

Covalent Immobilization of Lipase AY on Poly (-Glutamic acid

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ABSTRACT

Industrial application of lipase requires efficient methods to immobilize the enzyme for yielding a biocatalyst with greater than free lipase. Lipase AY from *Candida rugosa* was immobilized on Celite by adsorption and poly -glutamic acid (PGA) by covalent binding, respectively. Response surface methodology (RSM) and 3-level-3-factor fractional factorial design were adopted to evaluate the effects of immobilization variables, such as immobilized time, temperature and enzyme/support ratio on specific activity of immobilization lipase. For lipase AY was immobilized on Celite by adsorption. The optimum immobilization conditions were: immobilization time 59.1 min, immobilization temperature 10.7 °C, enzyme/support ratio 0.5 (w/w) and the highest specific activity was 18.2 U/mg-protein with activity yield 34.1%. In the study of covalent binding, lipase AY was immobilized on PGA using EDC as a activator. The optimum immobilization conditions were: immobilized time near to 2 h, immobilized temperature 0 °C, enzyme/support ratio about 0.1 (w/w) and the highest specific activity was 96.4 U/mg-protein with activity yield 180.9%. The results showed that the lipase- PGA has better activity than lipase AY-Celite and free lipase AY and demonstrated the possible industrial application of lipase AY- PGA for yielding higher production.

Keywords : Adsorption ; Covalent binding ; Immobilization ; Lipase AY ; Optimzation ; Poly -glutamic acid.

