

Lipid-transfer proteins from plants: Structure and binding properties

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Abstract

Plant cells contain lipid-transfer proteins (LTPs) able to transfer phospholipids between membranes *in vitro*. Plant LTPs share in common structural and functional features. Recent structural studies carried out by NMR and X-ray crystallography on an LTP isolated from maize seeds have showed that this protein involves four helices packed against a C-terminal region and stabilized by four disulfide bridges. A most striking feature of this structure is the existence of an internal hydrophobic cavity running through the whole molecule and able to accomodate acyl chains. It was thus of interest to study the ability of maize LTP to bind hydrophobic ligands such as acyl chains or lysophosphatidylcholine and to determine the effect of this binding on phospholipid transfer. The binding abilities of maize LTP, presented in this paper, are discussed and compared to those of lipid-binding proteins from animal tissues. (Mol Cell Biochem **192**: 157–161, 1999)

Key words: lipid, transfer, binding, proteins, plants, maize

Abbreviations: LTP – lipid-transfer proteins; FABP – fatty acid binding protein; ALBP – adipocyte lipid-binding protein; SCP-2 – sterol carrier protein - 2; PC – phosphatidylcholine; lyso-PC – lysophosphatidylcholine

Introduction

Lipid transfer proteins (LTPs) are defined by their ability to transfer phospholipids between membranes *in vitro*. They have been characterized in a large variety of organisms including mammalian tissues, plants, yeast, fungi and some bacteria. It has been suggested - but not yet well demonstrated - that these proteins participate in extracellular flux of lipids, essential for the biogenesis and renewal of endomembranes [1].

The situation is more complicated for plant LTPs which are still in search of an *in vivo* role. It is now clear that these proteins are present in numerous plants and share in common structural and functional properties: small size (about 9 kDa), high isoelectric point (9–10), presence of eight cysteine residues, among 90–93 amino acids, located in conserved positions and forming four disulfide bridges [2, 3]. Structural studies by NMR and X-ray crystallography have been carried out on plant LTPs. These proteins comprise a single compact

domain with four alpha-helices and a long C-terminal region. They have a tunnel-like hydrophobic cavity able to accomodate fatty acids [4, 5]. A complex between maize LTP and palmitic acid has been indeed recently crystallized [4]. This recent structural model of ns-LTPs gives a basis for understanding the function of LTPs in relation to the transfer and binding of molecules containing acyl chains (phospholipids, fatty acids, acyl CoA or other acyl group containing molecules).

As it was found that various cDNAs coding for LTPs contained a signal peptide suggesting a possible secretion of these proteins, a role in cell wall edification, and more precisely biosynthesis of cutin, has been proposed [6–8]. In this perspective it was of interest to study the interaction of LTPs with other ligands than phospholipids originally used to define this class of proteins, such as fatty acids which are necessary substrates for the formation of cutin. LTP isolated from maize seedlings is a good model for such studies. This

paper presents novel informations on the binding of acyl chains and of other ligands such as lysophosphatidylcholine on maize LTP and on the effect of this binding on the phospholipid transfer process.

Materials and methods

Materials

Maize seeds (*Zea mays* L., cv. Mona) were a gift from Pioneer France-Mais.

Protein preparation

LTP was prepared from maize seeds after 3 days of germination on moist vermiculite in the dark at 30°C. Steps of purification were as defined previously [9]. Purified LTP used was the isoform fully characterized [10].

Lyso PC binding assay

Radioactive lysophospholipids were obtained by action of phospholipase A1 on phosphatidylcholine biosynthetically radiolabelled by aging potato slices in the presence of (³H) methyl choline as precursor [9]. Seventy-three nmoles of (³H) lyso-PC were dried under nitrogen then 1.5 ml of pure LTP fraction (100 nmoles) was added in two steps, separated by a strong agitation in the presence of small glass beads. Volume was adjusted to 2 ml with elution buffer. After incubation under agitation at 30°C during 30 min, the sample was loaded onto a Sephadex G-100 column to separate the LTP/lyso-PC complex from free LTP and lysoderivative. Fractions of 4 ml were collected and 0.5 ml aliquots subjected to liquid scintillation counting. LTP elution was followed by ELISA with specific polyclonal IgGs directed against the maize LTP, purified by affinity chromatography, as described elsewhere [11].

