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TRANSGALACTOSYLATION ACTIVITY OF SWEET ALMOND α-GALACTOSIDASE: SYNTHESIS OF SACCHARIDES

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Abstract—Sweet almond α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) catalyses hydrolytic, synthetic (*de novo*) and transfer reactions. Transfer products were formed using *p*-nitrophenyl α-D-galactoside as the galactosyl donor and glucose, galactose, sucrose, maltose and lactose as acceptors; several of the products were identified. The enzyme also caused elongation of the oligosaccharide chain of two substrates (melibiose and raffinose). In addition, the enzyme catalysed condensation of free galactose, yielding oligosaccharides. The products were identified in all cases by chromatography.

INTRODUCTION

In 1950, Bacon and Edelman [1] and Blanchard and Albon [2] showed by PC that the hydrolysis of sucrose and melibiose, catalysed by invertase and α-galactosidase, respectively, yielded not only the hydrolytic products but also some new oligosaccharides. Further studies showed that the oligosaccharides were the products of enzymic transglycosylation; this property is now known to be displayed by most glycosidases, e.g. glucosidases [3–9], galactosidases [2, 10–14] and fructosidases [15, 16]. The process of transglycosylation is believed to proceed by transfer of a glycosyl moiety to acceptor molecules which are generally hydroxylic organic compounds. A glycosyl-enzyme complex is considered to be a transitory intermediate in the reaction,

$$\underset{\text{Donor}}{\text{Glycosyl—O—R}} + \text{Enzyme—H} \rightleftharpoons \underset{\text{Intermediate}}{\text{Glycosyl—Enzyme}} + \text{ROH}$$

$$\text{Glycosyl—Enzyme} + \underset{\text{Acceptor}}{\text{R'OH}} \rightleftharpoons \underset{\text{Product}}{\text{Glycosyl—O—R'}} + \text{Enzyme—H}$$

The process of hydrolysis will take place when the acceptor is a water molecule.

In a glycosidase-catalysed reaction in aqueous medium the equilibrium generally favours hydrolysis. However, some *in vivo* conditions such as a localized high concentration of the product and rapid removal of one of the reactants may reverse the reaction and induce synthesis. Thus, Hassid [17] suggested that the possibility of *in vivo* synthesis of some complex saccharides from low-energy donors should not be overlooked. Since the discovery of UDP-glucose by Leloir [18], it has been well established that in higher plants, the general pathway of carbohydrate synthesis is through high-energy intermediates, namely sugar nucleotides (also see refs. [19] and [20]). This report describes the *in vitro* formation and characterization of products of transgalactosylation catalysed by sweet almond α-galactosidase in presence of high concentrations of some naturally occurring sugars.

RESULTS AND DISCUSSION

Preliminary experiments

Galactosyl donors used in the experiments were *p*-nitrophenyl α-D-galactoside and α-D-galactose-1-phosphate. The latter compound failed to serve as a donor and no transfer products could be detected. This may be explained because of its lack of affinity for the enzyme [21]. The optimum concentration of *p*-nitrophenyl α-D-galactoside, as a donor, was 0.05 M in the incubation mixture. Similarly, out of several enzyme concentrations tested (20–320 μg protein/ml), the optimum was 160 μg/ml. At this concentration, all phases of changes in the transfer product could be observed in a period of 24 hr.

Acceptor specificity

Some monosaccharides and disaccharides were examined as acceptors and the results are shown in Table 1. Glucose yielded only one product (R_G 0.38) which was characterized as melibiose [21]; similar results were obtained by Petek and Dong [22]. The amount of the product formed increased as the glucose concentration was raised from 0.5 to 1 M but there was no further change at 2 M. The concentration of the product increased with time up to 4 hr beyond which it decreased, indicating enzymic breakdown of the compound on prolonged incubation. The nature of the transfer product further confirms earlier findings that α-galactosidases preferentially transfer galactosyl moieties to the primary alcoholic groups of acceptor sugars [23, 24].

