FEBS 17377 FEBS Letters 391 (1996) 333–335

Phosphatidylcholine transfer protein from bovine liver contains highly unsaturated phosphatidylcholine species

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Received 21 June 1996

Abstract The phosphatidylcholine transfer protein (PC-TP) from bovine liver contains one molecule of non-covalently bound PC. In order to gain more insight into the physiological function of PC-TP, PC was extracted from bovine liver PC-TP and its molecular species composition identified by fast atom bombardment mass spectrometry. The prevailing molecular species were C18:0/C18:1-, C18:0/C18:2-, C18:0/C20:4-, C18:0/20:5- and C18:0/C22:5-PC accounting for 85% of the PC species present. This molecular species composition is not representative for what is present in bovine liver where these species account for 43% of the total PC content [Montfoort et al. (1971) Biochim. Biophys. Acta 231, 335–342]. Another striking observation is that PC species carrying a palmitoyl chain at the *sn*-1 position are nearly absent, despite these species being abundantly present in bovine liver. This study suggests that PC-TP could play a role in the metabolism of highly unsaturated, stearoyl-containing PC species.

Key words: Phosphatidylcholine; Mass spectrometry; Phosphatidylcholine transfer protein; Bovine liver; Signal transduction

1. Introduction

In contrast to other phospholipid transfer proteins, the phosphatidylcholine transfer protein (PC-TP) is highly specific for phosphatidylcholine (PC) [1]. The protein was first isolated from bovine liver [2]. The primary structure of bovine liver PC-TP was elucidated by automated Edman degradation and consists of 213 amino acid residues with a calculated molecular mass of 24681 Da [3,4]. The determined amino acid sequence was confirmed by analysis of the cDNA encoding bovine PC-TP [5,6].

Extraction of bovine liver PC-TP by organic solvents showed that one PC-TP molecule contains one molecule of non-covalently bound PC [2,7]. These data indicate that PC-TP has a binding cavity for PC. Time-resolved fluorescence experiments showed that the fatty acyl chains attached to the *sn*-1 and *sn*-2 position of PC are immobilised at distinctly different sites in the protein [8]. Investigations with fluorescently labeled PC analogues showed that the binding affinity of PC-TP for PC is strongly dependent on the length of the fatty acyl chains [9]. Furthermore, in vitro PC-TP has the highest affinity for PC species with a palmitoyl (C16:0) chain at the *sn*-1 position and an unsaturated fatty acyl chain at the *sn*-2 position. This demonstrates that the characteristics of the binding site are ideally suited to bind natural PC species. In agreement with this study it was found that PC-TP prefers 1-palmitoyl-2-arachidonoyl-PC (C16:0/C20:4 PC) and 1-palmitoyl-2-docosahexanoyl-PC (C16:0/C22:6 PC) over any other naturally occurring PC species in vitro [10].

So far, the preference of PC-TP for certain PC species has been investigated using in vitro transfer activity and binding assays [9,10]. In order to gain more insight into the physiological function of PC-TP, the molecular species composition of PC bound to PC-TP in vivo should be determined. Here, we have isolated bovine liver PC-TP and identified the molecular species composition of bound PC by fast atom bombardment mass spectrometry (FAB-MS). We will show that the PC species bound are highly unsaturated and atypical as compared to the species present in bovine liver.

2. Materials and methods

PC-TP was purified to homogeneity from bovine liver according to the procedure described by Westerman et al. [11]. Its purity was determined by SDS-PAGE (data not shown). The pure PC-TP samples were desalted by chromatography using a Sephadex G-25 column (25 ml) and concentrated to a volume of 3 ml with Centricon 20000 filters. Lipids were extracted from the PC-TP samples by the method of Bligh and Dyer [12]. The lipid extracts were analysed by fast atom bombardment mass spectrometry (FAB-MS).

Positive- and negative-ion FAB mass spectra were acquired using a Jeol JMS SX/SX 102A four-sector mass spectrometer, operated at 10 kV accelerating voltage, equipped with a Jeol FAB gun set at 5 mA emission current producing a beam of 6 keV Xe atoms. Full mass spectra were recorded at a speed of about 15 s for the *m/z* 10–1000 mass range by averaging 5–10 scans and acquired and processed with an HP-9000 data system using Jeol Complement software. The samples were dissolved in 10–20 μl of a mixture of methanol and chloroform (50:50, v/v) and approx. 1 μl of the solution was mixed with a microdroplet of triethanolamine (TEA) on the FAB probe. Instrumental slit width settings corresponding to a resolution of about 1000 were chosen to obtain optimal sensitivities. Normal mass spectra were obtained by scanning MS-1, while collisional-induced dissociation (CID) product-ion spectra of selected precursor ions were acquired by scanning MS-2 in the B/E mode using a collision cell in the third-field-free region of the instrument and air as the collision gas. The pressure of the collision gas was adjusted to obtain a 50% intensity reduction of the main beam.

