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 $1 \rightarrow 3$ and/or $1 \rightarrow 6$. From lectin binding studies it was learnt that the $1 \rightarrow 3$ linkages possess $\beta$-configuration. The anomeric configuration of the $1 \rightarrow 6$ 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 ($1 \rightarrow 3$) and ($1 \rightarrow 6$) linked galactosyl residues [6].

A galactan, isolated from the African giant snail, *Achatina fulica* [7], and showing a broad lectin reactivity spectrum for $\beta$-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.

**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 [10]. 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].

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

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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 peracylation 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 peracylated 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\(_2\)H\(_4\) in \(\text{D}_2\)O. In glc/ms analysis 1,5-di-O-acetyl-2,3,4,6-tetra-O-methyl-(1-\(\text{H}\))galactitol, 1,3,5-tri-O-acetyl-2,4,6-tri-O-methyl-(1-\(\text{H}\))galactitol and 1,3,5,6-tetra-O-acetyl-2,4-di-O-methyl-(1-\(\text{H}\))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 \(\text{H}\)-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 \(\beta\)-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 \(\beta\)-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-\(\text{H}\))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 \(\beta\)-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 *Lymnaea* 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*, *Bioplumularia 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
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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