Volume 232, number 2, 372–376 FEB 05882 May 1988

Linolenic acid-induced release of Mn, polypeptides and inactivation of oxygen evolution in photosystem II particles

Maciej Garstka and Zbigniew Kaniuga

Institute of Biochemistry, University of Warsaw, Al. Zwirki i Wigury 93, PL 02-089 Warszawa, Poland

Received 22 March 1988

The release of both Mn and polypeptides as well as the inactivation of O_2 evolution in $C_{18:3}$-treated PS II particles was studied. An increase of incubation temperature above 20°C greatly accelerated depletion of both Mn and peptides and inactivation of O_2 evolution. At 25°C about 30% of Mn was released whereas following incubation at 30°C release of 50% of Mn, complete depletion of both 10 and 18 kDa peptides as well as complete inactivation of O_2 evolution were observed. Simultaneously, the release of 24 and both 33 and 43 kDa peptides was about 40 and 30%, respectively. At 25°C, $C_{18:3}$ released only one Mn/reaction centre from PS II particles depleted of 18 and 24 kDa peptides, while two Mn/reaction centre were released from particles devoid of 18, 24 and 33 kDa peptides. These observations suggest that the 33 kDa peptide stabilizes preferentially two of the four Mn atoms of the oxygen-evolving complex. The effect of $C_{18:3}$ on Mn release from PS II particles is thus similar to that of heat and urea treatments.

O_2 evolution; Mn; Polypeptide; Linolenic acid; Photosystem II

1. INTRODUCTION

In recent studies on the polypeptides and Mn in the oxygen-evolving complex and their function in photosynthetic O_2 evolution, several methods have been applied to liberate these components from membranes such as broken thylakoids, O_2-evolving PS II particles or inside-out thylakoids. The agents used for solubilization of Mn and polypeptides are active to various extents against one or several of these components. Monovalent salt-washing [1,2] and deoxycholate [3] liberate the 18 and 24 kDa polypeptides without release of both Mn and the 33 kDa peptide and cause partial inactivation of oxygen evolution. On the other hand, divalent salt-extraction [4,5] or treatment with urea + NaCl [6] completely liberate all three polypeptides and inactivate O_2 evolution, whereas all the Mn is preserved after removal of these proteins [4,6]. Tris- and/or alkaline-extraction of PS II particles [1,3,7] and inside-out thylakoids [8] liberates the 18, 24 and 33 kDa peptides accompanied by a release of Mn [1,9] and causes total inactivation of oxygen evolution. A similar effect is observed following heat treatment [3,10,11]. Incubation of PS II particles with concentrated urea [6,12,13] results in a depletion of these three peptides (to various extents) and about half [10,11] or only part [13] of the Mn content causing an inactivation of oxygen evolution. Hydroxylamine at 1.5 mM removes about 50% of the Mn from the particles and almost completely inactivates O_2 evolution whereas the 18, 24 and 33 kDa polypeptides are not liberated [9].

Recently we have reported [14] that similar to Tris and hydroxylamine, the inhibitory effect of both endogenous and exogenous $C_{18:3}$ on oxygen evolution depends on the release from chloroplasts of functionally active loosely bound Mn. These

Correspondence address: Z. Kaniuga, Institute of Biochemistry, University of Warsaw, Al. Zwirki i Wigury 93, PL 02-089 Warszawa, Poland

Abbreviations: Chl, chlorophyll; $C_{18:3}$, linolenic acid; Mes, 4-morpholineethanesulphonic acid; PS II, photosystem II

This is the 20th paper of the series: Photosynthetic apparatus of chilling-sensitive plants

Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/88/$3.50

findings suggested that the release of Mn from chloroplasts by this fatty acid may be accompanied by a depletion of some polypeptides involved in the O_2-evolution system. The present data show that treatment with $C_{18:3}$ of PS II detergent particles results in the complete depletion of the 10 and 18 kDa peptides and about one third of the 24, 33 and 43 kDa peptides as well as release of 50% of the Mn content accompanied by inactivation of oxygen evolution.

2. MATERIALS AND METHODS

Broken spinach chloroplasts were prepared as described in [15] and the PS II detergent particles were obtained by the procedure of Yamamoto et al. [16] with some modifications. The activity of PS II particles was about 220 ± 20 μmol O_2 evolved·h^{-1}·mg Chl^{-1}. Particles were stored at −60°C or in liquid N_2 until use.

