研究简报

去促甲状腺激素血清的研制

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摘要 采用亲和层析法. 经过固体硫酸铵预处理的人促甲状腺激素抗血清与 CNBr-Sepharose 4B 在碱性条件下偶联，偶联率大于90%. 亲和柱规格：1.2cm×40cm. 淋洗流速：3ml/min. 正常人血清上样流速：0.25ml/min. 淋洗液用0.01mol/L PBS（pH7.4）. 柱子再生液用6mol/L 盐酸胍溶液. 此法处理过的血清符合 TSH 免放药盒的要求.

关键词 亲和层析，促甲状腺激素（TSH），正常人血清

在目前检测激素的所有药盒中，促甲状腺激素（TSH）药盒作为检测甲状腺功能的重要手段，使用量较大，而 TSH 免放药盒则是 TSH 药盒中灵敏度最高的药盒. 去激素血清做为 TSH 免放药盒标准点中的“0”标准点，它的好坏直接影响到药盒的灵敏度. 文中报道了作者采用亲和层析法从正常人血清中去除 TSH 激素的工作.

1 实验材料

CNBr-Sepharose 4B 及 Sepharose 4B（美国 Sigma 公司），正常人血清（长春血站），TSH 抗血清（中国原子能科学研究院），甘氨酸，固体硫酸胺.

2 实验方法

2.1 亲和柱的制备及预处理

称取 6g CNBr-Sepharose 4B 在 150ml，1mol/L HCl 中浸泡15min，抽干，加入9ml 1mol/L NaCl，66mg $NaHCO_3$和经过预处理的人 TSH 抗血清，4℃下搅拌反应2h. 取出复合物1500r/min 离心15min，弃上清，将沉淀悬浮于0.01mol/L $NaHCO_3$-NaCl 溶液中加入甘氨酸饱和，室温过夜. 第二天上柱（柱子规格：1.2cm×40cm），并用0.01mol/L PBS（pH7.4，含 150mmol/L NaCl，0.02% NaN_3，0.1% BSA）反复洗涤至 A_{280}值小于0.02. 经计算，偶联率大于90%.

2.2 抗血清的预处理

取人 TSH 抗血5ml（滴度1∶40 000），加入5ml 生理盐水，边搅拌边加入固体硫酸铵，使其饱和度达到33%，4℃过夜. 第二天离心，3500r/min 15min. 取沉淀用适量生理盐水溶解，对0.05mol/L PBS（pH7.4）透析至外液中无硫酸根离子为止.

2.3 正常人血清的预处理

将正常人血清4000r/min 离心20min，过 Sepharose 4B 柱子（规格：5cm×10cm），收集流出液.

2.4 上柱

将经过预处理的正常人血清上亲和柱. 流速：0.25ml/min. 收集流出液即为所要血清.

2.5 亲和柱的再生

在正常情况下，用 0.01mol/L PBS（pH7.4）淋洗柱子即可. 若柱子已饱和，则先用6mol/L 盐酸胍溶液洗，再用0.01mol/L PBS洗柱子即可再用或于4℃保存，经此方法

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处理，亲和柱可连续使用数十次.

2.6 去激素血清的鉴定

2.6.1 用 TSH 放免药盒检测血清中 TSH 抗体含量.

2.6.2 用 TSH 放免药盒检测血清中 TSH 含量.

2.6.3 用紫外分光光度计检测血清中蛋白含量（血清稀释50倍，检测 A_{280}，A_{260}值）.

3 结 果

所得去激素血清用 TSH 放免药盒检测无 TSH 抗体存在. 用 TSH 免放药盒检测无 TSH 存在. 用紫外分光光度计检测 A 值，证明蛋白损失量较小（上柱前后蛋白变化量见表1）. 上述三方面实验证明，所得去激素血清符合 TSH 免放药盒的要求.

表1 上柱前后蛋白变化量

(mg/ml)

	A_{280}	A_{260}	蛋白量
上柱前	1.400	0.995	6.47
上柱后	1.120	0.750	5.33

4 讨 论

去激素血清的制备方法目前已知有三种，一为活性碳法，二为右旋糖苷包被的活性碳法，三为亲和层析法. 前两种方法激素去除的不完全，蛋白损失量较大，最大可达到50%. 由于 TSH 免放药盒对“0”标准点的要求非常高，只有亲和层析法去激素较为完全，且此法较为简便，蛋白损失量较小，处理过的血清符合 TSH 免放药盒的要求. 亲和柱可长期连续使用，适于大批量生产.

据文献报导，亲和柱上的抗体易脱落，作者在实验中也遇到这个问题，这与抗血清和珠子的偶联条件有关，作者用大量缓冲液及适量正常人血清淋洗柱子后，可将抗体的脱落降至最小(用现有方法检测不到 TSH 抗体的存在)，但这个问题仍有待于解决.

