

如果辅以图象分析、计算机处理,则可达到定量化,有希望成为生物剂量计,对环境中低剂量辐射或其它以基因为靶的毒性因子进行监测。

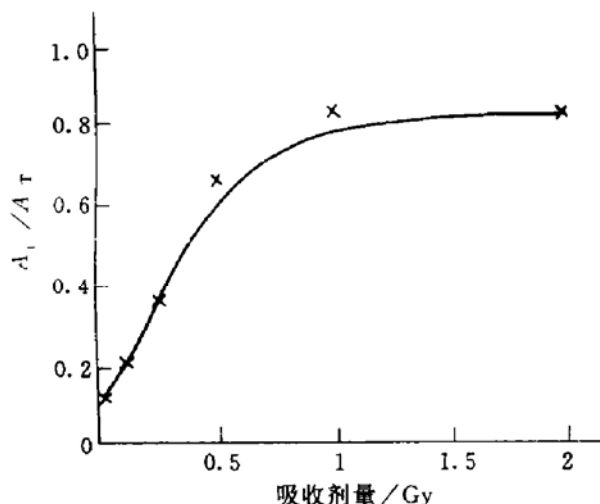


图2 剂量-效应关系曲线

A_t : 尾部积分光密度值; A_T : 总积分光密度值。(相关系数=0.84255)。

另外,在恶性肿瘤的放疗、化疗中,抗性细胞亚群的存在常常是肿瘤复发的根源。由于本法可检测出单个细胞的变化,可望用于肿瘤抗性细胞亚群的检出,为放疗、化疗效果评估

及预后提供科学依据^[5]。

本实验以人外周血淋巴细胞为研究材料,取材方便,利于临床应用。但由于体细胞的细胞间异质性较大,需进行大量细胞分析才能得到较稳定的结果,使用培养细胞则可得到重复性较好的结果(未发表)也说明了这一点。因此,如果推广应用到临床,正常细胞置信限的测定、取材的进一步优化等大量工作尚待进行。

本实验是针对低剂量损伤的检出而设计的,在较大剂量照射时,剂量-效应曲线可能转为平直,采用加大胶浓度、缩短电泳时间等措施可降低本法的检测灵敏度,提高检测剂量。

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一种改进的纯化和鉴定牛脑皮层 G_s 蛋白的方法

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摘要 用1%胆酸钠和15%饱和硫酸铵相结合的方法,从牛脑皮层细胞膜中抽提得到主要含激活型G-蛋白(G_s)和腺苷酸环化酶(AC)两种蛋白组分的制剂,然后通过Sepharose 6B柱将两者分开。将含G_s高活力的级分用庚胺-Sepharose 4B柱进一步分离,即可获得高活力的G_s, SDS-PAGE显示为分子量45 000和36 000的两条蛋白带。该法具有简便、快速、重复性好、产率高等优点,且可同时获得无G_s污染的AC。用无G_s污染的AC脂酶体测定G_s活力亦简便、可靠、灵敏度高。

