The Effect of Deoxycholate-Treatment to the Photoreactions of the Active Pigments in Photosynthesis

Günter Döring *

Max-Volmer-Institut für Physikalische Chemie und Molekularbiologie, Technische Universität Berlin

(Z. Naturforsch. **31 c**, 64–67 [1976]; received August 19, 1975)

Photosynthesis, Deoxycholate-Treatment, Electron Transport, Flash Excitation

In the heavy fraction of deoxycholate-treated spinach chloroplasts the chlorophyll a_{II} activity is high and the chlorophyll a_I activity is low when no artificial electron donor is added. The addition of the photosystem I donor system N-methyl-phenazonium sulphate plus sodium ascorbate (PMS+Asc) leads to a complete reactivation of the chlorophyll a_I reaction. The addition of the photosystem II donor system *p*-benzohydroquinone plus sodium ascorbate (HQ+Asc) leads to an inhibition of the chlorophyll a_{II} activity.

From these results we conclude:

1. Besides an interruption of the linear electron flow between the two photosystems deoxycholate-treatment leads to a block of the electron flow from water to photosystem II.
2. In deoxycholate-treated chloroplasts the linear electron flow in photosystem II just like in Tris-washed, heat-treated or aged chloroplasts, is replaced by a cyclic one.

Introduction

The detergent sodium deoxycholate has been found to be a very good agent to disintegrate chloroplasts [1]. However, Bril *et al.* [1] found neither a P700 bleaching nor HILL-activity (2,6-dichlorophenol-indophenol reduction) in the $10\,000 \times g$ fraction of deoxycholate-treated spinach chloroplasts. Van Gorkom *et al.* [2] reported that deoxycholate-treated spinach chloroplasts can be reactivated with different artificial electron donors and acceptors, but that they do not evolve O_2. In these subchloroplasts photosystem II has been studied by means of light minus dark difference spectra [2].

In this paper the photoreactions of P700 (chlorophyll a_I in our terminology) and P680 (chlorophyll a_{II}) have been investigated in the $10\,000 \times g$ fraction of deoxycholate-treated spinach chloroplasts by means of their absorbance changes. Gläser *et al.* [3] found that the chlorophyll a_{II} reaction has a biphasic kinetics. Besides the well known 200 μs absorbance change [4] they observed a fast component with a half-life of approx. 35 μs. In this paper only the 200 μs-component of chlorophyll a_{II} has been investigated.

Materials and Methods

Stripped spinach chloroplasts were isolated as described elsewhere [5]. Subchloroplast fragments enriched in photosystem II have been prepared as follows. Sodium deoxycholate (2 g) was added to 500 ml chloroplast suspension containing 100 μM chlorophyll, 0.4 M sucrose, 0.15 M KCl and 0.05 M Tris-HCl-buffer pH 8. This suspension was stored in the cold (0 °C) and the dark for 30 min. The pellet obtained by a 30 min centrifugation at $10\,000 \times g$ was resuspended in 25 ml Tricine-HCl-buffer 0.02 M, pH 7.4, containing 0.4 M sucrose, 10 mM NaCl, 2 mM $MgCl_2$ and 5% dimethyl sulfoxide.

The measurements of the absorbance changes were performed by the repetitive flash technique described in [4]. Excitation: 385 – 500 nm (2 mm BG 28 + 2 mm KG 2 from Schott) for measurements between 600 and 720 nm, 610 – 710 nm (4 mm RG 610 + 2 mm KG 2 from Schott) for measurements between 400 and 580 nm, saturating flashes of 20 μs duration, repetition rate 10 Hz. The electrical band width ranged from 0.1 Hz to 37 kHz. The optical path length through the cuvette was 1.2 mm. The band width of the monitoring light (grating monochromator) was 5 nm, and the intensity about 50 ergs · $cm^{-2} \cdot s^{-1}$. The temperature of the sample was 22 °C. The chlorophyll concentration was 100 μM. Except where noted the sample contained 0.05 M Tris-HCl-buffer pH 7.2, 100 μM benzyl viologen and 500 μM potassium ferricyanide resp. as electron acceptor, and 2 mM NH_4Cl as phosphorylation uncoupler. Further details are given in the legends of the figures.