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REFERENCES

- 中文部份 1. 王超群。2001。固定*Pseudomonas fluorescens* 脂肪酵素於幾丁聚醣之研究:6 – 8。國立清華大學化學工程學系碩士論文。新竹，台灣。2. 沈明來。2000。生物檢定統計法。第31 – 219 頁。九州圖書文物有限公司。台北，台灣。3. 洪哲穎和陳國誠。1992。回應曲面實驗設計法在微生物酵素生產上之應用。化工專論 39:3 – 18。4. 洪哲穎。1998。回應曲面品質工程技術。第64 – 269 頁。義守大學化學工程學系。高雄，台灣。5. 洪佃玠。2003。利用幾丁聚醣固定脂肪分解酵素之研究:36 – 78。國立清華大學化學工程學系碩士論文。新竹，台灣。6. 陳國誠。1990。生物固定化技術與產業應用。第125 – 164頁。茂昌圖書有限公司。台北，台灣。7. 簡家豪。2002。利用幾丁聚糖固定*Candida rugosa* Type VII脂肪分解酵素之研究:26 – 65。國立清華大學化學工程學系碩士論文。新竹，台灣。8. 鄭慈千。2005。固定化酵素生產果寡糖和半乳寡糖:46 – 58。私立大同大學生物工程學系博士論文。台北，台灣。9. 蕭介夫。2002。數種脂肪?基因之表達與應用。知識創新22:1 – 4。英文部分 1. Abdul Rahman, M. B., Tajudin, S. M., Hussein, M. Z., Abdul Rahman, R. Z., Salleh, A. B., Basri, M. 2005. Application of natural kaolin as support forthe immobilization of lipase from *Candida rugosa* as biocatalyst foreffective esterification. *Applied Clay Science* 29:111 – 116. 2. Amano international enzyme company. 2003. Product information of Lipase AY: Lipase AY “Amano ” 30. http://www.amano-enzyme.co.jp/pdf/food_e/cat_food_LAY-30_e.pdf. 3. Bayramo?lu, G., Kacar, Y., Denizli, A., Ar?ca, M. Y. 2002. Covalen timmobilization of lipase onto hydrophobic group incorporated poly(2-hydroxyethyl methacrylate) based hydrophilic membrane matrix. *Journal of Food Engineering* 52: 367 – 374. 4. Bommarius, A. S., Riebel, B. R. 2003. Biocatalysis. Wiley-Vch Verlag GmbH & Co. KGaA, Weinheim, USA, 30 – 571. 5. Cao, L., 2005, Immobilized enzymes: science or art ? *Current Opinion in Chemical Biology* 9:217 – 226. 6. Carta, G., Gainer, L. J., Gibson, M. E. 1992. Synthesis of esters using a nylon-immobilized lipase in batch and continuous reactors. *Enzyme Microbial Technology* 14: 904 – 910. 7. Chiou, S. H., Wu, W. T. 2004. Immobilization of *Candida rugosa* lipase on chitosan with activation of the hydroxyl groups. *Biomaterials* 25: 197 – 204. 8. Desai, P. D., Dave, A. M., Devi, S. 2005. Alcoholysis of salicornia oil using free and covalently bound lipase onto chitosan beads. *Food Chemistry* 95:193 – 199. 9. D ’ Souza, S. F. 1999. Immobilized enzymes in bioprocess. *Current Science* 77: 69 – 79. 10. Fuentes, I. E., Viseras, C. A., Ubiali, D., Terreni, M., Alcantara, A. R. 2001. Different phyllosilicates as supports for lipase immobilisation. *Journal of Molecular Catalysis B: Enzymatic* 11: 657 – 663. 11. Fujii, H. 1963. On the formation of mucilage by *Bacillus natto*. Part III. Chemical constitutions of mucilage in natto (1). *Nippon Nogeikagaku Kaishi* 37: 407 – 411. 12. Huo, C. T. 2002. Chap.18: Industrial uses of lipase. In Kuo T. K., Gardner H.W., Kou K. M. (Eds.), *Lipid Biotechnology*, p.387 – 398. Marcel Dekker, New York, USA. 13. Hung, T. C., Giridhar, R., Chiou, S. H., Wu, W. T. 2003. Binary immobilization of *Candida rugosa* lipase on chitosan. *Journal of Molecular Catalysis B: Enzymatic* 26: 69 – 78. 14. Kaewthong, W., Sirisansaneeyskul, S., Prasertsan, P., Kittikum, A. 2005. Continuous production of monoacylglycerols by glycerolysis of palm olein with immobilized lipase. *Process Biochemistry* 40:1525 – 1530. 15. Kaga, H., Siegmund, B., Neufellner, E., Faber, K. 1994. Stabilization of *Candida* lipase against acetaldehyde by adsorption onto Celite. *Biotechnology Techniques* 8:369 – 374. 16. Kamori, M., Hori, T., Yamashita, Y., Hirose, Y., Naoshima, Y. 2000. Immobilization of lipase on a new inorganic ceramics support, toyonite, and the reactivity and enantioselectivity of the immobilized lipase. *Journal of Molecular Catalysis B: Enzymatic* 9:269 – 274. 17. Khare, S. K., Nakajima, M. 2000. Immobilization of *Rhizopus japonicus* lipase on Celite and its application for enrichment of docosahexaenoic acid in soybean oil. *Food Chemistry* 68:153 – 157. 18. Knezevic, Z., Mojovic, L., Adnadjevic, B. 1998. Palm oil hydrolysis by lipase from *Candida cylindracea* immobilization on zeolite type Y. *Enzyme and Microbial Technology* 22: 275 – 280. 19. Jiang, D. S., Long, S. Y., Hung, J., Xiao, H. Y., Zhou, J. Y. 2005. Immobilization of *Pycnoporus sanguineus* laccase on magnetic chitosan microspheres. *Biochemical Engineering Journal* 25: 15 – 23. 20. Jonzo, M. D., Hiol, A., Zagol, I., Druet, D., Comeau, L. C. 2000. Concentrates of DHA from fish oil by selective esterification of cholesterol by immobilized isoforms of lipase from *Candida rugosa*. *Enzyme and Microbial Technology* 27: 443 – 450. 21. Liang, H. F., Chen, C. T., Chen, S. C., Kulkarni, A. K., Chiu, Y. L., Chen, M. C., Sung, H. W. 2006. Paclitaxel-loaded poly(-glutamic acid)-poly(lactide)nanoparticles as targeted drug delivery system for the treatment of liver cancer. *Biomaterials* 27: 2051 – 2059. 22. Moreno, J. M., Hernaiz, M. J., Sanchez-Montero, J. M., Sinisterra, J. V., Bustos, M. T., Sanchez, M. E., Bello, J. F. 1997. Covalent immobilization of pure lipases A and B from *Candida rugosa*. *Journal of Molecular Catalysis B:Enzymatic* 2:177 – 184. 23. Myers, R. H., Montgomery, D. C. 2002. Response surface methodology: process and product optimization using designed experiments, 2nd edition, John Wiley and Sons, New York. 24. Pefferkorn, E., Schmitt, A., Varogui, R. 1982. Helix-coil transition of poly(*L*-glutamic acid) at an interface: correlation with static and dynamic membrane properties. *Biopolymers* 21:1451 – 1463. 25. Peng, L., Xu X., Mu H., Hoy C. E., Adler-Nissen J. 2002. Production of structured phospholipids by lipase-catalyzed acidolysis: optimization using response surface methodology. *Enzyme and Microbial Technology* 31:523 – 532. 26. Pereira, E. B., DeCastro, H. F., DeMoraes, F. F., Zanin, G. M. 2001. Kinetic studies of lipase from *Candida rugosa*. *Applied Biochemistry and Biotechnology* 91 – 93:739 – 752. 27. Pernas, M., Lopez, C., Prada, A., Hermoso, J., Rua, M. L. 2002. Structural basis for the kinetics of *Candida rugosa* Lip1 and Lip3 isoenzymes. *Colloids and Surfaces B: Biointerfaces* 26: 67 – 74. 28. Richard, A., Margaritis, A. 2001. Poly(glutamic acid) for biomedical applications. *Critical Reviews in Biotechnology* 21:219-232. 29. Rodrigues, ?, R., Cabral, J. M., Taipa, M. ?. 2002. Immobilization of *Chromobacterium viscosum* lipase on Eudragit S-100: coupling, characterization and kinetic application in organic and biphasic media. *Enzyme and Microbial technology* 31: 133 – 141. 30. Sanchez, A., Rio, J.

