Gulose as a constituent of a glycoprotein

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The aldohexose gulose was identified as a constituent of a hydroxyproline-rich glycopeptide derived from the glycoprotein SSG 185. This glycoprotein is part of the extracellular matrix of the green alga *Volvox carteri*. The gulose residue occupies a terminal position in the corresponding saccharide.

Glycoprotein; Gulose; Extracellular matrix; *Volvox carteri*

1. INTRODUCTION

The extracellular matrix (ECM) of the multicellular green alga *Volvox carteri* contains insoluble fibrous layers that surround individual cells to form contiguous cellular compartments. A highly sulphated glycoprotein with an apparent molecular mass of 185 kDa (SSG 185) has been identified as the monomeric precursor of this distinct substructure within the ECM [1,2]. The polypeptide chain of SSG 185 exhibits a central domain consisting almost exclusively of hydroxyproline residues. This central domain is *O*-glycosylated. Arabinose, mannose and galactose are the main sugar constituents of the saccharides attached to this central domain [1,2]. Exhaustive digestion of SSG 185 with proteases yields a large glycopeptide with an apparent molecular mass of 145 kDa [1]. This protease-resistant fragment represents the central hydroxyproline-rich domain of SSG 185.

In this paper, we report the isolation of a small glycopeptide (12 kDa) derived from the 145 kDa fragment. This glycopeptide contains gulose at the non-reducing end, a hexose so far not observed as a constituent of glycoproteins.

2. MATERIALS AND METHODS

2.1. *Preparation of the 12 kDa glycopeptide*

The 145 kDa glycopeptide was isolated according to Ertl et al. [2]. A solution of the 145 kDa glycopeptide (3 µg/µl) was adjusted to 0.1 M NaOH and 1 M NaBH₄ and incubated for 16 h at 37°C. Subsequently, the solution was neutralized with 5 M acetic acid and applied to a QAE-Sephadex A25 column (bed volume 20 ml, acetate form; Pharmacia) to remove the degradation products carrying the sulphated polysaccharide of the original 145 kDa fragment. The flow-through fraction was desalted by passage through a Dowex AG-50W-X8 (H⁺) column. The effluent was neutralized with pyridine, con-

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centrated and evaporated repeatedly in the presence of methanol. This crude material was fractionated on a Sephadex G-25 column (95 × 1.5 cm) equilibrated in 0.1 M pyridinium acetate. Orcinol-positive [3] material was pooled and subjected to FPLC-chromatography on a Sepharose 12 column (Pharmacia). A single peak containing carbohydrates elutes at a position corresponding to a 12 kDa protein.

2.2. *Isolation of gulose from the 145 kDa fragment*

The 145 kDa glycopeptide was further purified by SDS-polyacrylamide electrophoresis (6% acrylamide). The eluted material was hydrolysed with 4 M trifluoroacetic acid for 2 h at 100°C. After evaporation, the material was applied on a Dowex AG-50W-X8 (H⁺) column to remove peptides. The monosaccharides were partially resolved on an amino-HPLC column (Lichrosorb, Merck) under isocratic conditions (82% acetonitrile, 2 ml/min). Eluting material was monitored at 193 nm.

2.3. *Identification of gulose*

Monosaccharides were analysed as peracetylated aldonitriles [4] with the following modifications: the dried samples were dissolved in dry pyridine (20 µl) containing 0.1 mg/µl hydroxylamine hydrochloride and incubated for 30 min at 80°C. After cooling, 20 µl of acetic anhydride were added and incubation continued for a further 30 min. After addition of 50 µl of water, the solution was extracted three times with 200 µl of chloroform. The combined extracts were dried by molecular sieve (4 Å; Merck), concentrated and subjected to analysis by GC-MS.

2.4. *Permethylation*

Permethylation of the 12 kDa glycopeptide was done according to Hakomori [5] with the modifications described by Waeghe et al. [6]. Sodium dimethylsulfinyl carbanion was prepared as described by Harris et al. [7]. The methylated product was purified with Sep-Pak C₁₈ cartridges (Waters Millipore Inc.) by elution with 90% acetonitrile. After hydrolysis in 4 M trifluoroacetic acid for 2 h at 100°C the methylated sugars were reduced with NaBH₄, peracetylated and analysed by GC-MS.

2.5. *GC-MS*

All GC-MS analyses were performed with a Hewlett Packard model 5995 GC equipped with a Durabond 1701 fused silica capillary column (30 m; J&W Scientific). The carrier gas (1 ml/min) was high-purity helium. The temperature gradient for methylated alditol acetates was 2°C/min, starting at 140°C.

Peracetylated aldononitriles were analysed with 2°C/min starting at 150°C.