Abbreviations: DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; PMS, N-methyl-phenazonium sulphate; TPB, tetraphenylboran.

* Requests for reprints should be sent to Dr. G. Döring, Bundesanstalt für Materialprüfung, Fachgruppe 5.4, Unter den Eichen 87, *D-1000 Berlin 45.*

Results

Chlorophyll a_{II}

In the 10 000 × *g* fraction of deoxycholate-treated chloroplasts the chlorophyll a_{II} reaction is fully active in the presence of ferricyanide as well as in the presence of benzyl viologen (Fig. 1). Compared

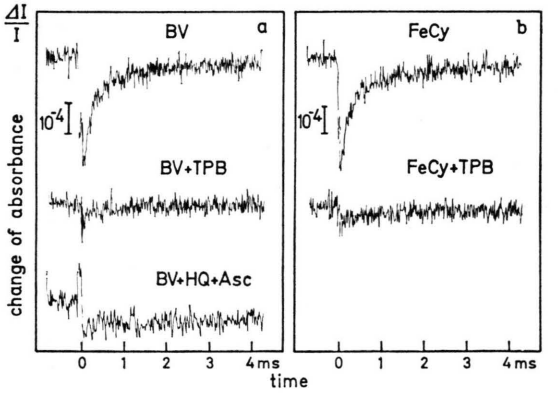

Fig. 1. Changes of absorbance at 690 nm in the 10 000 × *g* fraction of deoxycholate-treated spinach chloroplasts. a. In the presence of benzyl viologen (BV). Top: no artificial electron donor; center: 100 μM TPB as electron donor; bottom: 50 μM HQ+2 mM Asc as electron donor. b. In the presence of potassium ferricyanide (FeCy). Top: no artificial electron donor; center: 100 μM TPB as electron donor.

to untreated chloroplasts [6] the half-life time is increased from approx. 160 μs to approx. 200 μs. After the addition of 100 μM tetraphenylboran (TPB), which is an electron donor to photosystem II [7, 7a], the chlorophyll a_{II} absorbance changes are almost abolished. The concentration of TPB for half inactivation is 6 – 8 μM. Also the addition of 50 μM *p*-benzohydroquinone (HQ) plus 2 mM sodium ascorbate (Asc) leads to an almost complete inhibition of the chlorophyll a_{II} absorbance changes. The addition of 4 μM DCMU abolishes the absorbance changes of chlorophyll a_{II} completely (not shown in Fig. 1).

The difference spectrum of the absorbance changes with a half-life of approx. 200 μs in deoxycholate-treated chloroplasts is shown in Fig. 2. The absorbance changes are maximal at 434 nm and at 685 nm, which is characteristic for chlorophyll a_{II} [6, 8]. Compared with the difference spectrum in untreated chloroplasts [6] the red maximum is shifted 2 nm to a shorter wavelength.

The increase of the half-life of the chlorophyll a_{II} absorbance changes and the blue-shift of the red maximum of the chlorophyll a_{II} difference spectrum are in agreement with the results reported in [9].

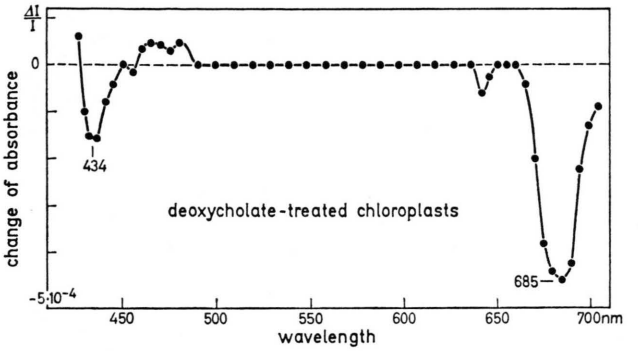

Fig. 2. Absorbance changes with a life time of approx. 200 μs as a function of the wavelength in the 10 000 × *g* fraction of deoxycholate-treated spinach chloroplasts in the presence of ferricyanide.

