described in Li (1975), but the buffer system was modified, using sorbitol to replace sucrose, and the pH of the buffer was 7.3 instead of 8. Method of determination of the concentration change of O_2 was the same as described in Li (1975). Actinic light intensity was 130 kerg/cm^2-sec.

Results and Discussions

Figure 1 shows that: (1) ascorbate stimulates O_2 uptake by chloroplasts in light mediated by methyl-viologen, the stimulation can be reversed by superoxide dismutase; (2) superoxide dismutase inhibits, slightly, the rate of O_2 uptake in control chloroplasts; (3) the rate of O_2 uptake of a sample having both superoxide dismutase and ascorbate is higher than that of a sample having superoxide dismutase alone, the difference is small (in agreement with the observation of Ort and Izawa, 1974) but can be observed in all experiments; (4) the percentage stimulation of (as well as the absolute increment in) rate of O_2 consumption induced by the addition of ascorbate is higher in the absence of gramicidin than in the presence of the uncoupler.

The O_2 uptake inhibition effect (in the absence of added ascorbate) of superoxide dismutase is not always observed (results not shown). In one case, the rate inhibited in light is just compensated by an increase of the rate of O_2 evolution in dark following illumination (Table 1).

Although it is not possible to know whether the small stimulation of O_2 consumption by ascorbate in the presence of superoxide dismutase (see also Ort and Izawa, 1974) is a real stimulation of electron transport or it is just a residual superoxide reaction, but the observation that the ascorbate-induced

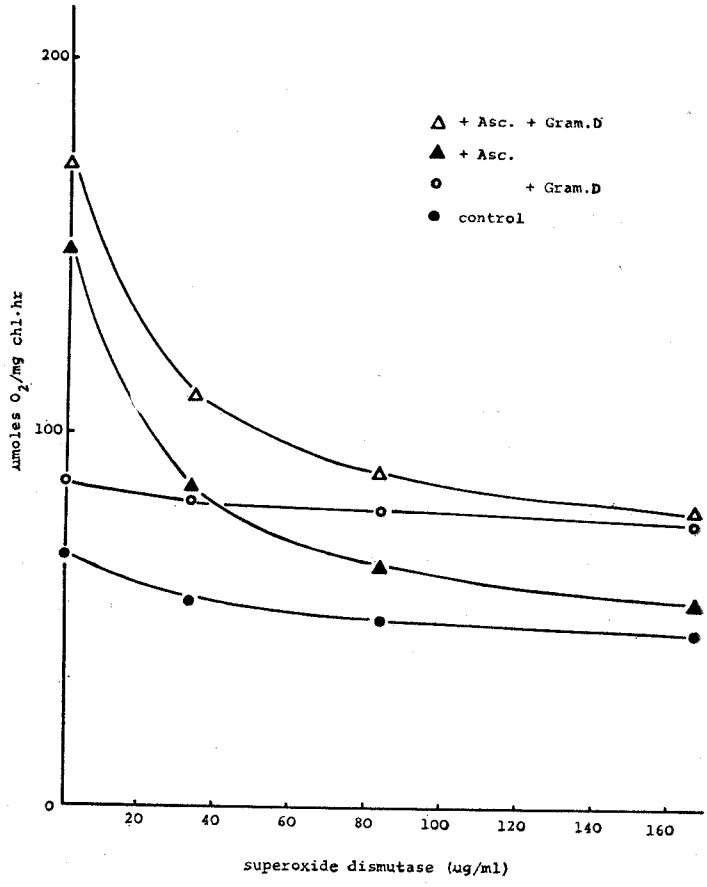

Fig. 1. Effects of superoxide dismutase on O_2 consumption in the absence of ascorbate or in the presence of ascorbate.

Oat chloroplasts: 10 μg chl/ml. Electron transport was mediated by methyl-viologen 0.1 mM. Ascorbate (asc) and gramicidin D (gram D) when added had concentration 3.3 mM and 3.3 μM, respectively.

increment of rate of O_2 consumption is higher in the absence of gramicidin D than in the presence of the uncoupler (Fig. 1) does provide an argument supporting indirectly the suggestion that ascorbate donates electron (by-passing partially a rate-limiting site) to Photosystem II, hence stimulates electron transport. The suggestion that ascorbate by-passes a limiting site and donates electrons to Photosystem II is also supported by an observation of Ort and Izawa (1974), showing that the P/2e ratio is lower for ascorbate supported electron transport (0.85) in treated chloroplasts than that for electron transport supported by other donors (1.00-1.15), or for H_2O supported electron transport (1.22), even though Ort and Izawa were reluctant to agree with the by-passing hypothesis. The fact that ascorbate stimulates fluorescence in the absence of an uncoupler (Li, 1978a), is another evidence support-

Table 1. *Superoxide dismutase's effects on O_2 consumption in the absence or in the presence of ascorbate*

Concentration of superoxide dismutase (μg/ml)	In the absence of ascorbate			In the presence of ascorbate (3.3 mM)	
	A O_2 uptake in light	B O_2 evolution in dark	Sum of A and B	O_2 uptake in light	O_2 evolution in dark
0.0	77	16	93	185	0
1.4	77	16	93	181	0
6.8	71	24	95	169	0
34.0	69	26	95	145	0
85.0	64	30	94	105	0

Lettuce chloroplasts: 10 μg chl/ml.

Electron transport was supported by methyl-viologen (0.1 mM) in the presence of gramicidin D (3.3 μM). Rate expressed in μmoles O_2/mg chl. hr. The dark rates were measured following the cessation of illumination.

ing the by-passing hypothesis of Böhme and Trebst (1969).

The differential effects of ascorbate on O_2 consumption in the absence and in the presence of gramicidin, in view of the above discussion, is consistent with the idea that in addition to the well-know coupling site located between the two Photosystems which limits the flow of electron there is an uncoupler-sensitive rate limiting site on the H_2O system side of Q (Böhme and Trebst, 1969; Cheniae, 1970; Li, 1973, 1975, 1978a, b), and ascorbate can bypass it and donates electron to Q.

In terms of electron donation, ascorbate may affect Photosystem II in several ways: (1) it may replace H_2O as the electron donor; and/or (2), it may donate extra electron to Photosystem II without affecting the H_2O donation reaction, and the site of donation can be either (a) prior to an uncoupler-sensitive rate limiting site; or (b), bypassing the mentioned limiting site; or (c) bypassing, partially, this limiting site.

Whether ascorbate can replace water as the electron donor (in which case it may or may not enhance the rate of electron transfer) is a matter can not be resolved by the present experiment. To know it one may compare ascorbate's effects on O_2 evolution supported by NADP with its effects on NADP reduction monitored by absorption changes, or with turnover measurements of electron carriers, such as Q, or plastoquinone, or cytochrome f, or P_{700} etc.

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維生素丙對於用 Methyl-viologen 為電子受子的光合電子傳送反應的影響

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在有 superoxide dismutase 存在的情形下，維生素丙略微促進光合電子傳送速率。該促進作用在沒有 uncoupler 時比在有 uncoupler 時爲大。該等現象顯示維生素丙能將其電子供輸給第二光合系統，其中一部份電子能超越一限制速率的反應步驟。