IMMOBILIZATION OF PHOTOSYNTHETICALLY ACTIVE INTACT CHLOROPLASTS IN A CROSSLINKED ALBUMIN MATRIX

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SUMMARY

Isolated higher plant chloroplasts with intact envelope membranes and bovine serum albumin were co-immobilized by treating the mixture with glutaraldehyde and then subjecting it to a freeze-thaw cycle. The immobilized chloroplasts are capable of photoinduced electron transport to lipophilic oxidants, but become compatible also with ionic oxidants after a transient hyposmotic shock.

INTRODUCTION

A general way to improve the stability of biological function, and at the same time to facilitate the handling of biological materials is to immobilize them by physical or chemical means. One chemical immobilization method, which compares favorably with others with respect to several stabilization indices, is the covalent entrapment of biological materials within a crosslinked protein matrix formed by deep freezing and then slowly rewarming a mixture of bovine serum albumin and glutaraldehyde. The method, originally developed by Broun et al. (1973) has been applied, so far, only to preparations of envelope-free thylakoids (Cocquempot et al., 1979; 1981 a) and to isolated bacterial chromatophores (Cocquempot et al., 1981 b; Larreta-Garde et al., 1981). Under the scanning electron microscope, the matrix appears as a network of homogeneous sheets having threadlike structures on their surface. Grana fragments, organelles, and cells are attached to these sheets with apparently intact morphology (Barbotin and Thomasset, 1981).

Isolated higher plant chloroplasts with unperturbed envelope membranes (intact chloroplasts) are known to be stabler than isolated broken chloroplasts with respect to temperature stress (Krause and Santarius, 1975)

Abbreviations: ASC, ascorbate; Chl, chlorophyll; DCIP, 2,6-dichlorophenol indophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethyl urea; FeCN, K_3 Fe(CN)_6; MV, methyl viologen; PD_Ox, FeCN-oxidized p-phenylene diamine
and prolonged storage (Kulandaivelu and Hall, 1976). Furthermore, glutaraldehyde treatment improves their thermostability and prevents the loss of soluble stroma proteins in aqueous suspension medium (Papageorgiou and Demosthenopoulou-Karaoulani, 1982). On the other hand, however, the thylakoids of intact chloroplasts are inaccessible to ionic and to several nonionic low molecular weight solutes due to the impermeability of the inner envelope membrane (Heldt, 1976). Thus, in order to make the isolated intact chloroplasts compatible with ionic electron transport cofactors, it is necessary to have their envelopes permeabilized.

In the present work, we extend the method of Cocquempot et al. (1979) to the immobilization of isolated intact chloroplasts, which we subsequently permeabilize in situ, and we examine the photosynthetic electron transport properties of these preparations.

MATERIALS AND METHODS

Intact chloroplasts were isolated from spinach leaves according to Nakatani and Barber (1977). The final preparation contained chloroplasts equivalent to 4 mg Chl/ml, suspended in a medium consisting of 330 mM sorbitol, and 0.5 mM Tris.HCl, pH 7.5.

The chloroplasts were immobilized in crosslinked albumin essentially as described by Cocquempot et al. (1979). The reaction mixture, made in the sorbitol-Tris buffer, contained per mL: bovine serum albumin 56 mg; glutaraldehyde 0.33% v/v (43 μmoles); and chloroplasts equivalent to 0.40-0.45 mg Chl. It was essential to maintain the electrolyte content of the reaction medium low in order to avoid the stripping of the envelope membranes. The mixture was frozen quickly to -25°C in a liquid bath consisting of 60% v/v aqueous acetone and dry ice, subsequently it was incubated for 2 h in a deep-freeze refrigerator at the same temperature, and finally it was allowed to rewarm overnight to O°C. The porous green mass which formed was washed with sorbitol-Tris, and it was stored under the same medium until use.

Photoinduced electron transport was measured with an O₂ concentration electrode as described elsewhere (Papageorgiou and Isaakidou, 1977). The assay buffer consisted of 330 mM sorbitol, 1 mM MgCl₂, 50 mM Hepes·KOH, pH 7.6, and 2 mM EDTA. Matrix immobilized chloroplasts were introduced after homogenization with a glass homogenizer. Chl was determined according to Arnon (1949) and glutaraldehyde according to Hesse (1973). Further details are given in the Results and Discussion.

RESULTS AND DISCUSSION

Table 1 lists electron transport activities of free and of matrix immobilized chloroplasts with respect to several electron donors and acceptors. The results shown are typical of several experiments. The free chloroplast samples had been subjected to the same freezing and rewarming protocol, as the immobilized chloroplasts, but in a minus-glutaraldehyde medium, and thus became permeable to FeCN. The permeabilized free chloroplasts retained however, their compact appearance under the light microscope. Accordingly, the higher activity they exhibit in the presence of PDox, relative to the activity in the presence of FeCN alone,