Chlorophyll a_I

In the absence of an electron donor the chlorophyll a_I reaction is inactivated nearly completely in deoxycholate-treated chloroplasts (see Fig. 3). The

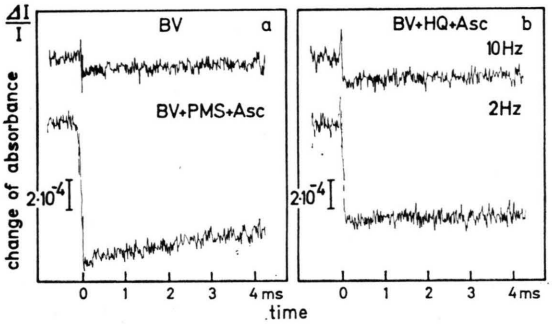

Fig. 3. Changes of absorbance at 705 nm in the 10 000 × *g* fraction of deoxycholate-treated spinach chloroplasts in the presence of benzyl viologen. a. Top: no artificial electron donor; bottom: 40 μM PMS plus 2 mM Asc as electron donor. b. 50 μM HQ+2 mM Asc as electron donor. Top: repetition rate 10 Hz; bottom: repetition rate 2 Hz.

addition of 40 μM PMS plus 2 mM Asc leads to a reactivation of the chlorophyll a_I reaction. When 50 μM HQ plus 2 mM Asc is added, the extent of the chlorophyll a_I absorbance changes is about 20% compared to untreated chloroplasts when the repetition rate is 10 Hz, but about 75% when the repetition rate is 2 Hz.

The difference spectrum of chlorophyll a_I in the 10 000 × *g* fraction of deoxycholate-treated chloroplasts is shown in Fig. 4. For these measurements the electron donor system PMS + Asc was used. The chlorophyll a_I absorbance changes are maximal at 434 nm in the blue region and at 685 and 705 nm in

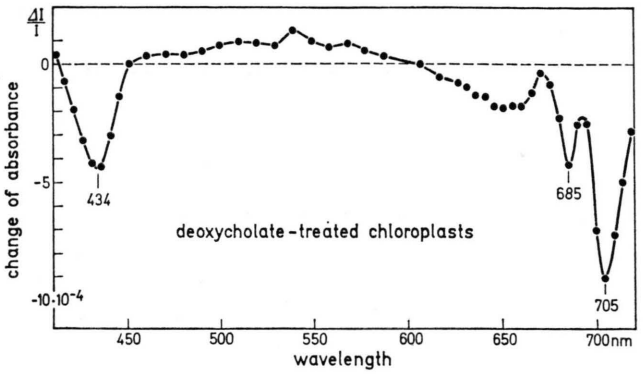

Fig. 4. Absorbance changes with a life time of approx. 10 ms as a function of the wavelength in the $10000 \times g$ fraction of deoxycholate-treated spinach chloroplasts. Electron acceptor: 100 μM benzyl viologen, electron donor: 50 μM PMS + 2 mM Asc.

the red. The shape of this difference spectrum with the characteristic double-band in the red agrees with previous work with digitonin-treated and Triton-X-treated chloroplasts [10–13]. The ratio $\Delta I/I$ (705 nm) : $\Delta I/I$ (685 nm) is approx. 2.

Ferricyanide reduction

The $10\,000 \times g$ fraction of deoxycholate-treated chloroplasts shows no ferricyanide reduction in the light. Even the addition of 100 μM TPB does not lead to an electron flow from this donor to ferricyanide *via* chlorophyll a_{II}.

