EFFECT OF FRUCTOSE AND GLUCOSE SUPPLEMENTATION ON INVERTASE MEDIATED SYNTHESIS OF OLIGOSACCHARIDES FROM SUCROSE.

Richard Idem Somiari* & Stanislaw Bielecki
Institute of Technical Biochemistry, Technical University of Lodz, Stefanowskiego 4/10, 90-924 Lodz, Poland.

SUMMARY:
The total amount of novel oligosaccharides synthesized by β-D-fructofuranosidase at pH 7.5 increased three-fold using a medium composed of 1.2M sucrose, 0.5M fructose and 0.1M glucose, as compared to that with only 1.8M sucrose solution. Using 0.6M of the three sugars did not increase yield but reduced rate of sucrose hydrolysis by 72.7%. Synthesis of fructosyl/glucosyl oligosaccharides based on β-fructofuranosidase mediated transglycosylation is enhanced by supplementation of sucrose solution with appropriate concentrations fructose and glucose.

INTRODUCTION
Enzyme catalyzed synthesis of oligosaccharides has a number of attractions (Rastall et. al., 1992; Christakopoulos et. al., 1994). However, most of the processes so far established at the laboratory scale have not been scaled up. This is partly because of low yield, high cost of appropriate enzymes (especially the transferases) and suitable substrates. β-D-Fructofuranosidase (invertase EC 3. 2. 1. 26) and sucrose are well known, readily available and relatively inexpensive, so should be ideal for large scale application. Though the transfructosylating capability of this enzyme has long been reported (Bacon, 1954; Anderson, et. al., 1969), information on its synthetic attributes is scanty (Ichikawa et. al., 1992) compared to information on its hydrolytic properties. Use of invertase as catalyst and sucrose as substrate; viz. fructose and/or glucose donor and acceptor, presents problems. Because water is the preferred acceptor (Cheetham et. al., 1986), hydrolysis of sucrose is still significant even at concentrations (>50% wt./vol.) normally expected to drive equilibrium towards synthesis. Increasing sucrose concentration up to 80 % (wt./vol.), does not correspondingly increase oligosaccharide yield (Straathof et. al., 1986), but significantly reduces enzyme activity, probably due to low water concentration (Bowski et. al, 1971), substrate inhibition (Besserdoch et al. 1977, Combes and Monsan, 1983) and substrate aggregation (Combes et. al., 1981). Considering that oligosaccharide synthesis by invertase should depend mainly on its transfructosylating capabilities, we presumed that starting with a sucrose concentration that will not significantly inhibit enzyme activity plus appropriate concentrations of fructose and glucose should increase the efficiency of transglycosylation, and hence yield.
MATERIALS AND METHODS

Source and some properties of the Enzyme:
β-D-Fructofuranosidase was obtained from Novo Nordisk Ferment Ltd., Switzerland (Batch S075). The preparation was suspended in 0.05M acetate buffer, pH 5.0 (used in all steps unless indicated) and subjected to ultrafiltration using PM 10 & 30 membranes (Amicon). Fractions retained by the PM 30 membrane were freeze dried and used for all experiments. β-Fructofuranosidase activity was estimated on the basis of glucose released (1 mM glucose/min. = 1 U) from 8% sucrose solution prepared with buffer, and incubated for 30 min. at 50°C. Glucose was determined enzymatically with a standard glucostat kit. Protein was estimated by the modified Lowry's method (Hartree, 1972). Activity of the enzyme treated as described above was 635.5U/mg protein.

Synthesis of Oligosaccharides:
Effect of pH on oligosaccharide synthesis was investigated from pH 4.0 - pH 8.0 with 1.8M sucrose solution. pH 6.0-8.0 was prepared with 0.05M phosphate buffer. Effect of 4 combinations of sucrose, glucose and fructose on oligosaccharide synthesis were investigated at pH 7.5. The molar ratios of sucrose, glucose and fructose used were (i) 1.2:0.5:0.1, (ii) 1.2:0.3:0.3, (iii) 1.2:0.1:0.5 and (iv) 0.6:0.6:0.6 respectively. In each case the reaction volume was 2 ml, consisting of 1.8 ml sugar solution and 0.2 ml of 1% w/v enzyme solution. Incubation was in a thermostated water bath (stationary) at 60°C. Controls with and without heat inactivated enzymes were set-up in each case.

Analytical procedures:
Samples were analyzed simultaneously by TLC and HPLC. Each reaction was stopped by addition of 100 μl of the reacted sample to 900 μl of acetonitrile. Proteins and impurities were removed by solid phase extraction using BAKERBOND spe Diol columns. Samples were concentrated to 1 ml at 40°C in a vacuum rotary evaporator before analysis. TLC was performed with precoated 20x20 cm aluminum plates (Silica gel 60-Merck), and ethyl acetate, pyridine and water (33:13:4 by vol.) as solvent. Spots were revealed by spraying with diphenylamine-aniline in acetone reagent and heating at 100°C for 10 min. (Bailey and Bourne, 1960). HPLC was with the BECKMAN System, consisting of Analog interface module 406, 2 μ-spherogel carbohydrate (7.5 x 300 mm) columns connected in series and R156 refractive index detector. Column temperature was 80°C and mobile phase was DDI HPLC grade water (0.6 ml/min.). Carbohydrates in the reaction mixtures were quantified from percent of peak areas as resolved by the Beckman (System Gold) software. All carbohydrates with retention times (HPLC) and relative front (TLC) different from those of sucrose, fructose and glucose were regarded as new oligosaccharides.

RESULTS AND DISCUSSIONS

Effect of pH:
Hydrolytic activity was more pronounced at pH 4.0-5.5 as compared to pH 6.0-8.0 using 1.8 M sucrose solution. Sucrose was not detected by TLC and HPLC in the reaction mixtures prepared with pH 4.0 - 6.5 buffers after 2 h. Oligosaccharides were not detected at pH 4.0, however, a disaccharide with retention time similar to that of sucrose was detected after 48 and 72 h but not at 96 and 120 h. A similar phenomenon was noted at pH 4.5 (48 - 120 h), and pH 5.0 (72 h). The occurrence of this disaccharide after the disappearance of sucrose suggests re-synthesis of sucrose or a closely related