Fructose did not function as a galactosyl acceptor at any of the concentrations examined (0.5–2 M). Similar results were obtained by earlier workers [23, 25]. Galactose was the best acceptor of the 3 monosaccharides tested; quantitatively, it yielded larger amounts and qualitatively, a greater number of products. These have been identified as α (1 → 6)-linked galactobiose,

Table 1. Acceptor specificity of sweet almond α-galactosidase

Acceptor	Concn (M)	Time of incubation (hr)*						No. of products	R_G
		1	2	4	8	12	24		
Glucose	0.5	+	+ +	+ +	+	tr	tr	1	0.38
	1	+ +	+ + +	+ + +	+ +	+	+	1	0.38
	2	+ +	+ + +	+ +	+ +	+	+	1	0.38
Galactose	0.5	+ +	+ + +	+ + + +	+ + + + +	+ + + + +	+ + + + +	3	0.30 0.37 0.49
	1	+ +	+ + +	+ + + +	+ + + + +	+ + + + +	+ + + + +	3	0.30 0.37 0.49
	2	+ +	+ +	+ + +	+ + +	+ + +	+ + + +	2	0.37 0.49
Fructose†	2	–	–	–	–	–	–	–	–
Sucrose	0.5	+ +	+ +	+	tr	—	—	1	0.36
	1	+ + +	+ + +	+ +	+	+	tr	1	0.36
	2	+ +	+ +	+	tr	—	—	1	0.36
Maltose	0.5	+ +	+ + +	+ +	tr	tr	—	3	0.09 0.19 0.33
	1	+ + +	+ +	+	tr	—	—	2	0.19 0.33
	2	+ +	+	tr	tr	—	—	2	0.19 0.33
Lactose	0.5	tr	+	+	tr	tr	tr	1	0.16
	1	+	+	tr	tr	—	—	1	0.16
	2	+	+	tr	—	—	—	1	0.16

**p*-Nitrophenyl α-D galactoside was used as galactosyl donor; + = presence of transfer product; tr = traces of transfer product; — = absence of transfer product.

†There was no transfer product at 0.5 or 1.0 M D-fructose.

galactotriose and galactotetraose [21]. At 2 M galactose, smaller amounts of transfer products were observed and also the compound with R_G 0.30 was missing. Evidently, this was the result of hydrolysis by the enzyme. It is of interest to point out that the acceptor specificity and the inhibitory effect (for enzymic hydrolysis of *p*-nitrophenyl α-D-galacoside) [21] of the 3 monosaccharides showed the same order, i.e. galactose > glucose > fructose. This must be, therefore, related to the degree of binding of the sugars to the enzyme active site.

The disaccharides, sucrose and maltose yielded *ca* equal amounts of transfer products, the former gave raffinose (Gal α-1 → 6 Glc α-1 → 2 β-Frc) [21] and the latter gave 3 compounds. As compared to the monosaccharides, the concentration of the products appeared to reach a maximum after a shorter time of incubation. The transfer product formed with sucrose seems to be hydrolysed more rapidly than that from glucose (this was identified as melibiose). This would be expected because the V_{max} value with raffinose was 10-fold higher than that with melibiose [26]. The formation of raffinose by transfer of galactose to C-6 of the glucose moiety of sucrose confirms the observation that fructose does not function as an acceptor and that the primary alcoholic group of glucose was preferred. The rapid disappearance of the 3 products formed from maltose on longer incubation indicate the presence of α-galactosidic linkages in them. The oligosaccharide with R_G 0.33 was earlier identified [21] as Gal α-1 → 6 Glc 4 ← 1 α-Glc. A small amount of the product with R_G 0.19 was isolated by preparative PC. Treatment of this compound with an α-glucosidase preparation [27] yielded glucose and a sugar which co-chromatographed with authentic manninotriose [28]. The tentative structure of this compound would therefore be, Gal α-1 → 6 Gal α-1 → 6 Glc 4 ← 1 α-Glc. The amount of the product with R_G 0.09 was insufficient for structural analysis.

