Structural Studies on the Galactan from the Albumin Gland of *Achatina fulica*

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The structure of the galactan isolated from the albumin gland of *Achatina fulica* (African giant snail) was investigated by methylation analyses. The molecule is highly branched. The linkages of the exclusively present D-galactose are 1 → 3 and/or 1 → 6. From lectin binding studies it was learnt that the 1 → 3 linkages possess β-configuration. The anomeric configuration of the 1 → 6 linkage is unknown. A tentative structure of the galactan is proposed.

Introduction

Several detailed structural investigations on some snail galactans have been reported [1–5]. The galactans from the snail species *Helix pomatia*, *Biomphalaria glabrata*, *Arianta arbustorum* and *Cepaea nemoralis* contain D- and L-galactosyl residues only, but in differing proportions [6]. In particular, the chemical and immunological studies carried out with these substances revealed species-specific differences with regard to the distribution of linear stretches and branches of the (1 → 3) and (1 → 6) linked galactosyl residues [6].

A galactan, isolated from the African giant snail, *Achatina fulica* [7], and showing a broad lectin reactivity spectrum for β-galactosyl specific lectins [8], had not been chemically characterized so far. In this paper the tentative structure of the *Achatina fulica* galactan, based on methylation analyses, is presented.

Abbreviations: DMSO, dimethylsulfoxide; glc, gas-liquid chromatography; glc/ms, gas-liquid chromatography/mass spectrometry; NMR, nuclear magnetic resonance.

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Materials and Methods

Achatina fulica (African giant snail) was obtained from local markets in Lagos, Nigeria. The isolation and purification of the galactan from removed albumin glands was carried out as described earlier [7].

Quantitative neutral sugar analyses was performed by gas-liquid chromatography (glc) of alditol acetate derivatives according to [9]. The quantitation of amino sugars and amino acids was performed on an automatic amino acid analyzer (Durrum D-500). The qualitative determination of uronic acids was carried out in high voltage paper electrophoresis (pH 2.8, 3 kV, 40–60 mA, 5–10 °C, 60 min). Electropherograms were stained with $AgNO_3$/NaOH according to Trevelyan *et al.* [10]. The phosphate content was determined according to Lowry *et al.* [11]. The determination of the enantiomers of galactose was done as described in [12]. Acetylation of the galactan was carried out with pyridine/acetic anhydride (1:1, by vol.) overnight at 100 °C. Such acetylated material was used for methylation analyses according to Lindberg [13]. Methylated alditol acetates were analyzed by combined gas-liquid chromatography/mass spectrometry (glc/ms) using a Finnigan MAT 1020 B automatic glc/ms-system (140 °C, temperature programming at 2 °C/min to 200 °C) with a SE 54 capillary column (30 m, 0.25 mm i.d.).

Results

Analyses of neutral and amino sugars, uronic acids, amino acids and phosphate of the isolated and purified glycosubstance from the albumin gland of *Achatina fulica* showed that besides traces of amino acids (0.7%), galactose is the only constituent of this molecule (about 99% of material dry weight). Therefore this glycosubstance represents a pure galactan. Using enantioselective gas chromatography, a technique which separates derivatives of D- and L-galactose [12], it was found that only D-galactose was obtained from the *Achatina fulica* galactan.

The galactan was almost insoluble in DMSO. For this reason methylation analyses resulted in considerable amounts of under- and non-methylated products. Furthermore, even peracetylation of the galactan was found to be difficult. Acetylation in pyridine/acetic anhydride (1:1, by vol.) for 1 h at 100 °C, or overnight at room temperature, failed. Overnight acetylation at 100 °C afforded acetylated galactan in satisfactory yields. No attempt was made to check whether the product was peracetylated or not. Such acetylated material was completely soluble in DMSO and allowed a methylation analysis according to Lindberg [13]. The reduction of the hydrolyzed methylated products was done with NaB^2H_4 in ^2H_2O. In glc/ms analysis 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-(1-^{2}H)galactitol, 1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-(1-^{2}H)galactitol and 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl-(1-^{2}H)galactitol in a ratio of 1:0.2:1 were identified indicating a 1,3-linked galactan backbone with monosaccharidic branches in 1,6-linkage. No indication for the presence of under- or non-methylated products was obtained. It was tried to determine the anomeric configuration of the linkages by ^{1}H-NMR-studies (Bruker 300 MHz, at 23 °C and 70 °C), but the results obtained were poor. The reason is probably associated with the low solubility of the material. From lectin binding studies [8] it is known that the 1,3-linkages possess β-configuration. The configuration of the 1,6-linkages is unknown so far.

Discussion

The structure suggested for the galactan from the albumin gland of *Achatina fulica* (Fig. 1) consists of a β-1,3-linked backbone of D-galactose with a high number of D-galactose branches in 1,6-linkage. Whether the non-substituted chain-linked galactose is part of a repeating unit or whether these units are statistically distributed in the chain is not known. A "repeating unit" should contain five backbone sugars of which four are branched. We favorize this structure and not a 1,6-linked backbone with 1,3-linked branches because no 1,5,6-tri-O-acetyl-2,3,4-tri-O-methyl-(1-^{2}H)galactitol could be identified in glc/ms, indicating rather a 1,3-linked backbone than a 1,6-linked one. The lectin binding studies carried out with the galactan with various lectins [8] suggest that the 1,3-linkages possess β-configuration whilst the configuration of the 1,6-linkages is still to be established.

Interestingly, this galactan consists only of D-galactose and resembles therefore galactans from the albumin glands of *Lymnea* spec. [14] and *Strophocheilus oblongus* [15]. The galactan from the latter organism also contains mainly 1,3- and 1,6-linked units of D-galactose. Furthermore, trimethyl derivatives were identified in glc/ms indicating unbranched residues in the backbone [15]. Therefore the structure of the galactan from *Strophocheilus oblongus* might show some similarities with that from *Achatina fulica.*

Other snail galactans investigated so far, for example those from *Helix pomatia*, *Biomphalaria glabrata*, *Arianta arbustorum* and *Cepaea nemoralis* contain D- and L-galactose in ratios of about 6:1 [6]. The analyses of these galactans indicate the presence of more complex structures than that found for the galactan of *Achatina fulica.* The highly branched comb-like structure of the galactan from *Achatina fulica* explains the difficulties in getting it dissolved in DMSO. For methylation of this galactan and other highly branched polysaccharides as well, a pre-acetylation step is surely of advantage. It should be noted additionally that methylation of this

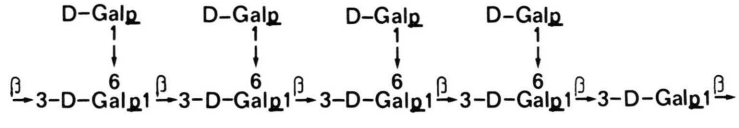

Fig. 1. Proposed structure of the galactan from the albumin gland of *Achatina fulica.* The configuration of the 1,6-linkage is unknown.

galactan according to techniques described by Prehm [16] or Ohno *et al.* [17] prior to the Hakomori method did not improve the extent of methylation. In both cases the low solubility of the galactan in either trimethylphosphate [16] or ethyl ether [17] should be the reason for these findings.

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