Incubation of PS II particles with $C_{18:3}$ (dissolved in ethanol at a final concentration lower than 1%) was performed for 1 h in room light in plastic tubes at a final Chl concentration of 1 mg/ml. After incubation in the temperature range between 15 and 30°C, the suspensions were centrifuged at 80000 × g for 30 min, pellets were suspended in a small volume of 20 mM Hepes buffer (pH 7.0) containing 0.33 M sorbitol, 15 mM NaCl, 4 mM $MgCl_2$ and used for oxygen-evolution activity and Mn and polypeptide content determinations.

For selective depletion of the 18 and 24 kDa peptides, particles were treated with NaCl [10], while for depletion of the 18, 24 and 33 kDa peptides either treatment with $CaCl_2$ [4] or urea + NaCl [6] was applied. Details are given in the legend to table 1.

Polypeptide composition in the PS II particles was analysed by SDS-urea electrophoresis following solubilization of samples as in [7]. The 12–22.5% polyacrylamide gradient, 0.4% bisacrylamide and 4 M urea was prepared as in [17]. The polypeptide patterns of control and pellets of $C_{18:3}$-treated samples were assayed using a Vitatron gel scanner. Peak areas of each peptide obtained on the densitogram were normalized using the 9 kDa peptide as inner standard. The amount of extracted polypeptides was calculated as the difference between their content in control and in $C_{18:3}$-treated samples.

Oxygen evolution was measured at 25°C under saturating tungsten light using a Clark-type electrode with 0.2 mM phenyl-

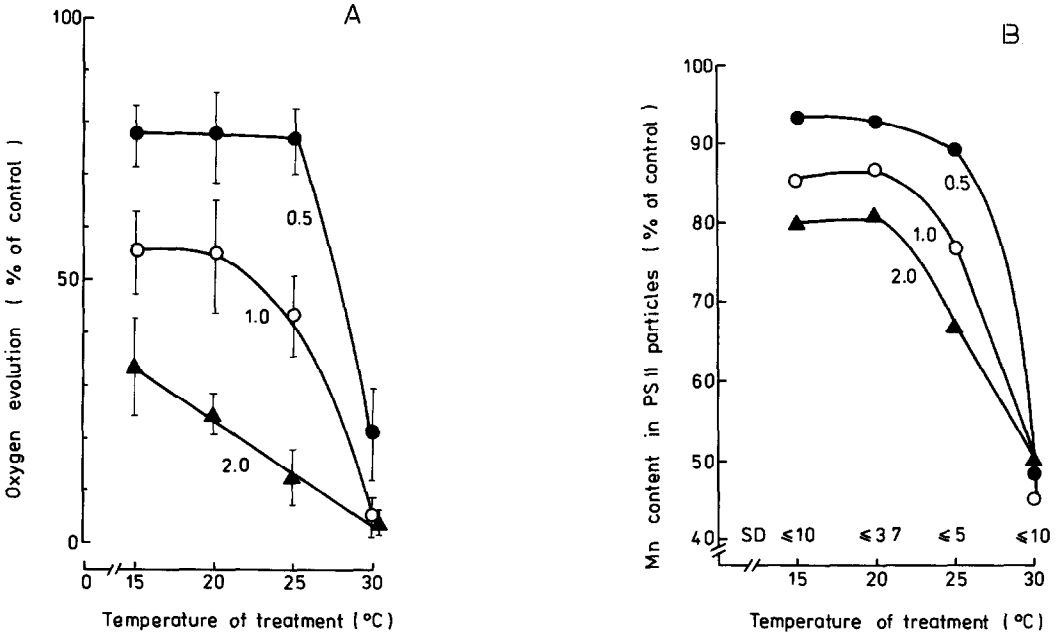

Fig.1. Effect of linolenic acid and incubation temperature on oxygen evolution (A) and Mn content (B) in PS II particles. Concentrations of $C_{18:3}$ were: (●) 0.5; (○) 1.0; (▲) 2.0 mg/mg Chl. The content of Mn and oxygen-evolution activity in control PS II particles were 4.3 ± 0.3 gatom/290 Chl and 210 ± 10 μmol O_2 evolved·h^{-1}·mg Chl^{-1}, respectively. The incubation of PS II particles over the temperature range 15–30°C in the presence of 1% ethanol but in the absence of $C_{18:3}$ results in both release of Mn and inactivation of O_2 evolution by 3, 6, 9 and 15% of the control at 15, 20, 25 and 30°C, respectively. Therefore for each temperature applied, 100% levels were calculated by subtraction of the appropriate values of both Mn content and O_2-evolution activity (measured following incubation of particles in the absence of $C_{18:3}$ at the temperature indicated) from the control levels determined in untreated particles. So, the net effect of $C_{18:3}$ treatment is presented in this figure. The results are means ± SD of 4–6 and 3–4 experiments in A and B, respectively. Error bars were omitted from B for clarity and replaced by numbers.

p-benzoquinone and 2 mM ferricyanide as an electron acceptor and buffer as in [18].

