Biochimica et Biophysica Acta, 724 (1983) 1–5
Elsevier

BBA 41319

FURTHER STUDIES ON THE ROLE OF CHLORIDE IN PHOTOSYNTHETIC O_2 EVOLUTION IN HIGHER PLANTS

CHRISTA CRITCHLEY

Department of Botany, The Faculties, Australian National University, G.P.O. Box 4, Canberra, A.C.T. 2601 (Australia)

(Received February 7th, 1983)

Key words: Oxygen evolution; Photosynthesis; Cl^-; pH effect; Salt tolerance; (A. eucalyptifolia, S. quinqueflora)

(1) Thylakoid preparations from two salt-tolerant higher plant species, *Avicennia eucalyptifolia* and *Salicornia quinqueflora*, were shown to require very high salt concentrations for Photosystem II electron transport at alkaline pH. (2) High concentrations (250–500 mM) of tetramethylammonium chloride were found to be as effective as NaCl in stimulating maximal O_2-evolution activity, supporting the evidence that the effect is mediated by chloride and not by cations. (3) Light-response curves of O_2 evolution at high and low concentrations of NaCl were indistinguishable in the light-limited region, providing further evidence for the O_2-evolving complex as the site of chloride action. (4) A considerable shift in pH optimum towards the alkaline region for O_2 evolution in the presence of increasing concentrations of NaCl, and pH-jump experiments in the presence and absence of NaCl indicate possible competitiveness or at least some form of interdependence of Cl^- and OH^- binding to the O_2-evolving complex. (5) The results are discussed in the light of recent evidence concerning the alkaline inhibition of O_2 evolution in non-halophytic thylakoids, and a comparison is made between the two systems.

Introduction

Chloride is an essential cofactor in photosynthetic O_2 evolution in thylakoids from both non-salt-tolerant and salt-tolerant higher plants [1,2]. In the former 10–20 mM Cl^- is required to restore fully the O_2-evolution activity [3], whereas 250–500 mM is optimal for the latter [2,4]. In both systems the O_2-evolving complex was shown to be the site of Cl^- action [4–6]. The overall similarities of the two systems, except for the actual Cl^- concentration required, are such that it seems reasonable to suggest the operation of a very similar mechanism. Therefore, reversible ionic binding, which was proposed to be the mode of Cl^- interaction with the O_2-evolving complex in halophytic thylakoids [4], may well be common to all O_2-evolving systems.

This reversible ionic binding of Cl^- to the O_2-evolving complex in halophytes was also shown to be strongly pH dependent [2,4] in a manner very similar to the pH- and uncoupler-dependent inactivation of O_2-evolution activity in non-halophytes [1,7–10], suggesting that Cl^- and OH^- binding is competitive or at least in some way interdependent in both systems.

In the present study this interaction of OH^- and Cl^- is investigated in more detail. Additionally, thylakoid preparations from two further salt-tolerant species are shown to have very similar properties with regard to Cl^- and pH dependence to those described previously [2,4].

Abbreviations: TMACl, tetramethylammonium chloride; PS, photosystem; Chl, chlorophyll; AMPD, 2-amino-2-methyl-1,3-propanediol; Tricine, *N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid; Mes, 4-morpholineethanesulphonic acid.

Materials and Methods

Young seedlings of *Avicennia marina* and *Salicornia quinqueflora* were collected from a mangrove swamp at Cullendulla Creek, Bateman's Bay, N.S.W., Australia, planted into 500-ml containers in sand and grown in a glasshouse under natural light conditions. The plants were kept flooded with Hoagland's solution supplemented with 250 mM NaCl (corresponding to approx. 50% seawater salinity). The experiments with *A. eucalyptifolia* were carried out at the Australian Institute of Marine Science in Townsville, North Queensland, on leaf material derived from large adult plants growing in the institute's shadehouse under natural environmental conditions.

Thylakoids were prepared from all three species essentially as described previously [2,4], except that 20% sucrose was used instead of 20% poly(ethylene glycol) in the grinding medium.

O_2-evolution activity was measured in a Clark-type O_2 electrode (Rank Brothers, Bottisham, U.K.) in red light (Schott RG 610 red cut-off filter) at a quantum flux density of 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. Assay temperature was 25°C and 30–35 μg Chl were used in a total volume of 3 ml. Assay buffers were adjusted for pH with NaOH or AMPD (Sigma) as specified in the figure legends. Final concentrations of ferricyanide and dimethylbenzoquinone were 1.5 and 0.5 mM, respectively. Chlorophyll was determined according to the method of Arnon [11].

