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ULTRASTRUCTURAL LOCALIZATION OF PHOTOSYSTEM I IN PLASTIDS OF SENESCENT SPINACH LEAVES

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Introduction

Detached leaves have often been used for experimental study of senescence, because in them senescence can be induced at will and can be either accelerated or delayed by changing experimental conditions. It is known that in detached leaves the processes of senescence progress much more rapidly in darkness than in light (Goldthwaite and Laetsch 1967). These processes affect mostly the chloroplasts. They soon lose chlorophyll and at the same time reduce their thylakoid system (Młodzianowski and Kwintkiewicz 1973). In spinach the reduction of the thylakoid system is especially well pronounced (Wrischer 1977). The aim of the present work was to establish whether these degraded plastids still contained some photosynthetic activity.

Photooxidation of diaminobenzidine (DAB) has been used as a tool for the detection of photosystem I in the thylakoids. DAB is now generally accepted as an electron donor for photosystem I (Chua 1972, Trebst 1974). The oxidized DAB polymers bind firmly and insolubly to the inner side of the thylakoid membrane and accumulate also inside the intrathylakoidal space (loculus). After an additional osmication the sites of this binding become electron opaque and are well visible on ultrathin sections in electron microscopy.

Material and Methods

Young spinach leaves, about 3 cm long, were kept in darkness for 1, 7 or 14 days on tap water or on a 0.05 M sucrose solution (in tap water). For the detection of oxidized DAB a somewhat modified method, de-

scribed by Nir and Seligman (1970) and Nir and Pease (1973), was used. Small pieces of leaves were fixed in 3% formaldehyde in 0.05 M phosphate buffer (pH 7.5) for 30 minutes in darkness. After fixation they were rinsed in the same buffer, to which 5% sucrose was added, for 20 minutes in darkness and then incubated in a medium containing 1 mg/ml diaminobenzidine (DAB) dissolved in 0.05 M phosphate buffer (pH 7.5). The incubation was performed in light (2 fluorescent tubes 20 W, 4500 °K, illumination intensity 4000 lx) for 45—60 minutes. After that the material was washed in phosphate buffer (containing 5% sucrose) for 30 minutes in darkness, and then postfixed in 1% OsO_4 in phosphate buffer (pH 7.2) for 60 minutes. After dehydration the leaf pieces were embedded in Araldite. Ultrathin sections were examined — without any further staining — in a Siemens Elmiskop I. As a control test, the incubation in DAB was performed in complete darkness instead of in light.

For comparison the same experimental material was fixed also in 1% glutaraldehyde in cacodylate buffer (pH 7.2), postfixed in 1% OsO_4 and embedded in Araldite. Ultrathin sections were stained with uranyl acetate and lead citrate.

Results

The chloroplasts of young, normal spinach leaves contain a well developed thylakoid system with grana and stroma thylakoids (Fig. 1). After incubation in DAB in light and subsequent osmication the membranes of both grana and stroma thylakoids become electron opaque. Dark are also the intrathylakoidal spaces (loculi). On the contrary, the membranes of the plastid envelope always remain faintly stained, due to their reaction with OsO_4 only (Figs. 2, 3).

After detachment of the leaves and their transfer to darkness considerable structural changes soon appear in the chloroplasts. After only one day in darkness small prolamellar bodies are present in the leaf chloroplasts. They lie between the grana and are in contact with them. After incubation in DAB only grana and stroma thylakoids are stained dark, but not the tubules of the prolamellar bodies (Fig. 4).

Fig. 1. Chloroplast of an intact young spinach leaf with well developed grana and stroma thylakoids. Fixation: glutaraldehyde/OsO_4; staining with uranyl acetate and lead citrate. 20,000 : 1.

Fig. 2. Chloroplast after incubation in DAB. Photooxidized DAB is present both in grana and stroma thylakoids, but not in the plastid envelope. 20,000 : 1.

Fig. 3. Part of a chloroplast with strong accumulation of photooxidized DAB polymers in the membranes of the thylakoids. The faint staining of the membranes of the plastid envelope is due only to their reaction with OsO_4. 70,000 : 1.

Fig. 4. Chloroplast of a detached leaf after one day in darkness. Grana thylakoids are dark due to photooxidized DAB, while the tubules of the prolamellar body (pb) and the plastid envelope are without any DAB reaction. Note the intratubular DAB staining in the mitochondria (upper part of the picture) due to cytochrome oxidase activity. 30,000 : 1.

