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Research article

Modeling, docking and dynamics simulations of a non-specific lipid transfer protein from *Peganum harmala* L.

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ABSTRACT

Non-specific lipid transfer proteins (ns-LTPs), ubiquitously found in various types of plants, have been well-known to transfer amphiphilic lipids and promote the lipid exchange between mitochondria and microbody. In this study, an *in silico* analysis was proposed to study ns-LTP in *Peganum harmala* L., which may belong to ns-LTP1 family, aiming at constructing its three-dimensional structure. Moreover, we adopted MEGA to analyze ns-LTPs and other species phylogenetically, which brought out an initial sequence alignment of ns-LTPs. In addition, we used molecular docking and molecular dynamics simulations to further investigate the affinities and stabilities of ns-LTP with several ligands complexes. Taken together, our results about ns-LTPs and their ligand-binding activities can provide a better understanding of the lipid–protein interactions, indicating some future applications of ns-LTP-mediated transport.

1. Introduction

Plant ns-LTPs were first isolated from spinach leaves over 30 years ago, and they possessed the abilities of transferring amphiphilic lipids (*e.g.*, phospholipids, fatty acids and glycolipids) and promoted the lipid exchange between mitochondria and microbody (Gincel et al., 1994; Kader et al., 1984; Pii et al., 2009). Hitherto, ns-LTPs have been discovered in various types of plants, including castor beans, tangerine, corn, rice, tobacco, peach, coffee (Takishima et al., 1988; García-Olmedo et al., 1995; Gomar et al., 1996; Liu et al., 2002; Da-Silva et al., 2006; Pasquato et al., 2006; Zottich et al., 2011), *etc.* Plant ns-LTPs can be divided into two subfamilies depending on the different molecular mass, namely ns-LTP1 with 9 kDa and ns-LTP2 with 7 kDa (Douliez et al., 2000; Kader, 1997). The typically structural characteristic of ns-LTP contains an internal hydrophobic cavity that can be stabilized by 8 Cys residues, and such a cavity is able to run through the whole protein to allow the binding of different lipids and hydrophobic compounds *in vitro* (Douliez et al., 2000; Pons et al., 2003). Therefore, ns-LTPs might play a pivotal role in the formation of protective hydrophobic layers on the surface of the plant aerial organs possibly stimulating the transfer of hydrophobic compounds to the extracellular environment (Trevino and Oconnell, 1998; Lee et al., 2009). All the relevant evidence provided a potential explanation for the ns-LTPs defense against viral, bacterial and fungal pathogens (Buhot et al., 2001; Salcedo et al., 2007). Furthermore, the transfer activity for drug delivery solves the problems involving lipid membrane permeability of drugs, which would be employed in pharmacology or medicine industries (Pato et al., 2001). Also, it could be recognized as plant pan-allergens present in foods and pollens (Salcedo et al., 2004).

The tertiary structures of ns-LTP from different origins (*e.g.*, wheat, rice, maize and barley seeds), either in free state or in complex with lipids, have recently been determined by X-ray crystallography or NMR method (Gincel et al., 1994; Gomar et al., 1996; Shin et al., 1995; Charvolin et al., 1999; Han et al., 2001; Heinemann et al., 1996; Lerche et al., 1997; Lee et al., 1998), suggesting the three-dimensional structure of ns-LTP consists four α-helices and a long N-terminal signal sequence, with four disulfide bonds interconnecting the secondary structure (Lee et al., 1998). They are also

Abbreviations: Ala, alanine; C, carbon; Cys, cysteine; Glu, glutamic acid; Leu, leucine; MD, molecular dynamics; MEGA, Molecular Evolutionary Genetics Analysis; NMR, nuclear magnetic resonance; nsLTP, non-specific lipid transfer protein; PHL-nsLTP, *Peganum harmala* L. non-specific lipid transfer protein; p*I*, isoelectric point; Ser, serine; Thr, threonine; RMSD, root-mean-square deviation; RMSF, root-mean-square fluctuation.

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characterized by a tunnel-like hydrophobic cavity into which a long fatty acyl chain could be inserted. A recent study has revealed that ns-LTP of maize can accommodate various types of saturated or unsaturated fatty acids, ranging in size from C10 to C18 (Han et al., 2001). These above-mentioned results would provide us more insightful clues to further understand the structure–function relationships of ns-LTP.

Peganum harmala L., distributed in Uyghur nationality in Xinjiang, China since ancient times, has been regarded as a traditional herb for medicinal or psychoactive therapy in water shortage deserts and grasslands (Ma et al., 2000). Moreover, it has been reported to possess a wide spectrum of pharmacological actions in different kinds of applications, including antibacterial, antifungal, antitumor treatments and effectiveness in treatment of dermatosis (Astulla et al., 2008; Song et al., 2004; Herraiz et al., 2010). To date, structure–function relationships of PHL-nsLTP are still under investigation, and therefore, it is of great significance to investigate its structure and ligand–protein interactions.

