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The specificity of an α-(1 → 2)-L-galactosyltransferase from albumen glands of the snail *Helix pomatia*

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Abstract

The specificity of an L-galactosyltransferase (L-Gal-T) from albumen glands of the snail *Helix pomatia* has been studied. This enzyme transfers L-Gal from GDP-L-Gal to various disaccharides with β-linked D-Gal in terminal non-reducing position, forming an α-(1 → 2) linkage. The subterminal residue and the type of interglycosidic linkage proved to be of minor importance. However, the branched trisaccharide β-D-Gal-(1 → 3)-[β-D-Gal-(1 → 6)]-β-D-Gal-(1 → O)Me is a very poor acceptor. The specificity of the L-Gal-T correlates well with the equimolar occurrence of L-Gal and the structural element → 2)-Gal-(1 → found in the storage polysaccharide of this snail. Since L-Fuc is also transferred from its GDP-activated form, the membrane preparations of the albumen glands can be used to synthesize fucosylated oligosaccharides. © 1997 Elsevier Science Ltd.

Keywords: L-Gal transferase; *Helix pomatia*, snail; Galactan

1. Introduction

Snails synthesize a highly branched polysaccharide which is composed entirely or predominantly of D- or D- and L-galactose (Gal) in their albumen glands. The D-Gal residues are β-(1 → 3)- or β-(1 → 6)-linked [1–5]; the polysaccharide is irregularly branched and contains linear sections, in which β-(1 → 3) prevail over β-(1 → 6) linkages [4,5]. The galactan is the exclusive carbohydrate source [6,7] for embryos or freshly hatched snails.

In 1885, Hammersten [1] observed the negative optical rotation ($[\alpha]_D = -23°$) of *Helix pomatia* galactan and therefore named it sinistrin; Bell and Baldwin [3] demonstrated that the negative optical rotation of this polysaccharide was due to the occurrence of L-Gal (~ 15%). So far it has been demonstrated that L-Gal is present only in galactans of some species, but there are too few studies to give a detailed picture of its occurrence within the different

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subclasses and families of *Gastropoda*. Among the species of the subclass *Pulmonata* it was exclusively detected in members of the family *Helicidae*.

When the galactans of the Helicidae were analysed by permethylation studies, 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methylgalactopyranose was detected among other partially methylated galactose derivatives indicative of a (1 → 2) linkage. This structural element was absent in all galactans devoid of L-Gal [4].

L-Gal is found exclusively in terminal non-reducing positions within the polysaccharide [8]. Since L-Gal is hydrolysed faster from the native galactan than D-Gal and due to the large negative optical rotation it was concluded that L-Gal is linked α-glycosidically [9–11]. Summarising both observations, it could be hypothesised that L-Gal is α-(1 → 2)-linked to a terminal D-Gal residue in the growing polysaccharide. However, it should be noted that Weinland et al. [9] detected minor quantities of L-Gal in a disaccharide preparation from the galactan of *H. pomatia*, constituted predominantly of β-D-Gal-(1 → 6)-D-Gal, and assumed that L-Gal was α-(1 → 6)-linked to D-Gal. An alternative explanation for the simultaneous occurrence in almost equimolar quantities of terminal L-Gal and the → 2)-Gal-(1 → structural element could be that the L-galactosyltransferase (L-Gal-T) requires a β-D-Gal-(1 → 2)-D-Gal acceptor structure for its activity. In this situation, L-Gal would be attached via a (1 → 3) or a (1 → 6) linkage.

To initiate the biosynthesis of snail galactans it is most likely that a D-Gal-based primer is required, onto which D-Gal residues are transferred from UDP-D-Gal [12]. More than one enzyme is probably involved in the formation of the β-(1 → 3) and β-(1 → 6) linkages as well as in the branching of the galactose polymer [13]. If a galactan contains L-Gal the cooperation of an additional enzyme must be assumed for its transfer to the growing polysaccharide. A further transferase would be required to introduce the D-Gal-(1 → 2) linkage if this is the acceptor structure for the L-Gal transfer.

