

与 PDI 相同, PPI 也不能决定蛋白质的折叠途径.

体内催化 Pro 顺-反异构物质主要是 PPI, 但这种酶在体内催化蛋白质折叠的机理尚不清楚. 最近发现越来越多的具有 PPI 活性的蛋白质存在, 如 Cyclopholin^[7] 和 FK506 rotamase^[8], 因为它们可分别与 Cyclosporin A 和 FK506 两种免疫抑制剂结合而被发现, 经部分氨基酸序列分析等结果表明, Cyclopholin 可能与 17 000 的 PPI 系同一种蛋白, 以上两种蛋白目前被认为是两种免疫抑制剂的受体. 蛋白折叠与免疫调节过程之间的内在联系还不清楚, 它们的异构酶活性在与抑制剂结合后也被完全抑制, 这样可能会造成免疫抑制的同时也抑制

了蛋白质正常的折叠反应.

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血管活性肠多肽结合核苷酸性质的研究

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摘要 采用光亲和技术检测了血管活性肠多肽 (VIP) 与核苷酸之间的相互作用, 发现 VIP 可以特异性结合同位素标记的 GTP, 还发现未标记的 GTP 以及其它三磷酸核苷抑制这种结合, 这意味着 VIP 与上述三磷酸核苷之间的结合是一种典型的可逆性的结合反应. 发现低浓度的 GDP, GMP 不但不抑制 VIP 与同位素标记 GTP 的结合反应, 反而增强这种结合. 联系到其它研究者关于 GTP 影响 VIP 与其受体结合反应的结果, 可认为 VIP 能可逆性地连接一种三磷酸核苷, 这种连接受反应系统中不同核苷酸比例影响, 通过这种连接来调节 VIP 与其受体之间的反应.

关键词 血管活性肠多肽, 光亲和技术, 核苷酸

血管活性肠多肽 (VIP) 是一种具有多种生物学效应的神经多肽^[1], 它由 28 个氨基酸组成, 广泛分布于人和哺乳动物的神经系统中, 发挥重要的效能. 它对局部血流、心血管反应、平滑肌张力、水盐代谢、免疫和神经内分泌功能、以至体内神经介质代谢有重要影响, 甚至参与调整动物的记忆程序^[2]. 现有的资料表明, 哺乳动物和人的 VIP 分子结构是相同的.

像许多其它的生物活性多肽一样, VIP 是通过其受体起作用. 用多种动物组织进行的研究表明, VIP 与其受体结合, 刺激离体膜腺苷酸环化酶使细胞内 cAMP 水平升高^[3,4]. 1987 年 Ho 等发现, VIP 与其受体结合, 可以改变松果体细胞的 cGMP 水平^[5].

虽然大量材料述及 VIP 通过结合受体激

活腺苷酸环化酶或鸟苷酸环化酶作用于 ATP 或 GTP, 但至今尚无材料报导 VIP 是否可直接与核苷酸反应以及与不同核苷酸反应的规律。

[γ - ^{32}P] $8\text{N}_3\text{GTP}$ 是 GTP 的类似物, 其特点是有高比活性的放射性同位素标记, 在鸟嘌呤第八位点上有一个叠氮基团, 可由适当波长光线照射而激活与蛋白或多肽的氮形成氮烯连接在一起, 最重要的是其结构与天然 GTP 极为类似可取代 GTP, 并参与 GTP 所能进行的反应。前人的大量工作证明, 同位素标记的 $8\text{N}_3\text{GTP}$ 可作为 GTP 的类似物, 取代 GTP 参与 GTP 的反应^[6]。本试验成功地运用这种光标记方法, 对 VIP 结合核苷酸性质进行研究, 检测了不同核苷酸、不同浓度核苷酸与 VIP 反应的规律, 为进一步研究提供了线索, 同时, 也探讨了这一现象可能的生理学意义。

1 材料和方法

1.1 材料 猪血管活性肠多肽 (VIP) 购自 Peninsula lab (CA), (γ - ^{32}P) 5' 三磷酸 8-叠氮鸟苷 (γ - $8\text{N}_3\text{GTP}^{32}$) 甲醇溶液由美国 Kentucky 大学化学系, Nobuyuki Imai 实验室赠送。其它试剂购自 Sigma 公司。

