A phospholipid transfer protein that binds long-chain fatty acids

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A phospholipid transfer protein, purified from spinach leaves, is able to bind long-chain fatty acids (oleic and linoleic acids) as well as oleoyl-CoA This binding was demonstrated by analytical isoelectric focusing and electrophoretic titrations The finding that a protein, known for its ability to catalyze intermembrane transfers of phospholipids, is also able to bind acyl chains, opens new perspectives for the physiological significance of this category of proteins in plant cells

Phospholipid Transfer protein Fatty acid Binding

1. INTRODUCTION

A highly purified phospholipid transfer protein (PLTP), able to catalyze intermembrane movements of phospholipids, has been recently isolated from spinach (*Spinacia oleracea*) leaves [1]. This protein has a high isoelectric point (about 9.0) and a rather low M_r (around 9000). These properties are similar to those recently attributed to a fatty acid binding protein (FABP) from etiolated oat (*Avena sativa*) seedlings (isoelectric point of 8.4 and M_r of 8700) [2]. This prompted us to consider the possibility that the spinach PLTP, in addition to its ability of transferring phospholipids may also act as a protein binding fatty acids. This paper shows that, indeed, the spinach protein strongly binds long-chain fatty acids as well as oleoyl-CoA.

2. MATERIALS AND METHODS

1-^{14}C-labelled fatty acids (51–60 mCi/mmol) oleoyl-[1-^{14}C]oleoyl-CoA (48 mCi/mmol) and dipalmitoyl-[1-^{14}C]dipalmitoyl phosphatidylcholine (95 mCi/mmol) were purchased from Amersham. Histone A II was obtained from Boehringer.

For the preparation of spinach PLTP, we have followed the recently published method [1], except that the last step of purification was omitted and replaced by diafiltration against distilled water (Amicon cell, YM2 membrane). After lyophilization, the white powder was suspended in distilled water at a concentration of 22 mg·ml^{-1}. The resuspended protein retained its phospholipid transfer activity, checked in liposome-mitochondria assays as previously described [1] (not shown); to our knowledge, it is the first time that a PLTP is reported to keep its activity after lyophilization.

Analytical isoelectric focusing was performed with 4.6% polyacrylamide gels (100 μm) containing 3.5% Pharmalyte 3–10. Gels were linked to polyester foils with Polyfix 1000 (Desaga) and prefocused for 30 min at 400 V. Radioactive ligands dissolved in 10 μl hexane were deposited on 0.5 × 0.5 cm pieces of Whatman 2MM paper and incubated with proteins as described earlier [3,4]. Papers were applied onto the surface of the gel and samples were focused first at 400 V for 30 min and then at 1000 V for 60 min at 4°C. Gels were dried, subjected to autoradiography (Kodak, no screen) and subsequently stained for protein (Coomassie blue, [4]).

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Electrophoretic titrations [5] were carried out on polyacrylamide gels prepared as above. After focusing the gels for 30 min at 400 V and 60 min at 800 V at 4°C, the electrode strips were removed and new electrode strips were applied perpendicular to the pH gradients. A trough was cut in the middle of the plate paralleling the electrodes, filled with protein/ligand solution and electrophoresis was carried out at 400 V for 30 min. Ligands and proteins were detected as outlined above.

3. RESULTS AND DISCUSSION

Here, binding of ligands by the spinach protein has been tested under conditions of isoelectric focusing, i.e., comigration of protein and ligand to the isoelectric point of the complex. All protein samples were incubated with the same amount of ligand (40000 cpm), except phosphatidylcholine, which contained a double amount of counts, due to its higher specific radioactivity. A comparison of the autoradiographic marks of ligands carried along with the protein (detected by Coomassie blue staining) indicates a strong binding of oleic acid and oleoyl-CoA (fig.1). In contrast, most of the phosphatidylcholine remained at the application spot. The PLTP from spinach leaves was thus able to transport the anionic ligands towards the cathode, in contrast to the p*I* 8.4 FABP from *Avena sativa* seedlings that upon binding of a fatty acid migrated to pH 4.8 [2].