In the present investigation, the activity of a L-Gal-T from the albumen gland of *H. pomatia* (*Helicidae*) is described and the structures of the corresponding reaction products are identified. From these studies, it can be inferred that the transfer of L-Gal is to oligosaccharides with D-Gal residues in terminal non-reducing position, irrespective of the linkage to the subterminal sugar moiety. This finding rules out a special structural requirement on the acceptor molecule for L-Gal-T.

2. Results

To identify L-Gal-T activity in *H. pomatia*, albumen glands were homogenised in Tris/HCl buffer (pH 7.6), centrifuged, and the pellet as well as the supernatant were recovered and treated further as described in the experimental section. Both fractions were incubated separately with GDP-L-Gal as a donor and β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me (A) as an acceptor. After 24 h the samples were centrifuged, the supernatants were concentrated, and the residues acetylated and analysed by TLC using 15:2 chloroform–acetone. When compared with the starting material the de novo synthesised product, migrating slower, was only detected in the reaction mixture with the membrane fraction, and not with the supernatant, indicative of a membrane-associated form of the enzyme.

In a time course study (1 mg acceptor, 2 mg GDP-L-Gal, membrane preparation from 200 mg of *H. pomatia* albumen glands) aliquots were taken at 24, 48, and 72 h, and analysed by TLC (Fig. 1). The complete conversion of the acceptor was achieved only after more than 48 h, a reaction time 50 to 100 times greater than seen with D-Gal-T [16].

As in earlier experiments with D-Gal-T [16] the L-Gal-T activity could be extracted at least partially

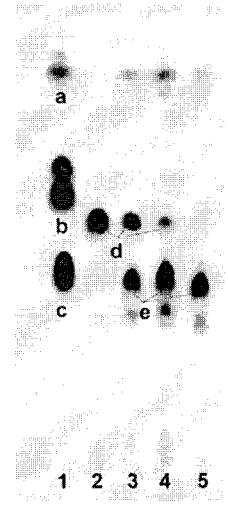

Fig. 1. Time course study of the L-Gal transfer from GDP-L-Gal to β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me by TLC using an albumen gland membrane preparation from *Helix pomatia*. Aliquots of the reaction mixture were dried, acetylated, and separated using 15:2 $CHCl_3$–EtOAc as solvent. (1) standard: galactose (a), lactose (b), stachyose (c); reaction mixture after: (2) 5 min, (3) 24 h, (4) 48 h, (5) 72 h; β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me (d), α-L-Gal-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me (e).

with a 1% solution of the non-ionic detergent CHAPS. However, the extraction was obviously incomplete as judged by residual activity in the once extracted membranes. Therefore, further studies on the L-Gal-T activity were performed only with the membrane fraction.

To analyse the structure of the synthesized oligosaccharide (B) a larger quantity was produced, acetylated, separated preparatively on TLC, and studied by one and two-dimensional ^{1}H-NMR spectroscopy. In Fig. 2 the 2D ^{1}H-NMR spectrum of (B) is shown. Chemical shifts and coupling constants are listed in Table 1. They were compared to the acceptor structure β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me (A). Almost all of the signals could be assigned.

The resonance signal for H-2′ of the newly synthesised trisaccharide (B) was detected by a COSY-experiment among a multiplet (δ 3.88–3.79). The chemical shifts and coupling constants J for H-1′, H-3′, H-4′, and H-5′ were identical to those of the corresponding protons for structure (A). The occurrence of a signal at δ 3.83 for H-2′ in the oligosaccharide studied, differing from that in (A) (δ 5.08) was indicative for the introduction of a (1 → 2) linkage. The resonance signal for H-1″ was observed at a downfield position at δ 5.28, and the coupling constant $J_{1'',2''}$ was found to be 4.0 Hz, typical for an α-linkage. These findings suggested that the L-Gal transfer had occurred to the terminal non-reducing D-Gal residue and that an α(1 → 2) linkage was formed.

