FEBS Letters 589 (2015) 177–181

FEBS Letters

journal homepage: www.FEBSLetters.org

Phosphatidylcholine is transferred from chemically-defined liposomes to chloroplasts through proteins of the chloroplast outer envelope membrane

Congfei Yin [a,b], Mats X. Andersson [b], Hongsheng Zhang [a,*], Henrik Aronsson [b,*]

[a] State Key Laboratory of Crop Genetics and Germplasm Enhancement, Nanjing Agricultural University, Nanjing, People's Republic of China
[b] Department of Biological and Environmental Sciences, University of Gothenburg, SE-405 30 Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 26 September 2014
Revised 24 November 2014
Accepted 24 November 2014
Available online 3 December 2014

Edited by Ulf-Ingo Flügge

Keywords:
Phosphatidylcholine (PC)
Chloroplast
Liposome
Lipid uptake

ABSTRACT

Chloroplasts maintain their lipid balance through a tight interplay with the endoplasmic reticulum (ER). The outer envelope membrane of chloroplasts contains a large proportion of the phospholipid phosphatidylcholine (PC), which is synthesized in the ER and also a possible precursor for thylakoid galactolipids. The mechanism for PC transport from the ER to chloroplasts is not known. Using isolated chloroplasts and liposomes containing radiolabeled PC we investigated non-vesicular transport of PC in vitro. PC uptake in chloroplasts was time and temperature dependent, but nucleotide independent. Increased radius of liposomes stimulated PC uptake, and protease treatment of the chloroplasts impaired PC uptake. This implies that the chloroplast outer envelopes contains an exposed proteinaceous machinery for the uptake of PC from closely apposed membranes.
© 2014 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

1. Introduction

Chloroplasts are vital organelles of plants as they harbor the photosynthesis machinery, nitrogen- and sulfur metabolism as well as produce, among other vital compound classes, amino- and fatty acids. The chloroplast contains an inner membrane system, the thylakoids, being surrounded by an aqueous stroma. The stroma is in turn surrounded by the envelope membrane, divided into an outer and inner envelope membrane. Chloroplast membranes constitute about 70% of the total plant mesophyll cell membranes, and 80% are galactolipids e.g. monogalactosyldiacylglycerol (MGDG) and digalactosyldiacylglycerol (DGDG) both being more common in the thylakoid membranes than in the envelope [12]. In the outer leaflet of the outer envelope membrane, facing the cytosol, the phospholipid phosphatidylcholine (PC) is a major constituent (32–44 mol%) [8,9]. Chloroplast galactolipids are synthesized in the envelope through a coordinated interplay with the endoplasmic reticulum (ER) e.g. chloroplasts export fatty acyl moieties to the ER and import diacylglycerol backbones that act as galactolipid precursors back from the ER [5].

In vivo pulse chase studies indicate that PC (detected in envelope/chloroplast) synthesized in the ER could be the immediate precursor for galactolipid synthesis in chloroplasts [15,16,17,30,31]. In addition, diacylglycerol (DAG) [36], phosphatic acid (PA) [41] and lyso-PC [23] have been suggested to be galactolipid precursors transported from the ER. Since chloroplasts lack capacity to synthesize the head group of PC, and both the outer envelope and the ER contain a significant amount of PC it seems likely that PC is transported from the ER whether or not it is the major precursor for galactolipids. However, the mechanism behind PC transport from ER to the envelope is unclear.