That the binding of fatty acids by the PLTP is not an artefact has been shown by the following control experiments:

(i) the native state of the spinach protein was ascertained as the protein alone migrated to the cathode in agreement with its basic p*I* (9.0–9.2, [1]) (fig.1, lanes a). Also, we have checked, as indicated in section 2, that the protein used in our experiments had a phospholipid transfer activity, even after the lyophilization step.

(ii) When the protein was omitted, the labelled fatty acids migrated toward the anode

(iii) Under identical conditions, basic histone II4 did not bind [1-^{14}C]oleic acid. As indicated in fig.1, no ^{14}C label (lane b) was found at the level of histone IIA band, revealed by Coomassie blue staining (lane a); the labelled fatty acid, which migrated toward the anode, at pH lower than 4, was not detected in the autoradiogram.

Electrophoretic titrations confirmed these observations since they also revealed strong binding of oleic and linoleic acids as well as oleoyl-CoA Fig.2

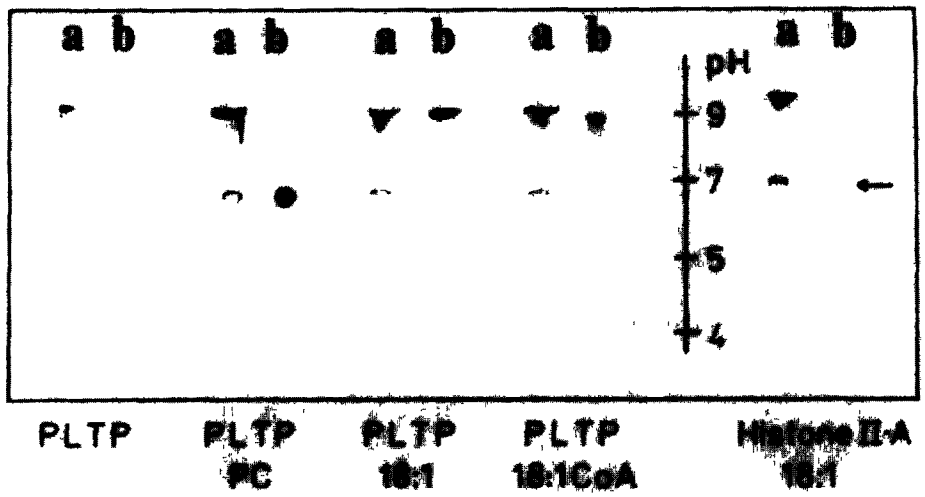

Fig.1 Binding of ligands by phospholipid transfer protein (PLTP) from spinach leaves. PLTP (85 ng) was incubated with either [1-^{14}C]dipalmitoyl-phosphatidylcholine (PC, 80000 cpm), or [1-^{14}C]oleic acid (18:1, 40000 cpm), or [1-^{14}C]oleoyl-CoA (18:1 CoA, 40000 cpm) and subjected to isoelectric focusing. Controls: PLTP (85 ng) alone and Histone II-A (440 ng) incubated with [1-^{14}C]oleic acid (18·1, 40000 cpm). a, lanes stained for protein; b, autoradiograms of ligands. The arrow indicates application spots of protein/ligand complexes

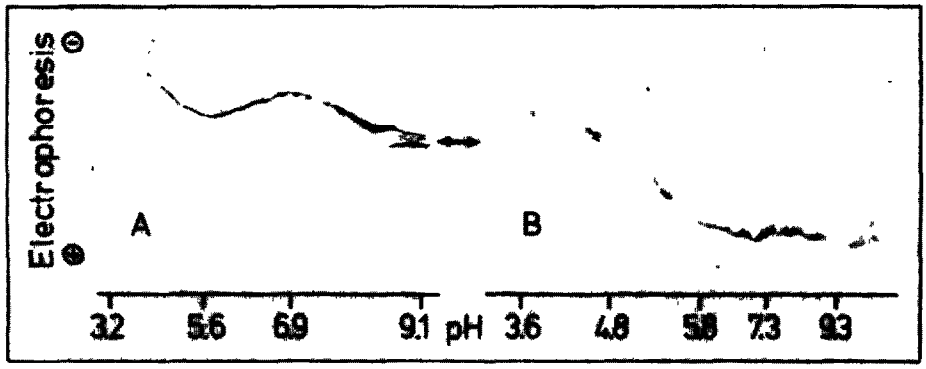

Fig.2. Titration curves of protein/ligand complexes. After incubation of proteins (900 ng) with ligands (600 pmol) complexes were filled into the trough and subjected to electrophoresis. Gels were stained for proteins. A, complex of [1-^{14}C]oleoyl-CoA with PLTP from spinach leaves B, complex of [1-^{14}C]oleic acid with FABP from etiolated oat seedlings The arrow indicates the position of the trough