To confirm the structure of (B), this material was also permethylated, hydrolysed, and acetylated. Analysis of the reaction products by GLC showed the formation of equimolar quantities of 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-α/β-galactopyranose, 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methyl-α/β-galactopyranose, and 1,3-di-*O*-acetyl-2,4,6-tri-*O*-methyl-α/β-galactopyranose. Identification of the different components was as follows. In comparison to the starting material, treated similarly, 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methyl-α/β-galactopyranose appeared de novo. For identification of the (1 → 2) linkage a sample of permethylated native galactan of *H. pomatia* galactan

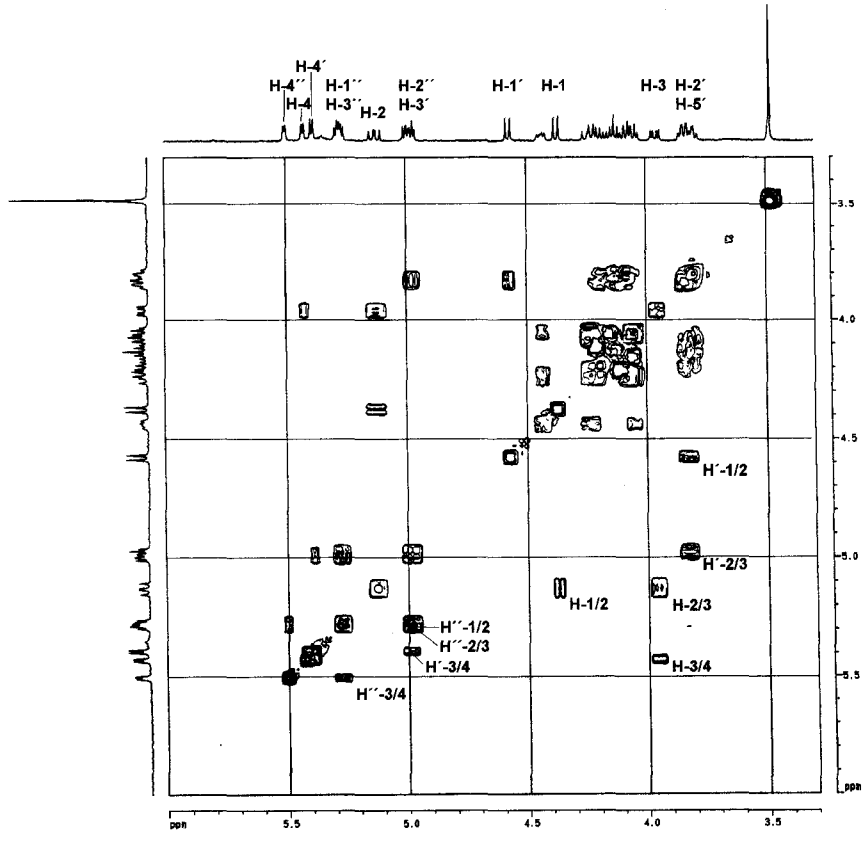

Fig. 2. COSY spectrum of the purified trisaccharide α-L-Gal-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me, obtained by galactosyl transfer from GDP-L-Gal to β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me using a membrane preparation from albumen glands of *Helix pomatia*. Assignments of the major resonances to specific protons are indicated, as well as the essential intraresidual cross-peaks of the different galactose residues.

