Ultrastructural Changes in *in vitro* Ageing Spinach Chloroplasts

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Ultrastructural changes in *in vitro* ageing spinach chloroplasts have been studied in detail. Prolonged storage caused swelling of the chloroplasts due to the increase in the thickness and spacing of the thylakoid membranes. The increase in the thickness of the membrane is partly accompanied by the release of lipids. Addition of crystalline bovine serum albumin was found to stabilize the membrane structures. Storage of the chloroplasts at 77 °K even though it resulted in complete breakage of the whole chloroplasts, maintained the thylakoid structures in a highly intact form.

Introduction

Inactivation of the photosynthetic capacity in chloroplasts stored under various *in vitro* conditions has been reported by some of the earlier workers [1–3]. Chloroplasts stored at room temperature were shown to loose their Hill reaction activity in 6–8 hours. This loss of photosynthetic capacity was found to be in parallel with an increase in the level of the free fatty acids and swelling of the chloroplasts. Addition of crystalline BSA was shown to reduce the level of the fatty acids release from the thylakoid membranes.

In a previous paper [4] we have shown that intact chloroplasts when stored at −5 °C in isotonic solution containing BSA were able to maintain a high level of Hill reaction activity for a maximum period of 10 days. Chloroplasts stored in the absence of BSA showed comparatively a rapid loss of photosynthetic reactions and a high level of uncoupled electron transport. Attempts were also made to prevent the swelling of the chloroplasts by storing them in high osmotic concentration solutions (0.66 and 1.2 M sorbitol). Irrespective of the storage medium ageing caused a high increase in the chloroplast volume and resulted in breakage of all the chloroplasts within 5 days. Storage of the chloroplasts at 77 °K even though it caused breakage, did maintain all the photochemical activities for a period of 15 days.

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This paper deals with the ultrastructural changes in the thylakoid membranes in ageing chloroplasts stored under different conditions.

Methods

For details on the preparation of intact chloroplasts, storage conditions and electron microscopy refer Kulandaivelu and Hall [4].

For microdensitometer studies, negatives at 50 000 magnification were traced with a 10 : 1 arm ratio on a recording densitometer with a 50 μm slit. The thickness of the lamellae was measured as described by Miller and Nobel [5] by measuring the full width at the half maximum region in microdensitometer traces (Fig. 3).

Results

Typical electronmicrographs of fresh and 10 day-old spinach chloroplasts suspended in isotonic (0.33 M sorbitol) and slightly hypertonic (0.66 M) solutions are shown in Fig. 1 *. Fresh preparations in 0.33 M contained mostly normal-sized (Type A) with a small percentage of broken and shrunken chloroplasts. High osmotic solutions resulted in complete shrinkage of all the chloroplasts. Irrespective of the sorbitol concentration in the storage medium, ageing caused complete breakage of all the chloroplasts. The extent of swelling was found to be comparatively lower at higher osmotic solutions.

Further attention was focussed on the nature of the granal thylakoid membranes, since swelling of the chloroplasts could be caused either by an

* Figs 1 and 2 see Plate on page 82 a and b.

