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基础医学

磷酸酶STEP的Q-loop中T541参与催化反应的机制

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摘要：

目的 研究纹状体蛋白质酪氨酸磷酸酶(STEP)pY-loop结构上第330位的苏氨酸(T330)和Q-loop结构上第541位的苏氨酸(T541)参与催化反应的作用机制。方法 构建STEP野生型(STEP-WT)及其突变体(STEP-T330D/T541A)的表达质粒；表达并纯化STEP-WT及其突变体蛋白，体外检测这些蛋白对小分子底物4-硝基苯磷酸二钠(pNPP)的催化活力，分析NaVO3对STEP-WT及其突变体酶活性的抑制作用；检测STEP-WT及其突变体催化反应的pH依赖性和对解离基团pKa的依赖性。结果 体外催化pNPP水解的过程中，STEP-T330D的催化性质较STEP-WT无明显变化，STEP-T541A的Km略有增加，kcat下降至STEP-WT的1/3以下。NaVO3对于STEP-WT及其突变体的抑制常数Ki无明显变化。在STEP的pH依赖性研究中，STEP-T541A的pK2app显著增加且它的(kcat)lim下降至野生型1/10以下。在STEP催化底物反应过程对底物解离基团pKa依赖性的研究中，STEP-T541A的β1g(kcat)较STEP-WT明显增大。结论 T541参与了STEP催化反应中从产物生成到磷酸根释放这一过程，靶向STEP治疗神经系统疾病的药物可以考虑通过与T541相互作用进行设计。

关键词： 蛋白质酪氨酸磷酸酶；纹状体蛋白质酪氨酸磷酸酶；纹状体；中枢神经系统；蛋白磷酸化

T541 in Q-loop of STEP plays a key role in the catalytical activity

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Abstract:

Objective To explore the essential role of Threonine at position 541 and 330(T541，T330)in the intrinsic phosphatase activity of striatal-enriched protein tyrosine phosphatase(STEP). Methods STEP wild type(STEP-WT) and its mutants STEP-T330D/T541A were sub-cloned into the PET15b vector. Expression and purification of STEP-WT and its mutants were performed by affinity column and liquid chromatography. The phosphatase activity was measured in vitro with 4 nitrophenyl phosphate (pNPP) as substrate. The inhibition by NaVO3 was measured to monitor the effects of mutants on protein folding. The pH-dependence and leaving-group pKa dependence of STEP catalysis were carried out to dissect the underlying molecular mechanism. Results STEP-WT and STEP-T330D displayed similar catalytic ability toward pNPP at pH 7.0. The kcat of STEP-T541A decreased 3 folds compared to STEP-WT.-STEP-WT and the two mutants had similar Ki for NaVO3. Examination of the kcat versus pH curve revealed that pK2app of STEP-T541A significantly increased and the (kcat)lim dropped by at least 10 folds. In consisitent with these observations, β1g(kcat) of STEP-T541A increased significantly. Conclusion T541 plays an important role in STEP catalysis, by participating the

processes from product formation to phosphate release. Future drugs targeting to STEP for therapeutic usage could be developed through modulating T541 conformations.

Keywords: Prtotein tyrosine phosphatase; Striatal enriched protein tyrosine phosphatase; Striatal; Central nervous system; Protein phosphorylation

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