Acyl-CoA binding assay

The binding of (¹⁴C) oleoyl-CoA to LTP was estimated after separation of LTP/ ligand complex by FPLC on a Mono S column. For binding assay, pure LTP (39 nmoles) was incubated with 1.8 nmole of (¹⁴C) oleoyl-CoA, in a final volume of 0.7 ml, at 0°C during 30 min. Elution from Mono S column was performed with a NaCl gradient from 0–250 mM and 1 ml fractions were collected. Radioactivity was measured on 0.5 ml aliquots.

PC transfer activity of LTP complexes

LTP complexes were formed with either lyso-PC or oleoyl-CoA under the conditions described above. PC transfer activity catalyzed by the different complexes was assayed as previously reported [12] by measuring the transfer of (³H)phosphatidylcholine from sonicated liposomes to maize mitochondria.

Results

Formation of a lyso-PC/LTP complex

After incubation of LTP with lyso-PC, the protein eluted from Sephadex G-100 column as two peaks (Fig. 1). Radioactivity associated to lyso-PC was detected in the second peak indicating that the LTP complexed with a lysoderivative was more retained than free LTP. Fifty-eight percents of the initial radioactivity of lyso-PC was found associated to LTP, indicative of a *n* apparent association constant (K_a) of ca. 50 nM⁻¹.

Transfer activity of lyso-PC/LTP complexes

PC transfer activity was impaired by a preincubation of LTP with lyso-PC (Fig. 2). Increasing amounts of lysoderivatives

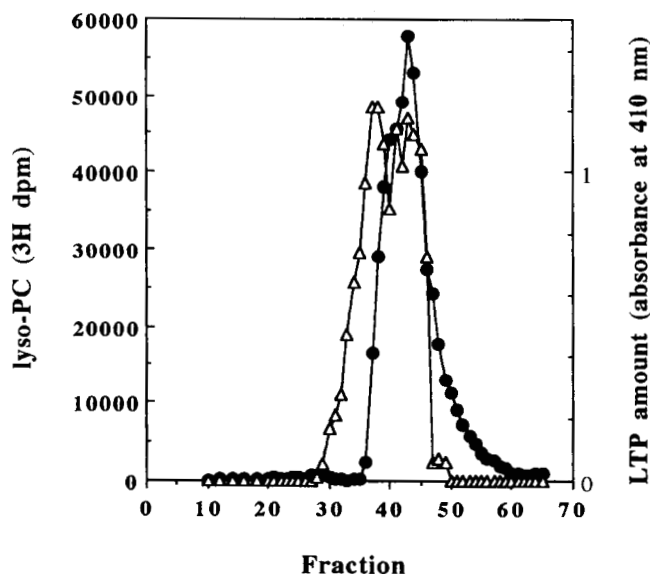


Fig. 1. Sephadex G-100 chromatography of lysoPC-LTP complex. Pure maize LTP (100 nmoles) was incubated for 30 min at 30°C with ³H-lysoPC (73 nmoles) then the different components were separated by gel chromatography. LysoPC was followed by its radioactivity (●). LTP was assayed using an ELISA test with affinity purified specific IgG (Δ).

inhibited the capability of LTP to transfer PC from liposomes to mitochondria. The dose-response was sensitive to the molecular category of lyso-PC. As can be seen, the transfer catalyzed by 50 nmoles LTP was 50% inhibited by approximately 0.6 nmoles lyso-palmitoyl-PC or 0.8 nmoles lyso-oleoyl-PC.

Formation of an oleoyl-CoA/LTP complex

The LTP/oleoyl-CoA complex was separated from the free forms of both components by FPLC on a Mono S column (Fig. 3). It was found to be associated with 33% of the initial radioactivity of oleoyl-CoA present in the assay.