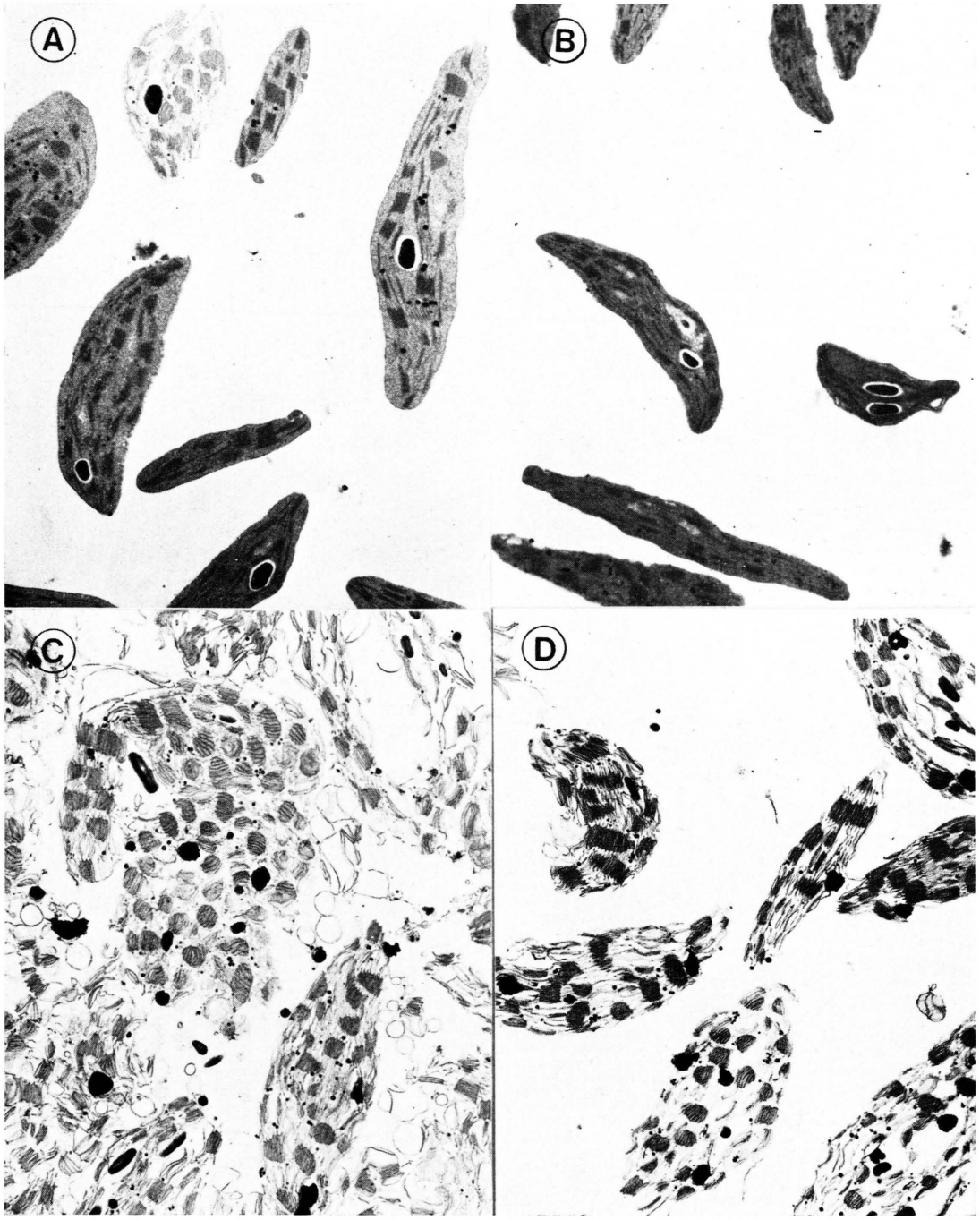

Fig. 1. Electron micrographs of typical fresh and 10 day-old spinach chloroplasts in 0.33 M (A. C) and 0.66 M (B, D) sorbitol solution. Chloroplasts were aged at −5 °C. × 7,500.

