# BIOSYNTHESIS OF PLANTEOSE IN *SESAMUM INDICUM*

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## 1. Introduction

Eight isomers of the trisaccharide, mono-*O*-α-D-galactosyl sucrose are possible according to the way in which the D-galactosyl group is linked to the sucrose molecule [1]. Six of these have been isolated and characterised from various plant sources [1]. Three of the isomers (unnamed members) are suspected to be formed as products of partial degradation of structurally related higher oligosaccharides [1–5]. The other three isomers, planteose, raffinose and umbelliferose are major reserve oligosaccharides of the seeds of a number of plant species [1]. The biosynthesis of the latter two members have been shown to proceed through a pathway involving UDP-galactose as the galactosyl donor [6–13]. However, in the synthesis of raffinose, galactinol [*O*-α-D-galactopyranosyl-(1 → 1)-myo-inositol] can also act as an important galactosyl donor [14,15]. As regards the biosynthesis of planteose [*O*-α-D-galactopyranosyl-(1 → 6)-β-D-fructofuranosyl α-D-glucopyranoside], only limited work has been reported in the literature; it was suggested that this oligosaccharide might be synthesised through α-D-galactosyl transfer to sucrose, catalysed by α-galactosidase [16]. Similar enzymic galactosylation involving low-energy galactosyl donors have long been demonstrated [17–20]. These reactions, however, require high concentrations of donors and acceptors. This communication describes identification of the planteose family of oligosaccharides and their synthesis through UDP-galactose mediated pathway in the seeds of *Sesamum indicum* (sesame).

*Abbreviations*: Pl, planteose; $Pl_1$ (sesamose) and $Pl_2$, the higher homologs of planteose, tetra- and penta-saccharides, respectively, containing additional α-D-galactose moiety attached to C-6 of the existing D-galactosyl group of the molecule

## 2. Materials and methods

### 2.1. *Enzyme preparation*

Seeds from the pods of sesame plants were collected at different stages of maturation (2, 4, 8 and 10 weeks after anthesis). The seeds (1 g) were homogenized in 2 ml 0.1 M phosphate buffer (pH 7.3) containing 10 mM β-mercaptoethanol in a pre-cooled pestle and mortar. The slurry was squeezed through two layers of cheesecloth and the resulting solution centrifuged for 20 min at 50 000 × *g*. The supernatant was dialysed against 1 litre of the extraction buffer for 4 h and used as crude enzyme preparation.

### 2.2. *Enzyme assay*

The incubation mixture (100 μl) for the assay of planteose synthesis contained 40 μl enzyme (400 μg protein), 40 μM [$^{14}$C]sucrose, 0.9 μCi, 5 mM UDP-gal, 40 mM phosphate buffer (pH 7.3) and 4 mM β-mercaptoethanol. After incubation at 35°C for the given time period, 20 μl of the mixture was transferred into a small centrifuge tube containing 80 μl 80% methanol. This was centrifuged to sediment the precipitated proteins and the entire supernatant was applied on a strip of Whatmann no. 1 paper. The chromatogram was developed for 72 h by descending chromatography using the solvent system, *n*-propanol/ethylacetate/water (7/1/2, by vol.) and then the paper was subjected to autoradiography. Unlabelled authentic samples of sucrose, Pl, $Pl_1$ and $Pl_2$ were also chromatographed and the sugars visualised with *p*-aminobenzoic acid reagent [21]. Radioactive areas corresponding to the standards were cut out and counted in vials containing 10 ml scintillant (5 g PPO/l toluene) in a Packard model-3390 liquid scintillation counter (efficiency, 50%). In an alternative assay UDP-gal was replaced by galactinol keeping the concentrations and the conditions constant.

2.3. *Isolation of oligosaccharides*

The seeds (1 g) were extracted with 80% methanol (2 ml) in a glass homogeniser. The slurry in the homogeniser was held in a boiling water bath for 3 min and then centrifuged. The sediment was reextracted twice and the pooled supernatant was concentrated to dryness in a rotary evaporator at 40°C. The residue was dissolved in 5 ml water, washed twice with diethyl ether and the aqueous layer freeze-dried. The residue was finally dissolved in 500 μl water and 100 μl of this was streaked along 8 cm of water-washed Whatmann no. 3 paper. The chromatogram was developed as in section 2.2. The corresponding areas for sucrose, Pl, $Pl_1$ and $Pl_2$ were cut out, eluted with water and the sugars estimated by the phenol–$H_2SO_4$ method [22].

## 3. Results and discussion

3.1. *Identification of the oligosaccharides*

Planteose was found to be the major oligosaccharide in mature–resting sesame seeds. In addition, Pl, $Pl_1$, $Pl_2$ and sucrose were also present (table 1). The relative mobilities of the oligosaccharides using the solvent system, ethyl acetate/pyridine/acetic acid/water (6/3/1/1, by vol.) and the system in section 2.2 were comparable to those of the authentic samples. Graphical analysis of the chromatographic mobilities according to [23] established that Pl, $Pl_1$ and $Pl_2$ were members of a regular homologous series. The oligosaccharides were also separated and identified using high-voltage electrophoresis in 0.1 M sodium tetraborate medium. The relative mobilities of the raffinose family of oligosaccharides were distinguishable from those of planteose family by both electrophoresis and chromatography. Raffinose and the related sugars were not detected in the sesame extract. The stained spots when viewed under UV (366 nm) [21] exhibited a pale-greenish fluorescence typical of the planteose family; the raffinose family of sugars show light brown fluorescence.

