M. A. Szarek and T. P. Anastassiades
Departments of Biochemistry and Medicine, Queen's University, Kingston, Ontario, Canada, K7L 3N6

OPN (osteopontin) is one of the sialoproteins of bone, moreover, it is one of the major phosphoproteins of bone. OPN was originally isolated from bovine bone by Franzén and Heinegård (F. & H. (1985) *Biochem. J.*, 232: 714–724). Recently, two forms of OPN, M_r = 44 kDa and 55 kDa, were isolated from rat calvaria (Wrana *et al.* (1991) *Biochem. J.*, 273: 523–531). Following the extraction and isolation procedure of Franzén and Heinegård, OPN was partially purified from bovine bone. The final purification and separation of three forms of OPN, M_r = 45, 72 and 85 kDa (SDS-PAGE, T = 12.5%), was achieved by preparative gel electrophoresis.

The amino acid compositions of all three glycoproteins were virtually the same and nearly identical to the composition of bovine OPN. Furthermore, the obtained partial *N*-terminal sequence, Leu-Pro-Val-Lys-Xxx-(Thr)-(Ser)-(Ser), where Xxx is undetermined and (Xxx) is possible assignment, for the 45 kDa and 72 kDa glycoproteins was identical to the bovine OPN sequence obtained by Prince *et al.* (Prince *et al.* (1991) *Biochem. Biophys. Res. Comm.*, 177: 1205–1210). It appears that post-translational modifications, such as glycosylation, phosphorylation and/or sulfation, may attribute to the differences in the three forms of OPN.

This work is supported by the Medical Research Council of Canada and an Arthritis Society Studentship to MAS.

S9.26

Structural Analysis of Carbohydrate Chains of Fish Gonadotropins

N. P. Arbatsky, H. A. Zenkevich*, V. P. Slanke*, Z. M. Latze*, A. O. Zheltova and V. A. Derevitskaya
*N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia; *Institute of Biology, Latvian Academy of Sciences, Salaspils, Latvia.*

The structure of carbohydrate chains of gonadotropic hormones, isolated from sturgeon and salmon pituitary glands was investigated with the use of $LiBH_4$/*t*-BuOH treatment for splitting off the oligosaccharides and HPLC for their identification. The majority of the oligosaccharides from both hormones was found to be biantennary chains (fucosylated or nonfucosylated), oligosaccharides from sturgeon hormone being terminated with GalNAc-GlcNAc units in one or both antennae [1], whereas those from salmon hormone bear usually NeuAc-Gal-GlcNAc sequence. Both α and β-subunits of sturgeon hormone contain the same oligosaccharides but in different proportion. No differences were detected in glycosylation of male and female hormones.

1. H. A. Zenkevich *et al.*, *Bioorgan. Khimiya*, 1992, v. 18, No 2, p. 226.

S9.27

The Structure of Carbohydrate Chains of Bean Storage Protein from *Phaseolus vulgaris*

L. M. Likhosherstov, I. A. Kalyakina, N. P. Arbatsky and V. N. Shibaev
N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, Moscow, Russia.

The plant glycoproteins can be considered as a convenient source of oligomannosidic and Xyl-containing *N*-linked carbohydrate chains. To isolate them, we used a bean storage glycoprotein, phaseolin, from *Phaseolus vulgaris*. Oligosaccharide fragments were split off by $LiBH_4$ method [1], reduced with $NaBH_4$, and separated by HPLC on Ultrasphere-C18 column in water. The structure of six oligosaccharides isolated was elucidated by ^{1}H-NMR. Three main oligosaccharides, $Man_9GlcNAc_2$, $Man_7GlcNAc_2$, and $Xyl_1Man_3GlcNAc_2$, were proved to be identical with those described earlier [2]. In addition, three minor oligosaccharides were identified, namely, $Man_8GlcNAc_2$, $Man_6GlcNAc_2$, and $Man_5GlcNAc_2$. For each oligosaccharide only one isomeric structure, typical for many *N*-glycoproteins, was detected.

1. L. M. Likhosherstov *et al.*, *Carbohydr. Res.*, 1988. v. 178, p. 155.
2. A. Stirm *et al.*, *J. Biol. Chem.*, 1988, v. 262, No 28, p. 13392.

S9.28

Isolation and Characterization of Glycoproteins which Cross-Reacts with Epiglycanin

T. Thingstad[a], H. Barsett[a], G. Skodbo[a], J. F. Codington[b] and *S. Haavik*[a]
[a]*Department of Pharmacognosy, Institute of Pharmacy, P.O. Box 1068 Blindern, N-0316 Oslo 3, Norway;* [b]*Department of Biological Chemistry and Molecular Biology, Harvard Medical School and Boston Biomedical Research Institute, 20 Staniford St., Boston MA 02114, USA.*

Mono- and polyclonal antibodies against a mucin-type glycoprotein, epiglycanin of the TA3-Ha mouse mammary carcinoma ascites cell [Codington, J. F. and Haavik, S. (1992) *Glycobiology*, **2**, 173–180] have been shown to cross react with an antigen present in the sera of patients with carcinomas. This human carcinoma (HC) antigen was isolated from spent culture medium of a human endometrial carcinoma cell line or ascites fluid of a patient with ovarian carcinoma. Each was concentrated on a Filtron™ Ultrasette, then fractionated by gel filtration on a column of Sepharose CL-2B. Fractions were monitored by an enzyme competitive binding assay performed on an immunoplate coated with epiglycanin. Activity was determined as ability to inhibit the binding between immobilized epiglycanin and a monoclonal antibody raised against epiglycanin. The final purification step involved affinity chromatography using an immobilized monoclonal mouse anti-epiglycanin IgM antibody. The majority of the epiglycanin cross-reacting material had M_r greater than 1000 kDa and a carbohydrate and amino acid composition consistent with that of a mucin-type glycoprotein. The purified glycoprotein had limited solubility in aqueous solvents, but smaller fragments, prepared by incubation with trypsin and Pronase possessed greater solubility. Proteolytic fragments of the HC-antigen were isolated by Superdex™ and immunosorbent FPLC. The glycopeptide structures of the isolated glycopeptides were studied.

(This work was supported by Epigen Inc., Wellesley, MA, USA.)

S9.29

Purification of Glycoproteins by Elution from the Polyvinylidene Fluoride Replicas of SDS-Page Gels

Z. Pilat, K. Bieńkowska-Szewczyk, G. Kochan and *B. Szewczyk*
Department of Biochemistry, University of Gdańsk, Gdańsk, Poland.

The preparation of a pure glycoprotein for structural studies or for inducing specific antibodies is very often a difficult