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THE OCCURRENCE OF PHOSPHATIDYL CHOLINE EXCHANGE PROTEIN IN LEAVES

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SUMMARY: The transfer of phosphatidyl choline between liposomes was stimulated by the protein fractions from spinach leaves, etiolated and greening leaves of Avena seedlings. This is confirmed by the transfer of [^{14}C]phosphatidyl choline or spin-labeled phosphatidyl choline between donor and acceptor liposomes. ESR spectrum changes also indicated that no spin-labeled phosphatidyl choline was released from donor liposomes by spinach leaf protein unless acceptor liposomes were present. [^{14}C]phospholipids were transferred from liposomes to both spinach chloroplasts and Avena etiochloroplasts by phosphatidyl choline exchange protein from germinated castor bean endosperms and further from liposomes to spinach chloroplasts by spinach leaf protein. These results support the view that phosphatidyl choline in the plastid is supplied from the synthesis site, the endoplasmic reticulum, by phospholipid exchange protein.

INTRODUCTION

Since the enzymes responsible for the synthesis of phosphatidyl choline (PC) are lacking in the plastid in higher plants, despite the occurrence of PC in this organelle, it is expected that PC contained in the plastid is delivered from the synthesis site of PC, the endoplasmic reticulum, and the transfer of PC is mediated by a phospholipid exchange protein (PLEP) which occurs in the cytosol and enhances the exchange of phospholipids (PLs) between membranes. Plant PLEPs have thus far isolated from non-photosynthetic tissues such as potato tubers(1), cauliflower inflorescences(2) and germinated castor bean endosperms(3,4), whereas there has been no evidence for the occurrence of PLEP in photosynthetic tissues. In addition, it has been unsuccessful that chloroplasts function as the acceptor membranes in PC transfer by PLEP, despite the fact that mitochondria function as the acceptor membranes(1,4).

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The purpose of this investigation is to examine the occurrence of PLEPs in leaves with special reference to the function of plastids as acceptor membranes in PC transfer by leaf PLEP.

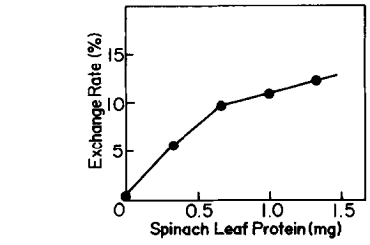
MATERIALS AND METHODS

Materials. Castor bean endosperms were harvested from 4 day-old seedlings grown at 28°C. Spinach leaves were purchased from a local market. Seeds of Avena sativa L. var. Victory I were germinated at 25° in the dark and the first leaves from 8 day-old seedlings were used as etiolated Avena leaves. The seedlings were illuminated at 1,500 lux under fluorescent light and harvested at intervals. These leaves were used as greening Avena leaves. [^{14}C]PLs, the mixture of radioactive PC, PE, PG, PA, PI and PS(44:33:9:9:3:2), were prepared from microsomes of germinated castor bean endosperms fed with [1-^{14}C]acetate according to Yamada et al.(3). Spin-labeled PC(PC*) having 12-nitroxide stearic acid at its β-position was a gift of Dr. K. Machida.

Preparation of plastids. Crude plastids were prepared from spinach leaves and greening Avena leaves harvested at 6 hr-illumination, according to Cockburn et al.(5), using the grinding medium supplemented with 0.1% bovine serum albumin. Crude plastids were further purified by Percoll gradient centrifugation according to Takabe et al.(6), except for the use of Percoll gradient containing 50 mM Hepes-NaOH buffer, pH 8.0. The crude plastids from 20 g of fresh leaves were put on a gradient. The purified plastids possessed no activities NADPH-cytochrome C reductase(a microsomal enzyme), cytochrome C oxidase(a mitochondrial enzyme), glyoxylate reductase and catalase(microbody enzymes).

Preparation of plant protein fractions. Crude protein fractions possessing phospholipid exchange activities were routinely prepared from castor bean endosperms, spinach leaves and greening Avena leaves, according to Wirtz and Zilversmit(7), and precipitated by 75% saturation of ammonium sulfate after the removal of pH 5.1 precipitate. Castor bean protein was further purified by DEAD-Sepharose CL-6B column chromatography. The fraction eluted from the column at 0.12 M NaCl in increasing concentrations was used as castor bean PLEP, Fraction II(8).