Mn was determined by atomic absorption using the graphite oven technique and a Perkin Elmer-300 spectrometer. The ratio of Chl to PS II reaction centres was estimated from the content of cytochrome *b*-559 (7.7 nmol/mg Chl) determined as described in [19] using a Specord M-40 (Carl Zeiss Jena) and assuming a stoichiometry of 2 cytochromes per reaction centre. Chlorophyll was determined as in [20].

3. RESULTS

Fig.1 shows the effects of temperature and concentration of $C_{18:3}$ on O_2 evolution (A) and release of Mn (B) following incubation of PS II particles. Inhibition of O_2 evolution and Mn depletion was small at a low (0.5) $C_{18:3}$/Chl weight ratio when incubation was carried out at temperatures up to 25°C. Much more effective were incubations at 25°C and 30°C and at higher $C_{18:3}$ concentrations. Following incubation of PS II particles at 25°C and 30°C and for a $C_{18:3}$/Chl ratio equal to 2, about 30% and 50% of the Mn were released, respectively, and O_2 evolution was completely inactivated. Thus, the effect of $C_{18:3}$ on O_2 evolution and Mn depletion was similar to that of urea [6,12] and heat [11] treatment.

Fig.2 shows the polypeptide electrophoretic profile of PS II particles before and after $C_{18:3}$ treatment. Incubation of PS II particles with $C_{18:3}$ at 15°C did not result in a release of polypeptides (not shown), whereas following incubation of particles at 25°C and 30°C peptides were released to a great extent. The optimum concentration of $C_{18:3}$ was 2 mg per mg Chl. As can be seen in fig.2, the treatment of PS II particles with $C_{18:3}$ at this concentration resulted in complete or partial liberation of five polypeptides: 10, 18, 24, 33 and 43 kDa, e.g. 10 and 18 kDa peptides were totally liberated, whereas release of the 24 and 33 kDa peptides from particles was about 40% and 30%, respectively. The 43 kDa band corresponding to the chlorophyll-carrying proteins was reduced by about 30%. It is noteworthy that $C_{18:3}$-induced release of the 10 kDa polypeptide from PS II particles resembles the depletion of this polypeptide by Tris-washing of the inside-out vesicles [21]. On the other hand, incubation of PS II particles with $C_{18:3}$ did not affect the light-harvesting chlorophyll-protein complex and proteins ranging from 20 to 25 kDa (except the 24 kDa polypeptide)

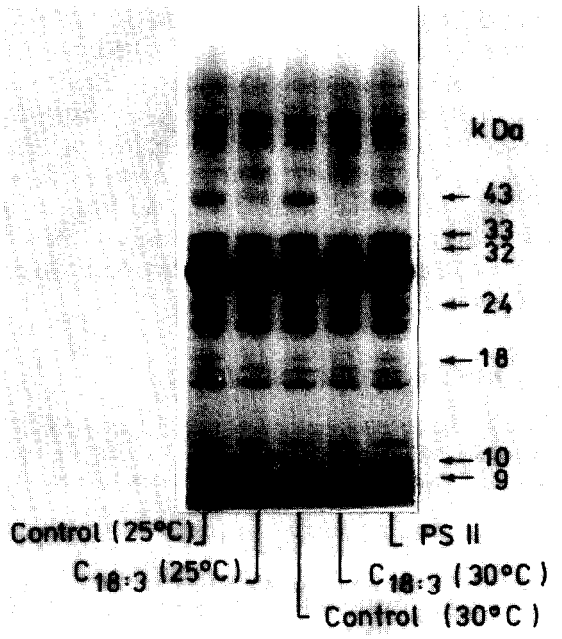

Fig.2. Polypeptide composition of SDS-urea PAGE of PS II particles following incubation with linolenic acid. PS II particles were washed once with buffer after storage at −60°C and incubated with $C_{18:3}$ (2 mg/mg Chl) either at 25°C or at 30°C for 1 h.

and the 32 kDa intrinsic protein. The effect of $C_{18:3}$ on peptide release from PS II particles is similar to the action of lauroylcholine chloride which extracted polypeptides of similar molecular masses [22].

The relationship between polypeptide and Mn release from PS II particles is shown in fig.3. It can be seen that for a $C_{18:3}$/Chl ratio of 1, 20% Mn depletion is accompanied by 100, 35 and 18% release of 18, 24 and 33 kDa polypeptides, respectively.