3. Results

Analysis of the lipid extract from bovine liver PC-TP by FAB-MS yielded several negative ions between *m/z* 650 and 850 which are related to the molecular mass (*M*) of the phospholipid species (Fig. 1). The positive ion mass spectra of this extract revealed various couples of $[M+H]^+$ and $[M+Na]^+$ ions (data not shown) which allowed the identification of the five high-mass anions observed at *m/z* 770, 772, 792, 794 and 820 as $[M-15]^-$ ions of PC species carrying different

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Abbreviations: PC-TP, phosphatidylcholine transfer protein; FAB-MS, fast atom bombardment mass spectrometry; CID, collisional-induced dissociation

PII S0014-5793(96)00770-3

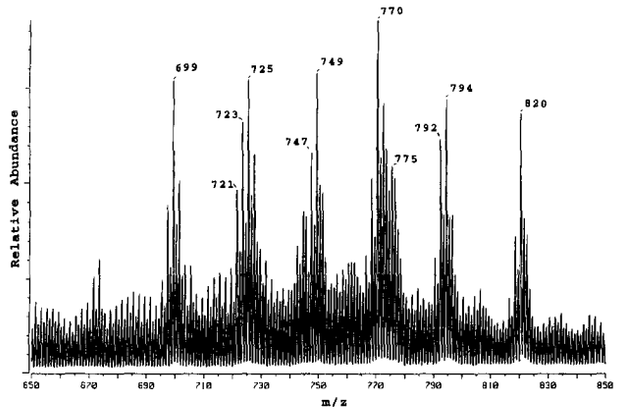

Fig. 1. Negative ion FAB mass spectrum of the extract from bovine liver PC-TP (range *m/z* 650–850).

fatty acyl substituents at the *sn*-1 and *sn*-2 positions. The formation of these $[M-15]^-$ ions corresponds to the loss of a methyl cation from the quaternary nitrogen of PC. The $[M-15]^-$ ion is one of the most characteristic peaks in the negative ion FAB mass spectra of diradyl-phosphocholines [13,14]. Two other important high-mass ions characteristic for PC species are $[M-60]^-$ and $[M-86]^-$, originating from the loss of a trimethylammonium cation and a vinyl trimethylammonium cation, respectively [13,14]. FAB desorption of the PC molecule with the molecular mass of 835 Da forms $[M-15]^-$, $[M-60]^-$ and $[M-86]^-$ ions at *m/z* 820, 775 and 749, respectively. The characteristic ions of the PC species present in the lipid extract are presented in Table 1.

The anions representing the different PC species were further analysed using tandem mass spectrometry. Fragmentation of the $[M-15]^-$ ion will yield as major fragments the carboxylate anions derived from the fatty acyl substituents at the *sn*-1 and *sn*-2 positions [13]. As an example, the carboxylate anions produced by fragmentation of the collisionally activated *m/z* 820 $[M-15]^-$ ions from the compound with a molecular mass of 835 Da are given in Fig. 2A. The two major carboxylate anions at *m/z* 283 and 329 correspond to the fatty acid chains C18:0 and C22:5, respectively. Inherent to the decomposition of the high-mass $[M-15]^-$ ion, the carboxylate anion corresponding to the *sn*-2 acyl chain is more abundant than the anion derived from the *sn*-1 acyl chain [13,15]. This means that the PC molecule with a molecular mass of 835 Da contains a C18:0 fatty acyl chain at the *sn*-1 position and the C22:5 fatty acyl chain at *sn*-2 position (Fig. 2A). The acyl chain at the *sn*-2 position can also be lost as a ketene molecule [13]. This explains the presence of a peak at *m/z* 508 in the CID spectrum of *m/z* 820, corresponding to the loss of a $C_{20}H_{31}$-CH=C=O molecule, in further support of the

Table 1
The characteristic anions of different molecular PC species after FAB desorption

M (Da)	$[M-15]^-$ (*m/z*)	$[M-60]^-$ (*m/z*)	$[M-86]^-$ (*m/z*)
835	820	775	749
809	794	749	723
807	792	747	721
787	772	727	701
785	770	725	699

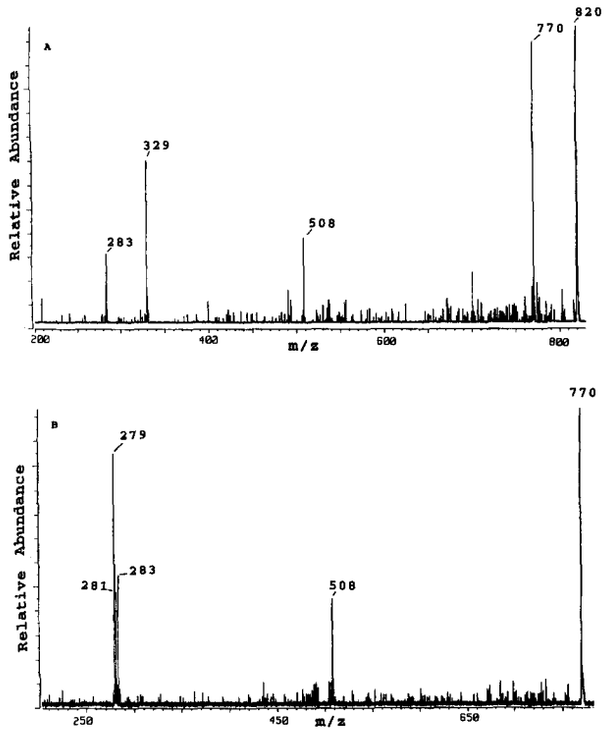

Fig. 2. Product ions following CID of the $[M-15]^-$ ion at *m/z* 820 (A) and 770 (B), derived from the bovine liver PC-TP lipid extract.

presence of the C22:5 fatty acyl chain. The CID spectrum of *m/z* 820 also exhibits a peak at *m/z* 770 which results from a CH_3Cl loss from the $[M+Cl]^-$ adduct ion in which M corresponds to the PC species with a molecular mass of 785 Da.

