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论著

山萘黄素是一种有效的体外重组人蛋白激酶CK2的抑制剂

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摘要 目的 为了筛选蛋白激酶CK2的抑制剂，观察山萘黄素对重组人蛋白激酶CK2的抑制效果及其酶动力学机制。方法 利用基因工程技术进行克隆、表达和纯化，获得重组人CK2的α′及β亚基在体外等摩尔混合构成CK2全酶，通过测定转移到CK2底物上的［γ-^{32}P］ ATP的^{32}P的放射性活性来检测CK2的活性。向反应体系中加入不同浓度的山萘黄素，观察其对CK2的抑制效果；通过固定酪蛋白浓度为2 g·L^{-1}, ATP浓度为10, 20, 40和80 μmol·L^{-1}或固定ATP的浓度为10 μmol·L^{-1}，改变酪蛋白浓度(1, 2, 4和8 g·L^{-1})，观察其酶动力学机制。结果 山萘黄素能显著抑制重组人蛋白激酶CK2的活性（IC_{50}=1.9 μmol·L^{-1}）。抑制作用强于已知的CK2抑制剂白杨素、桑色素和金雀异黄素。酶动力学分析表明，山萘黄素与ATP（K_i=1.1 μmol·L^{-1}）及酪蛋白（K_i=3.1 μmol·L^{-1}）均呈非竞争性抑制CK2的活性。初步的化合物结构分析表明，2′和3位上的羟基对山萘黄素及芹黄素发挥其抑制效果产生实质性的负面影响。结论 山萘黄素是一种新的体外蛋白激酶CK2的有效抑制剂。黄酮类CK2的抑制剂可能通过不同的位点作用于CK2，这种作用主要取决于其羟基的数目和位置。

关键词 酪蛋白类 蛋白激酶类 重组蛋白质类 山萘黄素 动力学

分类号 R963

Kaempferol is a potent inhibitor of recombinant human protein kinase CK2 holoenzyme *in vitro*

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Abstract

AIM In order to search inhibitors of protein kinase CK2, we observed the inhibitory effects of kaempferol on recombinant human protein kinase CK2 holoenzyme and its kinetics *in vitro*. **METHODS** Cloning, prokaryotic expression and purification of human protein kinase CK2 α′ and β subunits by gene engineering, the two subunits were mixed at equal molar ratio to reconstitute CK2 holoenzyme and identify its biological properties. The CK2 activity was assayed by detecting incorporation of ^{32}P of ［γ-^{32}P］ATP into the substrate. The inhibitory effect of kaempferol on CK2 was assayed in the presence of different concentrations of kaempferol. Kinetic analysis of kaempferol-induced inhibition was carried out in the condition that casein concentration was fixed at 2 g·L^{-1} and ATP was changed at various concentrations (10, 20, 40, 80 μmol·L^{-1}), or ATP was fixed at 10 μmol·L^{-1} and casein was changed at different concentrations (1, 2, 4, 8 g·L^{-1}). **RESULTS** Kaempferol was shown to strongly inhibit the holoenzyme activity of recombinant human protein kinase CK2 with IC_{50} of 1.9 μmol·L^{-1}, which was more effective than chrysin, morin and genistein which are both known as CK2 special inhibitors. Kinetic studies of kaempferol on recombinant human CK2 showed that kaempferol acted as a noncompetitive inhibitor with substrate ATP(K_i=1.1 μmol·L^{-1}) and casein (K_i=3.1 μmol·L^{-1}).

CONCLUSION Kaempferol is a novel potent inhibitor of protein kinase CK2 *in vitro*. Discussions indicate that flavonoid inhibitors of CK2 may adopt different orientations in the active site of CK2 and that these are determined by the number and position of their hydroxyl groups.

Key words caseins protein kinases recombinant proteins kaempferol kinetics

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