Vol. 154, No. 1, 1988 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
July 15, 1988 Pages 476-482

# A MASS SPECTROMETRIC STUDY OF THE STRUCTURE OF STEROL CARRIER PROTEIN $SCP_2$ FROM RAT LIVER

Howard R. Morris++, Barbara S. Larsen+,[1], and Jeffrey T. Billheimer*

* Medical Products Department
Experimental Station
E. I. du Pont de Nemours & Company
Wilmington, DE 19898

\+ Central Research & Development Department
Experimental Station
E. I. du Pont de Nemours & Company
Wilmington, DE 19898

++ Department of Biochemistry
Imperial College of Science and Technology
London SW7 2AZ
England

Received May 6, 1988

The amino acid sequence of Sterol Carrier Protein$_2$ ($SCP_2$) isolated from rat has been investigated. Using a novel mass spectrometric mapping approach, the C-terminus was found to be extended beyond the previously published sequence. Carbohydrate analysis of $SCP_2$ samples suggest the presence of tightly bound mannose oligosaccharide of 5-10 residues, although probably not in a glycoprotein linkage. 

Sterol carrier protein $_2$ (non-specific lipid transfer protein), a low-molecular weight cytosolic protein, facilitates the in vitro transfer of sterols and phospholipids between membranes. It is thought that $SCP_2$ may play an important role in intracellular lipid trafficking and/or sterol metabolism[1]. The primary structure of $SCP_2$ purified from bovine liver[2] and rat liver[3] has been determined and show greater than 90% homology between species.

In an independent mass spectrometric approach prior to the publication of the rat sequence, FAB mapping[4] was used to study

[1]To whom all correspondence should be addressed.

0006-291X/88 $1.50

the primary structure of rat liver $SCP_2$. A mass spectrometric protocol was also applied to determine the type of oligosaccharide present[5].

## Materials and Methods

### Purification

Sterol carrier protein $_2$ (non-specific lipid transfer protein) was purified according to the procedure of Trzaskos and Gaylor[6]. In brief, livers from twenty rats were perfused with 0.25 M sucrose, excised, homogenized in 0.1 M phophate, pH 7.4 containing 1 mM EDTA 1 mM glutathione, 20 uM leupeptin and 2 mM PMSF and the cytosolic fraction obtained by differential centrifugation. Purification protocol included acid precipitation (pH 5.1), ammonium sulfate precipitation, molecular exclusion chromatography on an ACA54 column, ion exchange chromatography on a MONO-S column, and heat-treatment at 90 degrees for ten minutes. The final preparation gave one band on SDS gel electrophoresis, one band on isoeletric focusing gels, and only one N-terminal amino acid sequence.

### Preparation of Samples

Tryptic digests were carried out by standard procedures in pH 8.5 ammonium bicarbonate buffer (50 millimolar) at an enzyme substrate ratio of 1:50 at 37°C for 4 hours. CNBr digests were carried out in 70% formic acid solution at room temperature in the dark for six hours.

FAB-MS - The mass spectra were acquired using either a VG analytical ZAB-H fitted with a Cesium ion gun[7] or a ZAB-HF fitted with an M-Scan Xenon ion gun.[8,9] Samples were dissolved in 5% acetic acid and 25µl aliquots were loaded into the glycerol/ monothio-glycerol matrix (1:1, v/v) on the FAB probe.

## Results and Discussion

The amino acid sequence for rat $SCP_2$ is shown in Figure 1. Mass Spectrometric analysis of a tryptic digest of $SCP_2$ resulted in the data shown in Figure 2. The molecular weights observed correspond to the peptides underlined in Figure 1. The assignments were checked by repeating the FAB map after a single cycle of Edman degradation. With the exception of small peptides not normally observed in FAB mapping[8] together with residues 39-46 over 80% of the molecule was assigned in this experiment.

