The Transferring Activity of β-Fructofuranosidase: Formation of two Disaccharides from Fructose

BJØRN ANDERSEN

Fysisk-kemisk Institut, Kemisk Laboratorium IV, Københavns Universitet, Copenhagen, Denmark

The formation of oligosaccharides as intermediates during the hydrolysis of sucrose by yeast invertase was described first by Bacon and Edelman [1] and by Blanchard and Albon.[2] Further work has established that the process is due to transfer of β-fructofuranosyl residues from the sucrose to glucose, fructose, and sucrose. It seems that the furanosyl group is transferred to primary alcohol groups only. Therefore formation of three trisaccharides and three disaccharides is possible. Since the purest β-fructofuranosidase preparations show oligosaccharide formation it seems that both hydrolysis and transfer are produced by the same enzyme. It has been shown that primary alcohol groups in other sugars can act as receptors for the fructosyl residue. Many authors have stated that the mechanism should be a two-step transfer reaction with formation of an active enzyme-fructose complex:

$$\begin{aligned} &EH + GOF \rightleftharpoons EF + GOH \\ &EF + HOH \rightleftharpoons EH + FOH \\ &EF + XOH \rightleftharpoons EH + XOF \end{aligned}$$

where XOH stands for the receptor sugar. This hypothesis has never been proved and is not generally accepted. The formation of the enzymefructose complex has been doubted because it did not appear to be a necessary condition for fructosyl transfer.

In hitherto described works the fructose donor has been a glycoside with a terminal fructose, *e.g.* sucrose and raffinose; however, the present paper establishes that free fructose itself, can act simultaneously as donor and acceptor, giving rise to two disaccharides. The enzyme preparation used has high purity.[3] Pure fructose, 5 g/100 ml solution, was incubated with the enzyme for 30 min. Paper chromatograms of the incubated mixture showed two spots in addition to that of fructose. The substances producing the new spots have R_F values normal for disaccharides; one moves like sucrose and the other somewhat further but not as far as glucose and fructose (Fig. 1). Both substances were then

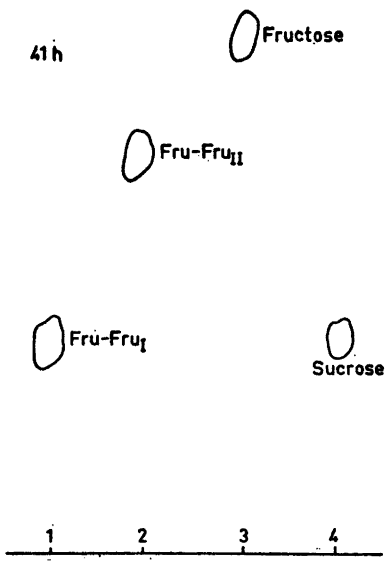

Fig. 1. Paperchromatogram of the two fructosides with sucrose and fructose as references (Solvent: CH_3-CO-C_2H_5/H_2O/HAc).

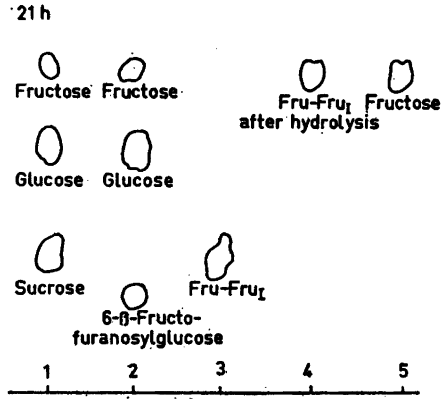

Fig. 2. 1. Sucrose partly hydrolyzed to glucose and fructose. 2. 6-β-fructofuranosyl-fructose partly hydrolyzed to glucose and fructose. 3. Fructoside$_I$. 4. Same after hydrolysis. 5. Fructose.

hydrolyzed with β-fructofuranosidase. Chromatography of the products of these reactions showed only a single fructose spot.

Fig. 2 shows a chromatogram of one of the substances (Fru-Fru_I) before and after hydrolysis, together with sucrose, and 6-β-fructofuranosylglucose and free fructose. No oligosaccharide formation was detectable when glucose was incubated with the enzyme preparation. The most reasonable mechanism of the reaction would seem to be:

$$EH + FOH \rightleftharpoons EF + HOH$$
$$EF + FOH \rightleftharpoons EH + FOF$$

Since fructose in glycosides always is present in the furanose form, and since we know that the β-fructofuranosidase transfers fructose to primary alcohol groups only, the two disaccharides probably are 6-β-fructofuranosylfructose (levanbiose) and 1-β-fructofuranosylfructose (inulobiose).

That fructose behaves as both donor and acceptor simultaneously supports the hypothesis of the existence of an intermediary enzyme-fructose complex, which, in turn, supports the hypothetical mechanism for sucrose hydrolysis mentioned above.

1. Bacon, J. S. and Edelman, *J. Arch. Biochem. Biophys.* **28** (1950) 467.
2. Blanchard, P. H. and Albon, N. *Arch. Biochem. Biophys.* **29** (1950) 220.
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Synthesis of DL-Heptadecane-1, 14-diol

BJÖRN LÜNING and LARS PAULSSON

Institute of Organic Chemistry, University of Stockholm, Stockholm, Sweden

During studies on Compositeae ene-ynes, Löfgren and Johansson[1] isolated the diacetate of oenanthotoxin, *trans*-heptadeca-2,8,10-triene-4,6-diyne-1,14-diol from *Centaurea montana* L. Oenanthotoxin had been previously found in *Oenanthe crocata* L. by Anet *et al.*[2] Complete hydrogenation of oenanthotoxin gives D-heptadecane-1,14-diol [2] and the enantiomer can be obtained from cicutoxin in the same way.[2] Although the racemate corresponding to oenanthotoxin has been synthesized by Bohlmann and Viehe,[3] no attempt has been made to synthesize the saturated diol in an unequivocal manner.

To synthesize DL-heptadecane-1,14-diol (I), the Grignard reagent of 11-bromo-1-undecene [4] (II) was reacted with oxirane to give 12-tridecene-1-ol (III), which was transformed to 13-bromo-1-tridecene (IV) by the action of phosphorus tribromide. Reaction of the Grignard reagent of IV with oxirane yielded 14-pentadecene-1-ol (V), which was oxidized to DL-pentadecane-1,2,15-triol (VI) by performic acid. Attempts to oxidize VI to tetradecanal-14-ol (VII) using periodate, periodic acid, or lead tetraacetate led to the formation of polymeric gums insoluble in most solvents except hot tetrahydrofuran (*cf.* Sisido *et al.*[6]). The method described by Raman [8] for cleavage of 1,2-glycols with "silver-iodide-dibenzoate" in benzene under anhydrous conditions, however, gave a satisfactory yield of VII. In air at room temperature, VII polymerizes rapidly, resulting in an increase of melting point and disappearance of the strong carbonyl band in the IR-spectrum. The hydroxyl group of VII was protected as a tetrahydropyranyl ether and the aldehyde group was allowed to react with propylmagnesium bromide; after hydrolysis DL-heptadecan-1,14-diol (I) was obtained. IR- and mass-spectra of I were identical with those of D-heptadecane-1,14-diol obtained by hydrogenation of oenanthotoxin,[2] kindly supplied by Dr. Lythgoe.