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Action of 3 tyrphostin derivatives on casein kinase Ⅱ from rat liver

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KEY WORDS tyrphostin; caseins; protein kinases; liver; enzyme inhibitors; kinetics

AIM: To study the action of tyrphostin on casein kinase (CK) Ⅱ. **METHODS**: CK Ⅱ was partially purified from rat livers by sequential DE52 and heparin-Sepharose chromatography. CK Ⅱ activity was assayed by incubating CK Ⅱ with dephosphorylated casein and [γ-^{32}P]ATP. **RESULTS**: AG34 inhibited the activity of CK Ⅱ with IC_{50} 33 (27 - 41) $\mu mol \cdot L^{-1}$. Both AG372 (121 $\mu mol \cdot L^{-1}$) and AG1112 (150 $\mu mol \cdot L^{-1}$) displayed inhibitory effects on the activity of CK Ⅱ. Kinetic studies of AG34 on CK Ⅱ showed that it was noncompetitive with casein and ATP. **CONCLUSION**: AG34, AG372, and AG1112 were potent inhibitors of CK Ⅱ, and the inhibitory action of AG34 was noncompetitive with casein and ATP.

Casein kinase (CK) Ⅱ is a multifunctional protein serine/threonine kinase ubiquitously distributed in the cytosolic, nuclear, mitochondrial and membranous fractions of eukaryotic cells. CK Ⅱ purified from various tissues is usually a tetrameric complex with $\alpha_2\beta_2$, $\alpha\alpha'\beta_2$, or $\alpha'_2\beta_2$ structures[1,2]. CK Ⅱ may play an important role in the physiological regulation of nuclear proteins related to oncogenic transformation and cell proliferation[3-5]. For example, CK Ⅱ phosphorylates a number of nuclear proteins including *Fos*, *Myb*, *Myc*, p53 tumor suppressor proteins.

Tyrphostins are synthetic compounds which are potent and selective tyrosine kinase (TK) blockers. They can serve as antiproliferative agents and molecular tools to investigate signal transduction pathways of TK[6-8]. It was suggested that some tyrphostin derivatives (ie, AG213) which are potent blockers of EGF receptor kinase and other TK possess an antiproliferative effect that may not be related to their inhibitory activity on EGF receptor kinase[8]. Some of them may serve as a new class of topoisomerase I inhibitors[9], and have been proposed to be the potent inhibitors of CK Ⅱ in our previous study[10]. But their mechanisms of action on CK Ⅱ are unknown. In the present study, the effects of 3 tyrphostin derivatives (AG34, AG372, and AG1112) on the activity of CK Ⅱ from rat livers were investigated.

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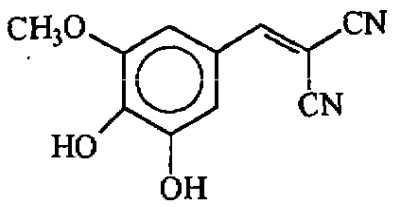
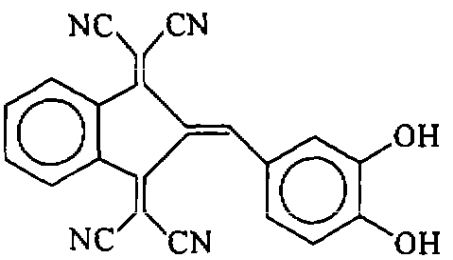
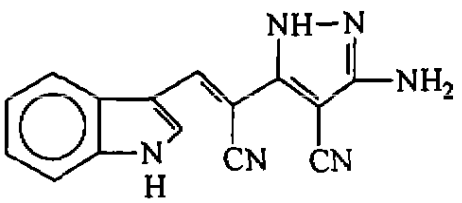


3-Methoxyl-5-(2,2-dicyanoethenyl)catechol (AG34)



1,3-Bis(dicyanomethenyl)-2-(3,4-dihydroxyphenyl)-methenylindane (AG372)



3-{2-[(3-Amino-4-cyano)pyrazol-5-yl]-2-cyano}-ethenylindole (AG1112)

MATERIALS AND METHODS

Heparin and phosphatidylserine (PS) (Sigma); ATP (Boehringer Mannhein); Sepharose 4B (Pharmacia); CNBr (Fluka); DEAE-cellulose (DE52) (Whatman); Casein (ICN); [γ-^{32}P]ATP (370 $GBq \cdot L^{-1}$, >185 $GBq \cdot mmol^{-1}$) was purchased from Yahui Biomedical Technology Co Ltd,

Beijing. All other chemicals were AR.

