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THE PRIMARY STRUCTURE OF THE NONSPECIFIC LIPID TRANSFER PROTEIN (STEROL CARRIER PROTEIN 2) FROM BOVINE LIVER

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The primary structure of the nonspecific lipid transfer protein (sterol carrier protein 2) from bovine liver has been determined. The protein consists of a single polypeptide chain of 121 amino acid residues with serine as the amino-terminal and alanine as the carboxy-terminal residue. The protein contains one single cysteine and tryptophan residue and lacks tyrosine, histidine and arginine.

Mammalian tissues contain a nonspecific lipid transfer protein which mediates *in vitro* the transfer of all common phospholipids, cholesterol and gangliosides between membranes (1-3). This protein has been purified from rat liver (3-5) and bovine liver (6) and shown to be identical to sterol carrier protein 2 (3,7). Inherent to its ability to transfer cholesterol, this protein stimulates the formation of cholesterol ester by microsomal membranes (3,8,9) and the synthesis of pregnenolone by adrenal mitochondria (10).

As part of our interest in the structure and mode of action of lipid transferring proteins, we have recently elucidated the primary structure of the phosphatidylcholine transfer protein from bovine liver (11). Here we report the complete amino acid sequence of the nonspecific lipid transfer protein from bovine liver.

Materials and Methods

Purification

The nonspecific lipid transfer protein was isolated from bovine liver by a modification of the method described in (6). After the CM-cellulose step the protein was purified to homogeneity by chromatography on Sephadex G-50 and hydroxyapatite followed by Fast Protein Liquid Chromatography on Mono S HR 5/5 (Pharmacia).

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Preparation of Peptides

Prior to fragmentation the transfer protein was reduced with dithiothreitol and carboxymethylated with [^{14}C]iodoacetic acid. Peptides were obtained by cleavage with cyanogen bromide (11), hydroxylamine (12), *Staphylococcus aureus* protease (11) and BNPS-skatol (13). The cyanogen bromide peptides were purified by chromatography on AcA 202 (LKB) in 0.1 M $(NH_4)HCO_3$ (pH 7.8) and high-voltage electrophoresis on cellulose MN300 sheets (Machery-Nagel) at pH 6.5 in pyridine/acetic acid/water (10:0.5:89.5, v/v). The other peptides were purified by chromatography on Sephadex G-50 in 0.1 M $(NH_4)HCO_3$ (pH 7.8). Further purification of the hydroxylamine peptides was carried out by chromatography on CM-cellulose using a linear gradient of 0.005-0.2 M $(NH_4)HCO_3$ (pH 7.8).

Amino Acid Analysis

Protein and peptide samples were routinely hydrolyzed with 6 N HCL for 24 hrs at 110°C under vacuum and analyzed on a Kontron Liquimat III.

Sequence Analysis

The sequence of protein and peptides was analyzed on a Beckman sequencer, model 890 C and the residues identified as described in (11). Repetitive yields varied between 93 and 97%. The carboxy-terminal sequence of the intact protein was determined by digestion with carboxypeptidase A and B (11).

Results and Discussion

The elucidation of the primary structure of the nonspecific lipid transfer protein is based on the automated sequence analysis of the intact protein and the peptides obtained by fragmentation with cyanogen bromide, *Staphylococcus aureus* protease and hydroxylamine (Fig. 1). The protein has five methionine residues at positions 75, 84, 88, 105 and 109. Cyanogen bromide digestion will yield a total of six peptides. We have isolated by molecular sieve chromatography and high-voltage electrophoresis the five small peptides representing the carboxy-terminal half (i.e., CB1, Ala^{76}--Met^{84}; CB2, Thr^{85}-Met^{88}; CB3, Asn^{89}-Met^{105}; CB4, Gly^{106}-Met^{109}; CB5, Lys^{110}-Ala^{121}). Digestion by staphylococcal protease yielded three peptides (i.e., SP1, Ser^{1}-Glu^{15}; SP2, Ile^{16}-Glu^{47}; SP3, Ala^{48}-Ala^{121}). Peptides due to cleavage of the bonds Glu^{17}-Lys^{18}, Glu^{21}-Asp^{22}, Glu^{23}-Gly^{24} and Glu^{25}-Gln^{26} were not identified. The protein has two bonds known to be particularly susceptible to cleavage by hydroxylamine (i.e., Asn^{56}-Gly^{57} and Asn^{102}-Gly^{103}). By molecular sieve and ion-exchange chromatpgraphy, we have isolated three peptides (i.e., HA1, Gly^{57}-Asn^{64}; HA2, Ser^{65}-Asn^{102}; HA3, Gly^{103}-Ala^{121}). The peptide HA2 resulted from cleavage at Asn^{64}-Ser^{65}

