Thesis Title Enzymatic Synthesis of Oligosaccharides in Aqueous Two-Phase System

Author Ms. Janjarus Panayong

M.S. Chemistry

Examining Committee :

Lecturer Dr. Dararat	Tongkao	Chairman
Assoc. Prof. Dr.Poonsook	Sriyotha	Member
Assist. Prof. Abhinya	Plikomol	Member

ABSTRACT

Oligosaccharides can be synthesized by glycosidase catalyzing reversed hydrolysis or glycosyltransfer reaction. Application of aqueous two-phase system in the synthesis is to immobilize the enzyme in bottom phase and separate the product from the reaction to top phase. Analysis of the oligosaccharides by chromatographic methods indicated that high performance liquid chromatography offered information for the oligosaccharides synthesized from both reactions, while thin layer and paper chromatography provided only from the reversed reaction.

The oligosaccharide synthesis from the reversed reaction was performed by incubation of 0.088 unit/ml α-mannosidase from Jack beans with 40% mannose at pH 6.0 and 60 °C for 4 days. At least one type of dimannoside was obtained in the amount of 4.3% of the mannose substrate. The synthesis in three types of the aqueous two-phase system:1) 8% poly ethylene glycol-8000/3% dextran-500, 2) 8.2% polyethylene glycol-8000/

9% Ficoll-400 and 3) 15% polyethylene glycol-8000/5% sodium sulphate, also yielded the same dimannoside as in the aqueous solution but in the amounts of 5.5, 6.2 and 9.1% of the mannose, respectively. Partition of the dimannoside in the polymer/polymer system was more or less the same concentration in the top and bottom phases, however, the partition in the polymer/salt system was less in the top. Optimization of the synthesis in the third system preferred 1.76 unit/ml α-mannosidase and the temperature of 50 °C.

The oligosaccharide synthesis from the transfer reaction was performed by incubation of 1.8×10^{-3} unit/ml α-mannosidase from jack beans with 2.5 mM p-nitrophenyl-α-D-mannopyranoside (pαM) and 50 mM p-nitrophenyl-β-D-galactopyranoside (pβG) at pH 6.0 and 25 °C for 2 days. At least three types of oligosaccharide (OS) were obtained in the amount ratio of OS-1 : OS-2 : OS-3 = 10 : 1 : 2 or the total OS of 5.4% of pαM, the mannosyl donor. The synthesis in five types of the aqueous two-phase system: 1, 2 and 3 as mentioned, 4) 8% polyethylene glycol-8000/7% Reppal-200 and 5) 20% polyethylene glycol-8000/7% potassium phosphate buffer, pH 6.0, also yielded the three OS in the same ratio as in the aqueous solution but in the total amounts of 6.5, 4.3, 3.8, 4.7 and 3.2% of the pαM, respectively. Partition of the three OS behaved similarly, i.e. it was almost the same concentration in the top and bottom phases of the polymer/polymer system but more in the top of the polymer/salt system. Optimization of the synthesis in the third system preferred 7.2×10^{-3} unit/ml α-mannosidase, concentration ratio of pαM : pβG = 1:10 and the temperature of 37 °C.