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# SHORT REPORTS

## PROPERTIES OF PLANT PHOSPHOLIPID-EXCHANGE PROTEINS*

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**Key Word Index**—*Solanum tuberosum*; *Solanaceae*; *Brassica oleracea* var. *capitata*; *Cruciferae*; *Helianthus tuberosus*; Compositae phospholipid exchange proteins.

**Abstract**—Proteins able to stimulate the exchange of phospholipids between natural membranes (mitochondria and microsomal fractions) or between artificial (e.g. liposomes) and natural membranes were isolated from cytosols of plant tissues: potato tuber, cauliflower inflorescence and Jerusalem artichoke.

### INTRODUCTION

Phospholipid exchange proteins (PLEP), soluble proteins able to stimulate the exchange of phospholipids between intracellular membranes, have been isolated from various animal tissues: rat liver [1–3], beef liver [4, 5], beef heart [6, 7], beef brain [8], rat intestine [9]. These animal PLEP also stimulate the exchange of phospholipids between artificial (e.g. liposomes) and natural membranes [10]. Similarly, exchange of phospholipids has been demonstrated *in vitro* between organelles of various plant tissues: cauliflower inflorescence and potato tuber [11]. However, phospholipid-exchange protein has been isolated from only one plant: the potato tuber [12]; this protein stimulated phospholipid exchange between mitochondria and microsomes. It is the aim of this work to study the properties of phospholipid exchange proteins in the cytosols prepared from potato tuber, cauliflower inflorescence and Jerusalem artichoke.

### RESULTS

*Properties of the phospholipid exchange protein of potato tuber*

The PLEP of potato tuber was isolated as previously described [12] except that DEAE-sepharose was used instead of DEAE-cellulose to purify the active protein after filtration of the cytosol proteins on a Sephadex-G 75 column. The PLEP activity was checked in the standard exchange assay involving labelled microsomal fraction and unlabelled mitochondria. When protein fractions of MW *ca* 22000, eluted from Sephadex-G 75, were introduced in this exchange mixture, the percentage of exchange, determined as previously indicated [12] was highly increased relative to the standard exchange assay without proteins. When the active fractions eluted from the Sephadex-G 75 column were pooled and chromatographed on DEAE-sepharose, the fractions able to stimulate the phospholipid exchange were eluted with the void volume. NaCl solutions (up to 1 M) eluted *inactive* proteins. The gain in purification, after DEAE-sepharose chromatography calculated as previously described, was found to be 4; the gain could not be determined relative to the pH 5.1 supernatant which inhibited the phospholipid exchange.

When introduced into an exchange mixture comprising $^{32}$P-labelled liposome and non-radioactive mitochondria, the active fractions eluted from the Sephadex column stimulated the exchange of phospholipids; the stimulation depended on the quantity of protein introduced in the incubation medium. The active protein fractions eluted from DEAE-sepharose had a higher sp. act. than those eluted from Sephadex-G 75. In these experiments, cross-contamination was determined by inserting in the liposomes cholesteryl oleate-[1-$^{14}$C] [5] or oestradiol-[$^{3}$H]; admitting that these steroid molecules are not exchanged, the maximum percentages of cross-contamination were *ca* 10%, while the percentage of phospholipid exchange was 50% at the maximum. When the exchange protein was treated at 70° for 15 min, the activity was almost suppressed.

*Isolation of phospholipid exchange proteins from various plants*

Elution patterns of total proteins isolated from the 2 cytosols of cauliflower inflorescence and Jerusalem artichoke showed a major peak corresponding to high MW compounds. When these fractions were introduced in a standard exchange assay, the high inhibition of the exchange intensity, previously found for potato tuber [12], was observed also with proteins from cauliflower inflorescence but only weakly with Jerusalem artichoke proteins; again, this inhibition was due to the presence of a lipid-acyl hydrolase active in these tissues [13]. A second major peak containing the lower MW proteins was observed in potato tuber cytosol [12] but not in cauliflower inflorescence and Jerusalem artichoke. More interestingly, when low MW fractions were incubated with $^{32}$P-labelled microsomes and unlabelled mitochondria, the exchange of phospholipid was highly stimulated. The calibration of the gel filtration column with protein markers allowed the calculation of the apparent MW of the active protein from Jerusalem artichoke and potato tuber was 22000, while a lower MW was found for cauliflower: 12000 ± 1000. These values were confirmed bySDS polyacrylamide electrophoresis.