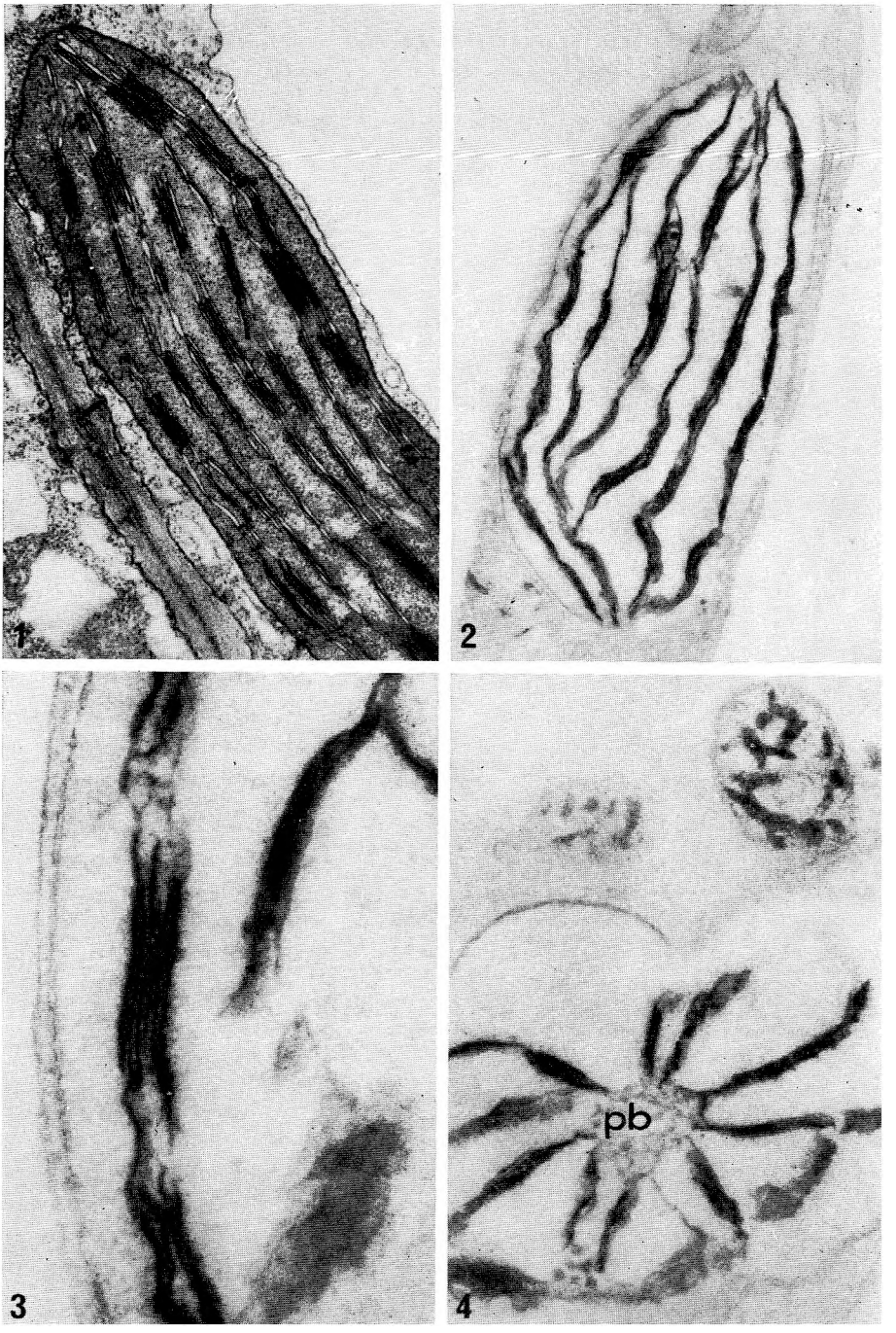

Fig. 1—4. — Sl. 1—4.