Results and Discussion

Thylakoid preparations from leaves of three salt-tolerant plant species, i.e., *A. marina*, *A. germinans* and *Aster tripolium*, were recently shown to require comparatively high NaCl concentrations for maximal O_2-evolution activity at high pH [2,4]. The results described in this paper were obtained using mainly *A. marina*, but also *A. eucalyptifolia*, a mangrove species from tropical Northern Queensland, Australia, and *S. quinqueflora*, a halophyte common to salt marshes of both hemispheres. As shown in Fig. 1, thylakoids from leaves of the latter two species show much the same characteristics for PS II electron transport as did the former [2,4], when assayed under similar conditions. TMACl was found to be equally as effective as NaCl, supporting the view that Cl^- is the active ion and not the cation (Fig. 1, inset). These thylakoids were also fully uncoupled. The dependence on NaCl concentration was markedly different at pH 7.0 as compared to 7.8. In Fig. 2 this is demonstrated for *A. eucalyptifolia* (A) and *A. marina* (B) thylakoids, where in both cases less Cl^- was required to obtain maximal O_2 evolution at neutral as compared to alkaline pH. These results support the earlier suggestion [2,4] that photosynthetic membranes of halophytes have some unusual features which are nevertheless common to a whole group of quite diverse salt-tolerant plants.

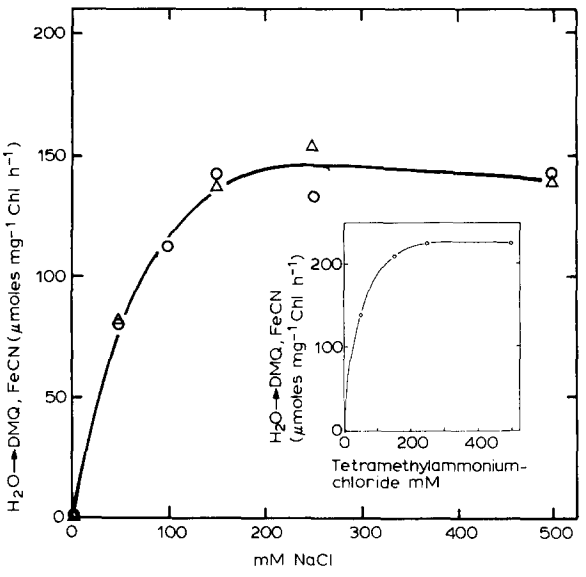

Fig. 1. Effect of NaCl on O_2 evolution with dimethylbenzoquinone (DMQ) and ferricyanide (FeCN) as electron acceptors in thylakoids from *A. eucalyptifolia* (○) and *S. quinqueflora* (△). Assay buffer was 50 mM Tricine-NaOH, pH 7.8. Inset: O_2 evolution in *A. marina* thylakoids as a function of tetramethylammonium concentration. Assay buffer was 50 mM Tricine-AMPD, pH 7.8.

The O_2-evolving complex has been previously shown to be the site of Cl^- action in the PS II electron-transport chain [4–6]. This result was confirmed by measurements of apparent quantum yield of O_2 evolution, since there was no difference in the light-limited rates of electron transport at low (25 mM) or high (250 mM) NaCl concentration (Fig. 3), and indicates that the PS II light-

