Biochemical Modification of Sucrose as Basis for New Products

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Sucrose is the most suitable raw material for processing to value-added products, as its easy accessibility and high purity, ease of transportation and storage ability in bulk quantities

Sucrose is a non-reducing disaccharide consisting of glucose and fructose, linked by a high energy oxygen bridge between the C_1 of the glucose and the C_2 of the fructose moiety. This high energy linkage makes glucosyl transfer reactions or reorganisation of the bonds within the sucrose molecule possible without having recourse to using coenzyme and coenzyme regeneration. This paper will discuss the fermentative and/or enzymatic modification of sucrose by conservation or reorganisation of the carbohydrate structure of sucrose. The first part will briefly describe the synthesis of oligosaccharides by the transfer of the glucose or fructose moiety to other saccharides with reference to the synthesis of dextrans or fructo-oligosaccharides. In the second part the reorganisation of the sucrose molecule is discussed with focus on the production of Palatinose, while the final part will illustrate the biotechnological synthesis of 3-keto sucrose and further chemical amination to 3-amino sucrose.

Synthesis of Oligo- and Polysaccharides from Sucrose

Dextransucrases from e.g. *Leuconostoc mesenteroides* or different *Streptococcus* species catalyse the synthesis of glucans, with different structures, from sucrose. Depending on the bacterial strain the dextrans differ from each other by the type of branching (α-1,2-; α-1,3- and α-1,4-), the percentage of branching and the length of the side chains. Beside the synthesis of dextrans the relatively unspecific dextransucrases are also able to synthesise e.g. the oligosaccharide α-1,6-glucosylsucrose from sucrose and maltose by the transfer of the glucose moiety from maltose to sucrose.

Fructose liberating enzymes such as β-2,1- and β-2,6-fructofuranosidases from e.g. *Aspergillus niger* or *Streptococcus salivarius* are able to synthesise the polysaccharides inulin and levan, respectively, by repeated transfer reactions of fructose units to sucrose. Similar to dextransucrases the rather unspecific fructofuranosidases can catalyse the synthesis of oligosaccharides. One example is the hydrolysis of sucrose and the transfer of the liberated fructose to xylose to produce xylosylfructosides. Another reaction performed by a fructofuranosidase from an *Aspergillus* strain is the transfer of fructose from a sucrose molecule to lactose, giving rise to the trisaccharide galactosylsucrose (lactosucrose).

Fermentative and/or Enzymatic Modification by Reorganisation of the Carbohydrate Structure of Sucrose

Enzymatic reactions resulting in a reorganisation of the sucrose molecule while maintaining the general carbohydrate structure seem to be most promising reactions for product diversification. Proteins able to catalyse the conversion of sucrose to the sucrose analogues trehalulose (1-O-α-D-glucopyranosyl-D-fructose), leucrose (5-O-α-D-glucopyranosyl-D-fructose) and isomaltulose (6-O-α-D-glucopyranosyl-D-fructose) have been detected in a variety of bacteria. *Protaminobacter rubrum* (CBS 574.77), *Serratia plymuthica* (ATCC 15928), *Erwinia rhapontici* (NCPPB 1578) and *Klebsiella planticola* (CCRC 19112)

are known to produce isomaltulose and trehalulose. With the exception of *Pseudomonas mesoacidophila* (Ferm 11808) and *Agrobacterium radiobacter* (Ferm 12397) these bacteria produce more isomaltulose than trehalulose. The enzyme that is responsible for the conversion of sucrose to the latter saccharides is named sucrose-glucosyltransferase, in literature the abbreviated name sucrose mutase or isomaltulose synthase (EC 5.4.99.11) is commonly used. The isomaltulose synthases produced by the different bacteria are quite similar with respect to their reaction mechanism. Sucrose is bound to the active side of the enzyme and is hydrolysed to generate glucose and fructofuranose. Only the glucose residue is strongly linked to the enzyme while the fructofuranose is ionically bound to the active side. After formation of the α-1,6-linkage between glucose and the fructofuranose isomaltulose is released. In case of tautomerisation of fructofuranose at the active side of the enzyme towards fructopyranose trehalulose is formed.

Leucrose, the α-1,5-isomer of sucrose is a by-product of dextran formation from sucrose catalysed by dextransucrase of *Leuconostoc mesenteroides* and *Streptococcus bovis*. The yield of leucrose formation can be increased by incubating a 65% aqueous solution of 1/3 sucrose and 2/3 fructose at 25°C with dextransucrase from *Leuconostoc*, which is secreted into the culture broth during growth in batch culture.

