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Protein Crystalloids in the Stroma of Bean Plastids

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Summary

The formation of protein crystalloids in the stroma of bean plastids has been studied. It has been shown that, besides crystallization of the protein already present induced by water loss (which cannot be inhibited by chloramphenicol), the crystalloids also appear when the leaves are fed—but only in light—with very low concentrations of sucrose or glucose for one or two days. In this case their appearance is completely inhibited by simultaneous treatment of the leaves with chloramphenicol but not with cycloheximide. The process of crystalloid formation and disappearance has also been studied. The possibility of their removal from the plastids into the cell vacuoles has been followed and discussed.

1. Introduction

Crystalloids have been repeatedly observed in the stroma of bean plastids of both normal and treated leaves. PERNER (1963) was first to describe such inclusions in bean plastids which were isolated in media containing high concentrations of sucrose. Later SHUMWAY et al. (1967) found such crystalloids in horse-bean plastids treated with hypertonic solutions. At the same time inclusions of the same type were described in normal untreated bean plastids (LEMOINE 1966, WRISCHER 1967), as well as in plastids of bean leaves treated with poisonous gases, in wilted and in plasmolysed leaves (THOMSON et al. 1965, 1966, DOLZMANN and ULLRICH 1966, GUNNING et al. 1968, WRISCHER 1970). It has also been indicated that the number of such inclusions can be raised by feeding normal leaves with very low concentrations of sucrose (WRISCHER 1967).

Since many points concerning the appearance and disappearance of protein crystalloids in bean plastids remained still unclear, a more detailed analysis of these structures has been attempted.
2. Material and Methods

Primary leaves of green or etiolated bean plants (*Phaseolus vulgaris*, var. Butterfisole) 8–9 days old, or exceptionally older, were used for the experiments. The detached leaves were either treated in light or in darkness with hypertonic solutions of sucrose (0.5 M or higher), mannitol (0.5 M higher), or KNO₃ (0.3 M or higher) for 2 to 8 hours, or were left to wilt in air for one to several hours. The leaves were also kept in Petri dishes on filter paper wetted with tap water (control) or with 0.1 M solution of sucrose, glucose, or mannitol, and were exposed to continuous illumination with white light (mercury bulb VTF—250 W, TT—Yugoslavia) of an illumination intensity of 5000 lux for 24 or 48 hours. In some experiments chloramphenicol ("Pliva", Zagreb, 4 mg/ml) or cycloheximide (TAAB, 0.1 μg/ml–10 μg/ml) was added to these solutions. Parallel experiments were also performed in darkness.

Small pieces of leaves were fixed in 1% glutaraldehyde in cacodylate buffer and embedded in araldite after dehydration. Ultrathin sections were stained with uranyl acetate and lead citrate (REYNOLDS 1963) and examined in the Siemens Elmiskop I (at the Institute of Biology of the University of Zagreb).

3. Results

The fine structure of protein crystalloids has been described in detail (e.g., LEMOINE 1966, WRISCHER 1967). According to the data obtained from ultrathin sections, the crystalloids consist of isodiametric particles (about 5 nm in diameter) arranged in more or less parallel lines lying 10 nm apart (Figs. 1 and 4). Sometimes yet another direction of striation is visible more or less perpendicular to the first (Figs. 2, 4, and 5). Crystal defects, i.e., missing particles, and dislocations of particle rows are also frequent (Fig. 2). The particles stain intensely with uranium salts, and can be successfully digested with pepsin (WRISCHER 1967).

In normal, untreated leaves fixed without addition of substances which could raise the osmotic potential of the fixative, protein crystalloids are rarely observed; according to the experience of the author this happens only in young green leaves with functioning cotyledons still attached.

The crystalloids can be, however, easily induced in the following two ways which are essentially different:

1. The crystalloids appear in the plastid stroma when a certain amount of water is removed from the tissue, and the plastids respectively, as by wilting or by plasmolysis, e.g., with sucrose, mannitol, potassium nitrate etc. (GUNNING et al. 1968, WRISCHER 1970). The present investigations have shown that under such conditions the crystalloids appear in green leaves as well as in etiolated leaves during their greening in light (Figs. 1 and 2). They are especially abundant in young primary leaves, but are rather scarce in old ones. They also appear in etioplasts, if treated in darkness, but are then remarkably smaller and fewer.

This process of crystalloid formation appears to be a crystallization of the proteins already present in the stroma, for it cannot be inhibited by a