

Inhibition of Photosynthetic Electron Transport in Tobacco Chloroplasts and Thylakoids of the Blue Green Alga *Oscillatoria chalybea* by an Antiserum to Synthetic Zeaxanthin

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An antiserum to synthetic *Zeaxanthin* inhibits photosynthetic electron transport on the oxygen-evolving side of photosystem II in tobacco chloroplasts and thylakoids of the filamentous blue-green alga *Oscillatoria chalybea*. The inhibition site lies for both species between the site of electron donation of water or tetramethyl benzidine and that of diphenyl carbazide or manganese II ions. Typical photosystem I reactions are not impaired by the antiserum. The effect of the antiserum concerning the inhibition site is practically identical to that of the earlier described antiserum to violaxanthin. However, the degree of inhibition seems to be generally somewhat lower with the antiserum to *Zeaxanthin*, than with that to violaxanthin which hints at a lesser accessibility of zeaxanthin, in the thylakoid membrane in comparison to violaxanthin.

In the course of these investigations new evidence was obtained that the oxygen-evolving side of the electron transport scheme is differently organized in *Oscillatoria chalybea* when compared to tobacco chloroplasts. Thus, the silicomolybdate reduction with water as the electron donor is sensitive to DCMU in these algae.

Introduction

In earlier publications we have reported on the inhibition of photosynthetic electron transport of tobacco chloroplasts by antisera to the carotenoids lutein [1] neoxanthin [2] and violaxanthin [3]. From these studies it appeared that all the antisera inhibited electron transport on the water-splitting side of photosystem II. With various artificial donor systems to photosystem II slight differences between these three antisera were found which placed the formal individual inhibition sites at three slightly different locations on the water-splitting side of the electron transport scheme. In general, the observed degree of inhibition was low which led us to the proposal that the carotenoids to which these antisera were directed were mainly located in partition regions of the lamellar system. This was substantiated by the observation of Trosper and Allen [4] who

found that 2/3 of the lutein, neoxanthin and violaxanthin were located in partition regions, and by Schmid *et al.* [5] who found that the degree of inhibition caused by the antisera to lutein and neoxanthin in thylakoids of the blue-green algae *Nostoc muscorum* and *Oscillatoria chalybea* were around 60 per cent in comparison to 10–20 per cent in tobacco chloroplasts. The lamellar system of these blue green algae consists of single unfolded thylakoids [6]. Occasionally, we asked the question whether the antiserum action was in reality unspecific and caused by a lipoic impurity contained in the antigen preparation injected into the rabbits. We therefore have prepared an antiserum to synthetic zeaxanthin. In the following we report on the effect of this antiserum on photosynthetic electron transport reactions in tobacco chloroplasts and thylakoids of *Oscillatoria chalybea*.

Materials and Methods

Zeaxanthin (3,3'-dihydroxy- β -carotene) was synthesized by Hoffmann, La Roche & Co., Basel [7]. The product was several times recrystallised from methanol-benzene. The spectrum of this product and

Abbreviations: DPC, Diphenylcarbazine; A-2-sulf, Anthraquinone-2-sulfonate; DCP, 2,6-Dichlorophenol indophenol; Mn, Manganese-II-ions; DCMU, NN': 3,4-Dichlorophenyl dimethylurea.

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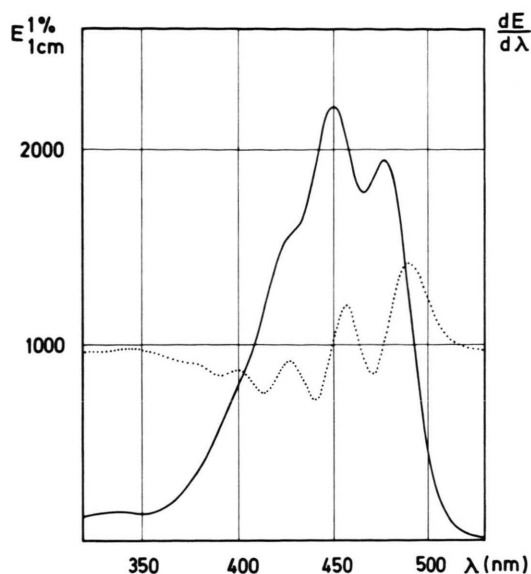


Fig. 1. Zeaxanthin spectra, solid line: spectrum of zeaxanthin in diethyl ether, the preparation which was used for injection into the rabbits. Dashed line: first derivative spectrum of zeaxanthin in diethyl ether.

the derivative spectrum are shown in Fig. 1; both spectra were obtained with a Cary-spectrometer model 118. The spectrum of the crystallized zeaxanthin dissolved in diethyl ether had maxima at 476, 449 and 425 nm, and dissolved in ethanol maxima at 479, 451 and 423 nm.

Preparation of the antiserum: Immunization of rabbits with zeaxanthin was carried out as described earlier [1, 2].

