

以可逆溶解型擔體固定澱粉糖化酵素之研究

洪嘉禧、陳鴻章

E-mail: 9015662@mail.dyu.edu.tw

摘要

本研究將澱粉糖化酵素 (GLUCOAMYLASE)，藉由幾種化學活性劑與腸胃藥用包覆劑 (ENTERIC COATING POLYMER) 形成可逆溶解型固定化澱粉糖化酵素。此固定化酵素會隨之於可溶狀態下進行反應，而於反應後藉著改變溶液之PH值，使酵素隨沉澱的擔體與溶液分離，而達到重複使用的目的。本研究主要分為兩部分。第一部份係利用一些具代表性之PH值型可逆溶解型高分子AS-L、HP-50及CAP三種，並且藉由幾種化學活性劑如戊二醛 (GLUTARALDEHYDE)、碳化二亞胺 (N-ETHYL-N-(3-DIMETHYLAMINOPROPYL) CARBODIIMIDE HYDROCHLORIDE, EDC) 及三聚氯化氰 (CYANURIC CHLORIDE) 對澱粉糖化酵素作固定化，而形成可逆溶解型固定化酵素，並根據研究分析所得固定化酵素之活性及固定量，來篩選適合的固定化擔體。第二部分則以最適當的擔體及最適化的操作條件來探討固定化酵素與游離酵素之基本性質、操作穩定性、熱穩定性及動力學性質的差異。就本研究結果發現，以使用CAP擔體之固定化酵素之酵素活性與蛋白質固定量效果最好；其中當CAP濃度在32 MG/ML時不論使用何種活化劑，所能固定之最大酵素量約為5 MG/ML，並且酵素活性約為173 U/ML。在基本性質方面，此游離與固定化酵素之最適作用溫度分別是50 和55。而最適作用PH值分別介於6.0~7.0與7.0~8.0之間。在熱穩定性方面，固定化酵素相較於游離酵素有較好之穩定度。在動力學性質方面，根據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不溶解型擔體上無明顯的質傳阻力影響，而擔體在固定化酵素之反應中存在非競爭性 (NONCOMPETITIVE) 之作用。本研究最後發現游離與固定化酵素之間反應活化能相類似，在不同澱粉濃度下反應也是如此。表示固定化酵素之反應不受擴散因素的影響，而為單純反應速率所控制。

關鍵詞：澱粉糖化酵素、可逆溶解性、固定化酵素

目錄

第一章 緒言--P1 1.1 研究背景--P1 1.2 研究動機--P2 1.3 研究大綱--P2 1.4 研究重要性--P3 第二章 文獻回顧--P4 2.1 澱粉簡介--P4 2.2 澱粉糖化酵素--P7 2.3 固定化酵素之發展歷程--P9 2.4 固定化酵素之種類及方法--P10 2.5 可逆溶解型擔體--P15 2.6 可逆溶解型高分子上羧基之活化及酵素分子接合--P19 2.7 以pH型可逆溶解性擔體做固定化研究--P22 2.8 穩定性的探討--P29 2.9 動力學參數探討--P36 第三章 實驗材料與方法--P41 3.1 實驗設備--P41 3.2 實驗材料--P41 3.3 實驗方法--P43 3.3.1 固定化酵素的製備--P43 3.3.2 酵素活性分析--P44 3.3.3 酵素固定量分析--P46 3.4 實驗設計--P49 3.4.1 可逆溶解性擔體與活化劑之篩選--P50 3.4.2 游離酵素與固定化酵素基本性質之比較--P51 3.4.3 固定化酵素與游離酵素動力學性質之比較--P52 第四章 結果與討論--P54 4.1 以AS-L為擔體固定澱粉糖化酵素的探討--P54 4.1.1 活化劑種類及濃度的影響--P54 4.1.2 擔體濃度的影響--P58 4.1.3 酵素用量的影響--P61 4.2 以HP-50為擔體固定澱粉糖化酵素的探討--P64 4.2.1 活化劑種類及濃度的影響--P64 4.2.2 擔體濃度的影響--P65 4.2.3 酵素用量的影響--P70 4.3 以CAP為擔體固定澱粉糖化酵素的探討--P73 4.3.1 活化劑種類及濃度的影響--P73 4.3.2 擔體濃度的影響--P76 4.3.3 酵素用量的影響--P79 4.4 固定化酵素基本性質的影響--P82 4.4.1 回收次數對酵素活性的影響--P82 4.4.2 最適作用溫度--P87 4.4.3 最適作用pH值--P89 4.4.4 熱穩定性--P95 4.5 動力學性質的探討--P100 4.5.1 動力學參數的變化--P100 4.5.2 活化能的變化--P101 第五章 結論--P115 第六章 參考文獻--P117