1.2 VIP 结合 GTP 饱和曲线 于 7 个 EP 管中加入不同量的 γ - $8\text{N}_3\text{GTP}^{32}$, 于 37℃ 水浴中蒸发掉甲醇, 然后每管加入 20 μl 水以溶解之, 再于管中加入 20 μl 的 8 $\mu\text{mol/L}$ VIP 混合, 于室温放置 30s 后置于冰浴上用紫外光 (245nm) 照射 45s, 立即加入 PMS 溶液 (50% 甘油, 0.05% 溴酚兰, 0.5% DTT, 1% SDS, 0.125mol/L Tris-HCl 缓冲液, pH6.8), 于 100℃ 加热 3min, 用 PAGE 法分离结合 VIP 的 γ - $8\text{N}_3\text{GTP}^{32}$ 和游离的 γ - $8\text{N}_3\text{GTP}^{32}$, 电泳后的凝胶经固定、染色、脱色、干燥后, 剪下蛋白带, 置于液体闪烁测定管中, 加入液体闪烁液 2.5ml, 于液体闪烁测定仪中测定放射强度。

1.3 不同核苷酸对 VIP 结合 γ - $8\text{N}_3\text{GTP}^{32}$ 影响曲线 在一系列 EP 管中, 加入 20 μl 8 $\mu\text{mol/L}$ VIP, 然后加入不同量的 ATP (或 GTP, UTP, TTP, GDP, GMP) 使其终浓度分别为 1,

10 $\mu\text{mol/L}$, 50 $\mu\text{mol/L}$, 250 $\mu\text{mol/L}$, 670 $\mu\text{mol/L}$ (或 1000 $\mu\text{mol/L}$)。于室温放置 45s 后, 加入 40 $\mu\text{mol/L}$ 的 γ - $8\text{N}_3\text{GTP}^{32}$ 10 μl 混匀, 放置 20s, 用 254nm 的紫外线照射 45s, 然后加入 PMS 溶液, 煮沸 3min, 用 PAGE 法分离, 固定, 染色, 脱色, 干燥后, 剪下蛋白带, 液闪计数。

2 结果与讨论

在一定体积的反应系统中, 不同量的 γ - $8\text{N}_3\text{GTP}^{32}$ 与 VIP 结合能力有不同, γ - $8\text{N}_3\text{GTP}^{32}$ 浓度越大, 与 VIP 结合能力越强, 直至 VIP 结合 γ - $8\text{N}_3\text{GTP}^{32}$ 能力达到饱和。在本实验中, 当 γ - $8\text{N}_3\text{GTP}^{32}$ 量达到 40 μmol 时, VIP 与 GTP 结合达到最高点, 继续增加 $8\text{N}_3\text{GTP}^{32}$, VIP 与 γ - $8\text{N}_3\text{GTP}^{32}$ 的结合不再增加 (图 1), 呈

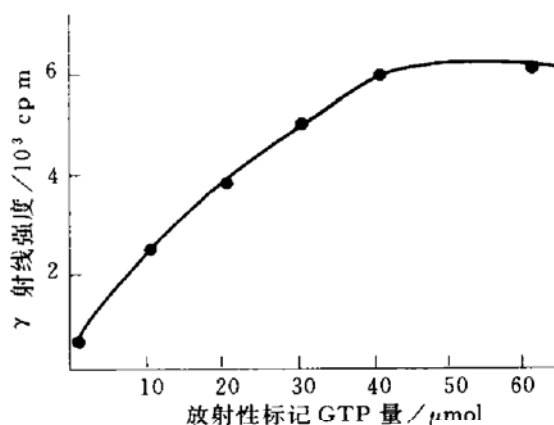


图 1 VIP 结合 GTP 饱和曲线

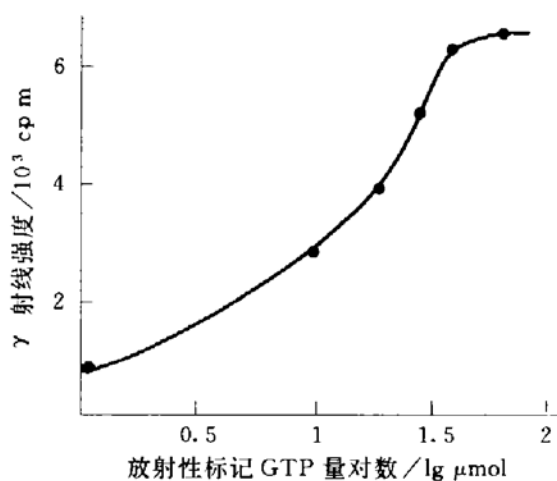


图 2 VIP 结合 GTP 半对数饱和曲线

现一典型的特异性结合曲线。将该结合曲线用半对数坐标表达, 则可看到一平滑的 S 型曲线

(图2),说明VIP与 γ - 3H -GTP的结合是单一位点的结合^[7].