Table 1
Levels of oligosaccharides during the development of sesame seeds

| Developmental stage of seeds[a] | mg oligosaccharide/g seeds | | | | Ratio of Pl/sucrose |
|---|---|---|---|---|---|
| | Sucrose | Pl | $Pl_1$ | $Pl_2$ | |
| 2 | 4.63 | 0.66 | n.d. | n.d. | 0.14 |
| 4 | 4.63 | 3.19 | 0.61 | n.d. | 0.69 |
| 8 | 4.43 | 8.95 | 1.30 | n.d. | 2.02 |
| 10 | 4.83 | 11.96 | 1.56 | 0.15 | 2.47 |
| Mature-resting | 6.00 | 18.48 | 1.86 | 0.51 | 3.08 |

[a] Expressed as weeks, following anthesis

n.d., not detected

Complete hydrolysis of Pl, $Pl_1$ and $Pl_2$ by purified α-galactosidase from *Vicia faba* [19,24] produced only galactose and sucrose; on partial hydrolysis of $Pl_2$ the intermediates (galactobiose, galactotriose, Pl and $Pl_1$) were also detected. The oligosaccharides were not hydrolysed by yeast invertase, probably because of the presence of substituted β-D-fructofuranoside groups in the sugars [5,25–27]. However, the action of this enzyme, following incubation of the sugars with α-galactosidase, resulted in complete degradation producing galactose, glucose and fructose. It is noteworthy that invertase action on the raffinose family of oligosaccharides cleaves the fructosyl groups and produces melibiose, manninotriose and verbascotetraose [1,28].

The identity of Pl (the simplest unit of the planteose family) obtained from sesame seeds was also confirmed by periodate oxidation followed by borohydride reduction as in [26,28].

3.2. *Biosynthesis*

The formation of Pl and its family of oligosaccharides during the development of sesame seeds is shown in table 1. In the second week, following anthesis, sucrose is the major sugar. Further maturation of the seeds result in deposition of Pl and its higher homologs; however, the increase in the level of sucrose is not marked during this period. This pattern of sucrose levels is typical of a metabolite which is rapidly used up in further biosynthetic steps. Sucrose in the developing seeds may initially come as a translocate from the leaves of the plant; this sugar is also known to be synthesised and turned over within the storage organ. On the other hand, levels of Pl and the higher homologs (table 1) show characteristics of reserve materials (increasing Pl/sucrose ratio during maturation). Similar observations were made in relation to the formation of the raffinose family of oligosaccharides in maturing seeds of *Phaseolus vulgaris* [29].

Enzyme extracts from the 8 and 10 week old seeds were able to catalyse the formation of Pl from both

Table 2
Synthesis of the planteose family of oligosaccharides by cell-free extracts of sesame seeds

| Galactosyl donor | Incubation (min) | [$^{14}$C]Oligosaccharides formed (cpm) | | |
|---|---|---|---|---|
| | | Pl | $Pl_1$ | $Pl_2$ |
| UDP-Galactose | 0 | 0 | 0 | 0 |
| | 10 | 920 | 0 | 0 |
| | | (550) | | |
| | 20 | 1630 | 140 | 0 |
| | | (1320) | (0) | |
| | 60 | 4200 | 730 | 330 |
| | | (3140) | (150) | (0) |
| | 120 | 6500 | 1100 | 720 |
| | | (4500) | (460) | (0) |
| Galactinol | 0 | 0 | 0 | 0 |
| | 10 | 0 | 0 | 0 |
| | 20 | 410 | 0 | 0 |
| | | (120) | | |
| | 60 | 1500 | 0 | 0 |
| | | (730) | | |
| | 120 | 2300 | 0 | 0 |
| | | (1700) | | |

Experimental conditions are in section 2.2.; 10 week old seeds (following anthesis) were used; results shown in parenthesis are from 8 week old seeds

UDP-gal and galactinol as the galactosyl donors, and [$^{14}$C]sucrose as the acceptor. Table 2 shows that, under conditions used in the assay, UDP-gal is a better donor, and that in the presence of the sugar nucleotide, $Pl_1$ and $Pl_2$ are also formed. Incubation with galactinol, on the other hand, resulted in the formation of Pl only. Compounds such as galactose, galactose-1-phosphate, melibiose and raffinose, failed to act as donors in the biosynthesis of Pl. The identities of the newly formed $^{14}$C-labelled Pl, $Pl_1$ and $Pl_2$ were established by the chromotographic, electrophoretic and enzymic methods as in section 3.1. It seems apparent from the results that the choice of the physiological state of seeds (stage of maturity) is important in demonstrating in vitro synthesis of the oligosaccharides; extract from 8 week old seeds produce lower levels of the labelled products (table 2). Enzyme preparations from the mature resting seeds and the immature seeds (2–4 weeks old) did not catalyse the synthesis of any of the oligosaccharides, either with UDP-gal or galactinol as donors. It is, therefore, likely that Pl and $Pl_1$ in the immature seeds (table 1) appear as a result of translocation from other parts of the plant [1]. However, in the resting seeds the enzyme(s) responsible for the synthesis of the oligosaccharides probably becomes dormant after it has completed its function in the final stages of seed maturity.

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