In the study described here, we reported for the first time that a new lipid transfer protein's nucleotide sequence information which was purified from *Peganum harmala* L. (PHL-nsLTP) seeds. Meanwhile, Sun and her colleagues demonstrated that this protein exerted lipid binding, antifungal, anticancer and anti-HIV-1 reverse transcriptase activities (Ma et al., 2013). Herein, based upon this nucleotide sequence information, we established an evolutionary relationship between PHL-nsLTP and ns-LTPs in the other species under the guidance of phylogenetic analysis and sequences alignments. Then, we modeled the three-dimensional structure of PHL-nsLTP and assessed its affinities and stabilities in complex with various fatty acids through molecular docking and dynamics simulations, thus concluding that the PHL-nsLTP may belong to ns-LTP1, which could form complex with a wide range of lipid ligands from C10 to C18.

In summary, our results demonstrate that ligand binding activities of ns-LTP can develop a better understanding of the biological functions of PHL-nsLTP, as well as the lipid–protein interactions, and thus might shedding light on future pharmacological or medical industrial implications.

Table 1
The detailed information of the ligands.

Ligand abbr.	Name	Formula
LP3	(7R)-4,7-Dihydroxy-N,N,N-trimethyl-10-oxo-3,5,9-trioxa-4-phosphaheptacosan-1-aminium 4-oxide	$C_{26}H_{55}NO_7P$
LAP	[2-((1-Oxododecanoxy-(2-hydroxy-3-propanyl))-phosphonate-oxy)-ethyl]-trimethylammonium	$C_{20}H_{43}NO_7P$
E2P	Prostaglandin B2	$C_{20}H_{30}O_4$
OLA	Oleic acid	$C_{18}H_{34}O_2$
PAM	Palmitoleic acid	$C_{16}H_{30}O_2$
PLM	Palmitic acid	$C_{16}H_{32}O_2$
MYR	Myristic acid	$C_{14}H_{28}O_2$
DAO	Lauric acid	$C_{12}H_{24}O_2$
DKA	Decanoic acid	$C_{10}H_{20}O_2$
CXS	3-Cyclohexyl-1-propylsulfonic acid	$C_9H_{19}NO_3S$

2. Materials and methods

2.1. Data preparation

PHL-nsLTP was isolated by cationic exchange chromatography on Resource S column and gel filtration on Sephadex 75 10/300 GL column (Ooi et al., 2008). Moreover, according to N-terminal amino acid sequence analysis results, degenerate primers were designed and the full length cDNA of PHL-nsLTP was acquired by rapid amplification of cDNA end (RACE) (Zhang and Frohman, 1997).

DNAMAN software (http://www.lynnon.com/) was used to translate the nucleotide acid sequences of PHL-nsLTP to the corresponding amino acid sequences. Moreover, PHL-nsLTP amino acid sequence was used to find its homology proteins through BLASTP (protein–protein BLAST) in a non-redundant protein sequences (nr) in the database (Altschul et al., 1990).

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ATGGCTACCATCAAGCTTGTTTGCGCCTTGGTTGCCTGCATGCTGGTGGCTGCACCGCTG
 M  A  T  I  K  L  V  C  A  L  V  A  C  M  L  V  A  A  P  L

ACCGAGGCGGCCATAGCATGCGGTACGGTGGTTTCAGCATTGTCTCCATGCATTGGTTAC
 T  E  A  A  I  A  C  G  T  V  V  S  A  L  S  P  C  I  G  Y

TTGAGGGCAGGTGGCAGCCCACCTCTGGCGTGCTGCAATGGAGTGAGAGCACTGAACAAT
 L  R  A  G  G  S  P  P  L  A  C  C  N  G  V  R  A  L  N  N

GCCGCCAGGACTACACCGGACCGCCAGGAGGCGTGCAGATGCTTGCAGAATGCGGCTAGA
 A  A  R  T  T  P  D  R  Q  E  A  C  R  C  L  Q  N  A  A  R

TCCATGGGCGGACTCAACGAAGCAAATGCTGGTTCTCTCCCTGGCAAGTGCGGCGTCAAC
 S  M  G  G  L  N  E  A  N  A  G  S  L  P  G  K  C  G  V  N

ATTCCATACAAGATCAGCACCTCCACCAACTGCTCTACGGTGAAGTGA
 I  P  Y  K  I  S  T  S  T  N  C  S  T  V  K  *
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Fig. 1. Nucleotide sequences of cDNA and deduced amino acid sequences for PHL-nsLTP. The first 23 amino acids was signal peptide which was shown as red letters. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)