Studies on the Use of Purified CBH I for Oligosaccharide Synthesis

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The importance of biologically active carbohydrates has been recognized over the last decade. The availability of cheap oligosaccharides for biological activity studies is very reduced. The isolation of these compounds from natural sources is almost impossible, because of their very high specific activity, and consequently very low concentration in nature. As chemical synthesis is a difficult and time consuming, the enzymatic synthesis has been regarded over the last years as a very attractive methodology for oligosaccharide production.

The main approach when utilizing glycanases for di- or trisaccharides synthesis has been the transglycosylation reaction. However, the isolation of products is quite complicated. On the other hand, the condensation reaction by reversed hydrolysis activity, which in many cases requires cheaper substrates, has a very low yield.

In this work, a purified exoglucanase CBH I from the fungus *Trichoderma reesei* was analyzed for its reversed hydrolysis activity. The enzyme was purified by conventional methodologies (preparative isoelectric focusing, gel filtration on Sephacryl 100 HR, anionic exchange on a Mono Q column and cationic exchange on a Mono S column), from a commercial cellulase, Cellulast, from Novo. The activity of the purified enzyme on a large set of substrates, such as lichenan, laminarin, filter paper, acid swollen Avicel, xylan and carboxymethylcellulose was characterized, suggesting that it is basically free of contaminant activities.

The enzyme was incubated in aqueous media with high sugar concentrations. Several mono- and disaccharides were used, in order to study the enzyme specificity. The obtained products were analyzed in a Dionex chromatographer using a CarboPac PA-100 column. The separated reaction products were analysed by NMR. The yields of the condensation reaction were in several cases considerably high.

Study of HMW Compounds in Sugar Using Gel Permeation Chromatography with an Avaporative Light Scattering Detector

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Raw sugars and other sugar process materials are studied by GPC (Gel Permeation Chromatography) using a Superose 12 column. As eluent was used a solution of 30% acetronitrile with 0.005 M ammonium acetate. As detector was used a spectrophotometric Diode Array Detector (DAD) and an Evaporative Light Scattering Detector, in series. By this arrangement both chromophoric and non chromophoric compounds are detected simultaneously. High sensitivity of both detectors allows a rapid detection of high molecular weight compounds without pre concentration of samples.

Synthesis and Reactions of Some Glycosidic Spiroacetal Derivatives of D-fructopyranose – Novel Quasi-di and Trisaccharides

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In connection with our interests in fructosides, the synthesis of an intramolecular fructopyranosyl spiro-acetal from allyl-β-D-fructopyranoside, and some of its reactions will be described. Little is known about glycosidic acetals from carbohydrates and glycol aldehyde. These derivatives were used to synthesize novel quasi-di and tri-saccharide derivatives.

Synthesis of Glycosyl Phosphatidylinositol Anchors and Phosphatidyl-inositol-(3,4,5)-triphosphate

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myo-Inositol appears widely in Nature, most frequently as phosphorylated or phospholipid derivatives, but *O*-methyl and glycosyl inositols have also been identified and synthesized. The discovery that inositol derivatives containing phosphates, phospholipids, glycans or glycan bound proteins are involved or act as "second messengers" in various cell regulation systems, have dramatically increased the synthesis of these compounds. Most eukaryotic cells utilize glycosyl phosphatidylinositols (GPIs) to anchor proteins to the cell membrane. Partial structural data, accumulated for over 100 GPI membrane-anchored proteins from a variety of organisms, have led to the proposal of the generalized anchor structure Glycan-Manα-4GlcNH$_2$α-6D-*myo*-inositol 1-phosphate. Only three of these structures have thus far been fully characterized, the variant surface glycoprotein (VSG) from *Trypanosoma* and *Leishmania*, and the Thy-1 glycoprotein anchor from rat brain. We are now synthesizing parts of the *Leishmania* structure. Parts of the structures of *Trypanosoma*, *Leishmania* and the Thy-1 glycoprotein anchor, have already been synthesized by others.

Inositol-(4,5)-diphosphate is a well-known precursor for a Ca^{2+}-mobilized "second messenger" inositol-(1,4,5)-triphosphate (IP_3). The metabolism and biological function of IP_3 have been described in detail during the last decade. More recently a phosphatidyl-inositol-(3,4,5)-triphosphate (PIP_3) has been found. PIP_3 is believed to initiate actin polymerisation in neutrophiles, respiratory burst, protein synthesis, secretion and glucose metabolism.

Synthesis of Oligosaccharides of Biomedical Relevance

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Oligosaccharide structures, usually linked to proteins in glycoproteins and proteoglycans or to lipids in glycolipids participate in a large number of biological processes. These involve, *inter alia*, bacterial and viral recognition of specific cell structures as well as immune system recognition of invasive organisms. They are also involved in autoimmune processes. The enormous structural variability possible in oligosaccharides structures is the probable reason for Nature using them for the purpose of molecular recognition.