Movement of lipids between cytosolic organelles may use vesicular transport e.g. endocytic or exocytic pathways or non-vesicular transport [18]. Lipid interchange between ER and chloroplasts has been proposed to occur by contact sites between the plastid envelope and a specialized ER domain coined plastid associated membranes (PLAM) [1,6]. Trigalactosyl diacylglycerol (TGD) proteins are located in the chloroplast envelope and facilitate lipid transport into chloroplasts from the ER [4,35,42]. TGD4 alone makes up a complex in the outer envelope and TGD1, 2 and 3 a complex in

Abbreviations: DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; ER, endoplasmic reticulum; MGDG, monogalactosyldiacylglycerol; PA, phosphatic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PLAM, plastid associated membranes; TGD, trigalactosyl diacylglycerol

* Corresponding authors. Fax: +46 31 7862540 (H. Aronsson).
E-mail addresses: hszhang@njau.edu.cn (H. Zhang), henrik.aronsson@bioenv.gu.se (H. Aronsson).

http://dx.doi.org/10.1016/j.febslet.2014.11.044
0014-5793/© 2014 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

the inner envelope [34]. TGD proteins are proposed to facilitate transfer of PA from ER to the chloroplast where it is used as a galactolipid precursor after dephosphorylation in the inner envelope [35]. Recently, it was proposed that the ER and the outer chloroplast envelope can form hemifusions at contact sites to facilitate transfer of lipophilic compounds [22].

It has been shown in vitro that radiolabeled phospholipids can be transferred from donor ER to isolated chloroplasts in a time and temperature dependent manner but without need for nucleotides or cytosolic factors [2,40]. Thus, available evidence suggests that chloroplasts can take up phospholipids from donor membranes, provided these make close contact to the organelle. Yeast mitochondria have been shown to be able to take up phospholipids from chemically defined donor liposomes by means of a protein machinery in their outer membrane [39]. In this case larger liposomes were more efficient donors and this was interpreted as those larger liposomes can contribute a "flatter" contact surface with the mitochondria. In this study we tested if isolated chloroplasts have the ability to take up PC from donor liposomes. We used an in vitro system using radiolabeled PC in liposomes mixed with isolated chloroplasts to test if chloroplast like mitochondria could take up phospholipids form chemically defined donor liposomes. We show that PC is absorbed by chloroplasts in a time and temperature dependent way, and that the PC uptake rate is related to the size of liposomes. Moreover, the process is independent of exogenous nucleotides, whereas, removal of proteins on the outer envelope by thermolysin impairs it, but which protein(s) responsible remains to be elucidated.

2. Methods and materials

2.1. Plant material and chloroplast preparation

Pea (*Pisum sativum* L. cv Kelvedon Wonder) were sown on soil, covered with vermiculite and grown at 22 °C and 12 h light. Isolation of chloroplasts from 10 to 12 days old peas was performed as previously described [3], and chlorophyll concentration was measured spectroscopically [21].

2.2. Liposome preparation

Lipids were extracted from 7 to 9 days old pea seedlings. They were cut with razor blade, and soaked in 12 ml ^{33}P ($2.66 * 10^8$ kdpm) (as $H_3^{33}PO_4$, Perkin Elmer) radiolabeled water for 48 h in a fume hood with lights on before lipid extraction. Thereafter total lipids were extracted [20] and a phospholipid fraction prepared by fractionation on a silica column. Total extracted lipids were loaded onto a 500 mg silica column (Supelco). Neutral lipids were eluted with $CHCl_3$:acetone (9:1, by volume), glycolipids with acetone:methanol (9:1, by volume), and total phospholipids with methanol.

The procedure of isolation of liposomes was adapted from [29] with some modifications as described [7]. Total phospholipids isolated from labeled pea seedlings or a mix of commercial 14C-dipalmitoyl phosphatidylcholine ([^{14}C]PC, specific activity 3.6 GBq/mmol, Perkin Elmer) or commercial L-3-phosphatidylethanolamine, 1-palmitoyl-2-[1-14C]linoleoyl ([^{14}C]PE, specific activity 2.04 GBq/mmol, Perkin Elmer) corresponding to 50 kdpm per assay with soybean PC and PE (Sigma–Aldrich) (mix 4:1 by weight, 10 μg/assay) dissolved in chloroform were dried under N_2. One ml of import buffer was added to the dried lipid film heated to 60 °C for about 10 s and vortexed thoroughly. After four cycles of freeze–thawing liposomes were pressure extruded through double 50, 100, 200, 400 or 1000 nm (as indicated) polycarbonate filters eleven times, and stored at 4 °C until use.

