以胰凝乳蛋白酵素催化二胜?早l生物(N-Ac-Phe-Gly-NH2)合成之最適化研究

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摘 要

二胜?衍生物，N-Ac-Phe-Gly-NH2，具有耐熱之特性，可以在細胞中預防蛋白質因熱產生聚集而造成細胞發炎與病變。本研究針對胰凝乳蛋白酵素(α-chymotrypsin)之固定化反應與其催化合成二胜?衍生物之最適化條件與產率探討。藉由實驗設計方式簡化實驗次數並找出最適的反應條件與反應值。本研究共分成三部份進行探討，分別為：(一)以α-chymotrypsin催化N-Ac-Phe-Gly-NH2合成之最適化條件研究。(二)以四氧化三鐵-幾丁聚醣奈米顆粒(Fe3O4-chitosan nanoparticles)為擔體，探討α-chymotrypsin以共價鍵(covalent binding)之固定化研究。(三)以磁場酵素反應器(magnetic field enzyme reactor)探討固定化酵素催化合成二胜?衍生物之最適化研究。 第一部分(第三章)，在緩衝溶液系統中，利用N-乙醯苯丙胺酸乙酯(N-acetyl-phenylalanine ethyl ester, N-Ac-Phe-OEt)和甘胺醯胺鹽酸鹽(glycinamide hydrochloride, Gly-NH2．HCl)作為基質以α-chymotrypsin進行催化合成。藉由反應曲面法(response surface methodology, RSM)和四變數五階層的中心混成實驗設計(central composite rotatable design, CCRD)來評估反應變數(反應時間、合成溫度、酵素用量及pH?)對於二胜?衍生物產率的影響。結果指出，pH?對於二胜?衍生物產率有非常顯著的影響；藉由脊型分析得知，最優化的合成反應條件在反應時間30.9分鐘，合成溫度35.8 ° C，酵素用量159.2 U及pH 8.98下，所得到預測的產率和實際的產率分別為98.0%和95.1%。第二部份(第四章)，將α-chymotrypsin以共價鍵結方式固定化在四氧化三鐵-幾丁聚醣奈米顆粒進行探討。藉由三階層三變數的Box-Behnken實驗設計方式，探討各時間(0.5-1.5 h)、溫度(25-45 ° C)和pH值(7.0-9.0)彼此之間對固定化酵素活性之影響。研究結果指出，溫度對於固定化酵素之活性影響較為顯著。四氧化三鐵-幾丁聚醣奈米顆粒在酵素固定化之前的平均粒徑為300 nm，然而，固定化後之平均粒徑增加到450 nm。經由之脊型分析結果顯示，在最適條件之溫度21.7 ° C、pH 7.6及時間1.1 h下，可得到較佳的固定化酵素活性分別為353.51 U/g-support (預測值)與346.76±46.51 U/g-support (實驗值)。另外，比較固定化酵素與可溶性酵素催化能力中，固定化酵素催化合成二胜?衍生物可以得到95%以上的產率。在不同pH環境下，固定化酵素比可溶性酵素更能在較廣的pH值範圍下仍具有不錯的催化能力。在使用率方面，固定化酵素經過12次的反應後，仍然具有50%以上的催化能力。自行固定化之酵素在催化能力、pH適應範圍及再使用率上皆有不錯的表現。第三部份(第五章)，在磁場酵素反應器中，以固定化酵素催化合成二胜?衍生物之最適條件與產率進行探討。在此藉由反應曲面法和三變數三階層的Box-Behnken實驗設計來評估反應變數(時間、溫度及pH?)對於二胜?衍生物產率的影響。結果指出，時間與pH?對於二胜?衍生物產率有非常顯著的影響；隨著時間增加或pH值的增加，所得到二胜?衍生物的產率也隨之增加。藉由正則分析(canonical analysis)與脊型分析(ridge of maximum)結果得知，本實驗具有最大反應值，其最優化的合成反應條件為反應時間92.3 min，合成溫度36.2 ° C及pH 8.7下，所得到預測的產率和實際的產率分別為84.22%和82.26±0.39%。 經由本研究三部份的實驗結果，本研究於第一部分(第三章)配合中心混成實驗設計成功找出α-chymotrypsin催化合成二胜?衍生物之最適條件與產率。在二部份(第四章)已具備成熟的酵素固定化技術，也配合實驗設計方式找出最適固定化條件與固定化後最佳酵素活性；並證明了固定化酵素除了具有可重複性利用外，還具備了耐酸性環境之優勢，且固定化後之催化能力與相同單位活性之可溶性酵素相較之下，催化能力並沒有明顯的差別。第三部份(第五章)利用固定化酵素在磁場酵素反應器中，配合Box-Behnken實驗設計，成功的在轉速150 rpm下得到最適合成條件與最佳產率。雖然與可溶性酵素(第三章)所得到之產率上約有10-15%的落差，但是，本章節所使用的固定化酵素可以重複再利用，對於有意想量化生產二胜?衍生物的業者，將可以大幅節省購買酵素的花費；且在產物與酵素在分離上也不需再多增加操作上的步驟。

關鍵詞 : 胰凝乳蛋白酵素、四氧化三鐵-幾丁聚醣奈米顆粒、酵素固定化、磁場酵素反應器、最優化

目錄

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