L., Valero, F., Lafuente, J., Faus, I., Sola C. 2000. Continuous enantioselective esterification of trans-2-phenyl- l-cyclohexanol using a new *Candida rugosa* lipase in a packed bed bioreactor. *Journal of Biotechnology* 84: 1 – 12. 31. Shaw, J. F., Wu, H. Z., Shieh, C. J. 2003. Optimized enzymatic synthesis of propylene glycol monolaurate by direct esterification. *Food Chemistry* 81:91 – 96. 32. Shieh, C.J., Chang, S. W. 2001. Optimized synthesis of lipase-catalyzed hexyl acetate in n-hexane by response surface methodology. *Journal of Agricultural and Food Chemistry* 49: 1203 – 1207. 33. Shih, I. L., Van, Y. T. 2001. The production of poly(-glutamic acid) from microorganisms and its various applications. *Bioresource Technology* 79: 207-225. 34. Shih, I. L., Wu, P. J., Shieh, C. J. 2005. Microbial production of a poly(-glutamic acid) derivative by *Bacillus subtilis*. *Process Biochemistry* 40: 2817 – 2832. 35. Siso, M. I. G., Lang, E., Carren-Gomez, B., Becerra, M., Espinar, F. O., M?ndez, J. B. 1997. Enzyme encapsulation on chitosan microbeads. *Process Biochemistry* 32: 211 – 216. 36. Soares, M. F., De-Castro, H. F., Santana, M. H. A., Zanin, G. M. 2001. Selection of stabilizing additive for lipase immobilization on controlled pore silica by factorial design. *Applied Biochemistry and Biotechnology* 91 – 93: 703 – 718. 37. Soares, C. M. F., Santana, M. H. A., Zanin, G. M., De-Castro, H. F. 2003. Covalent coupling method for lipase immobilization on controlled pore silica in the presence of nonenzymatic proteins. *Biotechnology Progress* 19:803 – 807. 38. Thomson, D. 1982. Response surface experimentation. *Journal of Food Processing and Preservation* 6:155 – 188. 39. Villeneuve, P., Muderhwa, J. M., Graille, J., Haas, M. J. 2000. Customizing lipases for biocatalysis: a survey of chemical, physical and molecular biological approaches. *Journal of Molecular Catalysis B: Enzymatic* 9: 113 – 148. 40. Wang, D. L., Nag, A., Lee, G. C., Shaw, J. F. 2002. Factors affecting the resolution of dl-menthol by immobilized lipase-catalyzed esterification in organic solvent. *Journal of Agricultural and Food Chemistry* 50: 262 – 265. 41. Wang, H. X., Wu, H., Ho, C. T., Weng, X. C. 2006. Cocoa butter equivalent from enzymatic interesterification of tea seed oil and fatty acid methyl esters. *Food Chemistry* 97: 661 – 665. 42. Wu, W. H., Akoh, C. C., Phillips, R. S. 1997. Stereoselective acylation of dl-menthol in organic solvent by an immobilized lipase from *Pseudomonas cepacia* with vinyl propionate. *Journal of the American Oil Chemists' Society* 74:435 – 439. 43. Wu, H. S., Tsai, M. J. 2004. Kinetics of tibutyryl hydrolysis by lipase. *Enzyme and Microbial Technology* 35: 488 – 493. 44. Yu, H. W., Chen, H., Yang, Y. Y., Ching, C. B. 2005. Effect of salts on activity, stability and enantioselectivity of *Candida rugosa* lipase in isoctane. *Journal of Molecular Catalysis B: Enzymatic* 35: 28 – 32. 45. Xi, W. W., Xu, J. H. 2005. Preparation of enantiopure (S)-ketoprofen by immobilized *Candida rugosa* lipase in packed bed reactor. *Process Biochemistry* 40:2161 – 2166. 46. Zaks, A., Russell, A. J. 1988. Enzymes in organic solvents: properties and applications. *Journal of Biotechnology* 8: 259 – 270. 47. Zeng, L., Luo, K., Gong, Y. 2006. Preparation and characterization of dendritic composite magnetic particles as a novel enzyme immobilization carrier. *Journal of Molecular Catalysis B: enzymatic* 38: 24 – 30. 48. Zhu, S., Wu, Y., Yu, Z. 2005. Immobilization of *Candida rugosa* lipase on a pH-sensitive support for enantioselective hydrolysis of ketoprofen ester. *Journal of Biotechnology* 116:397 – 401. 49. Zou, Y., Fu, H., Ghosh, S., Farquhar, D., Klostergaard, J. 2004. Antitumor activity of hydrophilic paclitaxel copolymer prodrug using locoregional delivery in human orthotopic non-small cell lung cancer xenograft models. *Clinical Cancer Research* 10: 7382 – 7391.