Table 1
^{1}H-NMR shift data (δ, ppm) and coupling constants (J, Hz) from the oligosaccharides synthesised in this study (B, C, D) using the albumen gland membrane preparation of *Helix pomatia* in comparison to the disaccharide A, which was the acceptor structure for B and D

Proton	Compound							
	A		B		C		D	
	chemical shifts (δ, ppm)							
1	4.30	d	4.38	d	4.48	d	4.33	d
2	5.18	dd	5.13	dd	4.95	dd	5.11	dd
3	3.85	dd	3.96	dd	5.28	dd	3.93	dd
4	5.39	dd	5.43	dd ~ d	3.88–3.79	m	5.45	dd ~ d
5	3.80–3.89	m	3.87–3.77	m	3.88–3.79	m		
6a	4.07–4.21	m	4.04–4.26	m	4.55	dd	4.22	dd
6b	4.07–4.21	m	4.04–4.26	m	4.26	dd	4.17–4.05	m
1′	4.57	d	4.58	d	4.40	d	4.60	d
2′	5.08	dd	3.83	dd	3.88–3.79	m	3.84–3.79	m
3′	4.94	dd	4.99	dd	4.98	dd	4.97	dd
4′	5.35	dd	5.38	dd ~ d	5.44	dd ~ d	5.29–5.26	m
5′	3.80–3.89	m	3.87–3.77	m	3.88–3.79	m	3.86–3.78	m
6a′	4.07–4.21	m	4.04–4.26		4.17–4.04	m	4.22	dd
6b′	4.07–4.21	m	4.04–4.26		4.17–4.04	m	4.17–4.05	m
1″			5.28	d	5.27	d	5.32–5.26	m
2″			4.99	dd	4.98	dd	5.23	dd
3″			5.27	dd	5.17	dd	4.97	dd
4″			5.51	d	5.43	dd ~ d	5.29–5.26	m
5″			4.44	ddd ~ dd	4.60	ddd ~ dd	4.30	dq ~ q
6a″			4.04–4.26		4.31	dd	1.15	dd
6b″			4.04–4.26		4.17–4.04	m		
Me	3.48	s	3.48	s	3.51	s	3.45	s
	Coupling constants (J, Hz)							
1,2	8.1		7.5		8.0		8.0	
2,3	9.5		10.0/10.5		10.0		10.0	
3,4	3.5		3.5		9.0		3.0/3.5	
4,5	≤ 1.0		≤ 1				1	
5,6a					1.5			
5,6b					5.0			
6a,6b					11.0			
1′,2′	7.5		7.5		7.5		7.5	
2′,3′	10.0/10.5		10.0/10.5		10.0		10.0	
3′,4′	3.5		4.0		3.5		3.5	
4′,5′	1		≤ 1.0		≤ 1.0		1.0	
5′,6a′								
5′,6b′								
6a′,6b′								
1″,2″			4.0		3.5		3.5	
2″,3″			11.0/10.5		11.0		11.0	
3″,4″			3.5		3.5		3.5	
4″,5″			≤ 1		≤ 1.0		1	
5″,6a″					7.0		6.5	
5″,6b″					7.0			
6a″,6b″					11.0			

A = β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me.
B = α-L-Gal-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me.
C = α-L-Gal-(1 → 2)-β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me.
D = α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me.

as well as of its reaction product after one cycle of Smith degradation, in which L-Gal is absent, were used. These were hydrolysed and acetylated, and the different components were identified by mass spectrometry and ^{1}H NMR [5,8]. This demonstrated clearly that the transfer occurred to the terminal nonreducing D-Gal residue forming a (1 → 2) linkage (Table 2, Fig. 3).

In order to test whether this transferase is able to utilise different acceptors, β-D-Gal-(1 → O)Me, β-D-Gal-(1 → 6)-β-D-Gal, and β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me (100 μg each) were incubated separately with GDP-L-Gal and membrane preparations of the albumen glands from *H. pomatia*. As judged by TLC, the methyl galactoside was unaltered and did not serve as an acceptor structure for L-Gal-T, whereas the reaction products of both disaccharides moved substantially slower than the starting material, as could be expected for the larger molecules. The substitution was presumably at the terminal D-Gal residue, as deduced from the transfer experiments with β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me. However, to prove this assumption unambiguously, at least for one component, a larger quantity of β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me was treated with GDP-L-Gal and the membrane preparation from *H. pomatia*. The resulting trisaccharide (C) was acetylated, separated by TLC and studied by 1D/2D ^{1}H-NMR spectroscopy.

