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研究论文

水稻谷胱甘肽磷脂氢过氧化物酶的表达、纯化及晶体生长条件初筛

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摘要 将水稻PHGPx(*OsPHGPx*)的编码序列克隆到表达载体pGEX-6P-1上, 并转化为大肠杆菌进行表达. 通过GST亲和层析、离子交换层析和凝胶过滤层析, 制备了可用于晶体学研究的OsPHGPx, 其纯度超过95%, 具备明显的PHGPx活性. 质谱显示OsPHGPx的精确分子量为19642.5553, 与理论分子量基本一致. OsPHGPx在多个晶体生长条件下出现微晶. 三维结构同源建模显示 OsPHGPx的结构为硫氧还蛋白折叠形式.

关键词 水稻 谷胱甘肽磷脂氢过氧化物酶 表达 纯化 晶体生长 三维结构建模

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Expression, Purification and Crystal Growing Conditions of Recombinant *Oryza sativa* PHGPx

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Abstract Phospholipid hydroperoxide glutathione peroxidase(PHGPx) is a unique antioxidant enzyme that directly reduces lipid hydroperoxides in biomembranes. It plays potential important roles in oxidative stress response. *Oryza sativa* PHGPx gene was cloned into expression vector pGEX-6P-1 and transformed into *E.coli* strain BL21(DE3). OsPHGPx for crystals was prepared with employing Glutathione Sepharose^{TM} affinity, cation-exchange and gel filtration chromatography. The purity of the purified OsPHGPx was over 95%. OsPHGPx showed an obvious PHGPx activity towards lipid hydroperoxides. MALDI-TOF analysis shows that the exact molecular weight of OsPHGPx was 19275.568, which was in accordance with the theoretical molecular weight. The microcrystals of OsPHGPx were obtained under several conditions. In addition, a tertiary structure model of the OsPHGPx generated from http://swissmodel.expasv.org/ displayed the thioredoxin fold.

Key words *Oryza sativa* Phospholipid hydroperoxide glutathione peroxidase Expression Purification Microcrystal growth of OsPHGPx 3D structure modeling

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