Assay of PLEP activity. Phospholipid exchange activity was determined by the transfer of [^{14}C]PLs or PC* between donor and acceptor liposomes. In the transfer of [^{14}C]PLs, the separation of acceptor liposomes from donor liposomes was performed by Con A method of Sasaki and Sakagami(9) using Con A-reactive acceptor liposomes with dimannosyl diglyceride and Con A-nonreactive donor liposomes with [9,10-^{3}H(N)]triolein and [^{14}C]PLs. The transfer of PLs was determined by exchange rate(^{14}C in acceptor after the incubation/^{14}C in donor before the incubation x 100) or ^{14}C/^{3}H(the ratio of ^{14}C to ^{3}H in acceptor after the incubation, provided that donor liposomes with [^{3}H]triolein and [^{14}C]PLs were incubated with acceptor liposomes with unlabeled PLs). Cross contamination in the separation of the donor from the acceptor was corrected by the transfer of ^{3}H from the donor to the acceptor. The transfer of PC* between donor and acceptor liposomes was measured by ESR spectrum changes according to Maeda and Ohnishi(10). For the transfer of [^{14}C]PLs from liposomes to plastids, the incubated mixture(0.5 ml) was layered on 0.75 ml of 50% Percoll(for the experiments as shown in Figs. 5A and 5B) or silicon oil(SH 550 Nakarai Chemical Ltd., for the experiment as shown in Fig. 6) in a plastic tube(1.5 ml) and centrifuged for 1 min with Eppendrof centrifuge 5412. Intact plastids were precipitated at the bottom, and broken plastids and donor liposomes floated at the upper phase. After freezing the tube, the bottom portion was cut and counted for the radioactivity with toluene scintillator-Triton X-100(2:1, v/v) after the dispersion. Of plastid fractions added to the incubation mixture, 70 to 90% of spinach chloroplasts and 60% of Avena etiochloroplasts were recovered from the bottom as intact plastids.



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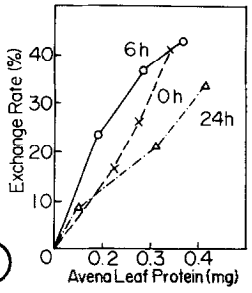

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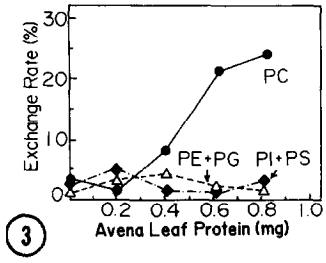
Fig. 1. Effect of spinach leaf protein on phospholipid transfer between liposomes. Donor liposomes with [^{14}C]PLs(50 μg, 6,000 dpm), egg yolk PC (50 μg) and [^{3}H]triolein(trace, 20,000 dpm) were incubated with acceptor liposomes with unlabeled egg yolk PC(0.25 mg) and dimannosyl diglyceride (0.5 nmol) in a final volume of 1.0 ml at 30° for 15 min.

Fig. 2. Effect of Avena leaf protein on PL transfer between liposomes. The incubation mixture and the conditions were the same as in Fig. 1, except for the addition of Avena leaf protein instead of spinach leaf protein. Avena protein fractions were prepared from etiolated leaves(0h), greening leaves after 6 hr-illumination(6h) or 24 hr-illumination(24h).

RESULTS

Fig. 1 shows that the transfer of [^{14}C]PLs between donor and acceptor liposomes was stimulated with increase of spinach leaf protein added. The similar stimulation was also found in case of both etiolated and greening Avena leaf proteins (Fig. 2). These results indicate the occurrence of PLEP in leaves. Fig. 3 demonstrates that the transfer of PLs by Avena leaf protein is specific for PC, since the increase of Avena leaf protein resulted only in the enhanced transfer of PC among PLs. Fig. 4 shows the changes in ESR spectra which represent time course of PC* transfer from donor to acceptor liposomes. The enhanced ESR peak height in the presence of spinach leaf protein indicates that PC is also preferable to be transferred by this protein. When donor liposomes with PC* were incubated with spinach leaf protein in the absence of acceptor liposomes, there was no change in ESR peak height (Fig. 4). This means that no PC* was released from donor liposomes by spinach leaf PLEP under the present condition unless acceptor liposomes were present in the incubation mixture. Figs 5A and 5B demonstrates that plastids functioned as acceptor membranes in PC transfer by PLEP, because the increase



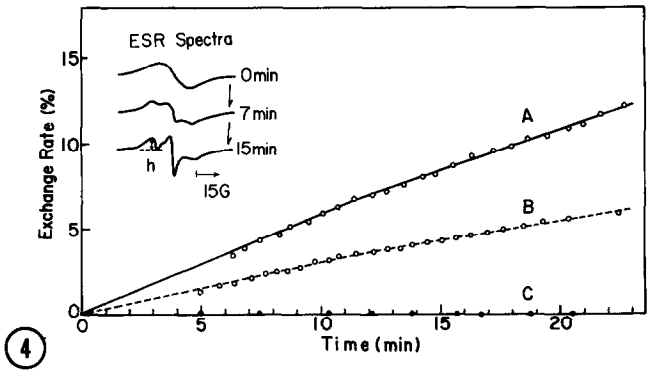


Fig. 3. Effect of Avena leaf protein on lipid classes transferred from liposomes to acceptor liposomes. The incubation mixture and the conditions were the sams as in Fig. 2. Avena leaf protein was prepared from greening Avena leaves after 6 hr-illumination. PLs were extracted from acceptor liposomes by the method of Bligh and Dyer(11), qualitatively analyzed by TLC(chlorofom-methanol-water, 65:25:4, v/v) and counted for the radioactivity with toluene scintillator, according to Tanaka and Yamada(4).

