Synthesis of Galβ1-3GlcNAc and Galβ1-3GlcNAcβ-SEt by an Enzymatic Method Comprising the Sequential Use of β-Galactosidases from Bovine Testes and Escherichia coli

LARS HEDBYS1, ELISABET JOHANSSON1, KLAUS MOSBACH1, PER-OLOF LARSSON1*, ALF GUNNARSSON2, SIGFRID SVENSSON2 and HANS LÖNN3

1 Department of Pure and Applied Biochemistry, Chemical Center, University of Lund, P.O. Box 124, S-221 00 Lund, Sweden
2 Department of Carbohydrate Chemistry, Sölvegatan 41, University of Lund, S-223 70 Lund, Sweden
3 Organic Synthesis Department, BioCarb AB, S-223 70 Lund, Sweden

Received January 23, 1989.

Key words: Glycosidases in carbohydrate synthesis, Galβ1-3GlcNAc, Galβ1-3GlcNAcβ-SEt, β-galactosidase

Galβ1-3GlcNAc (1) and Galβ1-3GlcNAcβ-SEt (2) were synthesized on a 100 mg scale by the transgalactosylation reaction of bovine testes β-galactosidase with lactose as donor and N-acetylglucosamine and GlcNAcβ-SEt as acceptors. In both cases the product mixtures contained unwanted isomers and were treated with β-galactosidase from Escherichia coli which has a different specificity, under conditions favouring hydrolysis, yielding besides the desired products, monosaccharides and traces of trisaccharides. The products were purified to >95% by gel filtration, with a final yield of 12% of 1 and 17% of 2, based on added acceptor. In a separate experiment Galβ1-6GlcNAcβ-SEt (3) was synthesized by the transglycosylation reaction using β-galactosidase from Escherichia coli. No other isomers were detected. Compound 3 was purified by HPLC.

With the increasing interest in biologically active carbohydrates has come an appreciation of the search for new methods of oligosaccharide synthesis, one of which utilizes glycosidases. Glycosidases can be used either by the reversal of the hydrolysis reaction [1, 2] or by the transglycosylation reaction [3, 4]. Because of the often limited aglycone specificity of these enzymes [5, 6], the product mixture can become complex [7, 8] and the purification of desired products cumbersome. In a previous study [9] we reported a new method which exploits the sequential use of β-galactosidases from bovine testes and E. coli. The latter enzyme with a slightly different specificity [10, 11], was used to hydrolyze unwanted structures formed by the bovine testes enzyme, thereby giving a mixture easily fractionated by gel chromatography.

*Author for correspondence.
In this paper we apply the method to the synthesis of the two related structures 1 and 2. The structure 1 is a constituent of the human milk oligosaccharides, e.g. lacto-N-tetraose, lacto-N-fucopentaose I and II, and also a component of the blood group determinants of the ABO-system [12]. The thioglycoside 2 can be used as a building block (after O-protection) in the synthesis of oligosaccharides [13] of which the structure, Galβ1-3GlcNAcβ is a part. Thus combining both chemical and enzymatic approaches it is possible to increase the efficiency and diversity of oligosaccharide synthesis.

Materials and Methods

Materials

*E. coli* β-galactosidase (E.C. 3.2.1.23) grade 6 (specific activity 80-150 U/mg protein, assayed as below), lactose, N-acetylglucosamine and o-nitrophenyl-β-D-galactopyranoside (ONPG) was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). GlcNAcβ-SEt was synthesized as published [14]. Bovine testes β-galactosidase (E.C. 3.2.1.23; 0.14 U/mg protein) was a crude preparation obtained as previously described [9]. Acetonitrile was obtained from Labscan Ltd (Dublin, Ireland) and was of Far u.v. grade. All other chemicals were of analytical grade or better.

Enzyme Assays

β-Galactosidase from bovine testes was incubated with 2 mM ONPG in 50 mM sodium phosphate-citrate buffer, pH 4.3 containing 0.02 % sodium azide. Samples were withdrawn at intervals and diluted eight times with 0.2 M Na₂CO₃, prior to spectrophotometric measurements at 420 nm. β-Galactosidase from *E. coli* was incubated with 2 mM ONPG in 50 mM sodium phosphate buffer, pH 7.0 with 1 mM MgCl₂ and 0.02 % sodium azide, and assayed directly at 420 nm. All assays were run at 37°C. One unit (U) was defined as the hydrolysis of 1 μmol ONPG/min under the above conditions.

HPLC Analyses

Transgalactosylation and hydrolysis reactions were monitored by HPLC on an LKB (Bromma, Sweden) equipment with either a 2151, variable wavelength monitor set to 195 nm, or a refractive index (RI) detector from Tecator (Höganäs, Sweden). Separations were performed on a Merck (Darmstadt, FRG) Lichrosorb NH₂-column with 70% aqueous acetonitrile. HPLC purifications were performed on a semi-preparative Lichrosorb NH₂-column. The reactions were normally followed by A₁₉₅-detection with the sensitivity of the monitor set in such a way that only acetamido structures were seen. This was more sensitive than RI-detection, but when detection of contaminating di- and trisaccharides was necessary, RI-detection was used. RI-detection was also used in the purifications by HPLC.

Transgalactosylation

Reactions were performed in 50 mM sodium phosphate-citrate buffer, pH 4.3 with 0.02% sodium azide, at 37°C, with 20% (w/w) lactose and 5% of the corresponding acceptor.