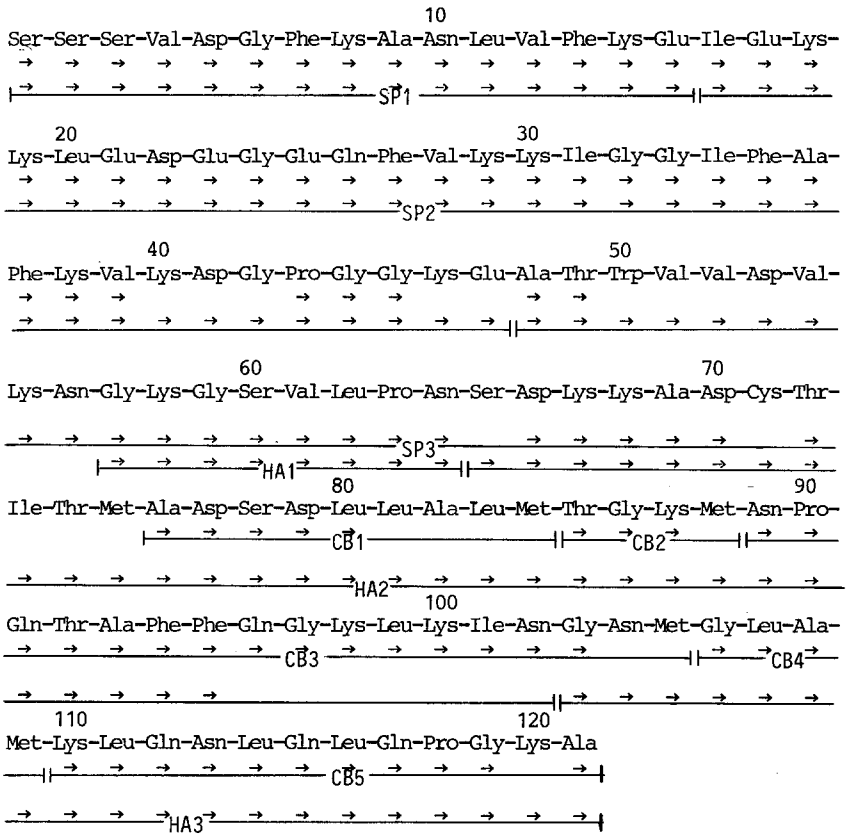

Fig. 1. Primary structure of the nonspecific lipid transfer protein from bovine liver. CB: cyanogen bromide peptides; SP: staphylococcal protease peptides; HA: hydroxylamine peptides; (→): amino acid determined by Edman degradation.

probably as a result of the prolonged time of incubation (16 hrs at 45°C instead of 3 hrs; see Ref. 12).

As indicated in Fig. 1, the amino acid sequence of the amino-terminal half was unambiguously identified by analysis of the intact protein and the peptides SP1, SP2, SP3 and HA1. The sequence of the carboxy-terminal half was established by the analysis of the peptides CB1, CB2, CB3, CB4 and CB5, and the overlapping peptides HA2 and HA3. Amino acid analysis showed that all cyanogen bromide peptides contained homoserine except CB5. This information firmly indicated that CB5 was the carboxy-terminal fragment. Carboxypeptidase digestion of the intact protein confirmed the identity of the carboxy-terminal residues (i.e., Lys^{120}-Ala^{121}). The peptides SP3, HA1 and

HA2 provided the overlap between the amino-terminal and carboxy-terminal half.

Analyses were routinely performed on peptides derived from the [^{14}C]-carboxy-methylated transfer protein. Identification of the single cysteine residue at position 71 was based on the release of radioactivity from peptide HA2 at cycle seven in the sequence analysis. The protein has a single tryptophan residue at position 50. Cleavage of the Trp^{50}-Val^{51} bond by BNPS-skatol (13) yielded two peptides. Analysis of the peptide Val^{51}--Ala^{121} confirmed the sequence up to Thr^{72}.

The amino acid composition as determined from the primary structure is given in Table I. The composition agrees very well with that reported in Ref. 6. A notable exception is the presence of four cysteine residues. Reduction of the transfer protein with dithiothreitol and carboxymethylation with [^{14}C]iodoacetic acid firmly established that the protein has only one cysteine residue. This residue is essential as modification with N-ethylmaleimide inactivated the transfer protein (unpublished observation).

TABLE I

Amino acid composition of the nonspecific lipid transfer protein

Amino acid	This study[a]	Previous study[b]
Lys	18 (14.9)	17
His	0 (0)	0
Arg	0 (0)	0
Asp	8 (6.6)	16 (Asx)
Asn	7 (5.8)	-
Thr	5 (4.1)	5
Ser	6 (5.0)	5
Glu	6 (5.0)	13 (Glx)
Gln	6 (5.0)	-
Pro	4 (3.3)	4
Gly	14 (11.6)	15
Ala	9 (7.4)	9
Val	8 (6.6)	8
Met	5 (4.1)	5
Ile	5 (4.1)	5
Leu	11 (9.1)	11
Tyr	0 (0)	0
Phe	7 (5.8)	7
Cys	1 (0.8)	4
Trp	1 (0.8)	1

[a]Composition based on primary structure; mol % in parentheses
[b]Data from Ref. 6

The transfer protein lacks tyrosine, histidine and arginine. It is of interest to note that a second nonspecific lipid transfer protein has been purified from bovine liver which differs from the one reported here, in that it contains one additional histidine and arginine residue (6). The protein has an excess of five positive charges in agreement with the isoelectric point of 9.55 (6).

To date, the primary structure of a number of cytosolic proteins involved in intracellular lipid transport and metabolism has been reported, that is the fatty acid binding protein (14), the retinol binding protein (15), and the phosphatidylcholine transfer protein (11). Possible relationships between these proteins and the nonspecific lipid transfer protein are currently under investigation. While this study was in progress, the amino-terminal half of the nonspecific lipid transfer protein was reported up to residue 52 (16).

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