Transfer activity of oleoyl-CoA/LTP complexes

Binding of oleoyl-CoA on LTP before PC-transfer assay diminished the transfer efficiency. However, oleoyl-CoA appeared to be less potent than lyso-PC in this role, as the transfer associated with 100 nmoles of LTP was only decreased by 25% after incubation with 50 nmoles of oleoyl-CoA (Fig. 4).

Discussion

Maize LTP was characterized by its *in vitro* capability to transfer phospholipids between two membranes [10]. Here

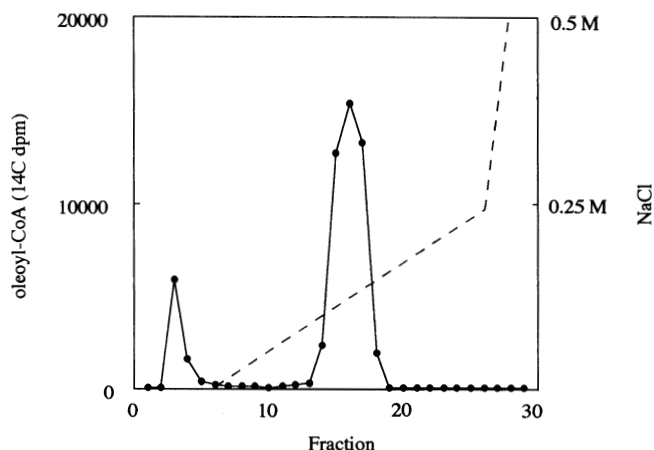


Fig. 3. FPLC chromatography on mono-S column of oleoyl-CoA/LTP complex. Maize LTP (39 nmoles) was incubated for 30 min at 0°C with ^{14}C -oleoyl-CoA then deposited on a mono-S column. The complex was then eluted by a NaCl gradient. Oleoyl-CoA was followed by its radioactivity (●).

we showed that it can also bind acyl-CoA esters and lyso derivatives. This property has been already described in the case of LTPs such as the ones from carrot [8], spinach [13], rapeseed [14] or castor bean [15].

The fact that the complexes could be separated from both the unbound ligand and the free protein was indicative of two properties. First, the complex life has to be long enough to allow the separation which indicates a relatively high

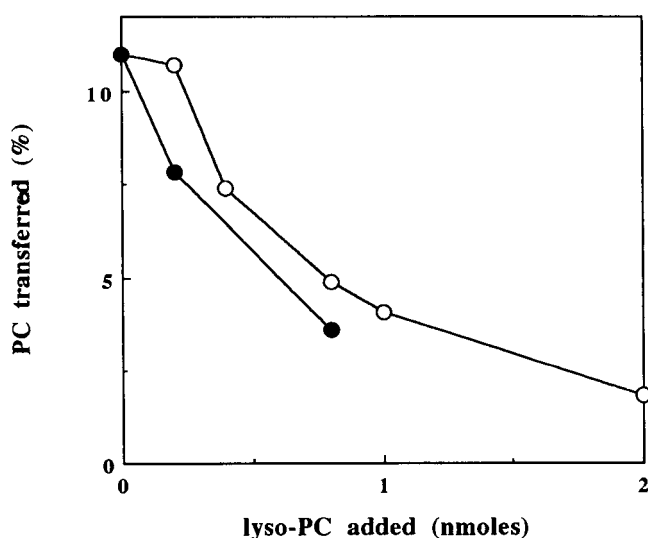


Fig. 2. Effect of lysoPC binding on PC transfer catalyzed by LTP. A complex of LTP and either oleoyl-lysoPC (O) or palmitoyl-lysoPC (●) was formed before the transfer assay. LTP amount was 50 nmoles and lysoPC amount was as indicated in abscissa. PC transfer was measured between sonicated small unilamellar vesicles and maize mitochondria as described previously [12].

