

THE EFFECT OF CHLORIDE AND OTHER ANIONS ON THE THERMAL INACTIVATION OF OXYGEN EVOLUTION IN SPINACH THYLAKOIDS

W.J. COLEMAN^a/I.C. BAIANU^b/H.S. GUTOWSKY^c/GOVINDJEE^{a,d}

1. ABSTRACT

The thermal inactivation of O₂-evolution in spinach thylakoids isolated without Cl⁻ and heated at 30° to 50°C for 3 minutes is decreased by the presence of 50 mM Cl⁻ or Br⁻ but is unaffected by 25 mM Na₂SO₄ or 50 mM NaNO₃. These results are similar to those reported for the specificity of the anions as cofactors for O₂-evolution in unheated thylakoids (see, for example, Critchley et al., 1982; Izawa et al., 1983). Both effects are attributed to selective reversible binding of the anions to or near the O₂-evolving complex. Binding of Cl⁻ or Br⁻ appears to stabilize the conformation of the complex.

2. INTRODUCTION

Previous work has demonstrated that Cl⁻ is a necessary cofactor for the oxidation of water to molecular oxygen (Izawa et al., 1983). Chloroplasts washed in Cl⁻-free medium are reversibly inactivated, the photosystem II function being restored by the addition of Cl⁻. Larger inorganic anions (NO₃⁻, I⁻) can substitute partially for Cl⁻ in Cl⁻-depleted spinach thylakoids (Hind et al., 1969). Br⁻, which is slightly larger than Cl⁻, restores nearly complete activity (Hind et al., 1969). But many other anions, such as PO₄³⁻, SO₄²⁻, and CNO⁻, are ineffective. It has been proposed (Critchley et al., 1982; Govindjee et al., 1983) that the active anions facilitate PS II electron transport by reversible ionic binding to the O₂-evolving complex or to the neighboring membrane. The anion specificity is thus the result of ionic field effects and steric factors which affect the binding.

Heat treatment of thylakoids, on the other hand, causes marked irreversible inactivation at or above 35°C (Cheniae, Martin, 1970; Yamashita, Butler, 1968). Prolonged treatment at lower temperature and high pH has been used to induce Cl⁻ depletion (Hind et al., 1969). Experiments with artificial PS II electron donors indicate that both heat treatment and Cl⁻-depletion inhibit electron transport from PS II to P680. However, the modes of action must differ in the two cases, because one is reversible (Kelley, Izawa, 1978) and the other is irreversible (Yamashita, Butler, 1968; Figure 7 in Critchley et al., 1982).

Anions are known to affect the thermal stability of enzymes in solution, with considerable specificity (Ginsburg, Carroll, 1965). This suggests a possible connection between the activity of an anion as a cofactor for O₂-evolution and its effect upon the thermal inactivation of PS II. This view is substantiated by the thermal inactivation curves we have determined for the four anions, Cl⁻, Br⁻, NO₃⁻ and SO₄²⁻.

^aDepartment of Plant Biology, ^bDepartment of Food Science/Physical Chemistry Laboratory, ^cDepartment of Chemistry, and ^dDepartment of Physiology and Biophysics, University of Illinois, Urbana, Illinois 61801 (U.S.A.)

3. RESULTS AND DISCUSSION

Figure 1 shows the relative effects of various anions on the loss of the Hill reaction rate (DCPIP reduction) due to heating for 3 minutes in the range 30°-50°C. The mean values of the Hill rate for all samples after heating at 25°C range from 530 to 660 $\mu\text{mol DCPIP reduced}\cdot\text{mg Chl}^{-1}\cdot\text{hr}^{-1}$, measured in 70 mM Cl^{-} . For unheated samples, thylakoids isolated and assayed without Cl^{-} show Hill activity amounting to 75% of the activity obtained in assay buffer containing Cl^{-} .

In agreement with a previous report for thylakoids in a Cl^{-} -containing medium, heat inactivation curves are sigmoidal (Cramer, et al., 1981). For all of our heating curves, the width of transition in the region from 25% to 75% activity is $5.3\pm 0.2^{\circ}\text{C}$. The presence of different anions affects only the relative positions of the curves, not the functional dependence or the steepness of the transitions. The steepness and sigmoid shape of the transition are similar to those found for many proteins in solution (Ginsburg, Carroll, 1965).