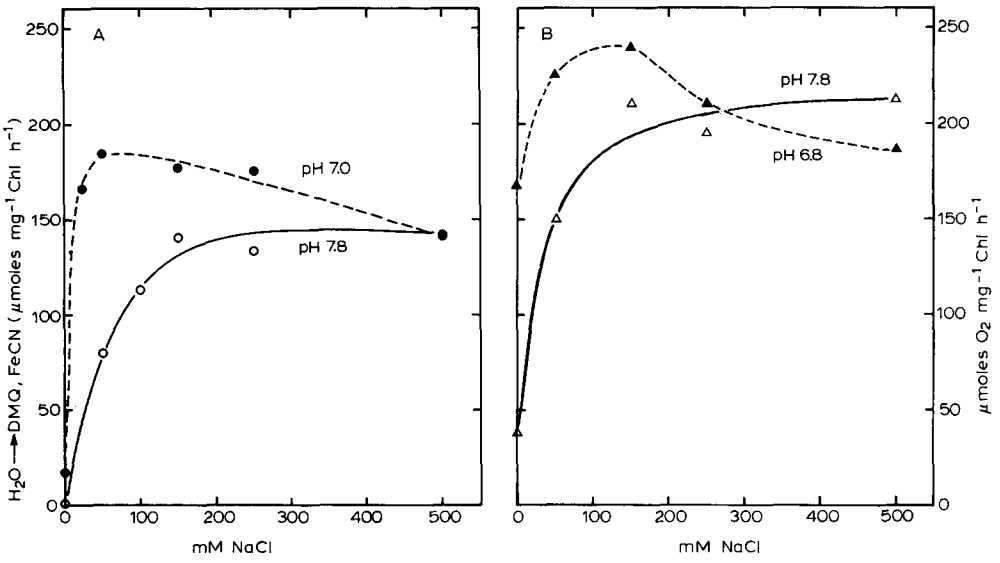

Fig. 2. Effect of pH on O_2 evolution activity as a function of NaCl concentration in (A) *A. eucalyptifolia* thylakoids and (B) *A. marina* thylakoids assayed at pH 7.0 (●, ▲) or pH 7.8 (○, △). Buffer as in Fig. 1.

harvesting and antennae functions were not limited by Cl^- concentration.

The pH effect on Cl^--dependent O_2 evolution reported previously [1,2,4] and in this paper (Fig. 2) was studied in more detail using halophytic

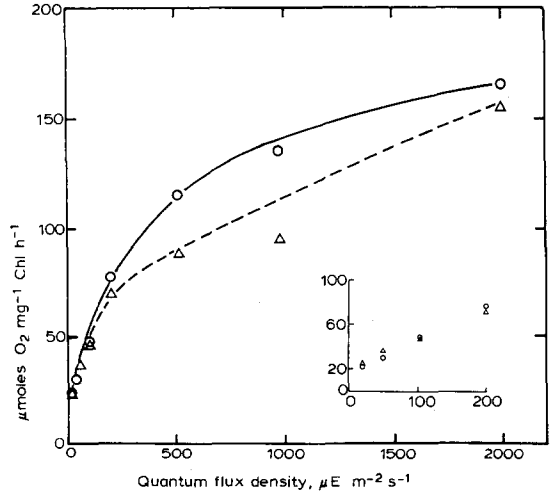

Fig. 3. Light-response curves of O_2 evolution in thylakoids from *A. eucalyptifolia* in the presence of 25 (○) or 250 (△) mM NaCl. Assay buffer as in Fig. 1 inset, pH 7.0. The *x*- and *y*-axes of the inset are the same as for the main figure.

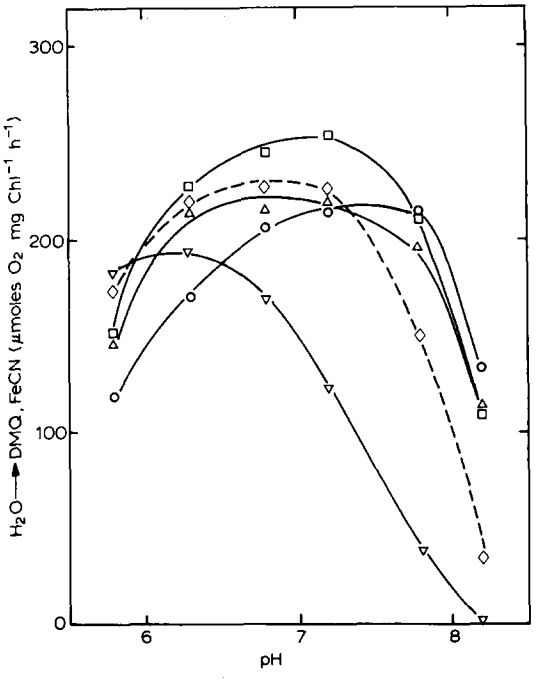

Fig. 4. pH dependence of O_2 evolution as influenced by NaCl concentration in *A. marina* thylakoids. Assay buffers were 25 mM Mes-AMPD, pH 5.8–6.8 and 25 mM Hepes-AMPD, pH 6.8–8.2, with no salt (▽) 50 (◇), 150 (□), 250 (△) or 500 (○) mM NaCl.

thylakoids. As can be seen from Fig. 4, the pH optimum for O_2 evolution in *A. marina* thylakoids was about 6.3 in the absence of added NaCl, shifting significantly towards the neutral or slightly alkaline region with increasing NaCl concentration. Beyond pH 7.8 the rates declined sharply in an NaCl-concentration-dependent fashion, such that they were highest at the highest concentration used and zero in the absence of added NaCl. It would appear that on the one hand the endogenous Cl^- is sufficient to stimulate high rates of O_2 evolution at low pH and that on the other hand high Cl^- concentrations prevent inhibition of this activity by alkaline pH. Unfortunately, we have been unable to determine the concentrations of this residual, endogenous Cl^- as yet.