參考文獻

- 1.ABDULLAH, M., CATLEY, B. J., ROBYT, J., WALLENFELS, K., AND WHELAN, W. J. (1966) THE MECHANISM OF CARBOHYDRASE ACTION. XI. PULLULANASE AN ENZYME SPECIFIC FOR THE HYDROLYSIS OF 1-6 BONDS IN AMYLACEOUS OLIGO AND POLYSACCHARIDES. CEREAL CHEM., 43, 111.
- 2.AHMED F. ABDEL FATTAH, OSMAN, MONA Y., MOHAMED A. ABDEL NABY (1997) PRODUCTION AND IMMobilIZATION OF CELLOBIASE FROM ASPERGILLUS NIGER A20. CHEMICAL ENGINEERING JOURNAL, 68, 189-196.
- 3.AXEN, R., HEILBRONN, E. AND WEETALL, H. H. (1969) PREPARATION AND PROPERTIES OF CHOLINE ESTERASE COVALENTLY BOUND TO SEOHAROUS. BIOCHEM. BIOPHYS. ACTA., 191, 478-481.
- 4.BALASUBRAMANIAM, K. AND VASANTHY ARASARATNAM (1989) KINETIC STUDIES ON SOLUBLE AND IMMobilIZED ALPHA AMYLASE AND GLUCOAMYLASE. JOURNAL OF THE NATIONAL SCIENCE COUNCIL OF SRI LANKA, 17 (1) 91-97, 9 REF.
- 5.BARKER, S. A., SOMERS, P. J. AND EPTON, R. (1968). PREPARATION AND PROPERTIES OF AMYLASE CHEMICALLY COUPLES TO MICROCRYSTALLINE CELLULOSE. CARBOHYDE. RES., 8, 491-493.
- 6.BARKER, S.A. AND EOTIB,

