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Kinetic Studies on Endo-β-galactosidase by a Novel Colorimetric Assay and Synthesis of Poly-*N*-acetyllactosamines Using Its Transglycosylation Activity

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Novel chromogenic substrates for endo-β-galactosidase were designed on the basis of the structural features of keratan sulfate. Galβ1,4GlcNAcβ1,3Galβ1,4GlcNAcβ-*p*NP (2), which consists of two repeating units of *N*-acetyllactosamine, was enzymatically synthesized by consecutive additions of GlcNAc and Gal residues to *p*-nitrophenyl β-*N*-acetyllactosaminide. In a similar manner, GlcNAcβ1,3Galβ1,4GlcNAcβ-*p*NP (1), GlcNAcβ1,3Galβ1,4Glcβ-*p*NP (3), Galβ1,4GlcNAcβ1,3Galβ1,4Glcβ-*p*NP (4), Galβ1,3GlcNAcβ1,3Galβ1,4Glcβ-*p*NP (5), and Galβ1,6GlcNAcβ1,3Galβ1,4Glcβ-*p*NP (6) were synthesized as analogs of 2. Endo-β-galactosidases released GlcNAcβ-*p*NP or Glcβ-*p*NP in an endo-manner from each substrate. A colorimetric assay for endo-β-galactosidase was developed using the synthetic substrates on the basis of the determination of *p*-nitrophenol liberated from GlcNAcβ-*p*NP or Glcβ-*p*NP formed by the enzyme through a coupled reaction involving β-*N*-acetylglucosaminidase or β-D-glucosidase. Kinetic analysis by this method showed that the value of V_{max}/K_m of 2 for *Escherichia freundii* endo-β-galactosidase was almost equal to that for keratan sulfate, indicating that 2 is very suitable as a sensitive substrate for analytical use in an endo-β-galactosidase assay. In addition, the hydrolytic action of the enzyme toward 2 has shown to be remarkably promoted by the presence of 2-acetamide group adjacent to *p*-nitrophenyl group in comparison with 4. In addition, enzymatic synthesis of GlcNAc-terminated poly-*N*-

acetyllactosamine β-glycosides GlcNAcβ1,3 (Galβ1,4GlcNAcβ1,3)$_n$ Galβ1,3GlcNAcβ-*p*NP (*n*=1-5) has been demonstrated using a transglycosylation reaction of *E. freundii* endo-β-galactosidase. The enzyme catalyzed a transglycosylation reaction on 1, which served both as a donor and an acceptor, and converted 1 into *p*-nitrophenyl β-glycosides GlcNAcβ1,3(Galβ1,4GlcNAcβ1,3)$_n$Galβ1,4GlcNAcβ-*p*NP (9, *n*=1; 10, *n*=2; 11, *n*=3; 12, *n*=4; 13, *n*=5). The efficiency of production of poly-*N*-acetyllactosamines by *E. freundii* endo-β-galactosidase was significantly enhanced by the addition of BSA and by a low temperature condition.

Key words: endo-β-galactosidase, enzyme assay, poly-*N*-acetyllactosamine, kinetics, transglycosylation

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