Short Communication

The Senescence of Isolated Chloroplasts

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Summary. Because previous work indicated that senescence of oat leaves in darkness probably centers in the cytoplasm, the senescence of chloroplasts isolated from the same material and carefully purified was studied. The rate of loss of chlorophyll was about one-tenth of that which takes place in the isolated leaves at the same temperature, while the loss of protein, though slightly more rapid, was still only 36% of the rate observed in the leaf after 7 days. This unexpected stability of the chloroplasts is matched by their photosynthesis, 81% of photosystem I and 35% of photosystem II being present after 3 days at 25°C. Traces of system I, but not of system II, were still detectable even after 7 days.

When the first leaves of oat seedlings are detached and left to senesce in the dark the sequence of events is as follows (Martin and Thimann, 1972). Within a few hours (at 25°C) proteolysis begins and about 18 h later the chlorophyll begins to decrease. After 3 days 55–60% of the chlorophyll and about 60% of the total protein has disappeared. Cycloheximide arrests both processes but chloramphenicol does not. Closely similar results were recently reported with barley leaves by Peterson and Huffaker (1974). In both cases the inference was drawn that hydrolytic enzymes, synthesized in the cytoplasm, initiate the senescence syndrome and the chloroplasts become involved subsequently. In order to test this idea, the chloroplasts were isolated and their senescence studied.

From oat (Avena sativa L. cv. Victory) seedlings 7 days old, grown under 6000 lx of light from white fluorescent lamps, the first leaves were cut off, iced, and minced twice in the isolation medium (all data in mM): 330 sorbitol, 30 N-tris-(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES), 5 dithiothreitol, 1 each MnCl₂, MgCl₂ and EDTA, plus 0.5% bovine serum albumin fraction V; the pH was brought to 7.4. After filtering through Millipore and Miracloth the residue was ground with 1/5 its weight of sterile acid-washed sand and the filtrate treated as above and added. All operations were carried out under aseptic conditions.

The chlorophyll contents of the isolated chloroplasts were estimated by the method of Bruinsma (1963) in 80% acetone supernatant obtained by centrifugation at 10000 × g for 20 min. The precipitates were homogenized with 1 ml of 10% TCA and centrifuged at 10000 × g for 30 min. The precipitated protein was measured by the method of Lowry modified by Miller (1959). Leaf chlorophyll and protein were determined as previously reported by Martin and Thimann (1972). Photosystem I was determined using methylviologen-Mehler reaction as described in Osmond (1974), while photosystem II was measured by a modification of the method of Boardman et al. (1972) using oxygen measurements instead of the spectrophotometer. In both procedures a Yellow Springs Instrument Co. (Yellow Springs, Ohio, USA) Model 53 oxygen electrode was used.

The time courses of proteolysis and of chlorophyll disappearance at 25°C in the dark are shown in Fig. 1. By comparison with the same processes in segments of whole leaves the chloroplasts appear to be relatively stable. After 3 days at
Fig. 1. Retention of total chlorophyll and protein in both chloroplasts and leaf segments, during senescence for 7 days. Chloroplasts were suspended in Medium B (see under Table 1) in the wells of spot plates below which were two filter papers saturated with sterile water in a large Petri dish, left in darkness at 25°. Initial values of chlorophyll: 200 µg/ml; protein: 800 µg/ml, respectively. Twelve apical 3 cm leaf segments were suspended on a gauze pad saturated with 15 ml sterile water in Petri dishes at 25° in darkness. Initial values of chlorophyll: 38 µg leaf⁻¹, protein: 309 µg leaf⁻¹. Circles (○), chlorophyll; squares (□), protein content.

25° not more than 5% of the chlorophyll has been lost. The decrease of protein is somewhat more rapid but still reaches only 12% after 3 days, compared to 57% in the leaf. Even after 7 days 70% of the protein is still present, while the leaves at this time are almost completely yellowed. Thus far we have not succeeded in inducing senescent changes as rapid as those in the leaf by adding various protease preparations, including one prepared from senescent oat leaves. Under the phase microscope about half of the chloroplasts showed high refractivity at the start, and of these about 80% had lost their high refractivity after 3 days, but otherwise all appear normal and intact. No bursting was observed and the only other visible change was the occasional appearance of a few bacteria after 5–7 days at 25°.

Because the chloroplasts can evidently preserve both their pigment and their form, as well as most of their protein, for several days, it was of interest to investigate the degree to which their ability to carry out photosynthesis might be preserved. Both photosystem I and photosystem II were highly active in the fresh chloroplasts. After 24 h at 25°, system I was still at almost full activity, while system II had decreased by 10%. Table 1 shows the activities in two different media: (a) the full medium used for isolation as described above, and (b) a simpler mixture of osmoticum, buffer and detergent. The composition of the suspending medium is evidently of overriding importance in determining the stability of these enzyme systems. It is notable, in view of the short duration of most experiments on photosynthesis, that 80% of photosystem I activity survives 3 days at 25°. Indeed, after 7 days at 25° system I could be still clearly detected, although system II had disappeared.

In the immediate context of the study of senescence, it is clear that the hypothesis that the senescent process is initiated, and indeed driven, by the cytoplasm is confirmed.