The resonance signal for H-2′ was detected at 3.83 ppm in the COSY experiment showing a similar shift from 5.08 to 3.83 ppm as found in the previous trisaccharide (B). Furthermore, the H-1″ signal was observed at the downfield position of 5.27 ppm; again, a coupling constant $J_{1'',2''} = 3.5$ Hz was indicative for an α-linked L-Gal moiety (Table 1).

To verify the formation of a (1 → 2) linkage, the trisaccharide obtained was subjected to permethylation, hydrolysis, and acetylation. When analysed by GLC, equimolar quantities of 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-α/β-galactopyranose, 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methyl-α/β-galactopyranose, and 1,4-di-*O*-acetyl-2,3,6-tri-*O*-methyl-α/β-glucopyranose were detected.

Identification of the different components was as mentioned above for the trisaccharide (B) with the partially methylated and acetylated galactose derivatives obtained from the native *H. pomatia* galactan and its 1. Smith degradation product as well as with those derived from disaccharide (A) and lactose (Fig. 3). Here too, the de novo appearance of the 3,4,6-tri-O-methyl-α/β-galactose derivative in equimolar quantities with 2,3,6-tri-O-methyl-α/β-glucose and 2,3,4,6-tetra-O-methyl-α/β-galactose proved the transfer of L-Gal to the terminal D-Gal forming a (1 → 2) linkage.

Since snail galactans are highly branched polysaccharides and L-Gal is found only in terminal nonreducing position, it was of interest to study whether this enzyme was able to transfer L-Gal to only one or to both D-Gal residues in terminal non-reducing position in a branched trisaccharide such as β-D-Gal-(1 → 3)-[β-D-Gal-(1 → 6)]-β-D-Gal-(1 → O)Me synthesised earlier with the branching enzyme of the snail *Biomphalaria glabrata* [16]. This trisaccharide was reacted with twice the equimolar amount of GDP-L-Gal in the presence of the albumen gland membrane fraction for 48 h. Surprisingly, analysis by TLC

Table 2
Retention times (relative to acetylated 2,3,4,6-Me_4-α-Gal) and molar ratios of the *O*-methylated and *O*-acetylated monosaccharide derivatives obtained from the various oligo- and poly-saccharides under investigation

	2,3,4,6-Me_4-Gal	3,4,6-Me_3-Gal	2,4,6-Me_3-Gal	2,3,4-Me_3-Gal	2,4-Me_2-Gal	2,3,6-Me_3-Glc	2,3,4-Me_3-Fuc
Retention time α/β	1/1.29	2.17/2.43	1.84/2.15	1.95/2.30	2.82/3.10	1.62/1.7	0.72/0.95
A	45.5	0.0	54.5	0.0	0.0	–	–
B	23.8	36.9	39.3	0.0	0.0	–	–
C	34.7	33.3	0.0	0.0	0.0	32.0	–
D	–	32.3	37.0	0.0	0.0	–	30.7
H.p. native galactan	41.1	10.2	11.6	1.6	35.6	–	–
H.p. after 1. cycle of IO_4^-/BH_4^-	32.1	0.0	15.5	20.3	32.1	–	–
Lactose	49.0	–	–	–	–	51.0	–
Fucose	–	–	–	–	–	–	100

The di- and tri-saccharides (A, B, C, D) are those listed in Table 1. The native galactan of the snail *Helix pomatia* (H.p.) as well as its reaction product after one cycle of Smith degradation were prepared as described in [4].