致谢 本工作得到我室王玉肖副研究员的指导，在此表示感谢.

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A direct micro determination of glutathione peroxidase (GSH-PX) activity with spectrophotometry was developed. Conditions of the assay were studied in detail. 10μl blood sample was diluted with distilled water and treated with 10% TCA to remove protein. There was a good linearity between DTNB product and concentration of GSH after 3 minutes enzymatic reaction at the temperature of 37℃ in pH 6.5 solution. The method had higher sensitivity, better reproducibility. It may became useful tool for analyzing GSH-PX in scientific research and clinical work.

Key words glutathione peroxidase (GSH-PX), spectrophotometry, assay

Production of Specific Antisera to Thyroid-stimulating Hormone (TSH). Zhou Ling. (*Deparment of Isotope, China Institute of Atomic Energy, Beijing* 102413). *Prog. Biochem. Biophys.* (China). 1994: **21** (4): 366—368

Specific high titre antisera to TSH were raised in two sheeps injected with 100μg (booster injection, 50μg) highly purified TSH preparation by the multi-site intradermal immunization technique. Blood were bled at two week intervals by cardiac puncture without killing the animals and solution of anti-anemia drug was given to sheeps after each leting blood. The antisera were monitored by TSH RIA Kit. Titres were range from 28×10^4 to 205×10^4 and no cross-reaction occured between TSH antisera and human LH, FSH, HCG and all antisera have the avidity more than 10^{10}L/mol.

Key words TSH, antiserum, RIA

Experimental Research on Naked DNA Gene Therapy of Parkinson's Disease. Cao Lei, Zheng Zhongcheng, Liu Xinyuan, Liu Zhenguo, Zhao Yingchun, Chen Shengdi, Jiang Zhihua, Zhou Changfu. (*Shanghai Institute of Biochemistry, Academia Sinica, Shanghai* 200031). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 369, 289

In vivo naked DNA gene transfer method was used in gene therapy of Parkinson's disease (PD). The complex of rat tyrosine hydroxylase (TH) expression plasmid and Lipofectin was injected stereotaxically into striatum of PD rat model. The asymmetric rotational behavior was reduced substantially and quickly. On the third day after injection, drug-induced rotation decreased 50% compared with pretreatment scores. Immunohistochemical staining showed TH-positive nerve cells in striatum of injection side, which indicated that TH gene was uptaked and expressed by nerve cells. These preliminary results have general implications for the application of naked DNA transfer technique in gene therapy of human neurological disease and specific implications for PD.

Key words Parkinson's disease, gene therapy, tyrosine hydroxylase

Production of TSH-Free Thyroid Stimulating Hormone Serum. Zhou Ling. (*Department of Isotope, China Institute of Atomic Energy, Beijing* 102413). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 370—371

Using affinity chromatography to eliminate TSH from normal human serum. Preliminary treatment to TSH with 33% saturated ammonium sulfate were coupled to agarose in alkaline condition and packed in column (1.2cm×

40cm). Human normal sera were applied to the column at a flow rate of 0.25ml/min. and the column was washed with 0.01mol/L PBS (pH7.4) at a rate of 3ml/min. Materials adsorbed to the immobilized antibodies were eluted with 6mol/L guanidine-HCl. Between experiments, the column was stored in PBS-azide at 4℃.

Key words affinity chromatography, TSH, normal human serum

Quantitative Method of Heparin. Zhang Changjing, Li Yihe, Li Xianbai. (*Department of Biochemistry, Chongqing Teachers Training College, Chongqing* 630047). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 372—373

It is known that the dropping rate of optical absorbing value at 300 nm wave length when ribonucleic acid is hydrolysed with ribonuclease is inhibited quantitatively by heparin. Upon this fact, a standard measuring line is plotted when the inhibition is quantitated by known quantity standard heparin. Any unknown can be determined conveniently by comparing with this standard.

Key words ribonuclease, ribonucleic acid, heparin from hog lung, optical absorbing value

Modifications on the Polarographic Oxygen Electrode Method for Superoxide Dismutase Activity Determination. Zhang Jiquan, Chen Youchun, Zou Yueqi, Yan Jinghui. (*Institute of Animal Science, CAAS, Beijing* 100094). *Prog. Biochem. Biophys.* (China), 1994; **21** (4): 374—375

The following modifications to the polarographic oxygen electrode method for SOD activity determination were made: (1) directly determining at room temperature, (2) introducing standard SOD as activity unit standard, (3) using phosphate buffer as reaction medium, and (4) increasing the pyrogallic acid used. These modifications result in: (1) the abolishment of the bubbles easily produced on the electrode surface which severely interfere with the determination, (2) increased sensitivity of determination and (3) broadened linear range of SOD activity.

Key words polarographic oxygen electrode, superoxide dismutase, activity determination