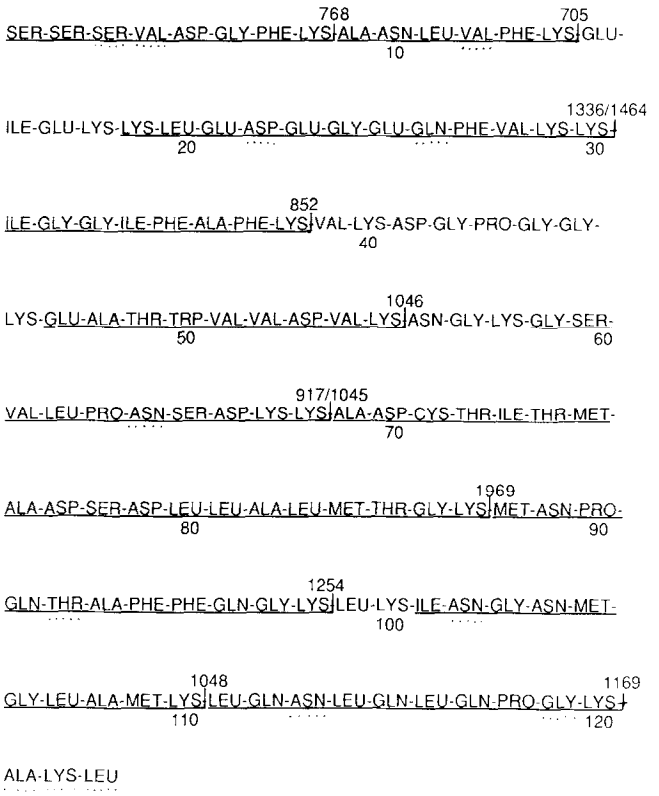


FIGURE 1 - Bovine liver $SCP_2$ Sequence showing the assignments for the masses observed from the tryptic digest on rat liver $SCP_2$. The dotted lives indicate the difference in rat $SCP_2$ from bovine $SCP_2$ (sequence from references 2 and 3).

The differences between the rat and bovine sequence are indicated by the dotted lines in Figure 1. These substitutions result from a single base mutation except for position 102 (AlA/ASN). The C-terminal peptide of the bovine $SCP_2$ sequence terminated at LYS 120.[2] The previously reported sequence for rat-$SCP_2$ indicated that the C-terminal peptide contained 75% LYS-ALA-LYS-COO- and 25% LYS-LEU-LYS-COO-[4]. The shortened protein observed in bovine liver could be explained by an additional proteolytic cleavage either in processing or during isolation and purification. Indeed in the initial purification from bovine liver, Crain and Zilversmit[10] isolated two proteins which differed in isoelectric point while having almost identical amino acid compositions. The 0.2 pKI difference could be