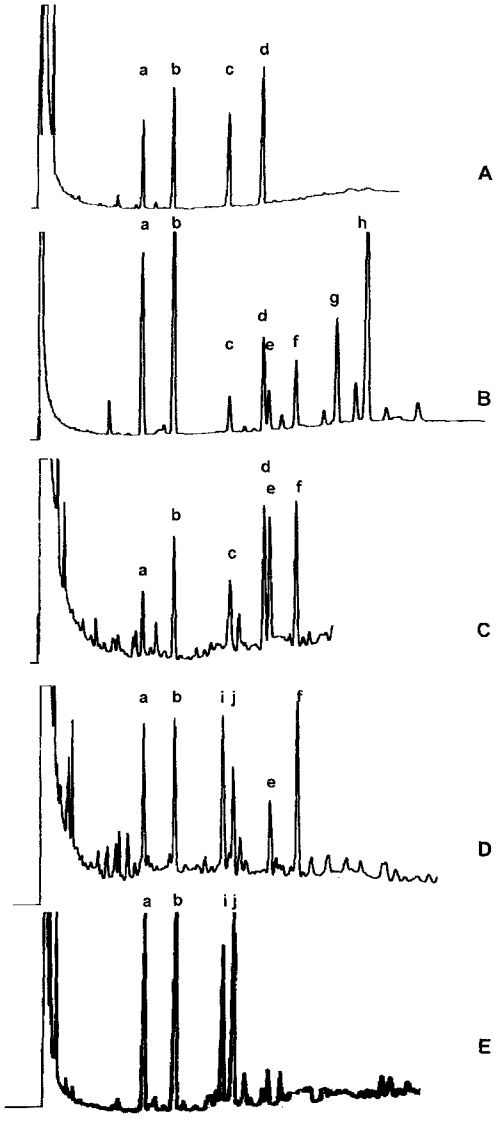

Fig. 3. Gaschromatographic analyses of the permethylated, hydrolysed, and acetylated products of: (A) β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me; (B) Native galactan of *Helix pomatia*. Identification of the different peaks was according to [8]; (C) and (D) Reaction products of β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me and β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me, respectively, with GDP-L-Gal and the membrane preparation of *H. pomatia*; (E) Lactose. a/b 1-*O*-Ac-2,3,4,6-*O*-Me_4-α/β-D-Gal; c/d 1,3-*O*-Ac_2-2,4,6-*O*-Me_3-α/β-D-Gal; e/f 1,2-*O*-Ac_2-3,4,6-*O*-Me_3-α/β-D-Gal; g/h 1,3,6-*O*-Ac_3-2,4-*O*-Me_2-α/β-D-Gal; i/j 1,4-*O*-Ac_2-2,3,6-*O*-Me_3-α/β-D-Glc.

showed that the main quantity of the starting material was unchanged and only two small slower migrating spots could be observed. Due to the low reaction rate no efforts were made to analyse the reaction products further.

Since L-Gal and L-Fuc are structurally closely related and are transferred from the corresponding GDP-activated moieties, we also tried to introduce L-Fuc residues into oligosaccharides using GDP-L-Fuc. GDP-L-Fuc was offered as a donor and β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me as an acceptor in a comparable set up. The acetylated reaction product (D) could be separated on TLC (1:1 *n*-hexane–ethyl-acetate), because it migrated faster than the acceptor material. Here too, ^{1}H-NMR and permethylation studies were performed.

As seen in the COSY spectrum in comparison with (A) the shift for H-2′ from 5.08 ppm to higher field (3.84–3.79 ppm) was indicative for a (1 → 2) linkage, whereas the downfield position for H-1″ and the coupling constant $J_{1'',2''}$ of 3.5 is associated with an α-configuration.

When the GLC chromatograms of the starting material and the reaction product were compared after permethylation, hydrolysis, and acetylation, four peaks appeared de novo. Two of them were identical with 1-*O*-acetyl-2,3,4-tri-*O*-methyl-α/β-fucopyranose and two corresponded to 1,2-di-*O*-acetyl-3,4,6-tri-*O*-methyl-α/β-galactopyranose, whereas the peaks for 1-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-α/β-galactopyranose had disappeared.

These findings prove the introduction of L-fucopyranose into the terminal non-reducing position of the disaccharide used as substrate, forming an α-(1 → 2) linkage.

