生物化学工程、制药、食品和天然产物加工

海因酶法制备D-苯丙氨酸的酶催化过程动力学

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收稿日期 2002-12-17 修回日期 2003-8-1 网络版发布日期 2008-9-1 接受日期

摘要 采用自行筛选的兼有海因酶和N-氨甲酰氨基酸水解酶活性的 $Burkhol\,deri\,a\,cepeci\,a\,1003$ 菌种,利用海因酶法大规模制备D-苯丙氨酸,对其中涉及的各过程的动力学参数进行了测定. 结果表明:L-苄基海因的消旋速率常数为3.975×10 $^{-3}$ min $^{-1}$; 海因酶的米氏常数为16.7894 mmol • L $^{-1}$,最大反应速率为0.6127 mmol • L $^{-1}$ • min $^{-1}$; N-氨甲酰氨基酸水解酶的米氏常数为0.82688 mmol • L $^{-1}$,最大反应速率为4.828×10 $^{-4}$ mmol • L $^{-1}$ • min $^{-1}$. 对DL-5-苄基海因的溶解、L-苄基海因的消旋、D-海因的水解开环及其中间产物(N-氨甲酰苯丙氨酸)的水解脱酰氨过程建立了动力学模型,并在此基础上进行了动力学参数显著性分析和优化.结果表明:对于这一级联酶转化反应,D-海因酶的水解反应是快速反应,而N-氨甲酰氨基酸脱氨甲酰的反应速率极小,是该过程的控制步骤. 提高氨甲酰水解酶的活力将有助于提高总体的转化速率,而L-海因的消旋速率则是影响外消旋苄基海因转化率的主要因素.

ENZYMATIC CATALYSIS DYNAMICS OF PREPARATION OF D-PHENYLALANINE

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Abstract

The *Burkholderia cepecia* 1003 screened by the authors' laboratory, which contains hydantoinase and *N*-carbamoylase activities, was used to prepare D-phenylalanine on a large scale. The dynamic parameters of the whole bioconversion process were measured, and the results showed that $k_{\rm r}$ was $3.975\times10^{-3}{\rm min}^{-1}$, $K_{\rm m}$ and $r_{\rm m}$ of hydantoinase and *N*-carbamoylase were 16.7894 mmol•L⁻¹, 0.82688 mmol•L⁻¹, 0.6127 mmol•L⁻¹•min⁻¹ and $4.828\times10^{-4}{\rm mmol}$ •L⁻¹•min⁻¹, respectively.Simulation was made including processes of dissolution, racemization, hydrolysis of D-BH and hydrolysis of *N*-carbamyl phenylalanine.The significance of parameters in this model was investigated and these parameters were optimized. The result showed that the reaction rate of D-BH hydrolysis was higher than that of *N*-carbamyl phenylalanine hydrolysis, the latter was the limiting step of the whole process. Promotion of *N*-carbamoylase activity was helpful to D-phenylalanine production. Another result was that the rate of L-BH racemization was the main factor, which influenced the conversion of racemic BH.

Key words hydantoinase D-phenylalanine enzymatic catalysis dynamics

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