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SHORT COMMUNICATION

SUBSTRATE SPECIFICITY OF FRUCTOSYL TRANSFERASE FROM CHICORY ROOTS

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Abstract—The substrate specificity of the enzyme, fructosyl transferase (sucrose-sucrose 1-fructosyl transferase) from chicory root was studied by incubating a number of sugars, alone and in combination, with the enzyme. Sucrose was found to be the true substrate. The oligosaccharide synthesized from sucrose was identified as the trisaccharide 1^F-fructosyl sucrose.

INTRODUCTION

FRUCTOSYL transferase (sucrose-sucrose 1-fructosyl transferase) is known to occur in a number of plants belonging to the Compositae.[1-7] Edelman and Bacon[1] first reported the presence of this enzyme in the extracts of Jerusalem artichoke, catalysing the transfer of the fructose moiety from inulin to sucrose. Dedonder[2] contradicted the above findings by suggesting that sucrose can act as both an acceptor and a donor. However, the detailed substrate specificity of the enzyme was not investigated to any great extent. In our earlier communication,[8] we have discussed the purification and the properties of the fructosyl transferase from roots of chicory (*Chiocrium intybus* Linn.). The present communication demonstrates the detailed substrate specificity of this enzyme.

RESULTS AND DISCUSSION

The enzyme catalysed the synthesis of an oligosaccharide when incubated with sucrose as the substrate. The oligosaccharide was isolated in a pure form, hydrolysed completely with HCl (1 N), analysed quantitatively, and the ratio of fructose to glucose was found to be 2:1.

Partial acid hydrolysis of the oligosaccharide gave fructose, glucose, sucrose and unchanged oligosaccharide detected by paper chromatography, whereas on complete hydrolysis only glucose and fructose were revealed. On hydrolysis with invertase, again only glucose and fructose were detected showing that the respective sugars in the oligosaccharide are linked by $\beta(2 \rightarrow 1')$ linkage. The presence of sucrose in the partial hydrolysate

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[1] J. EDELMAN and J. S. D. BACON, *Biochem. J.* **49**, 529 (1951).
[2] R. DEDONDER, *Bull. Soc. Chim. Biol.* **34**, 171 (1952).
[3] I. S. BHATIA and M. SRINIVASAN, *J. Sci. Ind. Res.* **13B**, 373 (1954).
[4] I. S. BHATIA, M. N. SATYANARAYANA and M. SRINIVASAN, *Biochem. J.* **61**, 171 (1955).
[5] I. S. BHATIA, T. SATYANARAYANA and K. V. GIRI, *Indian Sci. Congr. Abst.* Part IV, 135 (1959).
[6] R. W. SCOTT, T. G. JEFFORD and J. EDELMAN, *Biochem. J.* **100**, 23p (1966).
[7] R. W. SCOTT, Transfructosylation in higher plants containing fructose polymers, Ph.D. Thesis, University of London (1968).
[8] R. SINGH and I. S. BHATIA, *Phytochem.* (in press).

suggests that glucose is linked in the oligosaccharide with fructose in the same manner as it is in sucrose. All these results, therefore, confirm the nature of the oligosaccharide to be a trisaccharide, linked by β (2 $\rightarrow$ 1′) linkages, and the one which occurs naturally in chicory roots,[9] 1^F-fructosylsucrose (F_2G).

In coupled reactions with sucrose as the donor and a number of acceptors such as xylose, arabinose, rhamnose, glucose, fructose, mannose, galactose, maltose, lactose, melibiose, trehalose, melezitose and cellobiose, no other oligosaccharide except F_2G was synthesized illustrating that the fructose from sucrose is not transferred to any of the acceptors tested.

In coupled reactions in which inulin was used as the donor molecule and sucrose in addition to the earlier mentioned sugars were used as acceptors, no new sugar was formed except in the case of the sucrose–inulin mixture, where the trisaccharide (F_2G) was synthesized. The experiment was repeated by incubating either a mixture of inulin and sucrose, or sucrose alone with the enzyme. The amount of glucose released in the two cases was found to be the same, proving that the oligosaccharide synthesized in the coupled reaction of inulin and sucrose arises solely from sucrose and that fructose is not transferred from inulin to sucrose. Further, when inulin, chicory fructosan or onion fructosan alone were incubated with the enzyme, no hydrolytic activity was observed. This thus excludes the possibility of the hydrolytic action of the enzyme on inulin type polymers. Also when sucrose was replaced by other sugars such as maltose, lactose, melibiose, trehalose, cellobiose, melezitose and F_2G (trisaccharide of chicory) as the substrates of the enzyme, no activity was detected, demonstrating that sucrose is the only true substrate acted upon by the enzyme.