3. Discussion

A L-Gal-T was identified in the membrane fraction of the albumen gland of *H. pomatia* and its activity was studied in detail. It transfers L-Gal from GDP-L-Gal to the terminal non-reducing end of β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me forming an α-(1 → 2) linkage, as assigned by ^{1}H-NMR and permethylation studies. This observation fits in very well with structural studies on the galactan from *H. pomatia* [4,8] in which a → 2)-D-Gal-(1 → structural element was identified in almost equal quantities to L-Gal. At that time it was uncertain whether the L-Gal was (1 → 2) linked to the core of the polysaccharide or whether the L-Gal-T required a (1 → 2) linkage in the acceptor determinant. With the activity of the L-Gal-T described here, it is almost certain that the second of these two possibilities can be ruled out and that the first holds true.

The transfer rate of this enzyme appears to be substantially slower than that of the branching en-

zyme described by Stangier et al. [16], because for complete conversion of an equal quantity of acceptor the L-Gal-T needs about 50 to 100 times more time, regardless of whether the enzyme was membrane-bound or solubilised by CHAPS.

When β-D-Gal-(1 → O)Me, β-D-Gal-(1 → 6)-β-D-Gal, and β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me were offered, only the latter two served as acceptors and L-Gal was attached again to the terminal non-reducing D-Gal residue by an α-(1 → 2) linkage.

It thus appears that L-Gal can be transferred to terminal non-reducing D-Gal residues forming an α-(1 → 2) linkage, irrespective of how D-Gal is bound to the subterminal residue. However, no transfer occurred if the penultimate D-Gal residue was already substituted by a further D-Gal unit. Thus, during biosynthesis of the native galactan the L-Gal transfer would apparently require an unbranched linear section of the polysaccharide, comprising at least a disaccharide with D-Gal residues linked β-(1 → 3) or (1 → 6).

Once L-Gal is attached to this disaccharide structure, at least the D-Gal residue subterminal to the L-Gal is not substituted further, as deduced from the observation that 2′-fucosyllactose is a poor acceptor structure for the branching enzyme of *Biomphalaria glabrata* [16] and presumably also for the corresponding enzyme from *H. pomatia*. The failure to detect 1,2,6-tri-*O*-acetyl-3,4-di-*O*-methyl-galactopyranose in appreciable quantities from the native galactans during permethylation studies supports this inference [5].

Since the structural element → 6)-D-Gal-(1 → is almost absent in the native *H. pomatia* galactan and a branching enzyme introducing a β-(1 → 3) linkage was not found and presumably does not exist, the exclusive acceptor structure during biosynthesis is most likely β-D-Gal-(1 → 3)-β-D-Gal-(1 → and not the β-(1 → 6)-linked isomer.

If GDP-L-Fuc is offered as a donor, a corresponding reaction to the L-Gal transfer is observed, as demonstrated with the synthesis of α-L-Fuc-(1 → 2)-β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me (D). Whether L-galactosyl- and L-fucosyltransferase activity is mediated by the same enzyme, can only be clarified when the corresponding transferase is purified to homogeneity.

With the help of this L-Gal-T preparation, it is possible to synthesize blood group H active determinants as was demonstrated with the oligosaccharide (D). Thus, it may be possible to use the enzyme described here and others of invertebrate origin for a variety of syntheses, especially because they can be used as membrane preparations without any particular purifications.

4. Experimental

Snails.—Snails of the species *H. pomatia* were collected in their natural habitat near Hamburg or Schwandorf (Germany). A larger quantity of albumen glands from *H. pomatia* was obtained shortly before the main breeding season in May/June, when the highest enzyme activities were expected. At that time individual glands can have a weight of up to 1 g. They were stored at −20 °C before use. No substantial loss of activity was observed during storage for 2 years.

