Tetracaine Inhibition of Electron Transport in Pea Chloroplasts is Coupled to Calcium Displacement by the Local Anesthetic

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Abstract. Chloroplasts isolated from pea seadlings grown on water containing $^{45}Ca^{2+}$ were treated with local anesthetic tetracaine. Addition of tetracaine inactivated the electron transport activity of donor side photosystem II. This inhibition was accompanied by $^{45}Ca^{2+}$ release from the chloroplast membranes as the whole and destroyed by osmotic shock. No such effect was observed when Tris or hydroxylamine were used to inhibit the donor side photosystem II. Upon thermal inactivation of chloroplasts $^{45}Ca^{2+}$ release occurred but at temperatures above 80°. The functional role of Ca^{2+} in photosystem II is discussed.

Key words: Chloroplast — Photosystem II — Local anesthetic — Calcium

Introduction

The structural and functional organization of PSII-driven electron transport in photosynthetic membranes is the least studied in photosynthesis. The available experimental data point to the involvement of of Ca^{2+} in PSII functioning in both cyanobacteria and in chloroplasts of higher plants (Brand and Becker 1984). That Ca^{2+} plays an essential role in the operation of the water splitting system has been demonstrated in oxygen evolution reactivation experiments (Ono and Inoue 1983) and also in experiments using compounds which interact with Ca^{2+} or influence Ca^{2+}-dependent processes (Barr et al. 1980, 1982; Carpentier and Nakatani 1985). The PSII-driven electron transport has been shown to be inhibited by calmodulin antagonists (Barr et al. 1982) and agents blocking Ca^{2+}-channels (Carpentier and Nakatani 1985). Previously we reported that local anesthetics of the procaine series inhibit the water-splitting system in chloroplasts (Semin et al. 1989). From studies of the mechanisms of action of local anesthetics it is known that this compounds can influence membrane

Abbreviations: PSII, photosystem II; DCIP, 2,6-dichlorophenolindophenol

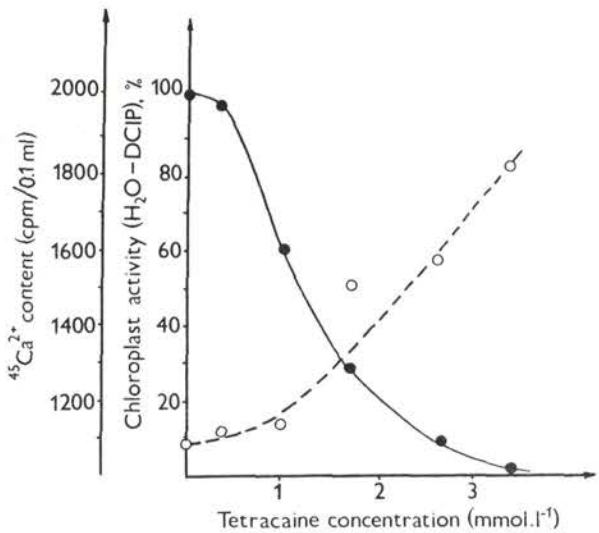

Fig. 1. Chloroplast electron transport activity ($H_2O \rightarrow$ DCIP) and $^{45}Ca^{2+}$ content in the incubation medium as functions of tetracaine concentration. Circles, chloroplast activity; empty circles — $^{45}Ca^{2+}$ content. Chlorophyll concentration, 100 μg/ml; pH of the incubation medium, 8.5.

function through Ca^{2+}-dependent processes by extruding Ca^{2+} (Low et al. 1979) from its binding sites. Since Ca^{2+} is supposed to be involved in water photolysis, it would be interesting to know whether inhibition of PSII electron transport by local anesthetics interfaces with membrane-bound calcium.

Materials and Methods

Pea seeds were soaked for 24 h in tap water and kept on filtration paper until the appearance of roots. The seedlings were grown in tap water in a growth chamber under 12 hours light/dark cycle and at 20 °C. The tap water contained $^{45}Ca^{2+}$ (2.96—4.14 × 10^4 s^{-1}/ml). Chloroplasts (class II) were isolated from 12—14-day old seedlings using the method according to Semin et al. (1989). Chloroplasts were prepared from all leaves since preliminary studies showed little difference in $^{45}Ca^{2+}$ content between leaves of different age. The incubation medium contained 40 mmol.l^{-1} Tris-HCl buffer, 2 mmol.l^{-1} $MgCl_2$, 10 mmol.l^{-1} KCl, 0.2 mol.l^{-1} sucrose. To study the effect of tetracaine, the anesthetic solution was added to chloroplast suspension. After 1 min incubation, DCIP was added and the preparation in a 1 mm thick cuvette was illuminated for 30 s. Chloroplast functional activity was estimated from absorbance within the absorption band of the artificial acceptor used (around 600 nm). The sample was then centrifugated and the supernatant was collected in a beaker for scintillation analysis in a Rackbeta liquid scintillation counter (LKB, Sweden). To induce osmotic shock the chloroplasts were placed in distilled water for 5 min. To inactivate the chloroplasts by Tris, the sample was washed with 0.8 mol.l^{-1} Tris buffer for 15 min (Trebst 1980).