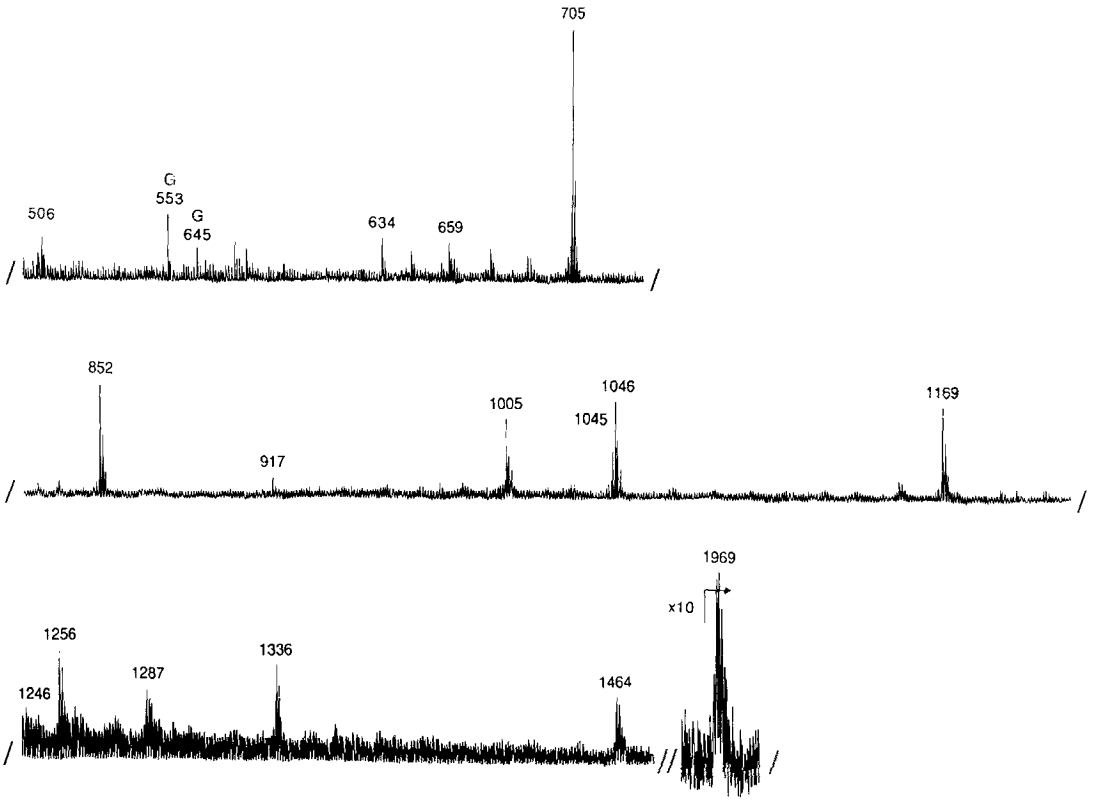


FIGURE 2 - Fast atom bombardment mass spectrum of the tryptic digest of 5 nmoles of $SCP_2$

due to the presence or absence of LYS-122. A mass spectrometric procedure designed to locate ragged ends[4,11] was employed to investigate the C-terminal sequence of $SCP_2$. The CNBr digest of $SCP_2$ was analyzed and is shown in Figure 3. The anticipated ions are indicated in Table I. The signal observed at m/z 1802 was predicted

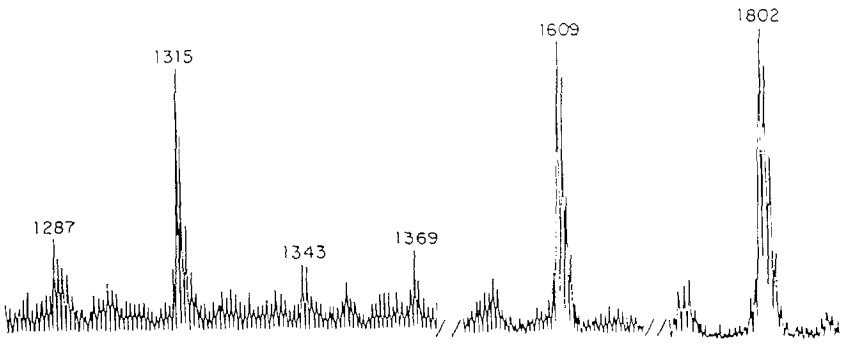


FIGURE 3 - Partial FAB-MS spectrum of a CNBr digest on 5 nmoles of $SCP_2$

TABLE 1
Predicted Signals for CNBr Digest

| Residues | Mass |
|---|---|
| 1 - 75 | >8000[a] |
| 76 - | 84921 |
| 85 - 88 | 390 |
| 89 - 105 | 1802 |
| 106 - 105 | 345 |
| C-terminus | |
| 110 - 120 | 1297 |
| 110 - 120 - ALA | 1368 |
| 110 - 120 - ALA - LYS | 1496 |
| 110 - 120 - LEU - LYS | 1538 |

a) Peptide beyond the mass range of instrumentation used.

and can be assigned to residues 89-105. The m/z 1287 signal corresponds to the peptide 76-88 with no cleavage observed at MET(84); it has been previously observed that MET-THR bonds are not cleaved by CNBr. The m/z 1315 signal arises from the formylation of the 76-88 peptide. The C-terminal peptide should give a single signal at m/z 1297 if the sequence matches the bovine sequence or two signals would be expected at 1496 and 1538 if $SCP_2$ has a micro heterogeneity as indicated by Scallen[3]. In fact, neither was observed but an additional signal at m/z 1609 was seen which was not anticipated. This is to 113 amu higher than the -LYS-ALA-LYS fragment, suggesting that rat $SCP_2$ terminates in an additional LEU or ILE. The isomers LEU and ILE cannot be distinguished by FAB-MS. Classical gas phase analysis then confirmed the presence of LEU in position 123.

