UNEQUIVOCAL EVIDENCE FOR A β-D-CONFIGURATION OF THE GALACTOSE RESIDUE IN THE DISACCHARIDE CHAIN OF EPIGLYCANIN, THE MAJOR GLYCOPROTEIN OF THE TA3-Ha TUMOR CELL

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1. Introduction

The presence of a disaccharide chain composed of D-galactose and 2-acetamido-2-deoxy-D-galactose residues attached by an *O*-glycosyl linkage to serine or threonine in the protein backbone has been reported for many animal glycoproteins, including epiglycanin from the TA3-Ha mammary carcinoma ascites cell [1], as well as glycoproteins from rat brain [2], rabbit brain [3], antarctic [4] and arctic [5] fish, and fetuin from fetal calf serum [6]. The determination of the configuration at the anomeric carbon atom of the D-galactopyranosyl residue (of the isolated reduced disaccharide) has often been equivocal, due to the resistance of the D-Gal→D-GalNAc-ol bond to cleavage by either α- or β-galactosidases [2,3,7]. Since the complete or partial cleavage by β-galactosidases reported in a few cases [4,6,8] was obtained only with difficulty, an unequivocal proof for the configuration of this linkage was necessary.

Following the work in [1,7], the presence of the β-anomer of the D-galactose residue of the disaccharide was considered likely. This was mainly on the basis of the strong inhibitory activity for the hemagglutination of human erythrocytes of N blood-group specificity by the lectin from *Vicia graminea* seeds, an activity that had been attributed to a structure of this type [9], although without strong evidence. In addition, the occurrence of a terminal sialic acid residue attached to an α-D-linked galactose residue had not, to our knowledge, been demonstrated to occur in mammalian systems [2], and the presence of an *N*-acetylneuraminic acid residue attached to a D-galactose residue in the disaccharide had been suggested as a second chain type in epiglycanin [1].

Because of the high proportion of the disaccharide chains in epiglycanin, it was possible to obtain a sufficient amount of material for a definitive determination of the configuration of the anomeric carbon of the D-galactose residue by NMR. These results have shown that the galactose residue is in the β-D-form, and that the disaccharide has the structure, 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)-D-galactose, as shown in fig.1.

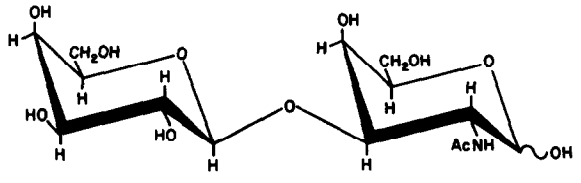

Fig.1

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2. Materials and methods

The reduced disaccharide, D-Gal→D-GalNAc-ol, was obtained by alkaline cleavage from epiglycanin [1] under reductive conditions ($NaBH_4$) and subsequent purification by ion-exchange and gel-filtration chromatography, as in [10]. The NMR spectra were recorded on a Varian FT-80 instrument at 30 rev./s and 22°C. Chemical shifts (δ) are relative to sodium 2,2-dimethyl-2-sila pentane-5-sulfonate. The spectrum of the reduced disaccharide (2.6 mg) in ^2H_2O was recorded after 1014 scans. This material was then dried and peracetylated with 0.1 ml acetic anhydride and 0.8 ml dry pyridine for 38 h at 22°C. The resulting amorphous product was dried extensively in vacuo, dissolved in C^2HCL_3, and its NMR spectrum recorded after 1030 scans, as above.

3. Results and discussion

Epiglycanin, the major glycoprotein of the TA3-Ha mammary carcinoma ascites cell, consists of ~530 carbohydrate chains attached to a single extended polypeptide chain of ~1300 amino acid residues [1,7]. Its molecular weight, as determined by sedimentation equilibrium, and confirmed by electron microscopic measurements [11], is ~500 000. By alkaline borohydride reduction of epiglycanin [1], followed by ion-exchange and gel-filtration chromatography, it has been possible to isolate from epiglycanin [1], five different reduced oligosaccharides, each representing a different chain structure [10]. The most abundant of the chains, a disaccharide of Gal and GalNAc in equimolar proportions, was found to comprise ~60% of the total number of the carbohydrate chains, isolated in reduced form. By permethylation, followed by gas–liquid chromatography–mass spectrometry, the Gal was shown to be 1→3-linked to the GalNAc-ol [10].

The results of NMR spectroscopy for the reduced disaccharide and its peracetylated derivative are presented in table 1. The $J_{1,2}$ values (7.25 Hz) are identical and are consistent with a β-D-configuration for the galactose residue [12]. The chemical shift of the H-1 signal of the unsubstituted disaccharide (4.46 ppm) is approximately at the expected position for the β-anomer. A chemical shift of 4.68 ppm for β-D-galactose was observed [13], whereas 5.34 ppm was obtained for the α-anomer. The downfield shift of the H-1 signal from 4.46–5.12 ppm upon acetylation was expected and was found to be identical (0.66 ppm) to that observed in [14] upon acetylating methyl 2-acetamido-2-deoxy-6-*O*-(β-D-galactopyranosyl)-α-D-glucopyranoside. These results clearly establish the β-configuration for the D-galactopyranosyl residue of the disaccharide chain of epiglycanin.

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Table 1
NMR results for 2-acetamido-2-deoxy-3-*O*-(β-D-galactopyranosyl)galactitol and its peracetylated derivative

Compound	Solvent	Chemical shift (δ) H-1 of Gal residue	Coupling constant ($J_{1,2}$)
Unsubstituted disaccharide	^2H_2O	4.46	7.25
Peracetylated disaccharide	C^2HCl_3	5.12	7.25

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