Inhibition of O_2 evolution by alkaline pH in the presence of uncouplers has been observed by several authors [9,10,12–14] and has been variously attributed to a conformational change of the thylakoid membrane [13], a direct effect on the dissipation of the H^+ gradient [9] and the inactivation of the S_2 state [14]. The latter authors also claimed that no conformational changes were involved, since glutaraldehyde fixation did not prevent the inactivation [10,14]. Incubation of *A. marina* thylakoids at pH 8.2 in the absence of added NaCl caused a severe inhibition of the rate of O_2 evolution measured subsequently at pH 6.8 (Fig. 5). The time required for 85% inhibition was 3 min, presenting some contrast to results reported recently for non-halophytic thylakoids, which, after 10 min at pH 9.3, were inhibited only 8% [10]. In the reverse experiment, where the pH was jumped from 6.8 to 8.2 it was seen that the inactivation by this change to alkaline pH was partially prevented by NaCl and directly dependent on the NaCl concentration (Fig. 6). From the results shown here and those reported for non-halophytic thylakoids it is apparent that halophytic thylakoids are much more sensitive to alkaline pH, which may be due to extensive loss of Cl^- during preparation. The affinity for Cl^- binding is much lower in halophytic thylakoids, which could be a consequence of the possibly considerable Cl^- concentrations prevailing in the vicinity of the O_2-evolving system in vivo. The loss of Cl^- during

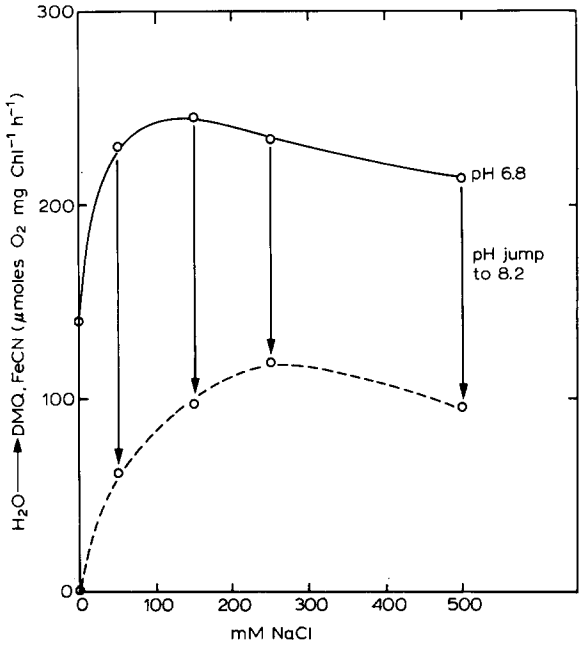

Fig. 6. Protective effect of NaCl on inhibition of O_2 evolution by alkaline pH in *A. marina* thylakoids. Buffer was 25 mM Hepes-AMPD, pH 6.8, which was jumped to pH 8.2 by injection of 20 mM AMPD.

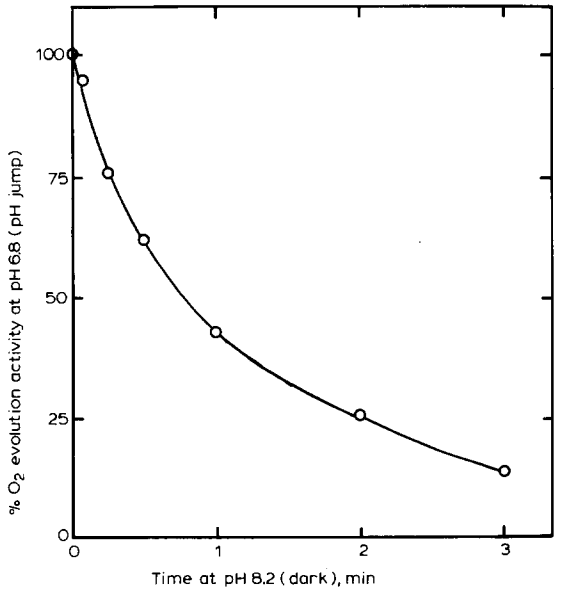

Fig. 5. Time-dependent inactivation of O_2-evolution activity by alkaline pH in *A. marina* thylakoids in the absence of added NaCl. Buffer was 25 mM Hepes-AMPD, pH 8.2, during incubation, and pH 6.8 during assay. pH was jumped by injection of 6.67 mM citric acid.