Thylakoids isolated and heated without Cl^{-} are inactivated significantly more than thylakoids isolated and heated in 50 mM Cl^{-} (or those isolated without Cl^{-} and heated in 50 mM Cl^{-}). All Hill assays were performed with 70 mM Cl^{-} , and therefore the differences reflect irreversible damage caused by heating in the absence of Cl^{-} . In both cases, this is approximately a 2-fold difference in Hill activity after heating at 39°C for 3 minutes. Addition of 25 mM Na_2SO_4 to thylakoids isolated without Cl^{-} does not affect the heating curve. This suggests that the effect is specific for certain anions, and does not depend simply on the ionic strength of the anion. Within the experimental uncertainty, addition of 50 mM NaNO_3 also has no effect, as in the unheated control. Addition of 50 mM NaBr , however, yields the same result, within the experimental uncertainty, as addition of 50 mM NaCl .

Compared to the transition temperature (T_m) for thylakoids heated without Cl^{-} (36.9°C), the presence of 50 mM Cl^{-} shifts the heating curve about 2.5°C to higher temperature (39.4°C). Addition of 50 mM Br^{-} shifts the curve about 1.7°C (38.6°C). Addition of SO_4^{2-} or NO_3^{-} produces no shift in T_m at the concentrations tested.

This behavior of Cl^{-} , Br^{-} , and SO_4^{2-} is consistent with their specificity in activating O_2 -evolution in Cl^{-} -depleted thylakoids at room temperature (Critchley et al., 1982). Although NO_3^{-} has been shown to partially substitute for Cl^{-} in spinach thylakoids (Hind et al., 1969), its relative effectiveness was considerably less than that of Br^{-} .

Earlier we proposed that the specificity of anions as cofactors for O_2 -evolution was determined by their ability to bind reversibly at or near PS II (Critchley et al., 1982). Such binding would serve to stabilize the active conformation of the oxygen-evolving complex and make it less susceptible to thermal change, in accord with the activity of the anion as a cofactor. (This is the case for all four anions we have studied so far.) Stabilization of the O_2 -evolving system through chloride binding suggests, therefore, that Cl^{-} plays the more specific role of a modifier even under physiological conditions.