3. RESULTS AND DISCUSSION

Upon mild alkaline treatment, the protease-resistant 145 kDa glycopeptide derived from SSG 185 is further degraded to a sulphated glycopeptide with an apparent molecular mass of 110 kDa (Fig. 1). In order to characterize the associated degradation product(s) with low molecular masses, the sulphated 110 kDa fragment was removed by adsorption to an anion exchange resin. In the flow-through fraction, a neutral glycopeptide was recovered and purified to homogeneity by gel permeation chromatography. Its molecular mass was estimated to be 12 kDa. This glycopeptide was submitted to amino acid sequence analysis on an automated gas-phase sequencer. A sequence of 18 amino acids could be determined matching amino acid positions 219–236 of glycoprotein SSG 185: Hyp-Ile-Gly-Hyp-Ala-Hyp-Asn-Asn-Ser-Hyp-Leu-Hyp-Hyp-Ser-Hyp-Gln-Hyp-Thr. In SSG 185, this particular sequence is N-terminally connected to the hydroxyproline-rich domain of SSG 185 [2] and therefore most probably represents the N-terminus of the protease-resistant 145 kDa fragment.
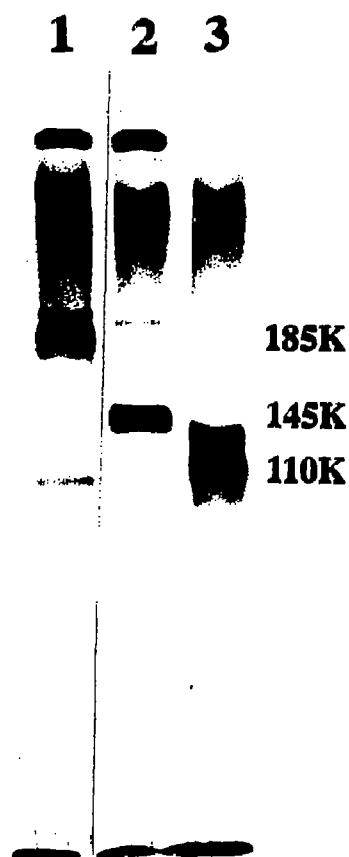

Fig. 1. Sequential degradation of glycoprotein SSG 185 as analyzed by SDS-polyacrylamide gel electrophoresis. (Lane 1) ^{35}S-labelled SSG 185; (lane 2) as lane 1 after digestion with subtilisin (50 μg/ml, 30 min); (lane 3) as lane 2 after alkaline treatment (0.1 M NaOH/1 M NaBH$_4$ for 16 h at 37°C).
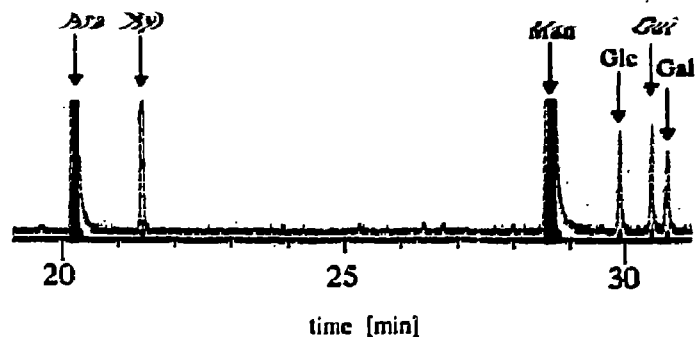

Fig. 2. Identification of gulose in an acidic hydrolysate of the 145 kDa glycopeptide. Sugars were analyzed by GC-MS as peracetylated aldononitriles. Fig. 2 shows the total ion profile. For details, see Materials and Methods.

The saccharide structure of the 12 kDa glycopeptide was analyzed by permethylation and subsequent GC-MS. The gas chromatogram revealed a terminally located hexose residue (1,5-di-O-acetyl-2,3,4,6-tetra-O-methylhexitol) that did not match any of the retention times of hexose derivatives usually found in glycopeptides. However, the retention time and mass spectrum of this unknown derivative was identical with synthetic 1,5-di-O-acetyl-2,3,4,6-tetra-O-methylgulitol. Preparation of the 12 kDa glycopeptide includes an incubation at high pH. In order to exclude the possibility of an artificial formation of gulose, an acid hydrolysate of the intact 145 kDa glycopeptide was analysed for the presence of gulose. Detection of gulose as the peracetylated alditol is not possible because of the identity of D-glucitol and L-gulitol. Therefore the sugars in the acid hydrolysate were analyzed as peracetylated aldononitriles [4].

Even by capillary gas chromatography, the derivatives of gulose and galactose are poorly resolved if the latter is present in excess. Therefore, in a first step the sugars of the hydrolysate were fractionated by HPLC on an amino-HPLC column. Under the conditions used, gulose elutes together with mannose. Therefore, the mannose-containing fraction was further analysed by GC for the presence of gulose. After this enrichment, all monosaccharides in question are excellently se-

Table I

Sugar composition in mol% of the 145 kDa and 12 kDa glycopeptide, respectively

Sugar	145 kDa	12 kDa
Ara	39.0	52.9
Xyl	8.6	7.9
Man	17.2	–
Glc	0.4	–
Gul	0.4	5.3
Gal	30.9	26.5
6-O-methyl-hexose	1.2	–
3-O-methyl-galactose	2.3	7.4

parated (Fig. 2): gulose is indeed present in the acid hydrolysate of the intact 145 kDa fragment. The quantification of all monosaccharides present in the 145 kDa fragment and its 12 kDa alkaline cleavage product is given in Table I. Gulose is significantly enriched in the 12 kDa glycopeptide. As mentioned above, the gulose residue is located in a terminal position as revealed by permethylation analysis.

As was shown previously [2], the 145 kDa glycopeptide contains a covalently linked polysaccharide with a core structure of 1,3-linked mannose residues which are connected to arabinose-containing disaccharides. In addition, most if not all of the hydroxyproline residues of the 145 kDa fragment are glycosylated and oligosaccharides of 1,2-linked arabinoses are a predominant structural element. Since mannose is absent in the 12 kDa glycopeptide, the gulose is not part of the polysaccharide. The gulose-containing saccharide remains attached to the peptide even after alkaline treatment, excluding an O-glycosidic linkage to serine or threonine. The only potential N-glycosylation site present in the 12 kDa glycopeptide (position 7–9 of the peptide is Asn-Asn-Ser) is not glycosylated as documented by the Edman degradation experiment. These facts favour a linkage of the gulose containing saccharide to a hydroxyproline residue.

To our knowledge this is the first demonstration of gulose as a constituent of a glycoprotein. However, gulose was found to be a constituent of the antibiotic bleomycin [9].

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