The addition of glucose and fructose to the reaction mixture (sucrose and enzyme) had no inhibitory effect on its catalytic action. Although free fructose occurs in the cytoplasm of plant cells, it occurs in the pyranose form and is relatively inactive.[10] This inertness applies to other sugars also present in free state in the cytoplasm, including glucose and galactose. It has been established that plants mobilize combined sugars more readily and will utilize these in preference to free sugars. This can be expected, because many enzymatic reactions in plants are of the transfer type and are catalysed by transglycosidases. Fructose occurs in the bound form as the more labile furanose ring and this explains the ready reactivity of sucrose as a donor and acceptor in the biosynthesis of glucofructosans.

The results presented in this report indicate that sucrose is the only true substrate of this enzyme. As higher oligosaccharides (F_2G, F_3G, F_4G ... etc.) are synthesized from sucrose, the ability of these to act as donor molecules seem to diminish. Thus when F_2G was used as a substrate, neither synthetic nor hydrolytic activity was observed under conditions when sucrose acted both as donor as well as acceptor of fructosyl residues. Whether the inactivity of the oligosaccharides to act as donor molecules lies in the altered steric configuration (compared to sucrose) or is due to some energetic factors is difficult to decide. It is not possible to get an accurate picture of the comparative energy of the F—G and F—F bond in sucrose and F_2G respectively. However, it seems logical to expect that the higher oligosaccharides and the fructosans will be poorer donor molecules compared to sucrose. The mechanism shown in Fig. 1 can, therefore, be proposed for the action of this enzyme.

As free fructose is not a product of the reaction, the enzyme, although a β-fructo-

[9] R. SINGH, The role of fructosyl transferase in the biosynthesis of fructosans in the roots of chicory (*Chicorium intybus* Linn.), Ph.D. thesis, Punjab Agricultural University, Ludhiana (1969)
[10] E. L. HIRST, *Proc. Chem. Soc.* 193 (1957).

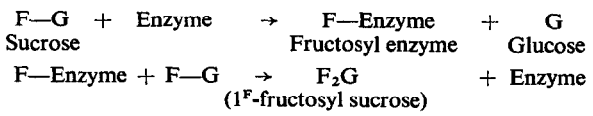

FIG. 1. MODE OF ACTION OF FRUCTOSYL TRANSTERASE.

furanosylase, is certainly not an invertase. It shows high specificity for sucrose, having no activity against the trisaccharide as donor, or with glucose as acceptor; thus its action is essentially irreversible, as also indicated for the enzyme of Jerusalem artichoke.[11] As the trisaccharide and higher polymers are practically inactive as acceptors for further transfer, therefore, the enzyme is unable to promote polymerization above the trisaccharide level.

EXPERIMENTAL

Isolation and purification of the enzyme. The enzyme was isolated and partially purified by the use of $(NH_4)_2SO_4$ and Sephadex column chromatography as described earlier.[8]

Assay of the enzyme activity. The activity of the enzyme in the present case was determined by incubating with various substrates (AnalaR grade) at 37° in the presence of $CHCl_3$ and under a layer of toluene. The reaction mixture contained $5{\cdot}84 \times 10^{-1}$ M substrates (donor and acceptor) in a final volume of 2 ml of buffered enzyme. A boiled control was used as control. Samples were drawn after 24 hr of incubation and heated at 100° for 10 min. The inactivated samples were cooled and filtered. The filtrates were analysed both qualitatively and quantitatively for various carbohydrates by the chromatographic methods reported in our earlier communication.[8]

Isolation of trisaccharide from the reaction mixture. The trisaccharide (F_2G) synthesized in a reaction mixture of sucrose and enzyme was isolated by fractionating on a column of carbon–aluminium oxide following the method of Stefanović.[12]

Hydrolysis of trisaccharide. (*i*) *Total acid hydrolysis.* The trisaccharide solution (2 ml) was hydrolysed with HCl (1 N) at 67° for 9 min. The hydrolysate was cooled, neutralized with $NaHCO_3$ and filtered. The filtrate was then subjected to paper chromatography with reference spots of glucose and fructose. (*ii*) *Partial acid hydrolysis.* The partial hydrolysis of trisaccharide (2 ml) was carried out with 0·01 N H_2SO_4 in a water bath for 1 hr and was further processed in the same way as given above. (*iii*) *Hydrolysis by invertase.* This hydrolysis was carried out in two ways. In one case the trisaccharide was treated with the enzyme invertase on the paper itself. The paper was kept for 3 hr at room temp. (25°) and was developed in the usual way.

In the second case, the trisaccharide solution was incubated with the enzyme invertase at 28° for 12 hr. The reaction products were then analysed by paper chromatography.

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[11] J. EDELMAN and T. G. JEFFORD, *New Phyto.* **63**, 517 (1968).
[12] V. D. STEFANOVIĆ, *J. Chromatography* **5**, 453 (1961).