Discussion

The experiments reported in this paper have shown:

1. When no artificial donor is added to the $10\,000 \times g$ fraction of deoxycholate-treated chloroplasts the chlorophyll a_{II} reaction is fully active and sensitive to DCMU in the presence of benzyl viologen as well as in the presence of ferricyanide. The addition of the photosystem II donors TPB and HQ + Asc resp. leads to an inactivation of the chlorophyll a_{II} reaction.

2. When no artificial donor is added to the $10\,000 \times g$ fraction of deoxycholate-treated chloroplasts to chlorophyll a_I activity is very low. The addition of the photosystem I donor PMS + Asc leads to a complete reactivation of the chlorophyll a_I reaction. The addition of HQ + Asc has the same effects, is, however, less effective.

Though the inhibition of the chlorophyll a_{II} reaction by artificial electron donors of photosystem II is in agreement with the results of van Gorkom *et al.* [2], this fact seems to be a contradiction in terms. However, this behaviour of chlorophyll a_{II} becomes intelligible in the light of the model of the electron flow in photosystem II, which we proposed in [6].

Because in the $10000 \times g$ fraction of deoxycholate-treated chloroplasts there is no linear electron flow from water to ferricyanide, the chlorophyll a_{II} reaction, however, is fully active, there must exist a cyclic electron flow in photosystem II. In our model of photosystem II the condition for this cyclic electron flow is that photosystem II is in the state $XChlY_1^+Y_2^+$ (resp. $XChlD^+Y_1^+Y_2^+$, when the 35 μs-component of chlorophyll a_{II} [3, 16] is taken into account) just before an exciting flash is fired (see Fig. 5) *. This condition applies to chloroplasts in

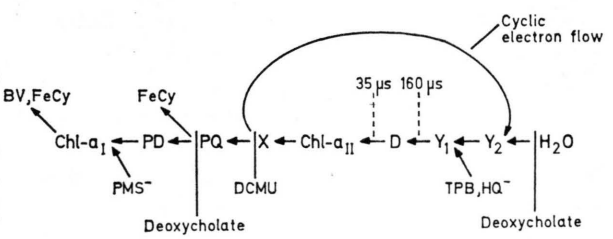

Fig. 5. Simplified scheme of the electron transport in the $10\,000 \times g$ fraction of deoxycholate-treated spinach chloroplasts. The primary electron donor pool of chlorophyll a_I, PD, includes cytochrome f and plastocyanin.

which the linear electron flow is blocked between the watersplitting site and photosystem II. Therefore, we conclude that deoxycholate-treatment just as Tris-treatment, heat-treatment, and aging of the chloroplasts leads to an interruption of the linear electron flow between the watersplitting site and Y_2 (see Fig. 5).

However, in contrast to Tris-washed, heat-treated or aged chloroplasts in deoxycholate-treated chloro-

* Reduced X has been identified as a special plastosemiquinone anion [14, 15], Chl is chlorophyll a_{II}, and Y_1 and Y_2 are unidentified electron carriers in photosystem II.

plasts the addition of the photosystem II donor HQ + Asc (or TPB resp.) does not lead to a linear electron flow from the donor *via* chlorophyll a_{II} and chlorophyll a_I to benzyl viologen. The reason for this behaviour is that a second reaction site of deoxycholate is located between the two photosystems [1, 2]. Van Gorkom *et al.* [2] have shown that in the presence of an artificial donor to photosystem II the plastoquinone pool PQ is reduced in the light, but the reoxidation of PQ in the dark is very slow even in the presence of ferricyanide. This has been confirmed by our finding that there is no ferricyanide reduction in the light under these conditions. Therefore, it follows that the reoxidation of PQ in the dark is obstructed in the $10\,000 \times g$ fraction of deoxycholate-treated chloroplasts (see Fig. 5). In this regard deoxycholate behaves similar as digitonin. The decisive difference to digitonin is, however, that deoxycholate-treatment interrupts the linear electron flow not only between the two photosystems but also between the watersplitting site and photosystem II.