关键词 激活型G-蛋白(G_s), 腺苷酸环化酶(AC), 牛脑

G-蛋白(GTP-binding protein)介导的信号跨膜传递体系是由细胞膜上受体、G-蛋白和效应器如腺苷酸环化酶(AC), 磷脂酶C和离

子通道等组成,体系中各组分在信号跨膜传递

中的作用及其相互关系是当前生物膜研究中的热点之一。G-蛋白种类繁多,牛脑中主要存在 G_s , G_i , G_o 三种类型,它们均含三个亚基

(α : $M_r=39\ 000-52\ 000$, β : $M_r=35\ 000-36\ 000$, γ : $M_r=8000-11\ 000$)。 α -亚基是其功能单位,可被 GTP, GTP 非水解类似物如 GTP γ S 和 AMF (Al^{3+} , Mg^{2+} 和 F^-) 激活。活化的 G_s 可激活 AC, 在细胞功能的调节中起着重要作用^[1]。按经典方法, G_s 的纯化须依次通过 DEAE-Sephacel, Ultrogel AcA 34, 庚胺-Sephadex 柱和羟基磷灰石四个柱, 这种方法除能分离 G_s 外, 也能获得高纯度、高产率的其他种类 G-蛋白^[2]。由于这种方法的分离过程复杂、周期长且条件要求高, 尤其是 G_s 产率低, 难以得到推广使用。本文介绍了一种较简便、快速分离和鉴定 G_s 的方法, 为进一步研究 G_s 的结构及其在信号跨膜传递中的作用提供了很

好的实验方法。

1 材料和方法

1.1 材料 新鲜牛脑购自北京市大红门肉联加工厂。Forskolin, GTP, GDP, GTP γ S 和大豆磷脂均为 Sigma 产品, ATP 为 Fluka 产品, Sepharose 6B 和 CNBr 活化的 Sepharose 4E 购自 Pharmacia 公司, 3H -cAMP 药盒由中科院原子能所提供。

1.2 方法

1.2.1 牛脑皮层细胞膜的制备 按 Sternweis 等^[3]的方法稍加改进。

1.2.2 庚胺-Sephadex 4B 柱的制备 按 Shaltiel^[4]的方法。

1.2.3 G_s 制备 按 Sternweis 和 Pang^[5]的方法进行了较大改进, 其基本步骤如图 1 流程所示。

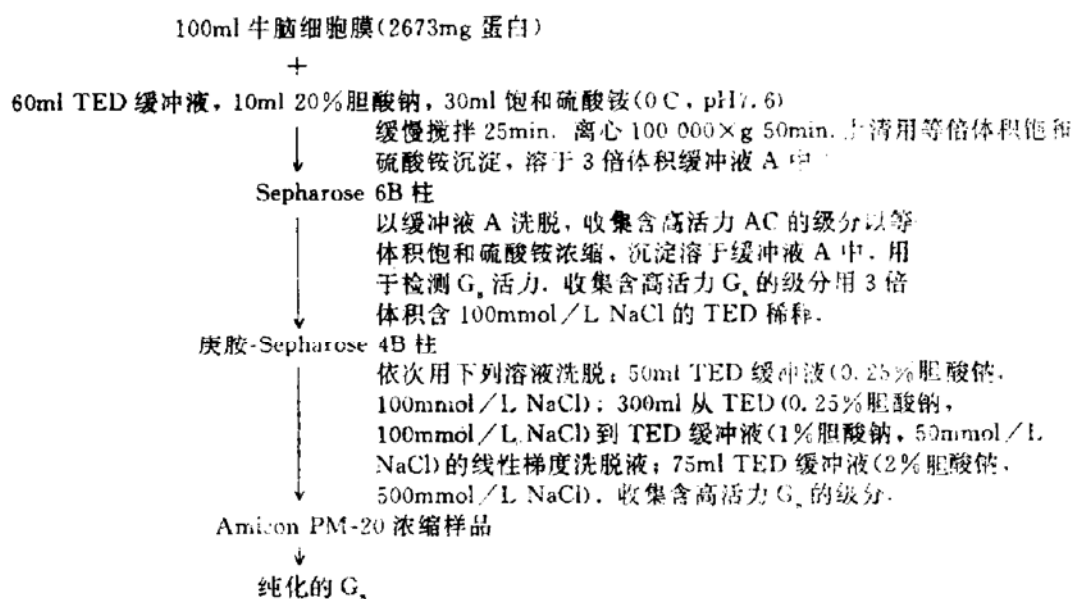


图 1 从牛脑皮层细胞膜分离和纯化 G_s 的流程图

TED 缓冲液: 20mmol/L Tris-HCl, 10mmol/L EDTA, 1 mmol/L DTT, pH8.0. 缓

冲液 A: 50mmol/L Tris-HCl, 1mmol/L DTT, 0.3mol/L 蔗糖, 15mmol/L $MgCl_2$.