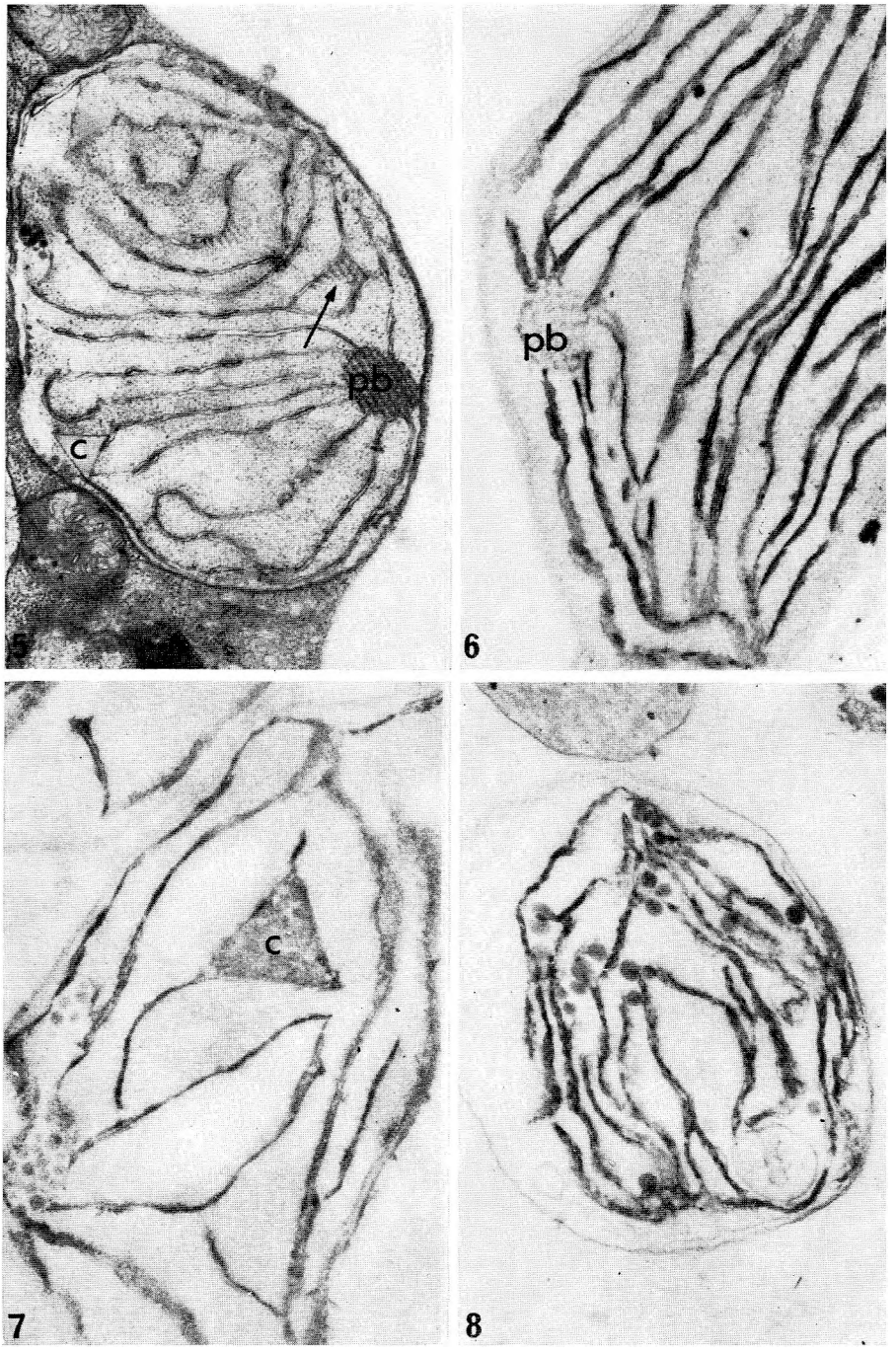

Fig. 5—8. — Sl. 5—8.

