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Note

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The preparation of a glycopeptide–Sepharose for affinity chromatography

Glycoproteins are conjugated proteins that contain as a prosthetic group one or more heterosaccharides with a relatively low number of sugar residues (5–20) without serially repeating units, covalently bound to the polypeptide chain[1].

The biological role of these heterosaccharides is not well understood. It has been suggested[2,3] that the glycans linked to glycoproteins, located on the outer surface of the cells, may be involved in the cellular association phenomena as well as in interactions with the macromolecules of the intercellular matrix.

Affinity chromatography is a useful method for the study of such interactions. The covalent coupling of various substances to insoluble polymers, activated by cyanogen bromide, has proved a useful and mild procedure for proteins[4] and for glycosaminoglycans[5].

This paper reports the preparation and characterization of the conjugates of some glycopeptides, obtained by proteolytic digestion of various glycoproteins, with agarose (Sepharose 2B and 4B). In these experiments, we used the glycopeptides obtained from bovine and human fibrinogen[6–8] as well as from the structural glycoprotein of calf cornea[9–11]. These glycopeptides contain glycanes of heterosaccharidic character composed of glucosamine, mannose, galactose and sialic acid, linked by an aspartamidoglucosaminic linkage to the peptide chain, (2–8 amino acids), as prepared by the procedures described earlier[6,9,10].

Tentative coupling experiments were also performed with glycopeptides acetylated at the N-terminal amino acid of the oligopeptide fraction.

Materials and methods

Preparation of the glycopeptides. Bovine fibrinogen was purchased from Sigma Chemical Co. Human fibrinogen was a gift from Prof. CAEN (Laboratoire d'Hemostase, Hôpital St. Louis, Paris).

The proteins were digested by pronase[6] and separated on Sephadex G-50 columns. A second chromatography on the same Sephadex column eliminated the peptides devoid of sugar. The purity of the glycopeptides was controlled by thin-layer electrophoresis. Sugars and peptides were detected on the same plate by successive application of ninhydrin and orcinol reagents[12].

Traces of pronase were precipitated by adding trichloroacetic acid (TCA) to the aqueous glycopeptide solution (final concentration of TCA = 10 %). Glycopeptides were isolated from the supernatant of the TCA precipitate by precipitation with ethanol (final concentration of ethanol = 90 %).

Glycopeptides of the structural glycoproteins were prepared from calf cornea as described elsewhere[9,10]. The fraction containing the heterosaccharides were repurified on Sephadex G-50 followed by a final chromatography on a Sephadex G-25 column. The final product was devoid of mucopolysaccharides and of hydroxylysine

glycosides, as well as peptides not containing sugars. Trace amounts of proteolytic activity were eliminated with TCA as above.

The sugar and amino acid contents of the glycoproteins prepared were determined as described earlier[7,9,10].

Acetylation of the N-terminal amino group of the glycopeptides. This reaction was performed essentially by the procedure of FRAENKEL-CONRAT[13]. The acetylated glycopeptides were purified on Sephadex G-50 and Amberlite IR-120 columns. Non-acetylated amino groups were determined by using trinitrobenzenesulphonic acid reagent[14]. More than 96 % of the available terminal amino groups were acetylated.

Binding of the glycopeptides to Sepharose. Sepharose 2B and 4B were activated by the cyanogen bromide method of AXEN *et al.*[4]. A 65-mg amount of the reagent was used per 1 ml of gel. The activated gel was washed with 0.1 *M* $NaHCO_3$ solution and 15 ml of the gel was stirred for 24 h at 4° with 15 ml of an aqueous solution of 100 mg of glycopeptide. The pH was adjusted to 7 with 0.1 *M* $NaHCO_3$ solution.

The glycopeptide–Sepharose conjugate was washed with water until no traces of hexoses could be detected in the washings, then successively with 0.1 *M* sodium bicarbonate solution, water, 0.002 *N* hydrochloric acid, 0.5 *M* sodium chloride solution and water.

The yield of conjugate was 8–20 %, depending on the nature of the glycopeptide used.

Unreacted glycopeptides were recovered unchanged from the aqueous washings.

Analysis of the glycopeptide–agarose conjugates. The most suitable method for the determination of the glycopeptides linked to the agarose beads is the determination of the hexosamines in the conjugate by the ELSON–MORGAN method[15]. To eliminate the interference of the deep brown colour of the hydrolysate, the glucosamine was purified on Amberlite IR-120 columns and eluted with 3 *N* HCl.

Results and discussion

The analytical results on the Sepharose–glycopeptide conjugates are given in Table I.

TABLE I

HETEROSACCHARIDE CONTENT OF THE GLYCOPEPTIDE–AGAROSE CONJUGATES, CALCULATED FROM THE HEXOSAMINE DETERMINATION IN THE GEL AFTER COUPLING AND IN THE STARTING MATERIAL

Origin of the glycopeptides	*Sepharose*	*Heterosaccharide content*	
		mg/ml of the wet gel	*μg/mg of dry weight*
Bovine fibrinogen	4B	0.7	53
Bovine fibrinogen, desialised	2B	0.45	28
Bovine fibrinogen, N-acetylated	2B	0	0
Human fibrinogen	2B	0.62	37
Structural glycoprotein of calf cornea	2B	1.11	76

Glycopeptides obtained from bovine fibrinogen contain N-terminal valine and aspartic acid residues[7,8], whereas the glycopeptides derived from the structural glycoproteins of bovine cornea contain aspartic acid and glycine in the N-terminal

positions[9]. The amino groups of the glycosamines in the sugar fraction are acetylated in all the glycopeptides studied[7–9].

It therefore seems reasonable to assume that covalent bounds are formed between the N-terminal amino groups of the glycopeptides and the activated agarose. This assumption is supported by the finding that glycopeptides acetylated at the N-terminal amino group did not react with the activated gels.

The electrophoretic pattern and the sugar composition of the glycopeptides that did not react with the activated gel are identical with that of the starting materials[6,7,9]. This shows that no structural modification occurs in the glycans of the glycopeptides during the fixation procedure.

The glycopeptide–Sepharose conjugates prepared by the method described can be used for the study of the interaction between glycopeptides and/or glycoproteins involving their sugar side-chains.

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