1.2mol/L 硫酸铵, 1%胆酸钠, 4mg/ml 大豆磷脂, pH 7.6.

1.2.4 AC 活力的测定 采用本实验室建立的 cAMP 蛋白竞争结合法^[5]。

1.2.5 G_s 活力测定 按 Cerione 等^[6]的方法将纯化的 AC 重建于大豆磷脂脂质体, 取 50 μ l (约 30—50 μ g) AC 脂酶体, 加入 0.35ml 的反应液 (50mmol/L Tris-HCl, 10mmol/L KCl,

1.0 mmol/L $MgCl_2$, 2.0 μ mol/L $AlCl_3$, 10mmol/L NaF, 2mg/ml BSA, 10U/ml 丙酮酸激酶) 和一定量的 G_s , 30°C 保温 10min, 再加 50 μ l 20mmol/L PEP-ATP, 30°C 反应 10min 后, 煮沸, 稀释 5 倍, 取 50 μ l 以 3H -cAMP 药盒测定 cAMP 生成量。 G_s 的比活力

定义为在 30℃, 每分钟使 1mg AC 样品催化 cAMP 生成的纳克分子数 ($1\text{U} = 1\text{nmol}/(\text{min} \cdot \text{mg})$).

1.2.6 蛋白质测定 采用 Lowry^[7]法.

1.2.7 SDS-PAGE 采用 Laemmli^[8]不连续垂直板电泳法. 凝胶梯度为 5%—15%, 考马斯亮蓝 R-250 染色.

2 结果和讨论

2.1 G_s 制备

2.1.1 胆酸钠增溶和硫酸铵沉淀 我们发现, 在用 1%胆酸钠增溶细胞膜的过程中, 虽可将 G_s 总活力的 80%从细胞膜上溶解下来(表 1),

表 1 胆酸钠与胆酸钠加硫酸铵增溶细胞膜的效果比较

	总蛋白量 /mg	G _s 活力 ¹⁾		总活力 回收率 /%	比活力 增加倍数
		比活力	总活力		
100ml 细胞膜	2430	1.10	2673	100	1.0
1%胆酸钠增溶	693	3.11	2155	80.6	1.8
1%胆酸钠增溶+15%饱和硫酸铵沉淀	345	6.14	2120	79.3	4.6

¹⁾ G_s 活力单位为: U/mg, 总活力=比活力×总蛋白量.

但增溶物中尚存较多杂蛋白, G_s 的比活力较低, 如果在这一步中加入 15%饱和硫酸铵沉淀增溶物, 实验结果表明, 加入的饱和硫酸铵不仅对 G_s 活力无明显影响, 而且, 对增溶物具有一定的纯化作用, 可使 G_s 的比活力提高近 1

倍, 由 3.11 增加至 6.14(表 1), 分离效果明显优于单纯以 1%胆酸钠增溶. 实验结果提示, 增溶并同时用 15%饱和硫酸铵沉淀增溶物相结合的方法对于再经两步层析得到高纯度 G_s 是至关重要的.

2.1.2 经 Sepharose 6B 凝胶过滤柱达到 AC 和 G_s 的分离 将上述硫酸铵浓缩后的增溶物溶于 3 倍体积缓冲液 A 中, 经鉴定主要是由 G_s 和 AC 两种蛋白组成的可溶性酶制剂, 已知 AC 的分子量约为 120000, G_s 的分子量约为 100000, 因此, 通过凝胶过滤可将两者分开. 从图 2 可见, 含高活力 AC 峰 (fractions 58—70) 无 G_s 污染, 收集含高活力 AC 各级分, 测得其基础活力为 355pmol/(min·mg). 对其反应特性的研究(表 2)显示, 它可被 Forskolin 和 Mn²⁺ 激活, 但 GTP, GTP_γS 和 AMF 对其活力无明显影响, 进一步说明分离下来的 AC 中已无 G_s 存在, 可用于 G_s 活力的检测.

