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论著

酪氨酸蛋白激酶抑制剂Tyrphostin AG114对重组人蛋白激酶CK2全酶的抑制作用

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摘要 目的 为了观察Tyrphostin AG114对重组人蛋白激酶CK2全酶的直接作用及其酶动力学机理。方法 利用基因工程克隆，表达和纯化获得重组人蛋白激酶CK2 α和β亚基，在体外等摩尔数混合构成有最大生物活性的重组CK2全酶，在不同条件下测定CK2的活性。CK2活性通过测定转移到CK2底物上的［γ-^{32}P］ATP或［γ-^{32}P］GTP的［^{32}P］放射活度来检测。结果 重组人CK2是一种Ca^{2+}、cAMP和cGMP等第二信使非依赖性蛋白激酶，与天然CK2的性质一致。AG114对重组人CK2全酶具有很强的抑制作用，IC_{50}为20.8 μmol·L^{-1}，抑制强度介于已知CK2抑制剂5，6-二氯-1-β-呋喃糖苯并咪唑(DRB)和*N*-(2-氨乙基)-5-氯萘-1-硫胺(A3)之间。AG114对重组人CK2的动力学研究表明：它与GTP呈混合竞争性抑制作用，与酪蛋白呈非竞争性抑制作用。结论 AG114不仅是酪氨酸蛋白激酶的抑制剂，而且是一种十分有效的蛋白激酶CK2的抑制剂。重组人蛋白激酶CK2可作为一种较为简便地筛选和开发有效的CK2抑制剂的分子靶点。

关键词 蛋白激酶 重组蛋白质类 全酶 Tyrphostins AG114 动力学 酶学

分类号 Q555.7, R963

Inhibitory effect of tyrphostin AG114 on recombinant human protein kinase CK2 holoenzyme

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Abstract

AIM To study the direct effect of tyrphostin AG114 on recombinant human protein kinase CK2 holoenzyme and its kinetics. **METHODS** [Recombinant human protein kinase CK2 α and β subunits were cloned and expressed by genetic engineering, and purified to homogene
ity. The two subunits were mixed at equal molar ratio and reconstituted CK2 holoenzyme, which exerted the maximum biological activity. The CK2 activity was as sayed by detecting incorporation of ^{32}P of ［γ-^{32}P］ATP or ［γ-^{32}P］GTP into the substrate in various conditions. **RESULTS** The recombinant human CK2 was a second messenger (Ca^{2+}, cAMP and cGMP) independent protein kinase, the characterization and function of the reconstituted holoenzyme were consistent with those of native CK2. AG114 strongly inhibited the holoenzyme activity of recombinant human protein kinase CK2 with an IC_{50} of 20.8 μmol·L^{-1}, which lay between IC_{50} of 5,6-dichloro-1-β-*D*-ribofuranosyl-benzimidazole(DRB) and *N*-(2-aminoethyl)-5-chloronaphthalene-1-sulfonamide(A3), known as CK2 special inhibitors. Kinetic studies of AG114 inhibition on recombinant human CK2 showed that the inhibition was mixed competitive with GTP and non-competitive with casein. **CONCLUSION** AG114 not only is an effective inhibitor of protein tyrosine kinases, but also is a novel potent inhibitor of protein kinase CK2. The recombinant human protein kinase CK2 might be used as a molecular target for simpler screening method and development of more effective inhibitors of CK2.

Key words protein kinase recombinant proteins holoenzyme tyrphostin AG114 kinetics enzymology

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