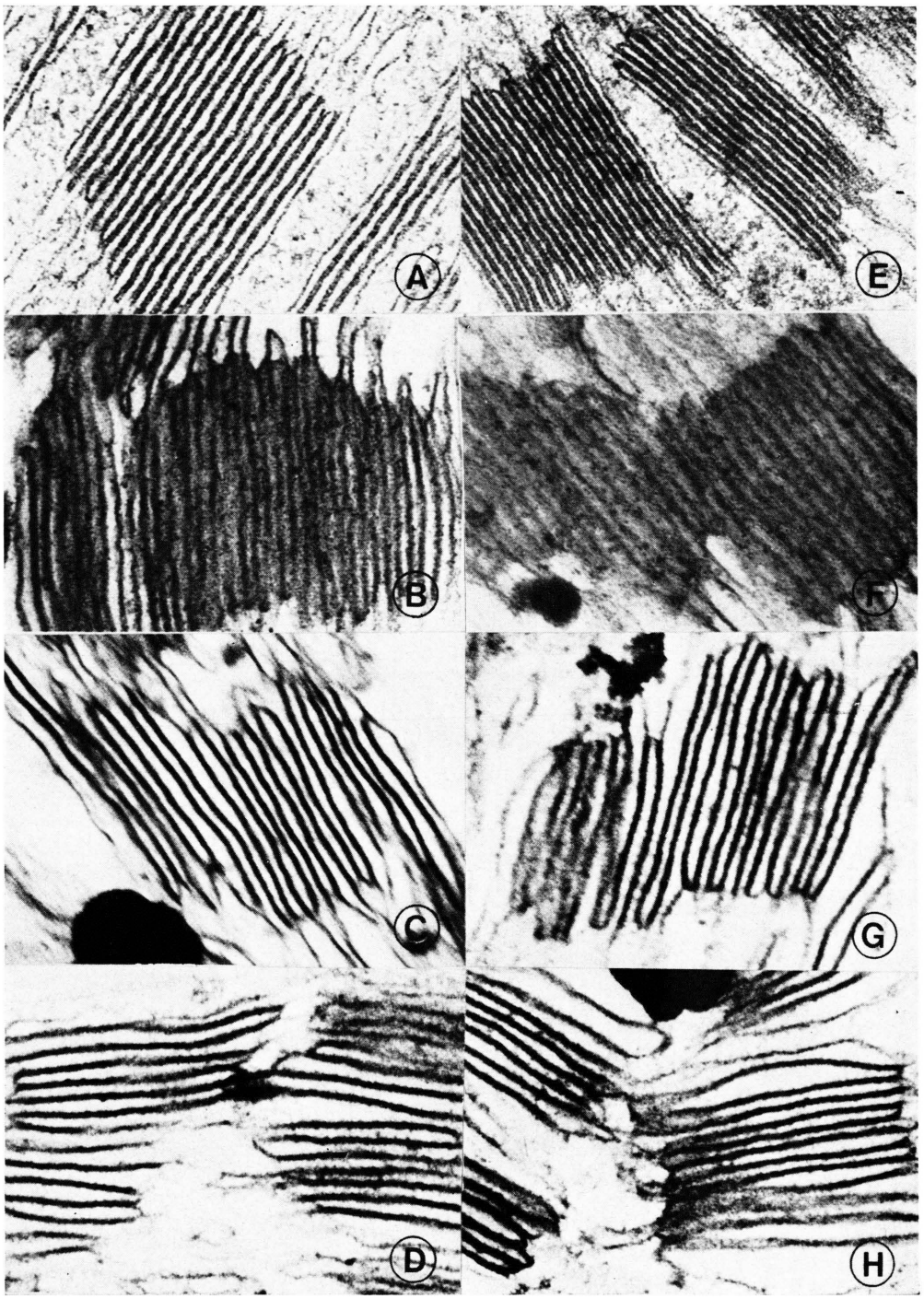

Fig. 2. Effect of ageing and storage conditions on the ultrastructure of the granal thylakoids in spinach chloroplasts. Chloroplasts were stored in 0.33 M (Left hand side A—D) or 0.66 M (Right hand side E—H); at −5 °C (A—C, E—G) or 77 °K (D and H). All, except B and F, contained 1% BSA in the storage medium. × 92,000.

increase in the thickness of the membranes or their moving apart from each other. Fine details of the granal thylakoid membranes in fresh and aged chloroplasts stored in 0.33 and 0.66 M sorbitol solutions at −5 °C and 77 °K are shown in Fig. 2. Higher osmotic concentration (1.2 M) reduced the spacing of the membranes in the fresh chloroplasts, but the aged samples did not show much difference. A large increase in the thickness and the spacing was generally observed in all the aged samples. Chloroplasts stored at −5 °C in the absence of BSA showed a much diffused thylakoid structure and the interthylakoid spaces were also found to be well contrasted; this could possibly be due to the high level of lipids released from the membranes (Fig. 2 B, F). In contrast to this, storage of chloroplasts at 77 °K resulted in a great increase in the spacing, but the thickness of the membranes was found to be comparatively less.

