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研究论文

羰基还原酶辅酶结合域位点突变对其不对称催化性能的影响

宫绪敏 1, 聂尧 1, 徐岩 1,*,肖荣 2, Gaetano T. MONTELIONE 2

1江南大学生物工程学院, 工业生物技术教育部重点实验室, 江苏无锡 214122; 2罗格斯大学高级生物技术与医学中心, 新泽西州 08854, 美国

GONG Xumin1, NIE Yao1, XU Yan1,*, XIAO Rong2, Gaetano T. MONTELIONE2

1School of Biotechnology, Key Laboratory of Industrial Biotechnology of Ministry of Education, Jiangnan University, Wuxi 214122, China; 2The Center fo Advanced Biotechnology and Medicine, Rutgers University, New Jersey 08854, USA

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摘要 基于同源模型的比较和分析, 发现羰基还原酶 SCR1 辅酶结合域 P124 和 W125 位点对辅酶 NADPH 的结合形成了一定的空间位阻效应. 通过对该位点进行小侧基氨基酸的取代突变, 该酶的底物专一性和立体选择性均发生了不同程度的改变, 表明该位点是酶与辅酶有效结合的关键位点, 而且它与辅酶结合的空间效应进一步影响了底物结合域活性中心对不同构型的底物及其对映体产物的亲和作用. 在底物专一性方面, 野生型酶对 2-羟基苯乙酮和 2-溴苯乙酮及其衍生物等底物表现出较高的催化活性, 而突变株 W125A, W125G, P124A/W125A 和 P124G/W125G 对苯乙酮及其部分衍生物和 2-辛酮等底物的催化活性均有所提高. 对于酶的立体选择性, 部分突变株发生了转化产物对映体构型反转的现象, 突变株 P124A/W125A 和 P124G/W125G 催化还原 2-羟基苯乙酮和 4-氯乙酰乙酸乙酯均生成了 (R)-型产物.

关键词: 羰基还原酶 定点突变 催化活性 底物特异性 立体选择性

Abstract: Based on homology modeling analysis, the sites, P124 and W125, in cofactor-binding domain of the carbonyl reductase SCR1 were found to have a steric effect on the binding of NADPH. The site-directed mutagenesis of these two sites using the amino acid residues with small side group showed that the substrate sepcificity and stereoselectivity of the enzyme were both changed in some level, indicating that these sites have a critical role in binding the cofactor, and the configuration of catalytic active center formed from enzyme/cofactor complex determines the recognition of the enzyme to substrate of different type and also the product of different stereo-configuration. Regarding the substrate specificity, the wild-type enzyme showed activity toward 2-hydroxyacetophenone, 2-bromoacetophenone and its derivatives, while the catalytic efficiency of mutants toward acetophenone and its derivatives and 2-octanone was enhanced. On the other hand, the stereospecificity of some mutants was even found to be inverted. The mutants P124A/W125A and P124G/W125G exhibited a shift of enantioselectivity toward 2-hydroxyacetophenone and ethyl 4-chloro-3-oxobutanoate to give the product in (R)-configuration.

Keywords: carbonyl reductase, site-directed mutagenesis, catalytic activity, substrate specificity, stereoselectivity

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