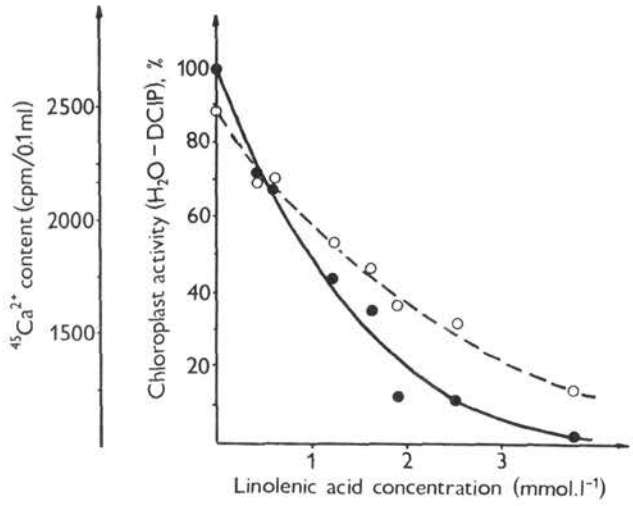

Fig. 2. Chloroplast activity (filled circles) and $^{45}Ca^{2+}$ content in the incubation medium (empty circles) as functions of linolenic acid concentration. Chlorophyll concentration, 200 μg/ml; pH of the incubation medium, 7.2.

Calorimetric measurements were carried out using a differential scanning calorimeter DASM-1M (USSR). The scanning rate was 2 K/min. Linolenic acid (Sigma, USA) was used without preliminary purification.

Results

Chloroplasts containing $^{45}Ca^{2+}$ were treated with the local anesthetic tetracaine. Fig. 1 shows the labelled calcium content in the incubation medium and chloroplast activity as a function of tetracaine concentration. The inhibition of electron transport by tetracaine is accompanied by an increase of the radioactive label content in the incubation medium: the stronger the inhibition, the larger the label content. The inhibitory power of tetracaine, which contains a tertiary amino structure in its molecule, largely depends on pH and is potentiated in the presence of its neutral form (Semin et al. 1989). The pH dependence of the tetracaine-induced Ca^{2+} displacement shows that the content of Ca^{2+} in its medium increases with the decreasing chloroplast activity (chloroplast concentration 200 μg/ml). Chloroplast exposed to osmotic shock exhibit a similar relation of the anesthetic concentrations and labelled calcium in the medium, suggesting calcium extrusion of the membrane rather than its leakage from the thylakoid interior. The amount of $^{45}Ca^{2+}$ released makes up about 17 percent of its total content in the chloroplast membrane.

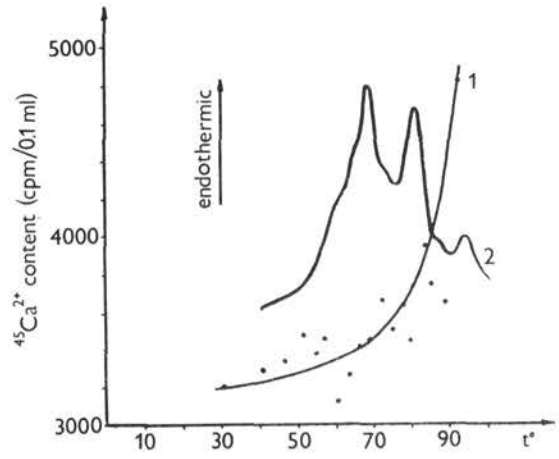

Fig. 3. The release of $^{45}Ca^{2+}$ from chloroplasts (1) and heat capacity of chloroplasts (2) as functions of temperature. Chlorophyll concentration 440 μg/ml (1) and 250 μg/ml (2); pH of the incubation medium, 7.2.

The results clearly show that electron transport inhibition on the donor side of PSII by tetracaine is accompanied by Ca^{2+} displacement from the chloroplast membrane. The question arises whether other inhibitors of electron transport linked to water photolysis produce a similar effect. To check this possibility experiments were conducted using Tris, hydroxylamine and linolenic acid. The latter is known to inhibit electron transport not only on the acceptor side of PSII but also on the donor side at the level of electron donor Z (Golbeck and Warden 1984). No release of calcium seems to occur in chloroplasts inactivated by Tris or hydroxylamine (5 mmol . l^{-1}). The inhibition by linolenic acid is accompanied by a decrease in the Ca^{2+} content in the incubation medium (Fig. 2). The effect can probably be explained by the formation of a complex of $^{45}Ca^{2+}$ in the medium and the fatty acid included in the chloroplast membrane (Herbette et al. 1984).