Attempts were also made to measure precisely the extent of swelling of the thylakoid membranes by scanning the electron micrographs in a microdensitometer. Table I shows the average values of the center-to-center spacing and the thickness of the membranes at half-maximum measured from the densitometer traces as described in Fig. 3. The values obtained with fresh chloroplasts suspended in 0.33 M sorbitol are in agreement of those reported by Miller and Nobel [5].

The *thickness* of the membranes in the fresh chloroplasts suspended in different osmotic solutions did not show much variation, whereas the *spacing* between the membranes was found to be increased at lower sorbitol concentrations. About a 2-fold increase in the spacing of the membranes was observed in all the aged chloroplasts. The thickness of the membranes also showed a large increase, but was comparatively less in chloroplasts stored at 77 °K.

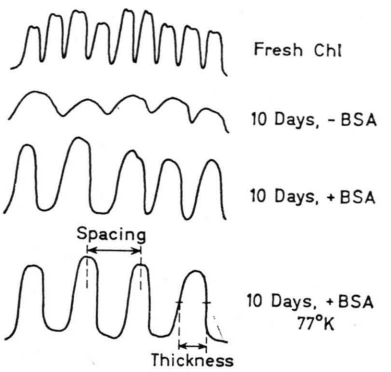

Fig. 3. Comparison of the thickness and spacing of the thylakoid membranes in fresh and aged chloroplasts stored in 0.66 M sorbitol at −5 °C. Traces were obtained by traversing the thylakoid membranes on electron microscope negatives using a recording microdensitometer.

Discussion

The fact that the photosynthetic capacity of the isolated chloroplasts decreases with an increase in the *in vitro* storage time has been known for many years. The decrease in the photosynthetic activities was reported to accompany an increase in the chloroplast volume and the level of the free fatty acids [1, 2]. Complete loss of the Hill reaction activity was shown when maximum chloroplast swelling had occurred [2]. However, recently we have observed that intact chloroplasts when stored under different conditions were able to retain a high percentage of their electron transporting capacity over a longer period [4]. To avoid breakage due to swelling, chloroplasts were

Table I. Effect of ageing and the conditions of storage on the thickness and spacing of the thylakoid membranes in spinach chloroplasts. Thickness and spacing of the membranes were calculated from the microdensitometer tracings as described in Fig. 3. The values are the average of more than 20 traces. All values in Å.

Sample	Sorbitol concentration in the storage medium					
	0.1 M		0.33 M		0.66 M	
	Spacing	Thickness	Spacing	Thickness	Spacing	Thickness
Fresh chloroplasts	194±11	159±3	156± 9	168±5	131± 4	155±4
10 days, −BSA −5 °C	···	···	269±12	218±7	275±11	210±9
10 days, +BSA −5 °C	325±15	214±7	311±15	192±6	268±14	196±4
10 days, +BSA 77 °K	318±19	193±5	327±16	184±5	315±15	176±5

stored under high osmotic solutions. Even though this preserved the intactness of the chloroplasts during the earlier period of ageing, there was a complete breakage within 5 days.

A more detailed analysis of the ultrastructural changes on the granal thylakoid membranes provided further support for the protective effect of BSA and the nature of the chloroplast swelling. A large increase in the thickness and spacing of the membranes was generally observed in all the aged chloroplasts. Changes in the osmolarity of the resuspending medium did not result in much variation of the thickness of the membranes in the aged chloroplasts. Higher osmotic solutions only caused a decrease in the spacing of the membranes. Much diffused and disintegrated thylakoid structures which were observed in chloroplasts stored in the absence of BSA provided again additional support to those earlier reports [1, 3].

In contrast to all these observations, chloroplasts stored at 77 °K showed a well preserved thylakoid organization even when they were broken. The thickness of the membranes was found to be comparatively low. The results presented here demonstrate that *in vitro* ageing of the chloroplasts causes an increase in the spacing and the thickness of the membranes. Increase in thickness of the membranes probably is accompanied by the release of fatty acids from the membranes which finally result in the complete loss of the photosynthetic capacity. The swelling of the chloroplasts due to the increase interthylakoid spacing showed little effect on the photosynthetic capacity.

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