After several days in darkness detached leaves yellow progressively. Grana become small and contain only a few thylakoids. In additon to that, there are also other structural changes in these plastids. The most obvious are protein crystals lying intrathylakoidally (Fig. 5), reported in detail in another communication (Wrischer 1977). Leaves which survived 14 days in darkness — predominantly those kept on sucrose solutions — contained plastids almost without any grana. The whole thylakoid system is reduced to single thylakoids, which overlap only here and there (Fig. 5). The photooxidation of DAB takes place in all these thylakoids (Fig. 6) and even in those which surround the protein crystals (Fig. 7). After a prolonged incubation in DAB the crystals also become dark. This is probably an unspecific reaction due to the accumulation of oxidized DAB polymers inside the dilated intrathylakoidal space; if the incubation in DAB is short, the crystals are not stained. Plastid envelops and prolamellar bodies always remain faintly stained (Figs. 6, 7). Special rows of tubules, which lie close to some thylakoids (Fig. 5), do not react with DAB either.

In still more senescent leaves the depositions of oxidized DAB in plastid thylakoids are not uniformly arranged any more, but become granulated (Fig. 8). In completely yellow leaves strongly damaged plastids, containing only vesicles instead of thylakoids, remain totally without DAB staining.

Control samples of leaf tissue incubated in DAB in darkness never show DAB staining of thylakoids.

Mitochondrial tubules are usually also intensely stained (Fig. 4), because the cytochrome oxidase of the inner mitochondrial membrane reacts with DAB (Seligman et al. 1968). This reaction is not dependent on light and occurs also in the control samples incubated in complete darkness.

Discussion

The results of the described experiments show that the photooxidation of DAB in the thylakoid membranes is a useful method to study different stages of plastid changes occuring in detached senescent leaves in darkness. In spinach these changes begin with a progressive degrada-

Fig. 5. Plastid of a detached leaf after 14 days in darkness on sucrose solution. The thylakoid system is reduced to single thylakoids, which are connected with rows of tubules (arrow). A prolamellar body (pb) and intrathylakoidal protein crystals (c) are also present. Fixation: glutaraldehyde/OsO_4; staining with uranyl acetate and lead citrate. 24,000 : 1.

Fig. 6. Part of a plastid from detached leaf after 14 days in darkness on sucrose solution. Oxidized DAB is present in the thylakoids, while the tubules of the prolamellar body (pb) and the plastid envelope are completely without reaction. 36,000 : 1.

Fig. 7. The same material as in Fig. 6. Single thylakoids contain oxidized DAB, which is present even in the thylakoid membrane surrounding the crystal (c). An accumulation of DAB oxidation products also appears inside the crystal. 36,000 : 1.

Fig. 8. Part of a plastid from a completely yellow leaf surviving after 14 days in darkness on sucrose solution. A positive DAB reaction is still detectable in the thylakoids, although it is not uniformly distributed. 28,000 : 1.

tion of the grana and end with a very poor membrane system reduced to a few single thylakoids. Although the leaves have been kept in complete darkness for days, these thylakoids still give a positive DAB reaction. On the other hand, mebraneous structures (prolamellar bodies and rows of tubules lying close to some thylakoids), which do not form until the leaves are put into the darkness, never react with DAB.

Because DAB is accepted as a specific marker for photosystem I (Nir and Pease 1973), it may be concluded that photosystem I is still present in the membranes of the reduced thylakoid system in plastids of senescent leaves. It is certain that these thylakoids originate directly from the thylakoid system of the chloroplasts, but it is impossible to establish at this stage whether they are remnants of the grana or of the stroma thylakoids, as both types contain photosystem I. On the contrary, it has been claimed that only the grana should contain photosystem II (Park and Sane 1971). Therefore, it would be worth while examining the plastids in senescent spinach leaves also with the cytochemical method for the detection of photosystem II, regardless of the fact that this method is not perfect yet (Hall et al. 1971, Nir and Pease 1973, Kirchanski 1976).

Summary

The localization of photosystem I in the thylakoids of plastids in senescent spinach leaves has been studied by photooxidation of diaminobenzidine (DAB). It has been shown that in progressively senescent leaves photosystem I is still present even in plastids with a much degraded thylakoid system.

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SADRŽAJ

ULTRASTRUKTURNA LOKALIZACIJA FOTOSISTEMA I U PLASTIDIMA OSTARJELIH LISTOVA ŠPINATA

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S pomoću fotooksidacije diaminobenzidina (DAB) proučavana je lokalizacija fotosistema I u tilakoidima plastida ostarjelih listova špinata. Istraživanja su pokazala da je i u plastidima vrlo ostarjelih listova s jako degradiranim tilakoidnim sistemom fotosistem I još uvijek prisutan.

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