R. (1970) WATER-INSOLUBLE ENZYMES. PROCESS BIOCHEM., 5, 14-18. 7.BARKER, S. A., DOSS, S. H., GRAY, C. J., KENNY, J. F., STACEY, M., AND YEO, T. H. (1971) α -D-GLUCOSIDASE CHEMICALLY BOUND TO MICROCRYSTALLINE CELLULOSE. CARBOHYDRATE RES., 20, 1-3. 8.CABRAL, J. M., NOVAIS, J. M., CARDOSO, J. P. AND KENNEDY, J.F. (1986) DESIGN OF IMMOBILIZED GLUCOAMYLASE REACTOR USING A SIMPLE KINETIC FOR HYDROLYSIS OF STARCH. J. CHEM. TECH. BIOTECHNOL., 36, 247-254. 9.CHEN, H. M., RELLY, P. J. AND FORD, C. (1992) SITE-DIRECTED MUTAGENESIS TO ENHANCE THERMOSTABILITY OF GLUCOAMYLASE FROM ASPERGILLUS: A RATIONAL APPROACH. ANNUAL BIOCHEM. ENG. SYMPOSIUM, 111-121. 10.CHEN, J. P. AND CHANG, K. C. (1994) IMMOBILIZATION OF CHITINASE ON A REVERSIBLY SOLUBLE-INSOLUBLE POLYMER FOR CHITIN HYDROLYSIS. JOURNAL OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY., 60(2), 133-140. 11.CLARK, K. M. AND GLATZ, C. E., (1987) POLYMER DOSAGE CONSIDERATIONS IN POLYELECTROLYTE PRECIPITATION OF PROTEIN. BIOTECHNOL. PROC., 3, 241-247. 12.CORDT, S., HENDEICKX, M., MAESMANS, G. AND TOBBACK, T., (1994) THE INFLUENCE OF POLYALCOHOLS AND CARBOHYDRATES ON THE THERMOSTABILITY OF α -AMYLASE. BIOTECH. AND BIOENG., 43, 107-114. 13.COUTINHO, P. M., DOWD, M. K., REILLY, P. J. (1997) AUTOMATED DOCKING OF MONOSACCHARIDE SUBSTRATES AND ANALOGUES AND METHYL-ACARVIOSINIDE IN THE GLUCOAMYLASE ACTIVE SITE. PROT. EINS., 27, 235. 14.D'AURIA, S., PELLINO, F., CARA, F. LA., BARONE, R., ROSI M., AND NUCCI, R. (1996) IMMOBILIZATION ON CHITOSAN OF A THERMOPHILIC BETA-GLYCOSIDASE EXPRESSED IN SACCHAROMYCES CEREVISIAE. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, 61 (1/2) 157-166, 9 REF. 15.DOMINIC W.S., AND WONG, PH. D. (1989) MECHANISM AND THEORY IN FOOD CHEMISTRY. 16.EASTMAN PUBLICATION EFC-205C, APRIL 1998. 17.FUJIMURA, M., MORI, T. AND TOSA, T. (1987) PREPARATION AND PROPERTIES OF SOLUBLE-INSOLUBLE IMMOBILIZED PROTEASE. BIOTECHNOLOGY AND BIOENGINEERING, XXIX, 747-752. 18.GERMAIN, P. AND CRICHTON, R. B. (1988) CHARACTERIZATION OF A CHEMICALLY MODIFIED α -AMYLASE IMMOBILIZED ON POROUS SILICA. J. CHEM. TECH. BIOTECHNOL., 41, 297. 19.GISELLA, M. ZANIN AND FLAVIO, F. DE MOORAES (1998) THERMAL STABILITY AND ENERGY OF DEACETIVATION OF FREE AND IMMOBILIZATION AMYLOGLUCOSIDASE IN THE SACCHARIFICATION OF LIQUEFIED CASSAVA STARCH. APPLIED-BIOCHEMISTRY AND BIOTECHNOLOGY, 70-72, 383-394, 28 REF. 20.GUNJA-SMITH, Z., MARSHALL, J. J. AND SMITH, E. E. (1971) ENZYMIC DETERMINATION OF THE UNIT CHAIN LENGTH OF GLYCOGEN AND RELATED POLYSACCHARIDES. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, 70-72, 383-394. 21.HOSHINO, K., TANIGUCHI, M. AND FUJII, M. (1992) PROPERTIES OF AMYLASE IMMOBILIZED ON A NEW REVERSIBLY SOLUBLE-INSOLUBLE POLYMER AND ITS APPLICATION TO REPEATED HYDROLYSIS OF SOLUBLE STARCH. JOURNAL OF CHEMICAL ENGINEERING OF JAPAN, 25 (5), 569-574. 