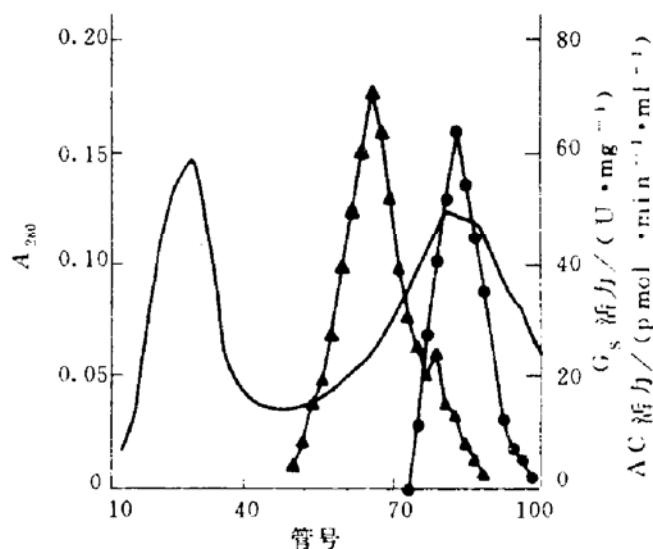


图 2 Sepharose 6B 凝胶过滤

柱体积 1.5cm×100cm, 2.5ml/管, 15ml/h.

—: A_{280nm}, —▲—: AC 活力, —●—: G_s 活力.

表 2 经 Sepharose 6B 柱凝胶过滤部分纯化的 AC 的反应特性

	基础活力	加 Forskolin	加 Mn ²⁺	加 GTP	加 GTP _γ S	加 AMF
酶活力 ¹⁾	355	1485	893	368	343	362
相对活力/%	100	418	252	104	96.6	102

¹⁾ 酶活力单位为: pmol/(min·mg).

2.1.3 用庚胺-Sepharose 4B 疏水层析柱 (C_7) 达到 G_s 的纯化 上述经 Sepharose 6B 凝胶层析柱分离得到的 G_s 级分 (fractions 78—88) 因有 G_i , G_o 以及少量 AC 等蛋白污染, 需经疏水层析柱进一步纯化. 疏水层析是一种特殊形式的亲和层析, 在固相载体上加入烷基链以提供疏水键的作用位点, 被结合的蛋白质通过逐渐增加洗脱剂的疏水性进行洗脱^[4]. 从 Sepharose 6B 分离下来的高活力 G_s 级分用不含胆酸钠的溶液稀释 4 倍, 使其胆酸钠的浓度为 0.25%, 先以含 0.25% 胆酸钠的高盐溶液洗脱未与 C_7 结合的以及以非疏水键与 C_7 结合的蛋白质, G_s 的分离则依赖于疏水性逐渐增加的线性梯度洗脱液而洗脱, 高活力 G_s 峰位于 0.6% 胆酸钠梯度处. 其洗脱曲线如图 3 所示, 收集各高活力 G_s 级分 (fraction 44—48),

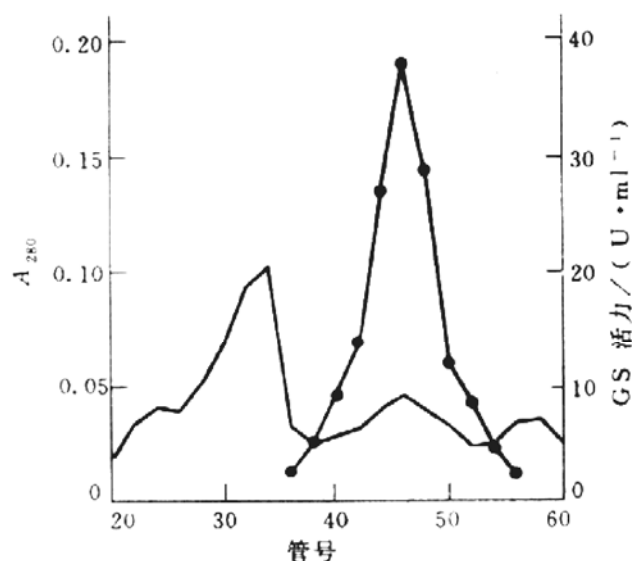


图 3 庚胺-Sepharose 4B 疏水层析

柱体积: 1.5cm × 30cm, 4ml/管, 20ml/h.