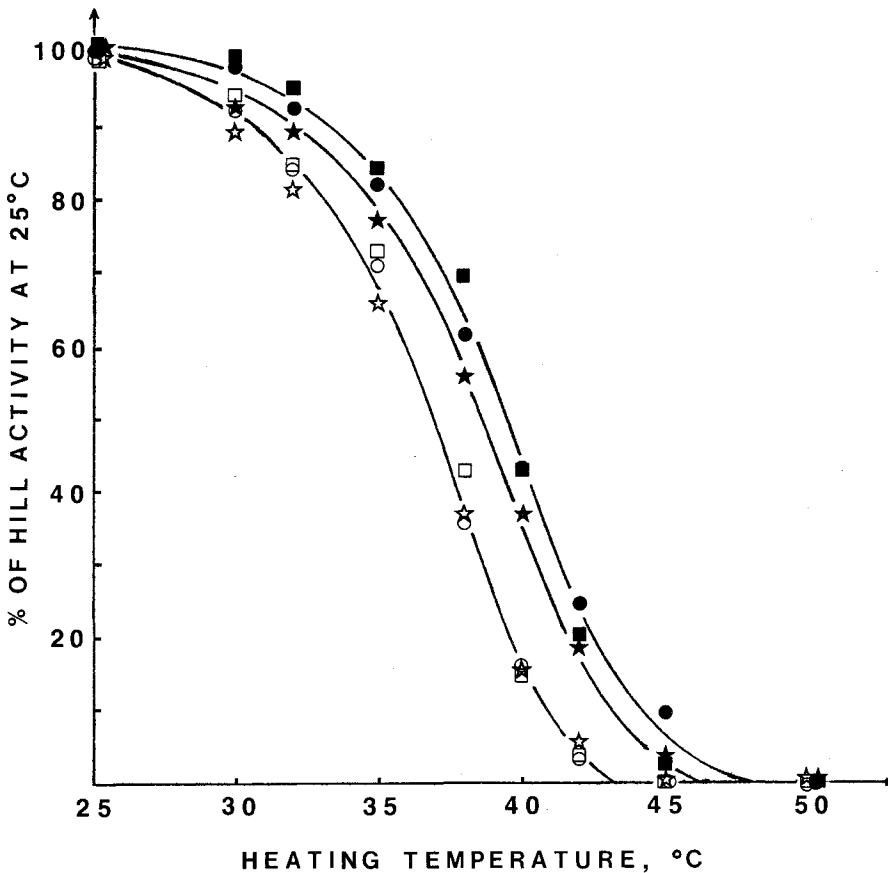


FIGURE 1. Effect of Anions on the Loss of Hill Activity in Heated Spinach Thylakoids. Thylakoids were (A) isolated and then heated in 50 mM NaCl (■); or (B) isolated without NaCl and heated in: 50 mM NaCl (●), 50 mM NaBr (★), 50 mM NaNO₃ (○), 25 mM Na₂SO₄ (□), or phosphate buffer (☆). Spinach (150 g) was homogenized in 300 ml of 2 mM EDTA, 2 mM ascorbate, 0.1% BSA, and 50 mM sodium phosphate (pH 7.2), \pm 50 mM NaCl. This was centrifuged and resuspended in 50 mM sodium phosphate (pH 7.2), \pm 50 mM NaCl, to 3.5 mg Chl ml⁻¹. Aliquots were removed, and anions were added as indicated. Samples were left in darkness for 5 minutes at room temperature (23°C) prior to heating. Each aliquot was heated for 3 minutes in darkness and immediately cooled on ice for 90s. Temperature was controlled \pm 0.1°C. After cooling, 5 μ l of suspension was diluted into 3 ml of 70 mM NaCl, 50 μ M DCPIP, 1.0 mM (NH₄)₂HPO₄, 0.01 μ M Gramicidin, and 50 mM sodium phosphate (pH 7.2) for Hill assay at room temperature. Data from two experiments were averaged for each treatment and normalized to the rates obtained after heating at 25°C. Each pair of measurements differed by no more than 10% in the relative Hill activity.

ACKNOWLEDGEMENTS

This work was supported by NSF Grant PCM 79-11148 to H.S.G., and by NSF Grant PCM 78-24532 and a University of Illinois Biomedical Research Support Grant to G.

REFERENCES

- Cheniae GM and Martin IF (1970) Sites of Function of Manganese within Photosystem II. Roles in O_2 -evolution and System II, *Biochim. Biophys. Acta* 197, 219-239.
- Cramer WA, Whitmarsh J, and Low PS (1981) Differential Scanning Calorimetry of Chloroplast Membranes: Identification of an Endothermic Transition Associated with the Water-Splitting Complex of Photosystem II, *Biochemistry* 20, 157-162.
- Critchley C, Baianu IC, Govindjee, and Gutowsky HS (1982) The Role of Chloride in O_2 Evolution by Thylakoids from Salt-Tolerant Higher Plants, *Biochim. Biophys. Acta* 682, 436-445.
- Ginsburg A and Carroll WR (1965) Some Specific Ion Effects on the Conformation and Thermal Stability of Ribonuclease, *Biochemistry* 4, 2159-2174.
- Govindjee, Baianu IC, Critchley C, and Gutowsky HS (1983) Roles of Chloride and Bicarbonate in Chloroplast Electron Transport. In Inoue Y et al., eds. *Photochemistry of Photosystem II and Photosynthetic Water Oxidation*, in press. Tokyo: Academic Press.
- Hind G, Nakatani HY, and Izawa S (1969) The Role of Cl^- in Photosynthesis. I. The Cl^- Requirement of Electron Transport, *Biochim. Biophys. Acta* 172, 277-289.
- Izawa S, Muallem A, and Ramaswamy NK (1983) Chloride Ion-Sensitive Inactivation of O_2 Evolving Centers. In Inoue Y et al., eds. *Photochemistry of Photosystem II and Photosynthetic Water Oxidation*, in press. Tokyo: Academic Press.
- Kelley PM and Izawa S (1978) The Role of Chloride Ion in Photosystem II. I. Effects of Chloride Ion on Photosystem II Electron Transport and on Hydroxylamine Inhibition, *Biochim. Biophys. Acta* 502, 198-210.
- Yamashita T and Butler W (1968) Inhibition of Chloroplasts by UV-Irradiation and Heat-Treatment, *Plant Physiol.* 43, 2037-2040.