22.IKEDI OBI, CO., STEVENS, M. AND LATINWO, L. (1998) IMMOBILIZATION OF LINAMARASE ON NON-POROUS GLASS BEADS. PROCESS BIOCHEMISTRY, 33 (5) 491-494, 12 REF. 23.LEE, J. C. (1971) PREPARATION AND PROPERTIES OF WATER-INSOLUBLE DERIVATIVES OF RIBONUCLEASE T1. BIOCHEM. BIOPHYS. ACTA., 235, 435-441. 24.LEONOWICZ, A., SAREKR, J. M. AND BOLLAG, J. M. (1988) IMPROVEMENT IN STABILITY OF AN IMMOBILIZED FUNGAL LACCASE. APPL. MICROB. BIOTECH., 29, 129-135. 25.LILLY, M. D. (1971) STABILITY OF IMMOBILIZED β -GALACTOSIDASE ON PROLONGED STORAGE. BIOTECH. AND BIOENG., 13, 589-592. 26.LINE, W. F., KWONG, A. AND WEETALL, H. H. (1971) PEP SIN INSOLUBILIZED BY COVALENT ATTACHMENT TO GLASS: PREPARATION AND CHARACTERIZATION. BIOCHIM. BIOPHYS. ACTA., 242, 194-198. 27.MANECKE, G. (1962) REACTIVE POLYMERS AND THEIR USE FOR THE PREPARATION OF ANTIBODY AND ENZYME RESINS. PURE APPL. CHEM., 4: 507-510. 28.MILLER, E. (1998) IMMOBILIZATION OF GLUCOAMYLASE ON POLYAMIDE NETS. ACTA-BIOTECHNOLOGICA, 18 (2) 135-146, 28 REF. 29.MONSAN, P. AND DUNZEL, G. (1971) NEW PREPARATION OF ENZYMES FIXED ON INORGANIC SUPPORT. C. R. ACAD. SCI. SER., 273: 33-36. 30.NORMAN, B. E. (1982) A NOVEL DEBRANCHING ENZYME FOR APPLICATION IN THE GLUCOSE SYRUP INDUSTRY. STARKE, 10, 340. 31.OHNO, Y. AND STAHMAN, M. A. (1971) POLYACRYLAMIDE DERIVATIVES OF AMINO ACID ACYLASE AND TRYPSIN. MICROMOLECULES, 4, 350-357. 32.O'NEILL, S. P., DUNNILL, P. AND LILLY, M.D. (1971) A COMPARATIVE STUDY OF IMMOBILIZED AMYLOGLUCOSIDASE IN PACKED BED REACTOR AND A CONTINUOUS FED STIRRED TANK REACTOR. BIOTECHNOLOGY. BIOENG., 13, 337. 33.PIETER, B. R., BARDELETTI, G., AND COULET, P. R. (1998) GLUCOAMYLASE IMMOBILIZATION ON MAGNETIC MICROPARTICLE FOR THE CONTINUOUS HYDROLYSIS OF MALTODEXTRIN IN A FLUIDIZED BED REACTOR. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, 32, 53. 34.ROBINSON, P. J., DUNNILL, P., AND LILLY, M. D. (1971) POROUS GLASS AS A SOLID SUPPORT FOR IMMOBILIZATION OF AFFINITY CHROMATOGRAPHY OF ENZYME. BIOCHIM. BIOPHYS. ACTA., 242, 659-665. 35.SALETAN, L. T. (1968) CARBOHYDRASES OF INTEREST IN BREWING WITH PARTICULAR REFERENCE TO AMYLOGLUCOSIDASE. WALLERSTEIN LAB. COMMUN., 31, 33. 36.SANROMAN, A., CHAMY, R., NUNES, M. J. AND LEMA, J. M. (1991) ENZYMIC HYDROLYSIS OF STARCH IN A FIXED-BED PULSED-FLOW REACTOR. APPL. BIOCHEM. BIOTECHNOL., 28, 527. 37.SCHINDLER, T. AND NORDMEIER, E. (1994) STUDIES ON THE FORMATION AND AGGREGATION OF POLYELECTROLYTE COMPLEXES FORMED BETWEEN POLY(STYRENESULFONIC ACID) AND POLY(DIALLYL-N, N-DIMETHYLAMMONIUM CHLORIDE). POLYMER JOURNAL. 26(10), 1124-1131. 38.SCOPE S, R. K. (1987) IN PROTEIN PURIFICATION: PRINCIPLE AND PRACTICE, 2ND EDITION, SPR

