

研究论文

细菌视紫红质多重突变体结构变化及其中间态的寿命

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摘要 采用基因定点突变的方法, 构建了细菌视紫红质(Bacteriorhodopsin, BR)的3种突变体蛋白, 即单突变体BR_{E194Q}、三突变体BR_{I119T/T121S/A126T}和四突变体BR_{I119T/T121S/A126T/E194Q}. 测定了突变体和野生型BR在水溶液和聚乙烯醇(PVA)膜中的紫外-可见吸收光谱和拉曼光谱, 采用显微视频录像技术记录了PVA膜中野生型和3个突变体样品的M态寿命. 与野生型BR相比较, 在水溶液中, 单突变体的可见吸收光谱的最大吸收峰发生了轻微红移, 三突变体和四突变体的最大吸收峰则分别发生了11.0和12.0 nm的明显蓝移. 在PVA膜中, 3个突变体BR的可见吸收光谱的最大吸收峰均发生蓝移, 四突变体BR的最大吸收峰为557 nm, 蓝移达15.0 nm. 四突变体BR在水溶液中的共振拉曼光谱不仅表现有与M态特征相关的1567和1573 cm⁻¹谱带, 还有L态特征带1334 cm⁻¹及N态特征带1200, 1328, 1530和1549 cm⁻¹. 在PVA膜中的样品与在水溶液中的比较, 四突变体共振拉曼光谱的1334和1549 cm⁻¹带消失, 同时1187 cm⁻¹带的强度下降. 显微视频录像技术记录的PVA膜中样品的M态寿命表明, 野生型BR的M态寿命最短, 单突变体的M态寿命小于1.0 s, 三突变体的寿命为3.0 s, 四突变体的寿命为2.0 s.

关键词 细菌视紫红质 突变体 共振拉曼光谱 聚乙烯醇

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Structure Change and Intermediate's Lifetime of Bacteriorhodopsin and Its Multipoint Mutants

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Abstract The site-specific residue replacements E194Q, I119T/T121S/A126T and I119T/T121S/A126T/E194Q were introduced into *bop* gene and expressed in *halobacterium salinarum* L₃₃ with the vector pXL-NovR, respectively. The photo-reaction of three mutants BR_{E194Q}, BR_{I119T/T121S/A126T} and BR_{I119T/T121S/A126T/E194Q} (the quadruple mutant) of bacteriorhodopsin (BR) and WT-BR (wild type BR) was investigated in solution and in PVA films at pH=7.0 by UV-Vis absorption spectrum and Resonance Raman spectra. The lifetime of M intermediate of the three mutants in PVA films were recorded by employing photomicrography. In distilled water, the visible absorption maximum of BR_{I119T/T121S/A126T} and BR_{I119T/T121S/A126T/E194Q} mutants were blue shifted notably by 11 and 12 nm respectively and the BR_{E194Q}'s was weakly red shifted in comparison with WT-BR. In PVA film, BR_{I119T/T121S/A126T/E194Q} mutant caused a blue shift of the visible absorption spectrum from 568 to 557 nm. The characteristic bands of Resonance Raman spectra of BR_{I119T/T121S/A126T/E194Q} mutant were observed at 1334 cm⁻¹ of the L intermediate, 1200, 1328, 1530 and 1549 cm⁻¹ of the N intermediate and 1567 and 1573 cm⁻¹ of M intermediate in solution. However, the 1334 and 1549 cm⁻¹ bands of the quadruple mutant disappeared

and the intensity of 1187 cm^{-1} band decreased in PVA film. The lifetime of M intermediate of BR_{E194Q} was less than 1.0 s by photomicrography. The lifetime of M intermediates of $\text{BR}_{\text{I119T/T121S/A126T}}$ and $\text{BR}_{\text{I119T/T121S/A126T/E194Q}}$ were 3.0 s and 2.0 s, respectively.

Key words [Bacterorhodopsin\(BR\)](#) [Mutant](#) [Resonance Raman spectrum](#) [PVA](#)

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