Tyrphostins (AG34, AG372, AG1112) (purities 99 %, Department of Organic Chemistry, The Hebrew University of Jerusalem, Israel) were derived from benzenemalonitrile and synthesized[6,7].

Two SD rats (♀, 2-month-old, weighing 250 ± 10 g) were obtained from the Animal Center of Guangdong Medical College.

Extraction and partial purification of CK Ⅱ[10].

CK Ⅱ activity assay The CK Ⅱ was assayed as described in our previous study[10], but the concentrations of [γ-^{32}P] ATP (7.4 GBq · mol^{-1}) and partially dephosphorylated casein were at indicated condition.

Kinetic analysis of AG34 on activity of CK Ⅱ When [γ-^{32}P]ATP was fixed (50 μmol·L^{-1}) and the concentrations of casein varied from 0.5 to 5 g·L^{-1}, or when casein was fixed (2 g·L^{-1}) and the concentrations of [γ-^{32}P]ATP varied from 12.5 to 100 μmol·L^{-1}, the activity of CK Ⅱ was assayed at 3 concentrations of AG34 (0, 20.8, and 41.6 μmol·L^{-1}). Lineweaver-Burk plot was used[11].

Statistical significance was analyzed by t test.

RESULTS

AG34 inhibited the activity of CK Ⅱ with IC_{50} 33 (27 − 41 μmol·L^{-1}). At 185 μmol·L^{-1}, the inhibitory rate of AG34 on the activity of CK Ⅱ was 83.2 % (Fig 1).

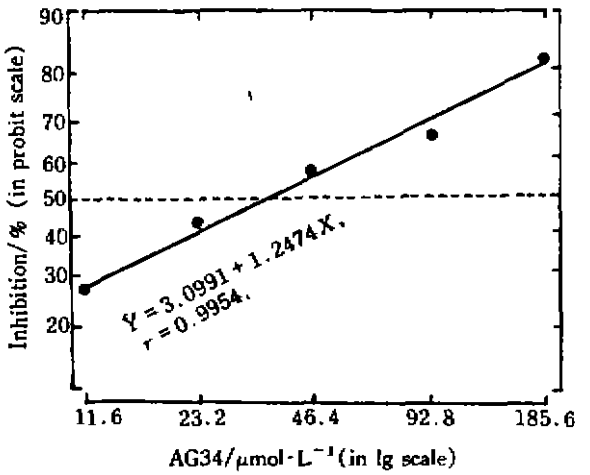


Fig 1. Effects of AG34 on the activity of CK Ⅱ from rat liver. $n = 3$, $\bar{x} \pm s$.

AG372 (121 μmol·L^{-1}) and AG1112 (150 μmol·L^{-1}) strongly inhibited the activity of CK Ⅱ (Tab 1).

The inhibition by AG34 on casein and ATP was noncompetitive (Fig 2).

Tab 1. Effects of tyrphostins on activity of CK Ⅱ from rat liver. $n = 3$, $\bar{x} \pm s$. $^cP < 0.01$ vs control.

	CK Ⅱ activity /Bq	Inhibition /%
Control	601 ± 16	-
AG34 (185 μmol·L^{-1})	100 ± 6^c	83.2
AG372 (121 μmol·L^{-1})	89 ± 4^c	85.2
AG1112 (150 μmol·L^{-1})	225 ± 59^c	62.5