Chloroplasts of tobacco were prepared according to Homann and Schmid [8] from the Connecticut cigar variety *N. tabacum* var. John William's Broadleaf. If necessary the oxygen evolving capacity of the chloroplasts was destroyed by washing with 0.8 M Tris pH 8 according to Yamashita and Butler [9].

Thylakoid preparations of *Oscillatoria chalybea* were obtained by osmotic disruption of protoplast preparations. The protoplasts were prepared by digestion of the algae in the light at 37 °C with 0.05% glucuronidase (Boehringer) for ½ h followed by a 1 h digestion with a mixture containing 0.05% lysozyme (Sigma) and 0.3% cellulase from Kinki Yakult, Japan. The entire procedure was carried out in 0.6 M manitol.

Electron transport reactions were carried out as described previously at different occasions [10, 11].

Rates of silicomolybdate reduction were calculated using the millimolar extinction coefficient of $8 \text{ mM}^{-1} \times \text{cm}^{-1}$ described by Barr *et al.* [12].

Results

The antiserum to zeaxanthin agglutinates stroma-free swellable tobacco chloroplasts, hence zeaxanthin is located in the outer surface of the thylakoid membrane.

The antiserum affects various electron transport reactions in the region of light reaction II in tobacco chloroplasts (Table I) and thylakoids of the filamentous blue-green alga *Oscillatoria chalybea* (Table II). The general observation is that the electron transport reactions in tobacco chloroplasts are on the average

Table I. Influence of the antiserum to zeaxanthin on photosynthetic electron transport reactions in wild type tobacco chloroplasts.

Electron transport system	Control rate $\mu\text{mol O}_2$ uptake or evolution/mg chlorophyll/h	% Inhibition
$\text{H}_2\text{O} \rightarrow \text{DCPIP}$	322	19
$\text{H}_2\text{O} \rightarrow \text{K}_3\text{Fe}(\text{CN})_6$	115	18
$\text{H}_2\text{O} \rightarrow \text{SiMoO}_4^{2-}$ ^a	87	21
$\text{H}_2\text{O} \rightarrow \text{A-2-Sulf}$	155	24
$\text{TMB/sc} \rightarrow \text{A-2-Sulf}$ ^b	437	12.5
$\text{Mn}^{2+} \rightarrow \text{A-2-Sulf}$ ^b	138	0
$\text{DPC} \rightarrow \text{A-2-Sulf}$	288	0
$\text{DCPIP/asc} \rightarrow \text{A-2-Sulf}$ ^{a, b}	577	+ 4

^a Reactions not sensitive to DCMU in the assay.

^b The degree of inhibition was unchanged when TRIS washed chloroplasts were used.

Table II. Influence of the antiserum to zeaxanthin on photosynthetic electron transport reactions of the lamellar system of *Oscillatoria chalybea*.

Electron transport system	Control rate $\mu\text{mol O}_2$ uptake or evolution/mg chlorophyll/h	% Inhibition
$\text{H}_2\text{O} \rightarrow \text{DCPIP}$	70	25
$\text{H}_2\text{O} \rightarrow \text{K}_3(\text{Fe}(\text{CN})_6)$	104	12
$\text{H}_2\text{O} \rightarrow \text{SiMoO}_4^{2-}$	97	11
$\text{H}_2\text{O} \rightarrow \text{A-2-Sulf}$	143	32
$\text{TMB/asc} \rightarrow \text{A-2-Sulf}$	209	20
$\text{Mn}^{2+} \rightarrow \text{A-2-Sulf}$	147	3
$\text{DPC} \rightarrow \text{A-2-Sulf}$	185	1
$\text{DCPIP/asc} \rightarrow \text{A-2-Sulf}$ ^a	504	0

^a Reaction not sensitive to DCMU.

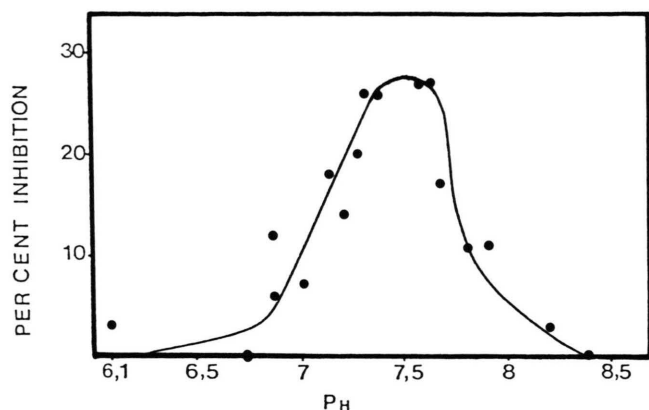


Fig. 2. pH dependence of the degree of inhibition of photosynthetic electron transport in tobacco chloroplasts caused by the antiserum to zeaxanthin. Electron transport system: $\text{H}_2\text{O} \rightarrow \text{anthraquinone-2 sulfonate}$.