—: A_{280nm} , —●—: G_s 活力.

用 Amicon PM-20 浓缩至 1ml, 得完全纯化的 G_s . 由 1g 牛脑皮层细胞膜蛋白可得到 0.31mg 纯化的 G_s , 其比活力为 1528U/mg, 比细胞膜 G_s 活力提高 1400 倍左右. 在 5%—15% SDS-PAGE 图谱上显示为两条电泳带 (图 4), 相对迁移率分别为 0.745 和 0.846, 其相对应的分子量分别为 45 000 和 36 000, 证实是 G_s 的 α -亚基和 β -亚基二聚体.

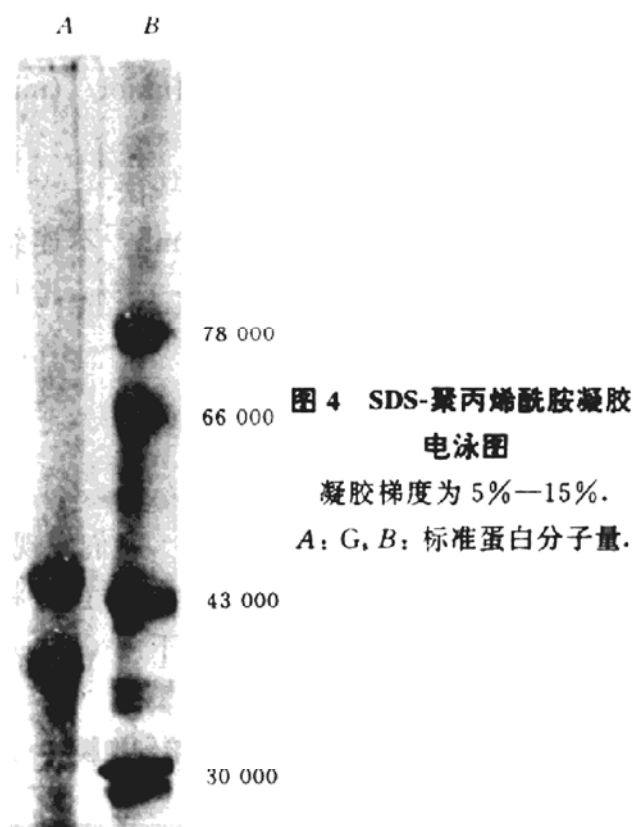


图 4 SDS-聚丙烯酰胺凝胶电泳图

凝胶梯度为 5%—15%.

A: G_s , B: 标准蛋白分子量.

2.2 G_s 活力测定方法的检验 G_s 活力测定的经典方法是含 G_s 的组分引入 G_s 缺陷型的 Sf9 淋巴瘤细胞株的细胞膜中, 以 AMF 或 GTP γ S 活化 G_s 后, 观察活化的 G_s 对 AC 的激活能力, 以 AC 催化 cAMP 的生成量推算 G_s 活力^[9]. 按本方法纯化的 AC 因无 G_s 的污染,