shows the titration curves of protein/[^{14}C]oleoyl-CoA complexes obtained either with spinach PLTP (fig.2A) or *A. sativa* FABP (fig.2B). In both cases, since the distribution of radioactivity exactly coincided with protein staining, only autoradiograms are presented. The titration curve observed for *A. sativa* protein indicated a p*I* around 4.8, since the protein was negatively charged above this value of pH and positively charged for pH lower than 4.8; this result is in agreement with the fact that *A. sativa* FABP, liganded with oleoyl-CoA, behaves as an acidic protein [2]. In contrast, the titration curve obtained with spinach PLTP indicated that the net electric charge was positive for all values of pH; this is in agreement with the basic character of this protein [1]. However, the shape of the curve was surprising since at pH around 5.6, the protein became less positively charged. This could be interpreted as the result of interactions between the ampholytes and the protein at this region; these interactions could add negative charges to the protein, leading to a decrease of the overall positive charge of the molecule. We have also observed identical titration curves with native or with oleate-complexed spinach protein; in contrast, native *A. sativa* FABP, having a p*I* of 8.4, exhibited a shift of its p*I* to 4.8 when charged with oleate [2]. Apart from analytical data derived for PLTP [1] and FABP [2], the shapes of titration curves are indicative of different protein species despite similar binding capabilities.

The physiological significance of the finding that PLTP is also able to bind long-chain fatty acids is unclear at this moment. To our knowledge, it is the first demonstration that a PLTP, defined by its properties of transferring phospholipids between membranes, shares in common with FABP an ability to bind acyl chains. Animal cells contain several categories of lipid-carrier or lipid-transfer proteins: phospholipid transfer proteins which are either specific (for phosphatidylcholine or phosphatidylinositol) or non-specific (reviews, [6–8]), fatty-acid binding proteins [9–12] and sterol-carrier proteins [13–16]. It is now accepted that the non-specific phospholipid transfer protein from rat liver is identical to the sterol-carrier protein (SCP2) [16]. However, it has not been demonstrated that phospholipid transfer proteins from animal tissues are able to bind fatty acids.

We have previously observed a binding of phosphatidylcholine on PLTP from spinach [1] or maize [17], after incubation of these proteins with liposomes and separation of liposomes and proteins by chromatofocusing. Here, only a weak binding of phosphatidylcholine was noted, far lower than that of fatty acids. This observation is somewhat surprising if it is supposed that PLTP acts as a phospholipid carrier. Wirtz and colleagues [18] have studied in detail the binding of phosphatidylcholine on the bovine liver specific protein which contains, when isolated, one mol of this phospholipid per mol protein. In contrast, no

phospholipid was detected in the non-specific protein purified from bovine liver [19]. In agreement with this observation, it has recently been suggested that this non-specific protein does not function by carrying phospholipids, but rather by increasing the spontaneous intermembrane transfer of lipids [20].

The finding that fatty acids are bound to the spinach protein opens new perspectives for the physiological significance of plant PLTP. We suggest that PLTPs, in addition to their role in the phospholipid movements between membranes, are involved in the intracellular dynamics of acyl chains and CoA esters. This novel property of PLTP may give new interpretations of previous experiments which consisted of adding spinach PLTP to an incubation mixture of chloroplasts, microsomes and labelled acetate; in addition to increase in label of chloroplast phosphatidylcholine, changes in fatty acid label were also noted [21,22]. Further experiments are now planned in order to elucidate the eventual participation of PLTP in the cellular utilization and metabolism of lipophilic substrates.

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