

电喷雾质谱法研究细胞色素**Tb5**及其**F35Y**突变体蛋白 肽链和辅基的相互作用

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摘要 本文通过牛肝线粒体细胞色素**Tb5**和它的**F35Y**突变体蛋白相对分子质量的外标法测定,得到细胞色素**Tb5**全蛋白的相对分子质量为10077.5,脱辅基蛋白的相对分子质量为

9461.4,**F35Y**突变体蛋白的相对分子质量为10093.6, 它的脱辅基蛋白的相对分子质量为9477.5,

不同nozzle电压下的电喷雾质谱结果表明, 该电压的大小明显影响蛋白肽链与血红素辅基之间的非共价结合, 随着电压的降低, 全蛋白谱峰强度逐渐增大, 然而,

过低的电压导致了 Na^+ , K^+ 离子加合峰相对强度的增加, 而不利于谱图分析。同时, 考察到细胞色素**Tb5**

在甲醇溶液和酸性溶液中的变性行为, 因此选择nozzle电压70V,10%的甲醇水溶液和pH=7

为得到全蛋白质谱峰的最佳条件。相同实验条件下得到的野生型Cyt**Tb5**和**F35Y**突变体全蛋白的质谱峰相比较, 其相对丰度有悬殊的差异,

表明**F35Y**突变体蛋白的血红素结合能力明显低于野生型蛋白。通过解离出的Heme**b**的分子离子峰进行解析, 证明铁仍以三价离子存在于血红素辅基中。

关键词 [电喷雾](#) [质谱法](#) [细胞色素](#) [突变体](#) [蛋白质](#) [非共价结合](#) [血红素](#) [相互作用](#) [线粒体](#)

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Studies on the interaction of polypeptide of cytochrome **Tb5** and its **F35Y** mutant with heme **b** by electrospray ionization mass spectrometry

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Abstract Cytochrome **Tb5** from bovine liver mitochondria and its **F35Y** mutant, digested from cyt **b5** membrane protein by trypsin, is a kind of electron transfer protein, which consists of 82 amino acid residues. The molecular weight of the **F35Y** mutant, 9 477.5, is obtained by ESI/TOFMS in external standard calibration method. Compared with molecular weight of the cyt **Tb5**, 9 461, this data is exactly coincided with the calculated value according to the mutant amino acid sequence. For studying the stability of heme binding to the **F35Y** mutant and cyt **Tb5**, a series of experimental conditions were selected such as nozzle potential, organic solvent, pH value, etc. It was found that the abundance of holoprotein content measured in the mass spectrum decreased with increase of the nozzle potential or/and increase of the composition of organic solvent of /and pH value deviated from neutral. Relatively, the holoprotein's mass abundance of cyt **Tb5** is higher than that of **F35Y** mutant that indicates stronger binding of heme **b** to polypeptide chain in the wild type cyt **Tb5**. However, we found that if the nozzle potential was too low it would produce more metal plus ion peaks, suppressing the sample's signals of mass spectrum, and meanwhile increasing organic solvent would reduce stability of the holoprotein. So, the optimal conditions chosen are those: the composition of methanol is 10% and the nozzle potential is 70V. In addition, it is verified that the heme **b** dissociated from the **F35Y** mutant is a Fe(III) porphyrin by means of internal standard method.

Key words [MASS SPECTROGRAPHY](#) [CYTOCHROME](#) [MUTANT](#) [PROTEIN](#) [HEME](#) [INTERACTIONS](#) [MITOCHONDRIA](#)

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