Restricted liberation of both the 33 kDa peptide and Mn from membranes (fig.3) led us to check the effect of $C_{18:3}$ on particles previously depleted of 18 and 24 kDa peptides by NaCl treatment [6] as well as on those depleted of all three peptides (18, 24 and 33 kDa) under conditions which did not affect Mn content, i.e. by $CaCl_2$ [4] or urea + NaCl [6] treatment. As can be seen in table 1, following $C_{18:3}$ incubation of NaCl-washed or intact particles about one Mn/reaction centre was released, similarly to Zn^{2+} treatment [23]. However, when the membranes were depleted of the 33 kDa

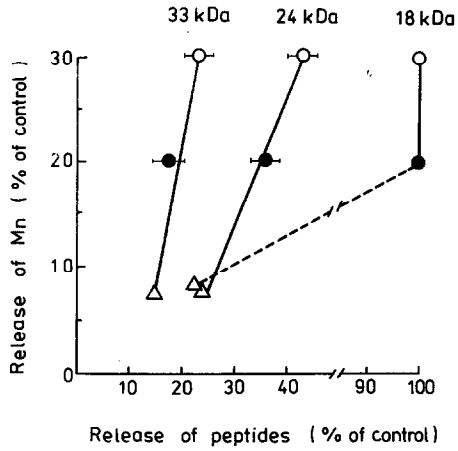

Fig.3. Relationship between Mn and polypeptide release from PS II particles following linolenic acid treatment at 25°C. Data for Mn are taken from fig.1B, while those for polypeptides are from an electrophoretic assay of the samples used in the experiments of fig.1B. Amounts of both Mn and polypeptides released were calculated as differences between their contents in control particles and pellets of $C_{18:3}$-treated samples.

polypeptide the amount of released Mn increased to 1.8–1.9 Mn/reaction centre. This suggests that the 18 and 24 kDa peptides did not enhance the release of Mn by $C_{18:3}$, while depletion of the 33 kDa peptide facilitated the removal of two Mn/reaction centre.

4. DISCUSSION

Extrinsic proteins (10, 18, 24 and 33 kDa) located at the inner surface of the thylakoid membrane are not released by Tris-washing of the thylakoid membranes and right-side out vesicles [21]. However, when PS II detergent particles or inside-out vesicles are used Tris treatment removes these proteins [24]. Similarly, incubation of PS II detergent particles with $C_{18:3}$ results in a release of the above mentioned peptides and Mn as well as an inactivation of O_2 evolution. Inhibition of O_2 evolution in chloroplasts by low $C_{18:3}$ concentrations (up to 100 μM) and short incubation time (up to 10 min) is reversible upon the addition of Mn^{2+} [25,26]. This is in accordance with the findings of Goldbeck et al. [26] that $C_{18:3}$ affects two sites in PS II: (i) a site of slow but irreversible loss of the loosely bound pool of Mn from the water-splitting complex and (ii) a site of fast but reversible inhibition of artificial donor reactions in PS II. In our experiments about 7-times higher concentrations of $C_{18:3}$ and a much longer time of incubation were applied than in those of Goldbeck et al. [26].

Table 1

The effect of linolenic acid on photosynthetic oxygen evolution and manganese content in oxygen-evolving PS II particles depleted of 18 and 24 kDa peptides

Treatment	Presence of the 33 kDa peptide	Oxygen evolution (μmol·mg Chl^{-1}·h^{-1})	Mn/290 Chl	Difference (effect of $C_{18:3}$)
Control, untreated	+	210	4.0	
Control, incubated, 1 h at 25°C	+	203	3.6	1.1
Control + $C_{18:3}$, 1 h at 25°C	+	22	2.5	
NaCl[a]	+	62	3.8	1.2
NaCl, followed by $C_{18:3}$	+	20	2.6	
$CaCl_2$[b]	−	38	3.2	1.8
$CaCl_2$, followed by $C_{18:3}$	−	9	1.4	
Urea + NaCl[c]	−	0	3.3	1.9
Urea + NaCl, followed by $C_{18:3}$	−	0	1.4	
Tris (pH 9.3), 4°C, 30 min	−	0	0.5	

[a] 1.0 M NaCl, 20 mM Mes-NaOH (pH 6.5)
[b] 1.0 M $CaCl_2$, 20 mM Mes-NaOH (pH 6.5)
[c] 2.5 M urea, 200 mM NaCl and 20 mM Mes-NaOH (pH 6.5)

Incubation with salts and urea was carried out at 4°C in room light for 1 h followed by treatment with linolenic acid (2 mg/mg Chl) for 1 h at 25°C where indicated

Under these conditions $C_{18:3}$ affects both Mn content and partially the peptide content. Their loss seems to be responsible for a decrease in O_2 evolution while an increase in the lipid/protein ratio of membranes in the presence of this fatty acid appears to be of secondary importance.