2.3. Liposome import assay

Liposomes corresponding to about 30 kdpm of ^{14}C- or ^{33}P-label were mixed with chloroplasts corresponding to 50 μg of chlorophyll in a total volume of 100 μl of import buffer (0.33 M sorbitol; 50 mM Hepes; KOH pH 8.0). Import assays were performed on ice or 25 °C water bath with 150 $\mu mol * m^{-2} * s^{-1}$ light using different time points. The reaction was stopped by adding 200 μl ice cold isolation buffer. The suspension was transferred to the top of a 1 ml 35% Percoll mixed in isolation buffer, centrifuged 5000×*g*, 8 min at 4 °C, and the pellet being washed 3 times in 1 ml isolation buffer. Finally the pellet was re-suspended in 200 μl isolation buffer and transferred to a scintillation vial containing 1 ml methanol, and 8 ml scintillation cocktail (Ready Protein$^+$, Beckman) was added before and the radioactivity counted in a Tri-Carb 2900 TR liquid scintillation analyzer.

Thermolysin treatment was performed according to [14] with slight modifications. Chloroplasts were incubated in 50 μg/ml thermolysin and 300 μM $CaCl_2$ for 30 min on ice in darkness. Control samples had thermolysin replaced with an equal volume of import buffer. Reactions were terminated by addition of an equal volume of 50 μM EDTA (final concentration 25 μM). The suspension was transferred to the top of a 1 ml "cushion" of 35% (by volume) Percoll™ in isolation buffer, centrifuged at 5000×*g*, 5 min at 4 °C, and the pellet washed twice in 1 ml import buffer, then finally re-suspended in 50 μl import buffer to continue the liposomes import assay.

For nucleotide experiments, ATP/GTP was added to the reaction to a final concentration of 0.5 mM or 1 mM (only for ATP). Control samples had ATP/GTP replaced with the same volume of import buffer. Reactions were incubated at 25 °C in a water bath with 150 $\mu mol * m^{-2} * s^{-1}$ light for 30 min or 60 min.

3. Results and discussion

3.1. Uptake of PC from liposomes to isolated chloroplasts is time and temperature dependent

As ER derived lipid transport to chloroplasts has been suggested to occur at contact sites between the ER and the chloroplast and yeast mitochondria apparently harbors a machinery for the uptake of phospholipids from protein free liposomes, we decided to test if intact plant chloroplasts could take up radiolabeled phospholipids from large unilamellar liposomes.

An initial experiment was performed with large (400 nm) unilamellar liposomes (LUVs) prepared from phospholipids isolated from pea seedlings pre-incubated with [^{33}P]orthophosphate. LUVs containing ^{33}P-labeled lipids were mixed with isolated chloroplasts isolated from pea seedlings. After incubation the chloroplasts were washed and the amount of radiolabeled transferred to the chloroplasts determined (Fig. 1A). There was a clear time dependent incorporation of radiolabel into the chloroplast fraction demonstrated by a more than 3-fold increase in label after 30 min compared to zero time (Fig. 1A). Moreover, phospholipid uptake was also temperature dependent; after 30 min at 25 °C uptake of phospholipids was approximately double that of samples incubated on ice (Fig. 1A). Unfortunately the amount of radiolabel transferred was too low to determine the exact composition of phospholipids transferred. We thus conclude that isolated chloroplasts can take up phospholipids from protein free liposomes in vitro. We next tested if PC, as this lipid is most likely transported from the ER to the outer chloroplast envelope (see above), could be transferred from chemically defined liposomes to isolated chloroplasts. To this end [^{14}C]PC was incorporated into liposomes (400 nm in diameter) made from a mix of soybean PC:PE (4:1).

Download English Version:

https://daneshyari.com/en/article/2047636

Download Persian Version:

https://daneshyari.com/article/2047636

Daneshyari.com