-INGER, BERLIN, HEIDELBERG, NY. 39.SCOUTEN, W. H. (1987) A SURVEY OF ENZYME COUPLING TECHNIQUES.METHODS IN ENZYMOLOGY, 135, 30. 40.SOHN, HEUNG-SIK, SEONG, MIN-PARK, BYUNG, YIL-SON HYEON MEE-CHOI AND KEUN, TAI-LEE (1998) IMMOBILIZATION OF AN ENZYME WITH CHITOSAN MICROBEADS.JOURNAL OF THE KOREAN FISHERIES SOCIETY, 32 (1) 83-87, 17 REF. 41.STANLEY, W. L., WATTERS, G. G., CHAN, B. G. AND MARCER, J. M. (1975) LACTOSE AND OTHER ENZYMES BOUND TO CHITIN WITH GLUTALDEHYDE. BIOTECH. AND BIOENG., 17, 315-319. 42.STANLEY, W. L., WATTERS, G. G., CHAN, B. G. AND KELLY, S. H. (1976) IMMOBILIZATION OF GLUCOSE ISOMERASE ON CHITIN WITH GLUTARALDEHYDE AND BY SIMPLE ADSORPTION. BIOTECH. AND BIOENG., 18, 439-442. 43.STASIW, R. O., BROWN, H. D. AND HASSELBERGER, F. X. (1970) CHLORINESTERASE BONDED TO PAPER. CAN. J. BIOCHEM., 48, 1314-1319. 44.STERNBERG, M. AND HERSHBERGER, D. (1974) BIOCHIM. BIOPHYS. ACTA., 342, 195. 45.STRANDBERG, G. W., BARCHLER, M. J. AND SMILEY, K. L. (1970) STARCH CONVERSION BY IMMOBILIZED GLUCOAMYLASE. BIOTECHNOL. BIOENG.,12, 1. 46.SUBRAMANIAN, A., KENNEL, SJ., ODEN, PI., JACOBSON, KB., WOODWARD, J. AND DOKTYCZ, MJ. (1999) COMPARISON OF TECHNIQUES FOR ENZYME IMMOBILIZATION ON SILICON SUPPORTS. ENZYME AND MICROBIAL TECHNOLOGY, 24 (1/2) 26-34, 19 REF. 47.SURINOV, B. P. AND MANOILOV, S. E. (1966) PRODUCTION AND PROPERTIES OF INSOLUBLE COMPOUNDS OF CERTAIN ENZYME WITH CELLULOSE. BIOKIMIYA, 31, 387-391. 48.SUYE, S., KUMON, Y. AND ISHIGAKI, A. (1998) IMMOBILIZATION OF GLUCOSE OXIDASE ON POLY-(L-LYSINE)-MODIFIED POLYCARBONATE MEMBRANE. BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, 27 (3) 245-248, 16 REF. 49.SYNOWIECKI, J., SIHORSKI, Z. E. AND NACZK, M. (1981) IMMOBILIZATION OF INVERTASE ON KRILL CHITIN. BIOTECH. BIOENG., 23, 231. 50.SYNOWIECKI, J., SIONDALSKA, A. S. AND BEDAWAY, EL. (1987) ADSORPTION OF ENZYME ON KRILL CHITIN MODIFIED WITH CARBON DISULFIDE. BIOTECH. BIOENG., 29, 352. 51.TANAKA, H., MASATOSHI, M. AND VELIKY, I. A. (1984). DIFFUSION CHARACTERISTICS OF SUBSTRATES IN CA-ALGINATE GEL BEADS. BIOTECHNOLOGY AND BIOENGINEERING, XXXVI, 53-58. 52.TANIGUCHI, M., TANAHASHI, S. AND FUJII, M. (1990) PROPERTIES AND REPEATED USE OF A REVERSIBLY SOLUBLE-INSOLUBLE YEAST LYTIC ENZYME. APPLIED AND MICROBIOLOGY BIOTECHNOLOGY, 33, 629-633. 