用不同剂量无放射标记的GTP与 γ - 3H -GTP同时作用于VIP,发现无放射标记GTP明显地竞争性抑制VIP与 γ - 3H -GTP的结合,若以无GTP存在时,VIP结合 γ - 3H -GTP能力为100%,随着GTP浓度增加,VIP与 γ - 3H -GTP的结合急剧下降.当GTP浓度为 γ - 3H -GTP浓度的7倍以上时VIP与 γ - 3H -GTP结合达到最低点(图3),这意味着VIP结合三磷酸腺苷是典型的可逆结合反应,它也表明VIP与 γ - 3H -GTP的结合是可被同类物竞争性取代的反应,同时这种竞争性抑制结果也表明本实验有较高的精确度,非特异结合只占总结合的6%左右.

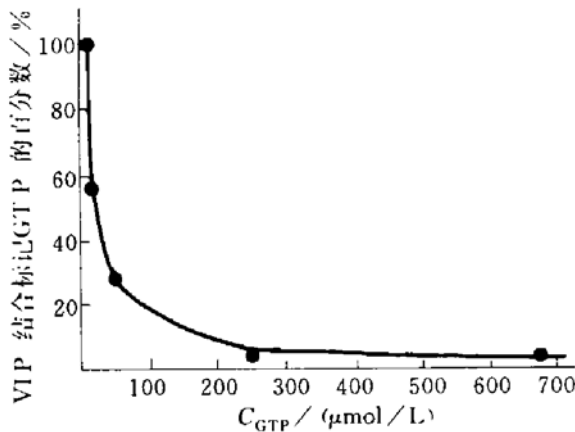


图3 未标记GTP对VIP结合标记GTP的保护曲线

实验发现,不仅GTP可以竞争性地抑制VIP与 γ - 3H -GTP的结合,其它三磷酸核苷也都有类似性质,可以抑制这种结合,表现出明显的竞争性抑制规律,只不过不同的三磷酸核苷抑制VIP与 γ - 3H -GTP结合的能力稍有不同.实验表明ATP,UTP和TTP都表现出与GTP相同的对VIP结合 γ - 3H -GTP抑制规律,但抑制强度较之GTP轻,抑制曲线下下降较缓(图4),这说明VIP结合三磷酸核苷没有严格的特异性.

较低能量级别的核苷酸GDP,GMP对VIP结合 γ - 3H -GTP的影响与三磷酸核苷大

有不同.当体系中存在有低浓度的GDP或GMP时,VIP与 γ - 3H -GTP结合能力不但

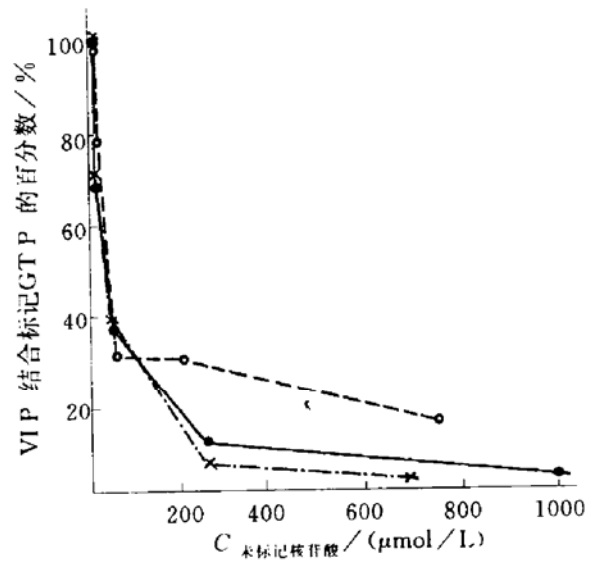


图4 未标记的UTP,ATP,TTP对VIP结合标记GTP的保护曲线

○---○: TTP; ●---●: ATP; ---x: UTP.