Lactose was a poor acceptor and yielded only trace amounts of a product at all concentrations. The amount of this product decreased rapidly on longer incubation, hence the presence of α-galactosidic linkage in the newly formed oligosaccharide.

Chain elongation

Melibiose and raffinose are substrates for the enzyme [26] and, therefore, can function as donors as well as acceptors. These were thus used in studies for elongation of oligosaccharide chain by transgalactosylation (Table 2).

With melibiose, 3 higher oligosaccharides were obtained. Analysis of the R_G values according to French [29, 30] clearly indicates that the chromatographic mobilities of the sugars are typical of a homologous series. The product with R_G 0.24 co-chromatographed with authentic manninotriose. On this basis, the tentative structures of the transfer products with R_G 0.19 and 0.15 may be written as, Gal α-1 → 6 Gal α-1 → 6 Gal α-1 → 6 Glc and Gal α-1 → 6 Gal α-1 → 6 Gal α-1 → 6 Gal α-1 → 6 Glc, respectively.

Three transfer products were also obtained with raffinose, their relative chromatographic mobilities were again typical of sugars of a homologous series [29, 30]. The products with R_G 0.20, 0.16 and 0.12 were identified by PC using authentic markers, stachyose, verbascose and ajugose, respectively [31].

Table 2. Chain elongation of melibiose and raffinose, catalysed by sweet almond α-galactosidase

Compounds*	Concn (M)	Time of incubation (hr)*						No. of products	R_G
		1	2	4	8	11	24		
Melibiose	0.5	—	tr	+	+ +	+ + + +	+ + +	3	0.15 0.19 0.24
	1	—	+	+ +	+ + +	+ + + +	+ + + + +	3	0.15 0.19 0.24
	2	—	tr	+	+ +	+ + +	+ + + +	3	0.15 0.19 0.24
Raffinose†	0.5	+	+ +	+ + +	+ + +	+ + +	+ + + +	3	0.12 0.16 0.20
	1	+	+ +	+ +	+ + +	+ + +	+ + + + +	3	0.12 0.16 0.20

*The compounds themselves acted as galactosyl donors as well as acceptors; + = presence of transfer product; tr = traces of transfer product; — = absence of transfer product.

†Experiments could not be carried out at 2.0 M raffinose because of solubility limitation.

Table 3. Condensation of galactose, catalysed by sweet almond α-galactosidase

Sugar concentration	Time of incubation (hr)*						No. of products	R_G
	1	2	4	8	12	24		
Galactose (0.5 M)	+	+	+ +	+ +	+ + +	+ + +	2	0.30 0.37
Galactose (0.5 M) and Glucose (0.5 M)	tr	+	+	+ +	+ +	+ +	2	0.30 0.37
Galactose (1 M)	+	+	+ +	+ +	+ + +	+ + +	2	0.30 0.37
Galactose (1 M) and Glucose (1 M)	tr	tr	tr	tr	tr	tr	2	0.30 0.37
Galactose (2 M)	..	+ + +	+ + +	..	+ + + +	+ + + +	2	0.30 0.37
Glucose (2 M)	—	—	—	—	—	—	—	—

* + = Presence of oligosaccharides; tr = traces of oligosaccharide; — = absence of oligosaccharide; .. = experiment not carried out.

Table 4. A comparison of hydrolytic and transfer reactions catalysed by sweet almond α-galactosidase†

Acceptor	Concn (M)		Time of incubation (hr)*				
			4	6	8	24	30
Glucose	2	Hydrolysis	50	..	82	100	..
		Transfer product	+ +	..	+ + +	+ + + +	..
Galactose	2	Hydrolysis	2.7	5.4	..	16.2	20
		Transfer product	+ +	+ +	..	+ + +	+ + + +
Fructose	2	Hydrolysis	90	100	100	100	100
		Transfer product	—	—	—	—	—
Sucrose	2	Hydrolysis	87	100	..	100	100
		Transfer product	+	+ +	..	+ +	+

* + = Presence of transfer product; — = absence of transfer product; .. = experiment not carried out.