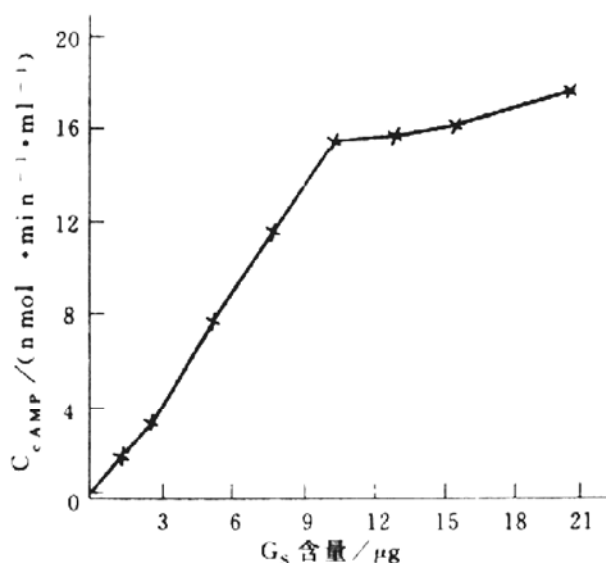


图 5 重建 AC 脂酶体在含 AMF 的反应体系中 cAMP 的生成量与 G_s 的加入量的对应关系

(下转第 469 页)

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将其和大豆磷脂重建形成脂酶体, 相似于在体外建立了一种 $G_s$ 缺陷的人工膜的测试体系, 且在一定量 $G_s$ 范围内, 重建的 AC 脂酶体在含 AMF 的反应体系中, 其所催化 cAMP 的生成量与 $G_s$ 加入量成直线关系 (图 5). 由此可知, 用这种重建脂酶体的方法检测 $G_s$ 的活力简便、快速、灵敏而且准确, 是一种可行的方法.

$G_s$  的分离、纯化及其与效应器的体外重建实验是研究信号跨膜传递机理的重要方法之一. 我们改进的纯化和鉴定 $G_s$ 的方法将为进一步探讨 $G_s$ 的结构和功能提供有用的实验手段.

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ethanol. The sample was applied on the column and equilibrated with pH4.0 acetic acid buffer. Then pH5.2 of different concentration acetic acid buffers were used for elution of MT. Two eluted peaks were obtained and identified as MT-2 and MT-1. Comparing with the traditional method—gel filtration and ion exchange chromatography, this method is simple and time-saving in laboratory-scale.

**Key words** metallothionein, metal chelating, isolation and purification

**Quantitative Analysis of DNA Structure Changes in Individual Irradiated Cells.** Luo Ying, Sun Zhixian, Yang Ruibiao, Zhang Zhensheng. (*Institute of Radiation Medicine, Beijing 100850*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (5): 451—453

Irradiated cells express DNA structural damages, such as DNA double-strand break, DNA-DNA crosslink and DNA-protein crosslink etc. These damages result to changes of DNA supercoil state, which trigger a series changes of DNA replication and expression. Many methods to test DNA damages have been established, roughly can be divided into two kinds according to the DNA materials used. The first use the naked DNA extracted from cells and free from DNA-binding materials existed in cell. The second one uses nucleoid for research, here the detergents and hypertonic salt buffers were used to remove nuclear envelope and a part of nuclear proteins, nuclear DNA remains appropriate tangled loop and binds to residual nuclearskeleton. This DNA structure is beneficial to research damage effects on DNA structure, single cell gel electrophoresis belongs to the latter. It also named comet assay because its

cell electrophoresis shape looks like a comet. It can test not only DNA stand break but also measure DNA structure changes resulted from stand break. According to oversea reports, with slight modification, single cell gel electrophoresis assay has been established. Employing image analysis system, fast quantitative measurement of DNA structure changes of single cell irradiated as low as 0.1Gy can be given with a well correlated dose-responses relationship. After further study, the method might be developed as a kind of biodosimeter for application of monitoring environmental low level irradiation.