The C-Terminal sequence GLY-LYS-ALA-LYS-LEU is almost identical to the last five amino acids of what is thought to be a

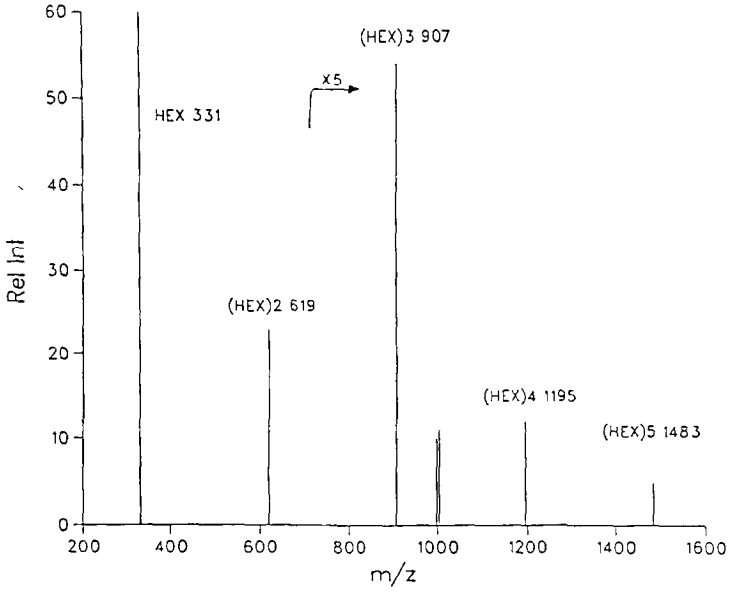


FIGURE 4 - Schematic of the FAB-MS spectrum of $SCP_2$ after 17 hours of acetolysis (isotopes not shown).

peroxisomal targeting signal.[12] Recently immuno-gold labeling studies suggest that $SCP_2$ is localized mostly in the peroxisomes.[13] Although originally purified from the cytosol, the cell-homogenization conditions normally used could result in peroxisomal disruption and subsequent appearance of the protein in the cytosolic fraction. The actual intracellular location of SCP2 will require additional work.

FAB-MS was used to investigate whether $SCP_2$ is a glycoprotein. A microchemical procedure which allows for the detection of fragments derived from cleavage of both labile and resistant bonds was used. The molecular ions present in the spectrum (Figure 4) uniquely identify the oligosaccharide as hexose containing. The data show evidence for a minimum of 5 hexose units which suggests an ASN linked high mannose attachment to $SCP_2$ or a chemically resistant hexose polymer impurity. The absence of signals above m/z 1483 suggests that the carbohydrate observed would be approximately 5 hexose units.

In summary, the application of FAB-MS to the analysis of $SCP_2$ suggests that this protein is either a glycoprotein or forms a close association with a high mannose oligosaccharide. Mass spectrometry was crucial for clear identification of the C-terminus of rat liver $SCP_2$ and in addition mapped a large portion of the molecule.

## References


1. Scallen, T.J., Pastuszyn, A., Noland, B.J., Chanderbhan, R., Kharroubi, A. and Vahouny, G. (1985) Chemo Phys. Lipids 38, 239-261.

2. Westerman, J. and Wirtz, K.W.A. (1985) Biochem. Biophys. Res. Comm.,127, 333-338.

3. Pastuszign, A., Noland, B.J., Bazan, J.F., Fletterick, R.J. and Scallen, T.J. (1987) J. Biol. Chem., 262/27, 13219-13227.

4. Morris, H.R., Panico, M.and Taylor, G.W. (1983) Biochem. Biophys. Res. Comm., 117, 299-305.

5. Naik, S., Oates, J.E., Dell, A., Taylor, G.W., Dey, P.M. and Pridham J.B. (1985) Biochem. Biohpys. Res. Comm., 132(1), 1-7.

6. Trzaskos, J.M. and Gaylor, J.L. (1983) Biochem. Biophys. Acta, 751, 52-65.

7. McEwen, C.N. (1983) Anal Chem., 55, 967-8.

8. McDowell, R., Morris, H.R. and Redfern, T. (1981) Proc. 29th ASMS, Minneapolis, U.S.A.

9. McDowell, R. and Morris, H.R. (1983) Int. Journal of Mass Spec. and Ion Physics 46, 443-446.

10. Crain, R.C. and Zilversmit, D.B. (1980) Biochemistry 19,1433-1439.

11. Greer, F. Morris, H.R., Fallon, A. and Brenner, S. (1987) Proc. 35th ASMS, Denver.

12. Gould, S.J., Keller, G. and Subramani, S. (1987) J. Cell Biol., 105, 2923-2931.

13. Keller, G., Scallan, P.J., Singer, S.J. and Krisans, S. (1987) J. Cell Biol., 105, 157.