†The galactosyl donor was *p*-nitrophenyl α-D galactoside (0.05 M) and the enzyme concentration was 40 μg/ml. Hydrolysis of the donor is expressed as percentage of the initial concn.

Enzymic condensation of galactose

Since it has been shown that galactose binds competitively to the enzyme, resulting in a diminished hydrolytic action on a substrate [21], it was thought possible that the enzyme may also bring about condensation of galactose units by a transfer reaction. Results of such experiments are shown in Table 3. Two products were obtained at all sugar concentrations; the amounts were maximal at 2 M galactose. Although glucose did not form any product, its presence inhibited the condensation of galactose. This agrees with an earlier observation that glucose is a poorer galactosyl acceptor and also inhibits the enzyme [21]. However, the presence of glucose in the incubation mixture has no effect on the nature of the product. The two oligosaccharides formed had the same R_G values, i.e. 0.37 and 0.30, as those when galactose was used as an acceptor (Table 1); these were chromatographically identified as α 1 → 6-linked galactotriose and galactotetraose [cf. ref. 21].

Hydrolysis, transfer and synthetic activities of the enzyme

The synthetic activity described in the preceding paragraph, raised the question whether the galactosyl transfer, as shown in Table 1, is due to *de novo* synthesis involving the acceptor and the galactose liberated on hydrolysis of the donor. As far as glucose (as acceptor) is concerned this possibility could be excluded by comparing the nature of the products (cf. Tables 1 and 3). Moreover, no oligosaccharide was formed when a mixture of 0.05 M galactose (the amount that would be liberated on complete hydrolysis of the donor, i.e. *p*-nitrophenyl α-D-galactoside) and 1 M-glucose, was incubated in the presence of the enzyme.

The relationship between the hydrolytic and the transfer activities of the enzyme was also examined by analysing a reaction mixture (containing donor and acceptor) for the amount of transfer products and the degree of donor hydrolysis (by estimating the liberated *p*-nitrophenol at 405 nm) at different time intervals. A comparison of the two reactions in the presence of glucose, galactose, fructose and sucrose is in Table 4. The results show that in each case, the hydrolytic and the transfer reactions proceed *ca* parallel to each other. There was no further transfer product after the hydrolysis of the donor had reached completion. The transfer products, however, started to diminish slowly on longer incubation; this was presumably due to enzymic hydrolysis.

In conclusion, it may be tentatively said that the hydrolytic and the transfer reactions are catalysed by the same site of the enzyme, presumably by identical mechanisms. These activities and the synthetic activity were also inhibited to the same degree by Ag^+ [21]. Moreover, the new linkage which was released or formed during hydrolysis or transfer reaction, had the α-configuration [21].

EXPERIMENTAL

Purified α-galactosidase [32, 33] was used in all expts. The enzyme was assayed using *p*-nitrophenyl α-D-galactopyranoside as substrate [34, 35]. Transgalactosylation reactions were carried out in 0.1 M acetate buffer, pH 5.5, at 30° in presence of 160 μg enzyme protein per ml, 0.05 mM *p*-nitrophenyl α-D-galactoside and varying concns of acceptor sugars. Aliquots were drawn at known time intervals and the reaction stopped by heating at 100° for 5 min. The samples, after deproteinization and deionization, were applied (10 μl) to Whatman No. 1 paper. The chromatograms were developed with BuOH–Py–H_2O (6:4:3). Marker spots of authentic samples were applied on chromatograms for comparison purposes. The spots were located by spraying the paper with aniline–phthalate (prepared by dissolving 0.97 g aniline, 1.66 g phthalic acid and 5 g TCA in 100 ml H_2O saturated BuOH) reagent and heating at 120–130° for 3 min. The relative migration of spots were expressed as R_G values. An approximate estimation of relative amounts of the cumulative transfer products was carried out by visual comparison of the intensity and the area of spots and expressed on an arbitrary scale in the respective tables.

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