Inactivation of the oxygen-evolving system by heating (to 45—55 °C) did not change the $^{45}Ca^{2+}$ content in the medium, similarly as did Tris and hydroxylamine. Release was seen only at temperatures above 80° (Fig. 3). At this temperature scanning differential microcalorimetry reveals denaturation of the PSII macromolecular complex (Ananieva et al. 1983).

Discussion

The present experiments show that inactivation of the donor side of PSII in chloroplasts is accompanied by Ca^{2+} release. It is difficult as yet to explain the observed correlation. Chloroplasts contain one Ca^{2+} per PSII reaction center (Shen et al. 1988), whereas free calcium is in excess. Owing to this, the effect of tetracaine on calcium involvement in PSII operation is largely masked under the experimental conditions used. Nevertheless, the analysis reveals that the increased Ca^{2+} release is accompanied by a stronger inhibition of electron transport. This correlation between the two processes is observed over the same concentration range of tetracaine. Treatment with Tris buffer or hydroxylamine induces no Ca^{2+} release; this agrees with recent reports concerning membrane PSII preparations (Shen et al. 1988).

The incorporation of tetracaine into chloroplast membrane is accompanied by Ca^{2+} release into the external medium. This does not interfere with the location of the oxygen-evolving complex (peripheral proteins of the complex) on the inner side of the thylakoid membrane: the action on the peripheral proteins (NaCl) is known to cause no Ca^{2+} release from PSII (Shen et al. 1988). By analysis of D2 amino acid sequence, an integral protein of the donor side of PSII, a calcium-binding site is revealed, which is similar to that in Ca^{2+}-binding proteins, e.g. calmodulin (Coleman and Govindjee 1987). The Ca^{2+} binding site is between amino acid residues 224—236. D2 protein has 5 hydrophobic helices spanning the membrane (Barber 1987) with predicted folding of the amino acid sequence. The calcium-binding sites on the stromal side of the thylakoid membrane. Obviously, when exposed to the anesthetic, the site will expell Ca^{2+} to the stromal side, just what has been seen in the present experiments. The data suggest that inactivation of PSII by tetracaine is presumably the result of the action of the drug on the Ca^{2+}-binding site. This effect may be employed in studies into the functional role of calcium in water photolysis.

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References

Ananieva L. K., Semin B. K., Ivanov I. I. (1983): Calorimetric investigation of structural transitions in pea chloroplast membranes. Fiziol. Rastenij **30,** 552—556 (in Russian)

Barber J. (1987): Photosynthetic reaction centres: a common link. TIBS **12,** 321—325

Barr R., Troxel K. S., Crame F. L. (1980): EGTA, a calcium chelator, inhibits electron transport in photosystem II of spinach chloroplasts at two different sites. Biochim. Biophys. Res. Commun. **90,** 206—212

Barr R., Troxel K. S., Crame F. L. (1982): Calmodulin antagonists inhibit electron transport in photosystem II of spinach chloroplasts. Biochim. Biophys. Res. Commun. **104,** 1182—1188

Brand J. J., Becker D. W. (1984): Evidence for direct roles of calcium in photosynthesis. J. Bioenerg. Biomembrane **16,** 239—249

Carpentier R., Nakatani H. Y. (1985): Inhibitors affecting the oxidizing side of photosystem II at the Ca^{2+} and Cl^--sensitive sites. Biochim. Biophys. Acta **808,** 288—292

Coleman W. J., Govindjee (1987): A model for the mechanism of chloride activation of oxygen evolution in photosystem II. Photosynth. Res. **13,** 199—223

Golbeck J. H., Warden J. T. (1984): Interaction of linolenic acid with bound quinone molecules in photosystem II. Biochim. Biophys. Acta **767,** 263—269

Herbette L. G., Favreau C., Segelman K., Napolitano Ch. A., Watras J. (1984): Mechanism of fatty acid effects on sarcoplasmic reticulum. II. Structural changes induced by oleic and palmitic acids. J. Biol. Chem. **259,** 1325—1332

Low P. S., Lloyd D. H., Stein T. M., Rogers J. A. III (1979): Calcium displacement by local anesthetics. Dependence on pH and anesthetic charge. J. Biol. Chem. **254,** 4119—4125

Ono T.-A., Inoue Y. (1983): Requirement of divalent cations for photoactivation of the latent water-oxidation system in intact chloroplasts from flashed leaves. Biochim. Biophys. Acta **723,** 191—201

Semin B. K., Tshudinovskich M. N., Ivanov I. I. (1989): Local anesthetic-induced inhibition of chloroplast electron transport. Gen. Physiol. Biophys. **8,** 233—244

Shen J.-R., Satoh K., Katoh S. (1988): Calcium content of oxygen-evolving photosystem II preparations from higher plants. Effects of NaCl treatment. Biochim. Biophys. Acta **933,** 358—364

Trebst A. (1980): Inhibitors in electron flow: tools for the functional and structural localization of carriers and energy conservation sites. In: Methods in Enzymology, v. 69, pp. 675—715, Academic Press, N.-Y.

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