The Application and studies of mass fermentative production of N-acetylchitooligosaccharides from hydrolysis chitin by l

陳思豪、王三郎、顏裕鴻

E-mail: 9300230@mail.dyu.edu.tw

ABSTRACT

The mian purpose of this thesis is to investigate the hydrolysis of colloiadal chitin by lysozyme to produce N-acetylchitooligosaccharides with low degrees of polymerization (DP) from 1 to 6. The best analysis method for N-acetylchitooligosaccharides (NACOs) is reverse phase High Performance Liquid Chromatography. The natural logarithm of the retention time of NACOs correlated linearly with the DP values. It was found that the optimum temperature and reaction time for production of NACOs were 37℃ and 1 hour. Longer reaction time lead to the generation of NACOs with lower DP's. The N-acetylchitooligosaccharides examined for their growth inhibition effects in human leukemia cell lines. K562 cells were treated with these compounds for 24, 48, 72 hours and their proliferation was determined by WST-1 reagent. N-acetylchitooligosaccharides had the highest growth inhibitory, and morphological changed. The lysozyme can be covalent immobilized on a polymeric support (hydroxypropyl methylcellulose acetate succinate, AS-L), which is soluble above pH 5.5 and insoluble below pH 4.5.This study used different activating reagent EDC & glutaraldehyde to bind on AS-L carrier. Efficiency of enzyme immobilization was 80% and 75%. The activation energy was 17.5 KJ/mole for free enzyme and was reduced to 15.5 KJ/mole and 12.7 KJ/ mole for EDC and glutaraldehyde immobilized enzyme respectively. The result found that the specific activity of glutaraldehyde immobilized enzyme increased to 223% of free enzyme. For glutaraldehyde immobilized enzyme, the optimum pH and temperature shifted to pH6.0 and 37℃.The storage stability was 5 days for free enzyme and impoved to 25 days and 20 day for EDC and glutaraldehyde immobilized enzyme respectively. Immobilized enzyme retained 70% of its original activity after 10 batches of reaction. According to Km and Vmax of free and immobilized lysozyme, the mass transfer of immobilized lysozyme was not obviously affected.

Keywords : lysozyme, N-acetylchitooligosaccharides, anticancer, immobilized enzyme.

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