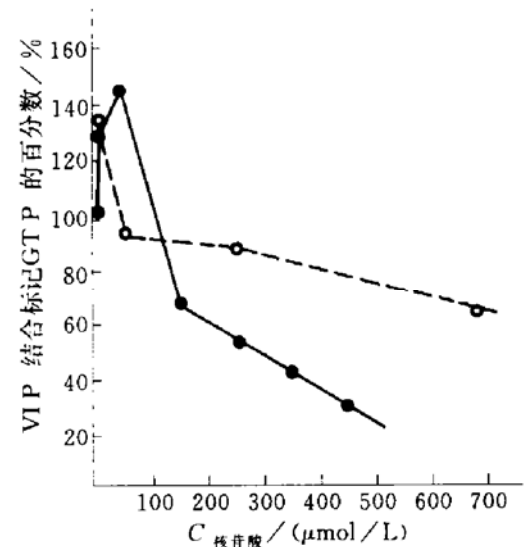


图5 GDP,GMP对VIP结合标记GTP的保护曲线

○---○: GMP; ●---●: GDP.

受抑制,反而明显增加,只有较高浓度的GDP或GMP存在时,才表现出对VIP结合 γ - 3H -GTP能力的抑制(图5).这种抑制能力的变化,可能是VIP在不同环境下构型变化所致,而构型的变化又反过来影响核苷酸与VIP结合.非常有意义的是,在接近生理浓度的低浓度条件下,三磷酸核苷抑制VIP与 γ -

$8N_3GTP^{32}$ 的结合,而二磷酸核苷及一磷酸核苷都促进VIP与 γ - $8N_3GTP^{32}$ 的结合,很明显,这是一种生物调节机制。

Couvineau 等人^[8]以及其它一些实验室的工作^[9,10],证实:一定浓度范围内GTP可以抑制VIP与其受体的结合。在该浓度范围内,GTP的浓度越高,VIP结合受体的能力越低。联系到本实验的结果,我们认为VIP与受体的结合,可能通过VIP与核苷酸结合来调节,而VIP与核苷酸的结合又受环境中不同能级的核苷酸成分比例调节。众所周知,包括VIP及其受体在内的无数内源性配体与受体的结合反应激活G蛋白,然后激发细胞内的第二信使,第二信使作用于多种效应系统产生生理或病理效应。一般认为,这一连串反应第一个调节点是配体与受体的结合反应,产生信号的大小依赖于配体浓度及受体数量^[11]。试验结果表明在VIP与受体结合之前,通过胞浆内不同能量级别核苷酸比例调节与核苷酸的结合从而影响与受体结合能力。在VIP一连串受体反应中,第一个调节点应该在配体受体结合反应之前。也就是说,不仅配体浓度影响反应强度,同时,配

体所处环境中各种核苷酸的成分及比例也会影响配体与受体的结合反应。

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Yang Luchuan. (*Department of Biochemistry, West China University of Medical Sciences, Chengdu 610041*). *Prog. Biochem. Biophys.* (China), 1994; **21** (3): 244

A series of DNA primers specific for human brain myelin basic protein (MBP) gene was designed and synthesized. MBP cDNA fragment about 600bp in length was amplified from human brain cDNA library by using polymerase chain reaction (PCR) with the specific primers P₁ and P₂. The recovered PCR product was flushed by klenow fragment and inserted into pGEM-3Zf (+) vector pretreated with Sma I and calf intestinal alkaline phosphatase. The recombinant plasmid was used to transform competent cell JM 109. The positive colonies were directly screened on indicator plates. The recombinant plasmid DNA and insert fragment isolated from four positive colonies were analyzed by digestion with EcoR I, Kpn I and Taq I. The different coding sequences including MBP exon I—VI, I—III, III—VI and I—V were amplified from these clones with their corresponding nested sets of primers respectively. These results show that these cDNA clones contain full-length coding sequence for 21.5kD human MBP.

Key words polymerase chain reaction (PCR), human brain cDNA library, coding sequence for myelin basic protein, vector pGEM-3Zf (+)

Studies on the Purification of PPI and its Catalyzing Activity for Folding of Recombinant Proteins. Xu Mingbo, Meng Wenhua, Ma Xiankai. (*Institute of Basic Medical Sciences, P. O. Box 130-3, Beijing 100850*). *Prog. Biochem. Biophys.* (China), 1994; **21** (3): 247
Proline isomerization catalyzed by peptidyl-

prolyl *cis-trans* isomerase (PPI) *in vivo* is a limited procedure in protein folding. In order to study the catalyzing activity of PPI on the refolding of recombinant proteins *in vitro*, PPI is purified from pig kidney, and is investigated the effects of the enzyme on catalyzing the refolding process. Results indicate that PPI increases the folding rate without increasing the correct folding ratio and specific activity, and PPI has a high catalyzing activity even at very low concentration.

