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细胞色素P450 BM-3羟基化吲哚能力的半理性改造

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摘要

为进一步改造细胞色素P450 BM-3酶对吲哚的羟基化能力，以P450 BM-3结构与功能关系的推测为指导，选择突变酶P450 BM-3 （A74G/F87V/L188Q/E435T）为父本，在可能影响P450 BM-3催化吲哚羟基化区域选择性的D168位点进行定点饱和突变，根据全细胞催化产物颜色及组成进行筛选，得到了产物组成、酶动力学性质与父本不同的两个突变酶。突变酶D168W的吲哚羟基化产物中90%是靛玉红，而另一个突变酶D168R的产物中87%是靛蓝，产物组成均不同于亲本。在催化吲哚羟基化时，D168W的k_{cat}与父本相当，但K_m却是父本的4.8倍，催化活力只有父本的20%；而D168R的k_{cat}是父本的1.9倍，K_m是父本的82%，催化活力比父本提高了1.37倍。结果表明，在E435T突变上叠加D168位氨基酸残基突变对酶的催化性质产生了单一位点突变所不具有的协同效应，对酶催化的区域选择性和催化活力都有显著影响，以致改变了催化产物组成。这种基于知识的半理性定向进化方法由于是在关键位点进行突变，因此突变目的性强、突变效果显著。

关键词

细胞色素P450 BM-3 定向进化 饱和定点突变

分类号

Semi-rational directed evolution in improving indole-hydroxylation ability of cytochrome P450 BM-3

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Abstract

Guided by postulations in the relationship between structure and functionality, a saturation mutagenesis was performed on the D168 position of a P450 BM-3 (A74G/F87V/L188Q/E435T) mutant which originally transformed indole largely into indigo. Two novel mutants have been obtained based on the differences in color and absorption spectrum of the indole-hydroxylation products followed by product composition analysis using HPLC. One mutant, P450 BM-3 (A74G/F87V/L188Q/E435T/D168W) catalyzes indole into indirubin in 90% with a decrease of k_{cat}/K_m in 80%, mainly owing to its K_m is increased by 4.8 times as compared with the parent. The other one, P450 BM-3 (A74G/F87V/L188Q/E435T/D168R) transforms indole into indigo in about 87%, which is higher than the parent and its k_{cat}/K_m has an increase in over 1.37 times as that of the parent. Such results indicate that the amino acid residue substitution on the D168 position combined with the mutation of E435T can influence the hydroxylation regioselectivity of P450 BM-3 as well as catalytic activity, which determines indole-hydroxylation product as indigo or indirubin. Such influence could not be caused by the mutation on a single amino acid residue site, neither E435 nor D168, because of the lack in some synergistic effect. In conclusion, the directed evolution guided by rational hypothesis for engineering the catalytic properties of P450 BM-3 has succeeded in getting two mutants exhibiting novel characteristics for indole-hydroxylation.

Key words

cytochrome P450 BM-3 directed evolution saturation mutagenesis

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