affected to a lesser extent than with the antiserum to violaxanthin [3] which could mean that zeaxanthin is exposed to a lesser extent in the membrane when compared to violaxanthin. This would fit into the results by Hager [13] and Lee and Yamamoto [14]. These authors propose for their reductive violaxanthin-zeaxanthin cycle that the latter carotenoid would be located more towards the inner membrane surface. This lesser accessibility of zeaxanthin to the antibodies is substantiated by the fact that electron transport in thylakoids of *Oscillatoria chalybea* is not

impaired to a greater extent than in tobacco chloroplasts. According to the literature the lamellar system of these blue-green algae consists of single unfolded thylakoids distributed in the stroma [6]. Due to this fact the inaccessibility of zeaxanthin to antibodies is not due to the differentiation of the tobacco lamellar system into grana and intergrana regions which seemed to affect the accessibility of lutein and neoxanthin to their respective antibodies [5]. Concerning the inhibition site, there is essentially no difference with the antiserum to violaxanthin and that to zeaxanthin. The inhibition of electron transport by the antiserum has a pH dependence (Fig. 2). At the optimal pH 7.5 we determined the inhibition site in the electron transport scheme. Just as the antiserum to violaxanthin, the antiserum to zeaxanthin inhibits electron transport on the oxygen-evolving side of photosystem II between the site of electron donation of the artificial donor tetramethyl benzidine on the one hand and manganese-II ions or diphenylcarbazide on the other hand (Tables I and II).

It should be noted that due to the fact that photosystem II is differently organized in the blue-green alga *Oscillatoria chalybea* when compared to tobacco chloroplasts [10, 15], a DCMU sensitivity is observed in the silicomolybdate reduction with thylakoid preparations of these algae (Table III). Otherwise, with tobacco chloroplasts silicomolybdate accepts electrons before the DCMU-block (Table IV) which is in agreement with the literature [12].

Table III. Influence of DCMU on the silicomolybdate reaction in normal an Tris-washed lamellar system of *Oscillatoria chalybea*.

Electron transport system	$\mu\text{mol O}_2$ evolution or SiMoO_4 reduction/mg Chl	% Inhibition
Normal		
$\text{H}_2\text{O} \rightarrow \text{SiMoO}_6^{2-}$	98	48
$\text{H}_2\text{O} + \text{DCMU} \rightarrow \text{SiMoO}_6^{2-}$	51	
Without Donor	0	
Tris-Washed		
$\text{DPC} \rightarrow \text{SiMoO}_6^{2-}$	32	0
$\text{DPC} + \text{DCMU} \rightarrow \text{SiMoO}_6^{2-}$	35	

(2.5 $\mu\text{mol DPC/assay}$).

Table IV. Influence of DCMU on the silicomolybdate reaction in wild type tobacco chloroplasts.

Electron transport system	$\mu\text{mol O}_2$ evolution/mg Chl/h	% Inhibition
$\text{H}_2\text{O} \rightarrow \text{SiMoO}_6^{2-}$	130	0
$\text{H}_2\text{O} + \text{DCMU} \rightarrow \text{SiMoO}_6^{2-}$	124	

Discussion

In previous publications we have shown that the xanthophylls lutein [1, 5] neoxanthin [2] and violaxanthin [3] are somehow involved in photosystem II

-activity of tobacco chloroplasts and thylakoids of the filamentous blue-green algae *Oscillatoria chalybea* and those of *Nostoc muscorum*. We were able to show that antisera to these carotenoids affected photosynthetic electron transport on the oxygen-evolving side of the electron transport scheme. Even though our observation fitted nicely into different reports of the literature [4, 16], we felt that in order to be unequivocal the following points had to be clarified or at least to be kept in mind. Firstly: due to the fact that the passive heme-agglutination test [1] is not functioning with carotenoids and other similarly stretched molecules, we were unable to demonstrate the specificity of our antisera [1]. The only way to demonstrate that we really had an antiserum was by the described effect on photosynthetic electron transport. Secondly: due to this circumstance described in the first point and due to the fact that all hitherto described carotenoid antigens were prepared from *Urtica* leaves, an unspecific action of an antiserum caused by an unknown minor lipoic but very immunogenic compound, contained in the *Urtica* extracts, could not be excluded. In this sense, driving the interpretation to an extreme the antisera action described [1–3, 5] could be due to such a lipoic im-

purity and was not necessarily due to an antiserum to a carotenoid. However, the just mentioned points can be fully rejected by the observations of the present paper, namely by the antibody action of an antiserum to synthetic zeaxanthin on photosynthetic electron transport. Our zeaxanthin was obtained from Hofmann La Roche Basel [7], and was prepared according to a procedure which yields an optically active carotenoid. The fact now that this antiserum acts essentially as the earlier described violaxanthin antiserum, but inhibits electron transport generally to a lesser degree, speaks in favour of an absolute specificity which might distinguish even between the epoxidized and unepoxidized 3-hydroxy- β -ion-ring. Moreover, it confirms perfectly the observation of Lee and Yamamoto [14] or Stransky and Hager [17] that in the reductive violaxanthin zeaxanthin cycle, zeaxanthin should be located more towards the inside of the thylakoid membrane.

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