Key words recombinant protein, refolding, PPI

The Research on Vasoactive Intestinal peptide Binding With Nucleotides. Zhu Ping, D. MANN, R. GREENBERG. (*Veterinary Institute, University of Agriculture and Animal Sciences of PLA, Changchun 130012*). *Prog. Biochem. Biophys.* (China), 1994; **21** (3): 251
The interaction between VIP and nucleotide was tested with advanced photo-affinity technique. It was found that VIP can bind radiolabelled GTP specifically and this binding could be competitively inhibited by cold GTP (unlabelled GTP). The experiment indicated that not only GTP could inhibit the binding between VIP and hot GTP, but also all other nucleoside triphosphates such as ATP, TTP, UTP could competitively inhibit this binding, although their inhibitions were a little weaker than GTP. It means VIP binding nucleoside triphosphate was a typical reversible binding reaction. It was found also that GDP, GMP at low concentration did not inhibit VIP binding hot GTP but enhanced the binding. Connecting with other researchers' results of GTP influencing on VIP-receptor interaction, it was considered that VIP could reversibly bind one of nucleoside triphosphates and this binding

was modulated by the ratio of different nucleotides in reaction system. Through this binding, the interaction of VIP and its receptor was regulated.

Key words VIP, photo-affinity, nucleotide

Study on Sensitivity Improvement of Dissociation Enhanced Lanthanide Fluoroimmunoassay. Zhao Qiren, Zhang Fuhua, Lu Jie, Lin Han. (*Institute of Radiation Medicine, Chinese Academy of Medical Sciences, Tianjin 300192*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (3): 255

Factors influencing the sensitivity, or the signal/noise ratio, of dissociation enhanced lanthanide fluoroimmunoassay (DELFA) have been studied. The fluorescence responses and signal/noise ratios for different europium amount were shown to be changed with the volume of enhancement solution, and there was an optimum volume at a certain europium amount. The smallest europium amount leads to the smallest optimum volume. 20% of the net fluorescence intensity was increased by using tinfoil reflection layer. Effective washing and drying methods of microtitration strips decreased background fluorescence have been developed.

Key words time-resolved fluoroimmunoassay, dissociation enhanced lanthanide fluoroimmunoassay, sensitivity

Purification of Cuprozinc Superoxide Dismutase From Human Erythrocytes by Cu^{2+} Chelate Affinity Chromatography. Lu Xing, Chen Jizhong, Li Peifeng, Yang Suhong, Fang Yunzhong. (*Beijing Institute of Radiation Medicine, Beijing 100850*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (3): 259

Cuprozinc superoxide dismutase (CuZn-SOD)

from human erythrocytes was purified by a procedure involving Cu^{2+} chelate affinity chromatography. It was shown in three experiments that the special chromatography held a number of important advantages for protein purification, such as a high rate of repeating performance and a large protein capacity. The purified enzyme, with a specific activity of 3073 U/mg protein, was tested for homogeneity by activity-stained and SDS gel electrophoresis. Accompanied by the study, a simple and efficient method was worked out for assessing the homogeneity of CuZn-SOD using its ratio of the absorbance at 260nm to that at 280nm.

Key words cuprozinc superoxide dismutase, metals-chelate affinity chromatography, human erythrocyte, purification

Determination of Isoenzyme 1 of Lactate Dehydrogenase by an Immunoprecipitation Method. Wu Xiyun, Yue Xiuling, Chen Yan, Si Xuezhong, Wang Zhongquan. (*Clinic Laboratory, Beijing TianTan Hospital, Beijing 100050*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (3): 262

Isoenzyme 1 of lactate dehydrogenase (LD1) was measured by an immunoprecipitation method. The antibody to M subunit of LD was added to the patient's serum and incubated for 5 min, at room temperature. The ratio of serum to antibody was 10 : 1. After incubation, a saturated ammonium sulphate solution was added with the same volume as serum. Then, centrifuged to precipitate all M-containing isoenzyme (LD2—LD5) as insoluble antigen-antibody complex. Determined the residual activity of LD in supernatant fluid. The relationship Between the LD activity and absorbance was linear up to 618U/L. Within-run