Short Communication

Biosynthesis of Stachyose in Phaseolus vulgaris

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Received August 22, 1966.

Stachyose, a sugar of the raffinose family, is present in a wide variety of plants (3, 5) and sometimes is the most abundant soluble carbohydrate including sucrose. The biosynthesis of stachyose is unknown, although galactose can be transferred from 1 raffinose molecule for example to another one by α-galactosidase giving rise to stachyose (10). α-Galactosidases, however, show little specificity for the donor as well as the acceptor molecule and they transfer with low efficiency as compared to their hydrolytic activity (2, 3, 10).

From physiological data evidence has accumulated that in a variety of plants stachyose arises by the transfer of galactose from a specific donor-galactoside to raffinose (8, 9). This galactoside has recently been identified as galactinol, a compound which was first found in sugar beets by Brown and Serro (1) and shown to be *O*-α-D-galacto-pyranosyl-(1 $\rightarrow$ 1)-*myo*-inositol (1, 6).

We have now obtained an enzyme from ripening seeds of dwarf beans (*Phaseolus vulgaris*) which transfers galactose specifically and with high yield from galactinol to raffinose giving rise to stachyose and *myo*-inositol. In addition it was found that galactinol constitutes the major galactoside in the beans during a certain maturation period and preceedes stachyose in this role (Tanner, unpublished).

The enzyme was prepared as follows: 177g of unripe seeds were homogenized in 250 ml of 0.1 M phosphate buffer, pH 7.0, with an Ultra Turrax at high speed for 2 minutes. The supernatant fraction obtained after centrifugation at 10,000 $\times$ *g* for 10 minutes was dialyzed 45 hours against 0.05 M phosphate buffer, pH 7.0. The enzyme was purified 10-fold by ammonium sulfate precipitation (35–56 %) and treatment with calcium phosphate gel.

The incubation mixture contained in a total volume of 0.04 ml: 0.5 μmole raffinose, 2.4 μmoles sodium phosphate buffer pH 7.0, enzyme as indicated, and galactinol-^{14}C (40,000 cpm where not indicated otherwise), isolated from Lamium leaves after 1 hour photosynthesis in $^{14}CO_2$ according to Senser and Kandler (9). Of the total radioactivity, 95.5 % of this galactinol-^{14}C was located in the galactose moiety. The reaction was run at 32° and stopped with 0.1 ml 99 % ethanol. The supernatant fraction and the washings were chromatographed in ethyl acetate: butanol: acetic acid: water (3: 4: 2.5: 4). The radioactive areas were located with a strip counter, and the radioactivity measured directly on paper with a methane flow counter Frieseke and Hoepfner 407 A.

The amount of stachyose formed was proportional to the amount of enzyme added and linear with time (table I). This linearity was maintained until 35 % of the galactose-^{14}C of galactinol had been transferred to raffinose, then the rate decreased. In 9 hours 80 % of the galactose-^{14}C was transferred by 0.14 mg protein. The reaction product was chromatographically identical to stachyose in 4 solvent systems (88 % phenol: water: 1 M Na_2 EDTA: acetic acid = 840: 160: 1: 10; *n*-butanol: propionic acid: water = 750: 352: 498; ethyl acetate etc. see above; *n*-butanol: pyridine: water: acetic acid = 60: 40: 30: 3).

When treated with α-galactosidase the only radioactive compound formed from the reaction product was galactose; in the presence of β-galactosidase no radioactive galactose was split off. Incubation of the reaction product with invertase yielded 1 radioactive compound with the same R_F in ethyl acetate-butanol-acetic acid-water as mannitotriose obtained from authentic stachyose in the same way.

The second reaction product was identified as *myo*-inositol by chromatography and with the help of inositol-dehydrogenase prepared from Aerobacter aerogenes according to Weissbach (11).

In the presence of raffinose as the acceptor

Table I. *Amount of Stachyose formed in Relation to Incubation Time and Protein Concentration*
Other conditions as given in the text.

Protein added mg	Incubation time hr	stachyose cpm
0.07	2	6005
0.07	4	12,360
0.14	1	5870
0.14	2	13,530

Table II. *A Comparison of the Enzyme's Activity to Hydrolyze Galactinol and to Transfer Galactose*

Incubation with 0.14 mg protein was carried out as given in the text, however, 60,000 cpm of predominantly galactosyl labelled galactinol-^{14}C were added. In each case 0.5 μmole of acceptor substances was added.

Acceptor added	Stachyose	Galactose	*myo*-Inositol	Other products
	cpm	cpm	cpm	cpm
Raffinose	31,855	1400	1345	none
None	0	8550	485	none
Glycerol	...	9260	580	none
Fructose	...	9815	610	none
Sucrose	...	10,590	725	none
Maltose	...	9110	640	none
Cellobiose	...	8365	500	none
Lactose	...	9275	740	445 (galactosyl-lactose)
Melibiose	...	5480	685	7500 (manninotriose)
Glucose	...	8345	815	2300 (melibiose)
Galactose	...	8800	890	1640 (galactosyl-galactose)
Stachyose	1890	3540	265	none

there was always 20 times more galactose transferred from galactinol to raffinose than to water. In the absence of any acceptor or in the presence of a wrong acceptor (eg. sucrose) the amount of galactose which was hydrolyzed off considerably increased, but still was only one third of that transferred to raffinose (table II). The enzyme possesses a high specificity towards the acceptor molecule. There was no radioactivity transferred from galactinol-^{14}C to glycerol, fructose, sucrose, maltose, cellobiose, gentiobiose, melizitose and trehalose. In the presence of stachyose no verbascose-^{14}C was formed either, however, an exchange reaction took place, yielding some radioactive stachyose. Slight acceptor activity was obtained with glucose (the product was chromatographically identical to melibiose), galactose, and lactose. These compounds, however, were 20 to 30 times less efficient as acceptors than raffinose. The best acceptor so far tested besides raffinose was melibiose, which was one-fourth as efficient as raffinose. The reaction product behaved chromatographically like manninotriose.

When nonradioactive galactinol, purchased from Calbiochem, was incubated with raffinose-^{14}C, obtained in the same way as galactinol-^{14}C, stachyose-^{14}C was also formed. The incubation of raffinose-^{14}C alone did not yield stachyose-^{14}C.

From these data it is quite obvious that this enzyme is not an α-galactosidase. It should be called galactinol: raffinose-6-galactosyl-transferase.

The enzyme was not stimulated by Mg^{++}, ADP, and UDP. Its pH optimum was found to be 7.0 and the reaction was inhibited to 98 % by 50 μM and to 40 % by 10 μM *p*-chloromercuribenzoate.

The crude extract from the ripening bean seeds also contained an enzyme which transferred galactose from UDP-gal-^{14}C, but not from ADP-gal-^{14}C, to *myo*-inositol. This enzyme has already been detected by Frydman and Neufeld in extracts of pea seeds (4). It was not possible, however, to find any galactosyl transfer activity from UDP-gal-^{14}C or ADP-gal-^{14}C to raffinose directly, analogous to the one described by Pridham and Hassid (7) to sucrose in the biosynthesis of raffinose. The pathway of stachyose biosynthesis, thus comprises the following 2 reactions:

I. UDP-gal + *myo*-inositol → *O*-α-D-galactopyranosyl − (1 → 1) − *myo*-inositol (= galactinol) + UDP

II. Galactinol + raffinose → stachyose + *myo*-inositol

Acknowledgments

This work was supported by a grant from the Deutsche Forschungsgemeinschaft. We gratefully acknowledge the skillful technical assistance of Mrs. Christa Kriebitsch and Mrs. Ingrid Appelmann.

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