Fig. 4. Time course of ESR spectrum changes in the transfer of PC* from donor liposomes to acceptor liposomes by spinach leaf proteing. Donor liposomes with PC*(30 μg) were incubated with acceptor liposomes with egg yolk PC(600 μg), and spinach leaf protein(500 μg) in 20 mM Tris-HCl buffer, pH 7.5 (in a final volume of 80 μl) at room temperature. A: the complete system, B: the complete system without spinach leaf protein and C: the complete system without acceptor liposomes.

of PC-specific exchange protein from castor bean endosperms enhanced the transfer of [^{14}C]PLs from liposomes to both spinach chloroplasts and Avena etiochloroplasts. Finally, Fig. 6 shows that the transfer of [^{14}C]PLs from liposomes to spinach chloroplasts was enhanced with increased amounts of spinach protein. These results are the strong support that leaf PLEP functions in transferring PLs, in particular PC, from the synthesis site, the endoplasmic reticulum, to the plastid membranes.

DISCUSSION

It should be avoided, in demonstrating the transfer of PC from donor liposomes to plastids by PLEP, that acceptor plastids are contaminated not only by mitochondrial and microsomal membranes, but also by broken plastid membranes, because mitochondrial and microsomal membranes serve as effective acceptor of PC(1,4) and broken chloroplasts also function as PC acceptor even when PC is not transferred to intact chloroplasts(12). Our chloroplast frac-

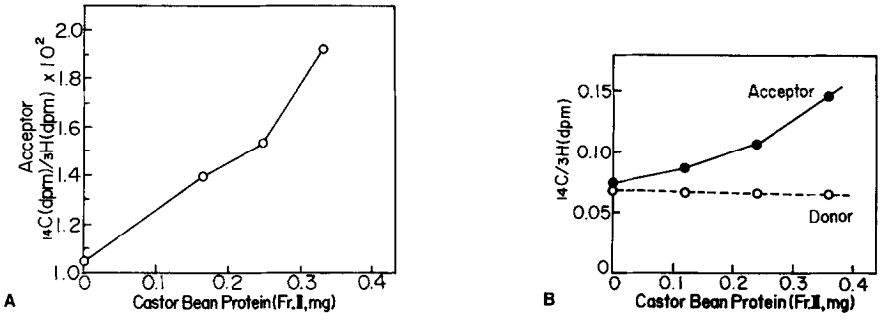


Fig. 5A. Effect of castor bean PLEP, Fraction II, on the transfer of PLs from liposomes to spinach chloroplasts. Donor liposomes with [14C]PLs(50 µg, 30,000 dpm) and [3H]triolein(trace, 300,000 dpm) were incubated with spinach chloroplasts(20 µg chlorophylls) and castor bean PLEP, Fraction II, in the medium containing 0.4 M sorbitol, 1 mM $MgCl_2$, 1 mM EDTA and Hepes-NaOH buffer, pH 8.0 (in a final volume of 0.5 ml) at 25°C for 15 min.

Fig. 5B. Effect of castor bean PLEP, Fraction II, on the transfer of PLs from liposomes to Avena etiochloroplasts. The incubation mixture and the conditions were the same as in Fig. 5A, except for the use of donor liposomes with [14C]PLs(50 µg, 50,000 dpm) and [3H]triolein(800,000 dpm), and Avena etiochloroplasts(2 µg chlorophylls) prepared from greening Avena leaves after 6 hr-illumination.

tion purified by Percoll gradient centrifugation contained over 90% of intact chloroplasts by phase-contrast microscopy and no membrane pieces of microsomes, mitochondria and microbodies as shown by the assay of the marker enzyme activities. Furthermore, care was taken to collect intact plastids, free from broken plastid membranes, after the incubation, because the occurrence of broken plastid membranes during the incubation will also raise an apparent transfer rate of PC. For the separation of intact plastids from

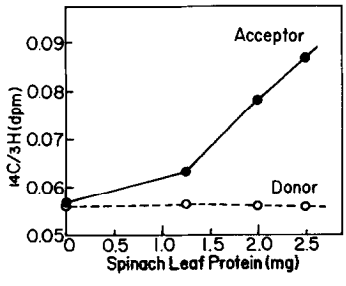


Fig. 6. Effect of spinach leaf protein on the transfer of PLs from liposomes to spinach chloroplasts. The incubation mixture and the conditions were the same as in Fig. 5A, except for the use of spinach chloroplasts(50 µg chlorophylls) and spinach leaf protein.

broken ones, the silicon method was as effective as the Percoll method. The transfer of PC from liposomes to plastid membranes was secured by the use of PC exchange protein from castor bean endosperms(Figs 5A and 5B).

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