53.TOLDRA, F., JANSEN, NB. AND TSAO, GT. (1990) MALTOSE HYDROLYSIS KINETICS WITH GLUCOAMYLASE IMMOBILIZED IN POROUS GLASS FIBERS IN A TABULAR FLOW REACTOR. NAHRUNG, 34(10), 945-951, 16 REF. 54.TSYN, H. Y. AND TASI, S. Y. (1988) COMPARISON OF KINETIC AND FACTORS AFFECTING THE STABILITY OF CHITIN-IMMOBILIZED NARINGINASES FROM TWO FUNGAL SOURCES. J. FERMENT TECH -NOL., 66, 193. 55.VERTESI, A., SIMON, LM., KISS, I. AND SZAJANI, B., (1999) PREPARATION, CHARACTERIZATION AND APPLICATION OF IMMOBILIZED CARBOXYPEPTIDASE A. ENZYME AND MICROBIAL TECHNOLOGY, 25 (1/2) 73-79, 19 REF. 56.VIRTO, D., MARIA, AGUD, ISABEL AND MONTERO, SOL (1995) KINETIC PROPERTIES OF SOLUBLE AND IMMOBILIZED CANDIA RUGOSA LIPASE. APPLIED BIOCHEMISTRY AND BIOTECHNOLOGY, 50, 127-136. 57.WANG, SAN LANG AND CHIO, SAU HWA (1998) REVERSIBLE IMMOBILIZATION OF CHITINASE VIA COUPLING TO REVERSIBLY SOLUBLE POLYMER. ENZYME AND MICROBIAL TECHNOLOGY, 22 (7) 634-640, 28 REF. 58.WEETALL, H. H. (1970) STORAGE STABILITY OF WATER-INSOLUBLE ENZYMES COVALENTLY COUPLES TO ORGANICS AND INORGANICS CARRIERS. BIOCHIM. BIOPHYS. ACTA., 212, 1-3. 59.WEIKY, N., BROWN, F. S. AND DALE, E. C. (1969) CARRIER-BOUND PROTEINS: PROPERTIES OF PEPTIDASE BOUND TO INSOLUBLE CARBOXYMETHYLCELLULOSE PARTICLES. BIOCHIM. BIOPHYS., 131, 1-4. 60.WHITE, J. S. AND WHITE, D. C. (1997) GLUCAN 1,4 GLUCOSIDASE.SOURCE BOOK OF ENZYME,BOCA RATON, NEW YORK, 419-422. 61.YAKUP, ARICA M., GURDAL, ALAEDDINOGLU, N. AND VASIF, HASIRCI (1998) IMMOBILIZATION OF GLUCOAMYLASE ONTO ACTIVATED PHEMA/EGDMA MICROSPHERES: PROPERTIES AND APPLICATION TO A PACKED-BED REACTOR. ENZYME AND MICROBIAL TECHNOLOGY; 22 (3) 152-157, 27 REF. 62.YANKOV, D., BESCHKOV, V. AND ROULEAU, D. (1997) KINETICS AND MODELLING OF THE ENZYME HYDROLYSIS OF MALTOSE WITH FREE AND IMMOBILIZED GLUCOAMYLASE. STARCH/STAERKE-, 49 (7/8) 288-293, 16 REF. 63.王三郎 (1997) 應用微生物學。高立圖書出版社。 64.李正吉 (1996) 澱粉切枝酵素固定於幾丁質與高分子共聚物之各種生化性質與穩定性之探討。國立台灣大學碩士論文。 65.祝鼎新 (1997) 熱敏感型高分子擔體固定澱粉水解酵素-AMYLAASE的探討和應用,元智大學碩士論文 66.張可昌 (1993) 可逆溶解型聚合物於固定化酵素的應用。成功大學碩士論文。 67.許政皓 (1997) 陽離子型熱感應性膠體之製被及吸水行為之研究。大同工學院碩士論文。 68.陳國誠 (1989) 酵素工程學。藝軒圖書出版社。 69.葉明杰 (1995) 可逆溶解性高分子在生化分離程序上的應用。台灣工業技術學院碩士論文。 70.劉英俊 (1993) 生物化學工程學。中央圖書出版社出版。 71.劉英俊、汪金追 (1987) 酵素工程。中央圖書出版社。 72.蘇大榮 (1999) 不同組成之熱敏感乳液粒子於酵素固定上之研究。長庚大學碩士論文。