Poly - and oligo - saccharides and the derivatives.—Snail galactans and their Smith-degradation products were those prepared and described earlier [4,5,14]. The oligosaccharides β-D-Gal-(1 → 6)-D-Gal, β-D-Gal-(1 → 3)-β-D-Gal-(1 → O)Me, β-D-Gal-(1 → 4)-β-D-Glc-(1 → O)Me as well as UDP-D-Gal and GDP-L-Fuc were purchased from Sigma (St. Louis, MO). GDP-L-Gal was prepared as described [15]. β-D-Gal-(1 → 3)-D-Gal and β-D-Gal-(1 → 6)-D-Gal were also prepared by partial hydrolysis of snail galactans as reported [14]. β-D-Gal-(1 → 3)-[β-D-Gal-(1 → 6)]-β-D-Gal-(1 → O)Me was obtained as reported recently [16].

Galactosyltransferase activity in membrane preparations and extracts.—Albumen glands (200 mg wet wt) were homogenised with 2 mL Tris/HCl-buffer (50 mM, pH 7.6) in a Potter–Elvehjem homogeniser, centrifuged at 3000 rpm for 30 min, and the supernatant separated from the pellet. The pellet was washed three times in 10 mL buffer and used for the transferase activity studies. The supernatant was centrifuged again at 40.000 rpm for 3 h to remove the galactan in order to avoid a competition with the acceptor added. All these procedures were carried out at 4 °C.

Since there was only a restricted quantity of GDP-L-Gal available, the experiments were carried out according to earlier investigations [16] where conditions had been optimised with respect to pH-value and temperature for the D-Gal-T from albumen glands of the snail *Biomphalaria glabrata*.

3-[(3-Cholamidopropyl)dimethyl-ammonio]-1-propansulfonat (CHAPS) extracts were prepared by incubating the washed pellet from 200 mg of albumen glands with 300 μL of a 2% CHAPS solution in Tris/HCl buffer (pH 7.6) for 2 h at 4 °C.

When the different acceptors were tested for the transfer of L-Gal, typically 1 mg (2.8 μmol) of the corresponding disaccharide and 2 mg (3.5 μmol) of GDP-L-Gal were either dissolved in 0.3 mL CHAPS-extract with a protein concentration of about 1 mg/mL or dissolved in 300 μL Tris/HCl-buffer (50 mM, pH 7.6) and added to the pellet prepared from about 200 mg of homogenised albumen glands. The mixture was stirred at 25 °C for the indicated period, ranging from 1 to 72 h. Subsequently the samples were concentrated by rotor evaporation, acetylated, and separated by TLC on Silica Gel 60 (Merck, Darmstadt) in 15:2 $CHCl_3$–acetone or 1:1 *n*-hexane–EtOAc, if the oligosaccharides contained L-Fuc. To detect the samples on TLC plates, these were sprayed with 2:5:2 H_2SO_4–EtOH–H_2O and heated to 150 °C. For preparative work marker strips on each side of the plate were charred and appropriate portions of the gel were extracted with EtOAc.

Permethylation studies.—Permethylation of the oligosaccharides was carried out by the procedure of Hakomori [17], however, methyl lithium (Merck, Darmstadt) instead of sodium hydride was used to generate the methylsulfinyl anion [18]. The procedure was adapted to smaller scale, such that samples of 0.5 to 1 mg could be applied.

Permethylation of the *H. pomatia* galactan and its I. Smith degradation product was performed also by the Hakomori method, however, sodium hydride was applied for the formation of the methylsulfinyl anion [4]. The resulting products were hydrolysed in 2.5 M CF_3COOH for 2 h at 100 °C and the reaction mixture was treated with 0.2:1 pyridine–Ac_2O at 80 °C for 2 h. The partially methylated sugar-acetates were subjected to GLC on a Shimadzu A-14 apparatus equipped with a 30 m RT_x-225 0.53 mm capillary column; temperature program: 150–200 °C at 2 °C 1 min, with an initial isothermal period of 5 min.

^1H - NMR spectroscopy.—^{1}H-NMR spectra (400 MHz) of acetylated oligosaccharides were recorded with a Bruker WM 400 instrument for solutions in $CDCl_3$. Chemical shifts (δ) are expressed in ppm relative to internal Me_4Si.

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