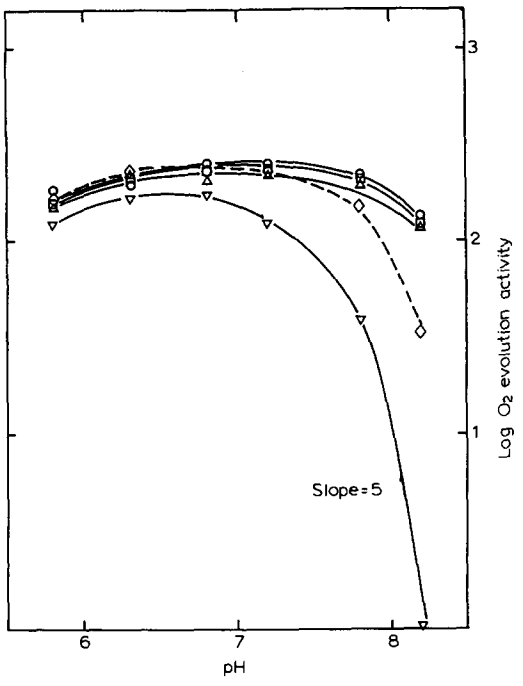

Fig. 7. Log plot of O_2 evolution vs. pH. Values taken from Fig. 4.

preparation of thylakoids would leave the O_2-evolving system partly inactivated as well as more sensitive to alkaline inhibition. It should be noted here again that thylakoids from halophytes are fully uncoupled after preparation which, in analogy to the glycophyte situation [3,6,7], would assist the Cl^- loss. It was proposed earlier that alkaline inhibition might be due to a replacement of Cl^- by OH^- [4]. The results presented in Figs. 4 and 6, however, indicate that OH^- may not be directly competing with Cl^- for binding sites, but that Cl^- binding may instead protect inactivation groups from titration by OH^-. A log plot of O_2-evolution activity vs. pH (Fig. 7) shows that possibly as many as five positive charges are titrated.

It is suggested that Cl^- binding, in halophytic and glycophytic systems alike, affords protection of titratable positive charges, possibly by inducing a conformational change of the O_2-evolving complex in the thylakoid membrane. The reduced binding capacity for Cl^- in a halophytic photosynthetic membrane could be due to alterations in the protein structure or to a change in the lipid environment of the halophytic membrane, containing more negatively charged lipids for example.

Acknowledgements

This research was supported by a grant from the Rural Credits Development Fund. I wish to thank Ron Pace for helpful discussions, Neda Plovanic for assistance with plant culture, Warwick Bottomley, Dean Price and Ivan Ryrie for critical reading of the manuscript, and Anne Gallagher for drawing the diagrams.

References

1 Hind, H., Nakatani, H.Y. and Izawa, S. (1969) Biochim. Biophys. Acta 172, 277–289
2 Critchley, C. (1982) Nature 298, 483–485
3 Kelley, P.M. and Izawa, S. (1978) Biochim. Biophys. Acta 502, 198–210
4 Critchley, C., Baianu, I.C., Govindjee and Gutowsky, H.S. (1982) Biochim. Biophys. Acta 682, 436–445
5 Heath, R.L. and Hind, G. (1969) Biochim. Biophys. Acta 172, 290–299
6 Izawa, S., Heath, R.L. and Hind, G. (1969) Biochim. Biophys. Acta 180, 388–398
7 Theg, S.M. and Homann, P.H. (1982) Biochim. Biophys. Acta 679, 221–234
8 Theg, S.M., Johnson, J.D. and Homann, P.H. (1982) FEBS Lett. 145, 25–29
9 Cohn, D.E., Cohen, W.S. and Bertsch, W. (1975) Biochim. Biophys. Acta 376, 97–104
10 Maison-Peteri, B., Vernotte, C. and Briantais, J.-M. (1981) Biochim. Biophys. Acta 637, 202–208
11 Arnon, D.I. (1949) Plant Physiol. 24, 1–15
12 Harth, E., Reimer, S. and Trebst, A. (1974) FEBS Lett. 42, 165–168
13 Reimer, S. and Trebst. A. (1975) Biochem. Physiol. Pflanzen 168, 225–232
14 Briantais, J.-M., Vernotte, C., Lavergne, J. and Arntzen, C.J. (1977) Biochim. Biophys. Acta 461, 61–74