**Key words** DNA structure damages, single cell gel electrophoresis, image analysis, low dose irradiation

**A Modified Method for Purification and Identification of G<sub>s</sub> from Bovine Brain Cortex.** Fan Gaofeng, Huang Youguo. (*National Laboratory of Biomacromolecules, Institute of Biophysics, Academia Sinica, Beijing 100101*). *Prog. Biochem. Biophys. (China)*, 1994, **21** (5): 453—456, 469

Soluble proteins mainly containing G<sub>s</sub> (stimulatory GTP-binding protein) and adenylate cyclase (AC) from cell membranes of bovine brain cortex were extracted with 1% sodium cholate and 15% saturated ammonium sulfate. Separation of G<sub>s</sub> and AC was carried out by Sepharose 6B gel filtration. Purified G<sub>s</sub> can be obtained by passing the fractions containing G<sub>s</sub> from Sepharose 6B column through a heptylamine Sepharose 4B hydrophobic column. The purity of G<sub>s</sub> was identified by its highly stimulated activity to AC and SDS PAGE which showed two bands of 45kD and 36kD. The procedure described above is characterized by simplicity, rapidity, repeatability and high

yield. At the same time, AC, a by-product which was not contaminated by  $G_s$ , can be used for assay of  $G_s$  activity after reconstituting it into asolectin vesicles. This method of assaying  $G_s$  activity has been proved to be simple, reliable and sensitive.

**Key words** stimulatory GTP-binding protein, adenylate cyclase, bovine brain

**FOS/JUN Mediates Endothelin-1 Gene Expression Induced by Phorbol Ester in Endothelial Cells.** Wen Jinkun, Hu Jing, Qiao Yaming, Zhang Chenhui, Zhou Airu, Tang Jian. (*Institute of Basic Medicine, Hebei Medical College, Shijiazhuang 050017*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (5): 457—458

Gel shift of electrophoresis and Northern and Western blotting analysis were used to detect the effect of c-jun antibody on the interaction between AP-1 site of ET-1 gene and nuclear proteins as well as the effect of TPA on c-fos/c-jun gene expression. The results showed that AP-1 binding activity in vascular endothelial cells was stimulated by c-fos/c-jun, whose expression was induced by TPA, and that the electrophoretic mobility of band of DNA-protein complexes was altered by the antibody against c-jun. These results suggest that ET-1 gene expression induced by TPA is mediated by c-fos/c-jun.

**Key words** ET-1 gene, endothelial cells, c-fos/c-jun, TPA

**A Rapid and Reliable Method for Direct Sequencing of PCR Products.** Wang Liang, Zhang Jinsan, Zhu Dan, Yin Luo, Wang Xiuqin, Wu Min. (*National Laboratory of Molecular Oncology, Cancer Institute, Chinese Academy of Medical Sciences and Peking Union Medical*

*College, Beijing 100021*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (5): 458—459

A simple, rapid and reliable sequencing method for double stranded PCR products is described. This method presented utilizing the unique property of T7 DNA polymerase which remains active at low temperature to allow the sequencing reaction performed at low temperature. Excellent sequencing results have been obtained by this method for various PCR products.

**Key words** polymerase chain reaction (PCR), DNA sequencing, T7 DNA polymerase

**Determination of the Content of AchE in the Plasma of Patients With PNH.** Xu Caimin, Lu Hong, Pan Huazhen, Zhang Zhinan. (*National Laboratory of Medical Molecular Biology, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, Beijing 100005*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (5): 460

The contents of AchE in plasma and erythrocyte membrane of patients with PNH were determined by ELISA. The results show that the content of AchE is low in PNH erythrocyte membrane but high in plasma when it is compared with that in normal.

**Key words** paroxysmal nocturnal hemoglobinuria (PNH), soluble acetylcholinesterase (soluble AchE), plasma, erythrocyte membrane

**Computer Design of Murine Adenosine Deaminase mRNA Specific Ribozyme.** Chen Hua, Chen Nongan, Lu Changde, Qi Guorong. (*Shanghai Institute of Biochemistry, Academia Sinica, Shanghai 200031*). *Prog. Biochem. Biophys. (China)*, 1994; **21** (5): 461—463

With computer analysis of the adenosine