Liberation of polypeptides in the presence of $C_{18:3}$ is similar to that caused by lauroylcholine chloride [22] and deoxycholate [3] which release 16 and 24 kDa peptides from thylakoid membranes. Whereas deoxycholate treatment of thylakoid membranes did not affect Mn content, the incubation of chloroplasts [14] and PS II particles (fig.1B and table 1) with $C_{18:3}$ results in a depletion of up to 50% of the Mn content. Moreover, the 10 and 18 kDa peptides are released completely while about one third of the 24, 33 and 43 kDa polypeptides are liberated. Thus in contrast to salt-washing [1,2,4,27] or urea + NaCl [6] treatment, $C_{18:3}$ releases Mn in addition to polypeptides. It is noteworthy that like in the case of urea-treated [9,12], urea + NaCl-treated [6] or heat-treated [11] particles, incubation of PS II particles depleted of the 33 kDa peptide by $C_{18:3}$ released two of the four Mn atoms together with a loss of O_2-evolution activity.

Acknowledgements: The authors are grateful to Professor A. Hulanicki and Dr E. Bulska at the Faculty of Chemistry, Warsaw University, for allowing the use of the atomic absorption spectrometer and for advice on measuring manganese, respectively. Linolenic acid and phenyl-*p*-benzoquinone were kindly provided by Professors S. van den Bergh and John L. Howland, respectively. This work was supported in part by the Department of Plant Physiology, Polish Academy of Sciences (CPBP 05.02, project 1.09).

REFERENCES

[1] Kuwabara, T. and Murata, N. (1982) Plant Cell Physiol. 24, 741–747.
[2] Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10.
[3] Franzen, L.-G. and Andreasson, L.-E. (1984) Biochim. Biophys. Acta 765, 166–170.
[4] Ono, T. and Inoue, Y. (1983) FEBS Lett. 164, 255–260.
[5] Ono, T. and Inoue, Y. (1984) FEBS Lett. 166, 381–384.
[6] Miayo, M. and Murata, N. (1984) FEBS Lett. 170, 350–354.
[7] Yamamoto, Y., Doi, M., Tamura, N. and Nishimura, M. (1981) FEBS Lett. 133, 265–268.
[8] Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232.
[9] Murata, N., Miyao, M. and Kuwabara, T. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp.213–222, Academic Press, Tokyo.
[10] Yamamoto, Y. and Nishimura, M. (1983) in: The Oxygen Evolving System in Photosynthesis (Inoue, Y. et al. eds) pp.229–238, Academic Press, Tokyo.
[11] Nash, D., Miyao, M. and Murata, N. (1985) Biochim. Biophys. Acta 807, 127–133.
[12] Miyao, M. and Murata, N. (1984) Biochim. Biophys. Acta 765, 253–257.
[13] Tang, X.-S. and Satoh, K. (1986) FEBS Lett. 201, 221–224.
[14] Kaniuga, Z., Gemel, J. and Zabłocka, B. (1986) Biochim. Biophys. Acta 850, 473–482.
[15] Berthold, D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234.
[16] Yamamoto, Y., Tabata, K., Isogai, Y., Nishimura, M., Okayama, S., Matsuura, K. and Itoh, S. (1984) Biochim. Biophys. Acta 767, 493–500.
[17] Chua, N.-H. (1980) Methods Enzymol. 69, 434–446.
[18] Seibert, M. and Lavorel, J. (1983) Biochim. Biophys. Acta 723, 160–168.
[19] Matsuura, K. and Itoh, S. (1985) Plant Cell Physiol. 26, 537–542.
[20] Arnon, D. (1949) Plant Physiol. 24, 1–15.
[21] Ljunberg, U., Åkerlund, H.-E. and Andersson, B. (1984) FEBS Lett. 175, 255–258.
[22] Wydrzyński, T. and Huggins, B.J. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp.265–272, Academic Press, Tokyo.
[23] Miller, M. (1985) FEBS Lett. 189, 335–360.
[24] Åkerlund, H.-E. (1983) in: The Oxygen Evolving System of Photosynthesis (Inoue, Y. et al. eds) pp.201–208, Academic Press, Tokyo.
[25] Siegenthaler, P.-A. (1974) FEBS Lett. 39, 337–340.
[26] Goldbeck, J.H., Martin, I.F. and Fowler, C.F. (1980) Plant Physiol. 65, 707–713.
[27] Kuwabara, T., Miyao, M., Murata, T. and Murata, N. (1985) Biochim. Biophys. Acta 806, 283–289.