### EXPERIMENTAL

*Purification of exchange proteins.* Total proteins of the supernatant were isolated from potato tubers, cauliflower inflores-

* Part 2 in the series: 'Proteins and the Intracellular Exchange of Lipids'. For part 1 see ref. [12].

cence or Jerusalem artichoke according to the technique described in the refs [12, 14, 15]. They were then chromatographed on a Sephadex-G 75 column. The active fractions were pooled and chromatographed on DEAE-sepharose equilibrated with NaPi (50 mM, pH 7.2) containing mercaptoethanol (10 mM); the proteins were eluted from the column by increasing concentrations of NaCl. The protein content of eluted fractions was determined by the method of ref. [16].

*Isolation of intracellular membranes.* Mitochondria or microsomes containing $^{32}$P-labelled phospholipids were isolated as previously described [12] and stored at −80°. The protein content was determined by the biuret method [17].

*Preparation of radioactive liposomes.* Thin slices of tissue were incubated in $Na^{32}Pi$ (10 μCi/ml) or in [1-$^{14}$C]-NaOAc (15 μCi/ml) or in choline-[$^{3}$H] (5 μCi/ml) for 16 hr at 25°. After extraction of total lipids by the method of ref. [18], the phospholipids were separated from the other components by TLC [19] and eluted from the plates by $CHCl_3$–MeOH–$H_2O$ (16·7:1). The phospholipids were dissolved in $C_6H_6$–EtOH (4:1) when storage was necessary. To prepare liposomes, the solvent was evapd and small glass beads were introduced in the flask [20]; after addition of exchange medium: sucrose (0.25 M), NaPi (50 mM, pH 7.2) and mercaptoethanol (10 mM), the soln was shaken 3 min with a vortex-mixer, then sonicated 30 min at 0° under $N_2$. A centrifugation for 1 hr at 20000 *g* gave a clear soln of liposomes.

*Exchange assays.* All expts were performed at 30° for 15 or 30 min; the total vol. was 4 ml. Unlabelled mitochondria (3 mg protein) and radioactive microsomal fraction (1 mg protein) or labelled liposomes were incubated in the exchange medium described above, with or without phospholipid exchange proteins. After incubation, the mitochondria were pelleted by centrigugation at 15000 *g* for 10 min and washed twice. The lipids of the mitochondria and supernatant were then extracted [18]. Phospholipids phosphorus was determined by the method of ref. [21].

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# ACTIVATION OF CHLOROPLAST ATPase BY REDUCED THIOREDOXIN

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**Key Word Index**—Thioredoxin; ATPase; $CF_1$; chloroplasts.

**Abstract**—Chloroplast thioredoxin that is reduced by dithiothreitol activates the ATPase that is associated with solubilized preparations of chloroplast coupling factor ($CF_1$).

We have recently described a mechanism of light-actuated enzyme regulation that depends on two newly identified soluble chloroplast proteins [1–4]. One of these new proteins, ferredoxin-thioredoxin reductase, catalyses the reduction of the second protein, thioredoxin, in a reaction that utilizes photoreduced ferredoxin (equations 1 and 2).

$$H_2O + 2\ \text{Ferredoxin}_{\text{oxidized}} \xrightarrow[\text{chloroplast membranes}]{\text{light}} 2\ \text{Ferredoxin}_{\text{reduced}} + \tfrac{1}{2}O_2 \quad (1)$$