利用溶菌酵素水解幾丁質生產N-乙 幾丁寡醣之分析應用及量產條件之研究

陳思豪、王三郎、顏裕鴻

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

摘 要

懸浮態幾丁質經溶菌酵素水解可得聚合度 1至6 之N-乙醯幾丁寡醣，並利用RP-HPLC分析可得最適分析條件。由結果發現N-乙醯幾丁寡醣在HPLC 中之滯留時間之自然對數值與其聚合度呈現線性關係。在37°C反應1小時可得較多的N-乙醯幾丁六醣，隨著時間的增長可得較多更低聚合度的N-乙醯幾丁寡醣。 藉由細胞週期停滯、誘導細胞凋亡與細胞分化以達到抑制癌細胞的增殖，是目前用來篩選有效抗癌藥的重要指標，以WST-1偵測經N-乙醯幾丁寡醣處理24、48、72小時後K562之細胞增殖率，結果發現N-乙醯幾丁寡醣有抑制細胞增殖率的作用，觀察以N-乙醯幾丁寡醣處理之細胞形態，發現細胞形狀有皺縮的現象。 溶菌酵素可共價鍵結於hydroxypropyl methylcellulose acetate succinate，（AS-L）上，其為一種可隨pH值改變而產生可逆溶解型的高分子聚合物。所得固定化酵素可在pH5.5以上時完全溶解，在pH4.5以下則完全不溶解。本研究利用不同的化學活性劑EDC及glutaraldehyde與AS-L鍵結，由結果發現分別使用EDC及glutaraldehyde做固定化研究時，可達80%及75%固定化率，且固定化酵素的活化能由游離酵素的17.5 KJ/mole，降低為EDC 15.5 KJ/mole 、glutaraldehyde12.7 KJ/ mole，且使用化學活性劑glutaraldehyde 其比活性較游離酵素高出2.23倍。至於最適pH和最適溫度方面，游離酵素的為pH5.0，37°C、使用EDC及glutaraldehyde固定化酵素的則分別為pH5.0，37°C、pH6.0，37°C。在儲藏穩定性方面使用EDC及glutaraldehyde固定化酵素由游離酵素的5天延長至25天及20天。固定化酵素在批次反應下水解懸浮態幾丁質，經10次回收後均可維持70%之比活性。在動力學性質方面，固定化酵素與游離酵素之Km與Vmax變化結果，顯示酵素固定於可逆溶解型擔體上並無明顯的質傳限制。

關鍵詞 : 溶菌酵素、N-乙醯幾丁寡醣、抗癌、固定化酵素

目錄

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