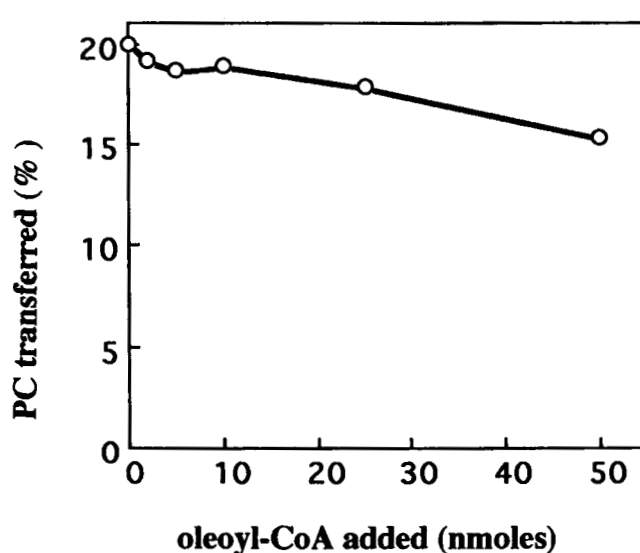


Fig. 4. Effect of oleoyl-CoA binding on PC transfer activity of maize LTP. A complex between LTP (100 nmoles) and the indicated amount of acyl-CoA was formed before PC transfer activity was assayed as indicated in Material and methods and in legend of Fig. 2.

binding constant. Secondly, since the complex was eluted from the chromatographic support after the free LTP, their structure should be different. This suggests that binding of the acyl chain should induce a change in conformation of the protein. The association between maize LTP and acyl-CoA esters or lyso derivatives appeared to be tighter than between the protein and a phospholipid, based on previous studies with PC showing a weak binding [12] and on the inhibitory effect of lyso-PC or oleoyl-CoA binding towards the phospholipid transfer rate. However, lysoderivatives were more potent inhibitors than acyl-CoA. In the case of LTP isolated from rape seedlings, oleoyl-CoA could completely abolish the PC transfer towards the mitochondrial membrane [14]. It is thus interesting to note that the relative affinities for the different ligands vary according to the source of LTP.

This acyl binding ability of maize LTP is in agreement with the structural studies made by NMR or X-ray crystallography on maize LTP indicating the presence of an hydrophobic cavity able to accommodate one or two acyl chains [4–5]. Recent NMR studies performed on barley LTP have led to similar conclusion: The structure of the complex between barley LTP and palmitoyl-CoA revealed a hydrophobic binding site that can expand to accommodate both large and small ligands [16].

It is of interest to note that several lipid-binding proteins have been characterized in mammalian cells. Fatty-acid binding proteins constitute a family of low molecular mass proteins that are suggested to function as intracellular transporters of fatty acids [17]. In contrast with plant LTPs, FABPs exhibit a predominantly beta-sheet structure [18]. Several structural studies have been carried out on the various FABP categories. For example, crystallographic studies have recently established that the fatty acid binding site of adipocyte lipid-binding protein (ALBP) is within an internalized water-filled cavity [18]. Rat liver FABP (L-FABP) isoforms I and II have two fatty acids or fatty acyl CoA binding sites [19]. This is coherent with the crystal structure of the recombinant form of rat liver fatty acid-binding protein complexed with oleic acid; two fatty acid molecules were showed to bind within a central cavity [20].

Other types of mammalian proteins which are able, like plant LTPs, to interact both with phospholipids and fatty acids, have been found. This is the case with sterol carrier protein-2 (SCP-2) which has been showed to bind fluorescent fatty acyl-CoAs at a single site with high affinity [21]. It remains to establish the relations between FABPs, SCP-2 and acyl-CoA-binding proteins (ACBP) [22]. In addition, it is to be noted that plant cells contain ACBP-like proteins which have been characterized in rape seeds [23]. It is not known if maize seeds contain similar proteins.

Future studies on maize LTP will involve recombinant approaches to help progress in the knowledge of the binding process of lipids on LTP and of the interactions of LTP with membranes. At a longer term, LTP gene antisense or over-

expression strategy will be followed in order to reduce or to increase LTP amount in maize tissues and thus to study the consequences on lipid metabolism and physiology of the transgenic plants.

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