Isomaltulose/isomalt

Isomaltulose, the α-1,6-structural isomer of sucrose is in small quantities naturally found in honey. Several bacterial strains are known to produce isomaltulose when they are cultivated with sucrose. SÜDZUCKER developed a process for the production of isomaltulose in large quantities. For that purpose *Protaminobacter rubrum* CBS 574.77 is cultivated in a 10 m^3-scale. Cells grow optimally within 12 h at 30°C and an aeration rate of 1 vvm in a molasses based medium to an optical density (OD_{578}) of about 50. During the fermentation process the supplied sucrose is converted within 5 h accompanied by the production of isomaltulose. Maximum activity of the enzyme converting sucrose to isomaltulose (sucrose mutase) can be obtained after 10-12 h of cultivation. However, as the yield of isomaltulose excreted to the culture broth during batch fermentation is rather low, a continuously operating process for the production of isomaltulose by immobilized non-viable cells was developed by SÜDZUCKER. High cell densities can not be achieved in a stirred or fluidized reactor, thus a packed bed reactor is the obvious choice of reactor configuration.

After the fermentation of *Protaminobacter rubrum* the cells are recovered and entrapped into calcium-alginate beads followed by a drying step. Higher sucrose concentrations have a stabilising effect on catalyst half-life. Sucrose is pumped down a column containing the immobilized cells at temperatures of about 20-30°C.

The sucrose mutase is not strictly specific so that sucrose is converted to about 78-85% isomaltulose, 8-12% trehalulose, <3% fructose, <3% glucose, <3% sucrose, <3% isomaltose and <2% tri- and tetrasaccharides on total solids. From this solution isomaltulose (Palatinose) is recovered by crystallisation. The molecular weight of the *Protaminobacter rubrum* sucrose mutase is about 69,000-70,000, the isoelectric point is 9.9 and the temperature optimum at pH 6.0 was measured to be 45°C. As the stability of the enzyme at that temperature is too low and the production of by-products such as invert sugar or isomelezitose increases with elevated temperatures, the industrial scale conversion of sucrose to isomaltulose is carried out preferably at temperatures below 30°C. The enzyme can be inhibited quite efficiently by 6-deoxy-α-D-glucose and 6-chloro-α-D-glucose.

The non cariogenic and non-hygroscopic Palatinose is used as precursor for Isomalt, which is pro-

duced by hydrogenation at elevated temperatures and pressure with Raney type nickel. In addition it is a raw material for the production of other sweeteners. In the non-food area it can be used as a reducing agent e.g. for vat dyes.

Biochemical Modification of Sucrose by Conservation of the Carbohydrate Structure

3-keto-sucrose

An example for the biochemical modification of sucrose by conservation of the carbohydrate structure is its oxidation at the 3-OH of the glucose moiety to the corresponding keto-derivative. *Agrobacterium tumefaciens* is a plant pathogenic, Gram-negative, nonsporeforming bacterium able to perform this oxidation. In contrast to sucrose, the 3-keto-derivative has one specific site for selective chemical synthesis and therefore offers promising potential as starting material for carbohydrate-based products.

The enzyme, responsible for this oxidation reaction has been identified as a flavin adenine dinucleotide (FAD) dependent oxidoreductase, with the trivial name glucoside-3-dehydrogenase.

As during batch cultivation of the cells only minor amounts of 3-keto sucrose are excreted into the culture medium it is advantageous to use resting cells with high enzymatic activity. The synthesis of 3-ketosucrose is therefore performed in two steps, the cultivation of the biocatalyst on sucrose in order to express the glucosyl-3-dehydrogenase and the final oxidation step with resting cells in buffer. Maximum sucrose conversion of 45% can be achieved within 12-15 h in a stirred bioreactor at pH-values of about 7.5 8.0 and an aeration rate of 4 vvm.

Isolated 3-keto sucrose is rather reactive and can thus easily be modified after recovery from the solution. An example is the reductive amination using ammonia, hydrazine or other amines catalysed by classical Raney type nickel in aqueous solution.

A candidate molecule is 3-amino sucrose which has inhibitive effects on digestive enzymes of the human intestinal tract such as sucrase/isomaltase or glucoamylase/maltase. These enzymes rapidly hydrolyze oligosaccharides from dietary carbohydrates consisting of glucose and partially fructose moieties. The resulting glucose is readily absorbed causing the typical blood glucose insulin-controlled response curve. For management of disorders of blood glucose/insulin regulation such as diabetes melitus, enzyme inhibitors like the known pharmaceutical acarbose can be applied or digested together with rapidly digestible carbohydrates. 3-amino sucrose would be an alternative substance with principally similar properties. Through its inhibitive effect glucose liberation and respective blood response from dietary carbohydrates would be attenuated, thus opening applications for 3-amino sucrose as a drug for diabetes melitus and helping patients in tolerating a normal diet.

Conclusion

Sucrose as the world's most abundantly produced renewable organic compound is available in high purity for a reasonable price. Today several products made from sucrose by biochemical reactions are sold. Nevertheless, the application of more advanced biochemical reactions will give sugar a new chance as raw material to synthesise a variety of new products for different markets. Further research and development work in close co-operation between academia, scientific institutes and sugar industry is necessary.