Fig. 2B shows the CID spectrum of the anion at *m/z* 770. The major carboxylate anions at *m/z* 279, 281 and 283 correspond to the fatty acid groups C18:2, C18:1 and C18:0, respectively. This shows that the PC species with a molecular mass of 785 Da consists of a mixture of two species. One PC species has the C18:0 chain at the *sn*-1 position and the C18:2 chain at the *sn*-2 position while the other species has the C18:1 chain at the *sn*-1 and *sn*-2 positions. Thus, the distribution of the fatty acyl chains in the PC species can be unambiguously established by tandem mass spectrometry. The other PC species were identified similarly (see Table 2).

As shown in Table 2, the major PC species in PC-TP are C18:0/C18:1-, C18:0/C18:2-, C18:0/C20:4-, C18:0/C20:5- and C18:0/22:5-PC which account for 85% of the total PC species bound. In bovine liver C18:0/C18:1-, C18:0/C18:2- and C18:0/C20:4-PC account for 43% of the total PC species present, whereas C18:0/C22:5-PC is not detected (see Table 2 [16]). Furthermore, PC species carrying very long unsaturated fatty acyl chains at the *sn*-2 position (C20:4, C20:5 and C22:5) account for more than 55% of the total PC species bound whereas these species in bovine liver account for about 11%. It is striking that PC species with the palmitoyl chain at the *sn*-1 position are nearly absent from the lipid extract while these species are very common in bovine liver (32%).

4. Discussion

PC-TP has a lipid binding site which is highly specific for

Table 2
Identification of the phospholipids bound by bovine liver PC-TP[a]

[M]	Lipid	Molecular PC species (R1,R2)	PC-TP (%)	Bovine liver (%)[b]
785	PC	C18:0,C18:2	19 ± 1	16
785	PC	C18:1,C18:1	6 ± 0.3	n.d.
787	PC	C18:0,C18:1	18 ± 2	16
807	PC	C16:0,C22:5	4.5 ± 0.3	n.d.
807	PC	C18:0,C20:5	9 ± 0.7	n.d.
807	PC	C18:1,C20:4	4.5 ± 0.3	n.d.
809	PC	C18:0,C20:4	21 ± 2	11
835	PC	C18:0,C22:5	18 ± 1	n.d.

n.d., not detected.
[a]These data were obtained by analysing independent pure PC-TP fractions, obtained from three different purifications from bovine liver.
[b][16].

PC. Upon purification, the protein contains PC in a molar ratio of 1:1 [2]. So far, the preference of PC-TP for certain PC species has been determined using in vitro binding and transfer activity assays. Here, we have been able to determine the molecular species composition of PC bound to PC-TP upon purification from bovine liver. Given the very small amounts of PC available, tandem mass spectrometry was very effective in identifying the prevailing molecular species of the bound PC. These species were C18:0/C18:1-, C18:0/C18:2-, C18:0/C20:4-, C18:0/C20:5- and C18:0/22:5-PC, accounting for 85% of the PC species identified (Table 2). This PC species composition is not representative for what is present in bovine liver where these species account for 43% of the total PC (Table 2). Another striking observation is that the bound PC contains a very low level of PC species carrying a palmitoyl chain at the *sn*-1 position, species which are abundant in bovine liver and for which PC-TP expresses the highest affinity in binding assays in vitro [8,9,17,18]. In view of this specificity in vitro, the distinct molecular species composition of the bound PC suggests that in situ there are distinct PC pool(s) from which PC-TP extracts its PC molecule. Evidently, these PC pool(s) consist primarily of PC species with the C18:0 acyl chain at the *sn*-1 position and very long unsaturated fatty acyl chains at the *sn*-2 position. In this context it is also important to establish where PC-TP is localised in the cell. Another possibility could be that PC-TP is part of the deacylation/reacylation mechanism responsible for the formation of these highly unsaturated species [19,20].

It is striking that the majority (57%) of the endogenous bound PC species contain fatty acyl chains (C20:4, C20:5 and C22:5) which are precursors in the prostaglandin/leukotriene biosynthesis [21]. This may indicate that PC-TP is involved in the delivery of these species to sites containing receptor-controlled phospholipases A_2 [10]. According to this model PC-TP plays a role in signal transduction similar to what is observed for PI-TP [22]. In this sense it is important to note that PC-TP is expressed at all stages of mouse embryo development as early as the embryonic stem cells [5].

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