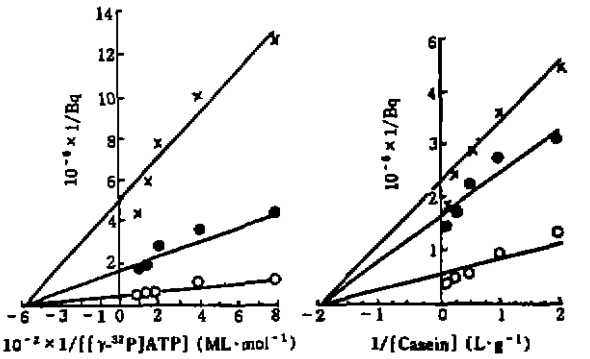


Fig 2. Lineweaver-Burk plot for AG34 on CK Ⅱ from rat liver. $n = 3$. AG34 0 μmol·L^{-1}(○), 20.8 μmol·L^{-1} (●), 41.6 μmol·L^{-1}(×).

DISCUSSION

Tyrphostins are a series of synthetic chemicals, which have been proved to inhibit tyrosine kinase activity[6-8]. In the present study, we demonstrated that AG34, AG3272, and AG1112 strongly inhibited the activity of CK Ⅱ, and the inhibition of AG34 on CK Ⅱ was noncompetitive with casein and ATP. The results provide further support for the conclusion, which has been made in our previous study[10], that some tyrphostins might not be specific tyrosine kinase inhibitors, and might be the potent inhibitors of CK Ⅱ.

CK Ⅱ is a cyclic nucleotide and calcium-independent serine/threonine-specific protein kinase. The physiological substrates of CK Ⅱ include metabolic enzymes, cytoskeletal proteins, transcription factors, as well as products of several oncogenes and tumor suppressor[1,3-5]. CK Ⅱ was stimulated in response to various growth factors in cultured cells[12-14]. These findings suggest that CK Ⅱ may occupy a key site in cell

proliferation. A recent report[9] shows that some tyrphostins are very potent inhibitors of topoisomerase I which is involved in DNA replication and transcription. In our previous study[10] and present work, we found that some tyrphostins inhibited CK Ⅱ activity. These suggest that the antiproliferative effect of some tyrphostin derivatives is due to their effect on TK, topoisomerase I and/or casein kinase Ⅱ activities. However, whether a particular tyrphostin actually works by inhibiting a TK, topoisomerase I or CK Ⅱ activity needs to be established for each compound.

In the review[8], Levitzki pointed out that the inhibition of tyrphostins on TK may either be competitive with ATP, or with ATP and its special substrate, slight modification on the structure of tyrphostins can transform the kinetic behavior to be competitive with the substrate and noncompetitive or mixed competitive with ATP. Our kinetic studies of AG34 on CK Ⅱ shows that its inhibition on CK Ⅱ is noncompetitive with casein and ATP. But kinetic studies of other tyrphostin derivatives on CK Ⅱ are not investigated. Whether will tyrphostins exhibit the same kinetic behavior on CK Ⅱ as those on TK? It is a problem worthy of being studied further.

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三种 tyrphostin 对大鼠肝酪蛋白激酶Ⅱ的作用

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关键词 tyrphostin；酪蛋白类；蛋白激酶类；肝；酶抑制剂；动力学

目的：研究 tyrphostin 对酪蛋白激酶Ⅱ（CK Ⅱ）的作用。**方法**：依次采用 DEAE-纤维素和肝素-Sepharose 层析将大鼠肝 CK Ⅱ进行了部分纯化，通过将去磷酸化的酪蛋白和[γ-^{32}P]ATP 与 CK Ⅱ保温的方法测定 CK Ⅱ的活性。**结果**：AG34 对 CK Ⅱ有强烈的抑制作用 IC_{50} 33 $\mu mol \cdot L^{-1}$(27 - 41 $\mu mol \cdot L^{-1}$)，AG372 (121 $\mu mol \cdot L^{-1}$) 和 AG1112 (150 $\mu mol \cdot L^{-1}$)对 CK Ⅱ有明显的抑制作用。AG34 对 CK Ⅱ的动力学研究表明：它与酪蛋白和 ATP 均呈非竞争性抑制作用。**结论**：AG34、AG372 和 AG1112 是 CK Ⅱ的抑制剂，AG34 的抑制作用与酪蛋白和 ATP 均呈非竞争性。