Under these conditions it is clear that the addition of the photosystem II donor HQ + Asc or TPB resp. to deoxycholate-treated subchloroplasts leads to an inhibition of cyclic electron flow in photosystem II: the electron donor keeps Y_1 in its reduced state, so that the state $XChlY_1^+Y_2^+$, ($XChlD^+Y_1^+Y_2^+$ resp., see above) which is a necessary condition for the cyclic electron flow in photosystem II, is not realized just before an exciting flash is fired.

As in the $10\,000 \times g$ fraction of digitonin-treated spinach chloroplasts the chlorophyll a_I reaction can be reactivated by photosystem I donors. The difference spectrum of chlorophyll a_I observed under these conditions is the same as in digitonin-treated chloroplasts [10]. This is in contrast to the results of Bril *et al.* [1] and van Gorkom *et al.* [2]. Bril *et al.* did not observe any P700 bleaching in the heavy fraction of deoxycholate-treated chloroplasts, and van Gorkom *et al.* found no absorbance changes of P700 in the blue region.

The reason for the reactivation of photosystem I by the photosystem II donor HQ + Asc (see Fig. 3 a) is that Asc is a donor for photosystem I. The effectiveness of ascorbate alone is much lower than that of PMS + Asc and therefore, the reactivation of chlorophyll a_I by HQ + Asc is better if the dark-time between the exciting flashes is longer.

The author wishes to thank Mrs. Barbara Sander for drawing the figures.

[1] C. Bril, D. J. van der Horst, S. R. Poort, and J. B. Thomas, Biochim. Biophys. Acta **172**, 345–348 [1969].
[2] H. J. van Gorkom, J. J. Tamminga, and J. Haveman, Biochim. Biophys. Acta **347**, 417–438 [1974].
[3] M. Gläser, Ch. Wolff, H.-E. Buchwald, and H. T. Witt, FEBS Letters **42**, 81–85 [1974].
[4] G. Döring, H. H. Stiehl, and H. T. Witt, Z. Naturforsch. **22 b**, 639–644 [1967].
[5] G. D. Winget, S. Izawa, and N. E. Good, Biochem. Biophys. Res. Commun. **21**, 438–443 [1965].
[6] G. Döring, Biochim. Biophys. Acta **376**, 274–284 [1975].
[7] P. H. Homann, Biochim. Biophys. Acta **234**, 336–344 [1972].
[7a] K. Erixon and G. Renger, Biochim. Biophys. Acta **333**, 95–106 [1974].
[8] G. Döring, G. Renger, J. Vater, and H. T. Witt, Z. Naturforsch. **24 b**, 1139–1143 [1969].
[9] G. Döring, Proceedings of the IIIrd International Congress on Photosynthesis Research, pp. 149–158, Rehovot, Israel, 1974.
[10] G. Döring, J. L. Bailey, W. Kreutz, J. Weikard, and H. T. Witt, Naturwissenschaften **55**, 219–220 [1968].
[11] B. Ke, Arch. Biochem. Biophys. **152**, 70–77 [1972].
[12] L. P. Vernon, H. Y. Yamamoto, and Z. Ogawa, Proc. Nat. Acad. Sci. U.S. **63**, 911–917 [1969].
[13] N. Murata and A. Takamiya, Plant Cell Physiol. **10**, 193–202 [1969].
[14] H. H. Stiehl and H. T. Witt, Z. Naturforsch. **24 b**, 1588–1598 [1969].
[15] K. Witt, FEBS Letters **38**, 116–118 [1973].
[16] Ch. Wolff, M. Gläser, and H. T. Witt, Proceedings of the IIIrd International Congress on Photosynthesis Research, pp. 296–305, Rehovot, Israel, 1974.