段的 cDNA 文库，经过一次简单的免疫亲和层析，得到任何一种相应半抗原或抗原的抗体片段应用到临床免疫诊断和科学研究的时代已指日可待.

致谢 承我校免疫教研室分子生物学实验室马大龙教授审阅，特此致谢!

参 考 文 献

1 Yakiw R, Berson S. Nature, 1959; **184**: 1648

2 Köhler F, Milstein C. Nature, 1975; **256**: 495

3 Orlandi R, Fussow D. Proc Natl Acad Sci USA, 1989; **86**: 3833

4 Huse W, Sastry L. Science, 1989; **246**: 1275

5 McCafferty J, Griffiths A, Einter A *et al*. Nature, 1990; **88**: 4363

6 Kang A, Barbas C. Proc Natl Acad Sci USA, 1991; **88**: 4363

7 Winter G, Milstein C. Nature, 1991; **349**: 293

8 Hoogenboom G, Griffiths, Johnson K *et al*. Nucleic Acids Res, 1991; **19**: 4133

9 Steele E, Tauylor L, Both G. Todays Life Science, 1991; **2**: 28

10 Babbs C F, Bain J D, Hickstra D M *et al*. Proc Natl Acad Sci USA, 1992; **89**: 4557

11 Gram H, Marconi L. Proc Natl Acad Sci USA, 1992; **89**: 3576

12 Herlitze SKeonin K. Gene, 1990; **91**: 143

13 Fowler R, Schapper R. J Barc, 1986; **167**: 130

14 Takeuchi Y, Nagomo T. J Virol, 1988; **63**: 3900

目前最高分辨率的电泳
——固相 pH 梯度等电聚焦

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摘要 固相 pH 梯度等电聚焦是国际上 80 年代的新型电泳技术. 利用一系列具有弱酸和弱碱性质的丙烯酰胺衍生物滴定时，在滴定终点附近形成的 pH 梯度并参与丙烯酰胺的共价聚合，从而形成固定的不随环境电场等条件变化的 pH 梯度. 该方法具有比传统载体两性电解质等电聚焦更高的分辨率、更大的上样量. 可用于分析和制备相近 p*I* 的蛋白质，多肽等.

关键词 等电聚焦，固相 pH 梯度，载体两性电解质

30 多年前瑞典科学家 Svensson-Rilbe[1,2] 建立了等电聚焦 (isoelectrofocusing，IEF) 的理论和 Vesterberg[3] 合成了能在凝胶中形成多种范围 pH 梯度的载体两性电解质(carrier ampholyte，CA) 后，等电聚焦技术由于它的高分辨率已被世界上农、林、牧、副、渔、医、法医等领域的成千上万个生化实验室广泛使用[4]. 常规电泳由于仅仅根据在碱性缓冲液中蛋白质荷负电的多少进行分离，分辨率受到了限制，即使采用带有分子筛效应的梯度凝胶也难以将荷负电差别不大和分子量相差不多的蛋白质分子分开，而 IEF 是根据蛋白质的等电点的不同在一个连续的、线性的、稳定的 pH 梯度中进行分离，只要在凝胶中的 pH 梯度范围足够窄，便可将等电点相近的蛋白质分开. 目前有两种 pH 梯度可供选择，一种是前面所述的 CA pH 梯度，大多在 2 个 pH 或大于 2 个 pH 范围，个别范围可达 1 个 pH，分辨率一般为 0.01pH，另一种便是本文要介绍的由 Gasparic

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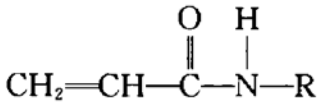
等人[5]在1975年合成了丙烯酰胺衍生物，它能在凝胶中形成固相pH梯度（immobilized pH gradient，IPG）. pH梯度范围最窄可为0.01pH/cm，且范围可以任选，分辨率可达0.001pH.

1 原 理[6]

固相pH梯度可用瑞典Pharmacia LKB公司生产的Immobiline（商品名）形成. 它是一些具有弱酸或弱碱性质的丙烯酰胺衍生物. Immobiline与丙烯酰胺和甲叉双丙烯酰胺具有相似的聚合行为. 它的结构式是：



(R代表羧基或4级氨基)，在分子的一端有一个双键. 在聚合过程中，它可以通过共价结合镶嵌到聚丙烯酰胺介质中，所以即使在电场中它也是“固相”的. 在分子的另一端是一个缓冲基团R，R为弱酸或弱碱，它可在聚合物中形成弱酸或弱碱的缓冲体系. 利用缓冲体系滴定终点附近一段pH范围就可形成近似线性的pH梯度，所以固相pH梯度等电聚焦（IPG IEF）与载体两性电解质等电聚焦（CA IEF）的区别在于前者不是两性分子，在凝胶聚合时便形成pH梯度. 后者是两性分子，在电场中两性分子迁移到自己的等电点而形成pH梯度.

Immobiline有六种不同的pK值. 二种弱酸性pK3.6，4.6（R基为羧基），四种弱碱性pK6.2，7.0，8.5，9.3（R是第四氨基）. 把两种或多种不同pK值的Immobiline贮液按重力梯度混合，并在聚合过程中完成共价结合，就可在聚丙烯酰胺凝胶内形成pH梯度.

2 实验方法[7]

2.1 pH梯度的选择和溶液配制

通常由两种Immobiline滴定产生pH梯度，一种起缓冲作用，另一种不起缓冲作用. 此时非缓冲Immobiline完全离子化，若同时知道缓冲和非缓冲Immobiline的pK值和它们的摩尔比，就可根据Henderson-Hasselbalch公式计算pH. 如果缓冲基团是酸性Immobiline，电离常数为pK_A它与非缓冲Immobiline摩尔浓度分别为C_A，C_B，则

$$pH = pK_A + \lg \frac{C_B}{C_A - C_B}$$

同理，如缓冲成分是碱性Immobiline，则

$$pH = pK_B + \lg \frac{C_B - C_A}{C_A}$$

图1是用pK3.6滴定pK7.0的Immobiline. 在pK7.0±0.5范围内形成一条逼近线性的滴定曲线，便形成pH6.5—7.5的线性梯度. 为了滴定时线性明显，常要求滴定Immobiline的pK与pH范围中间值越远越好，至少大于3个pH.

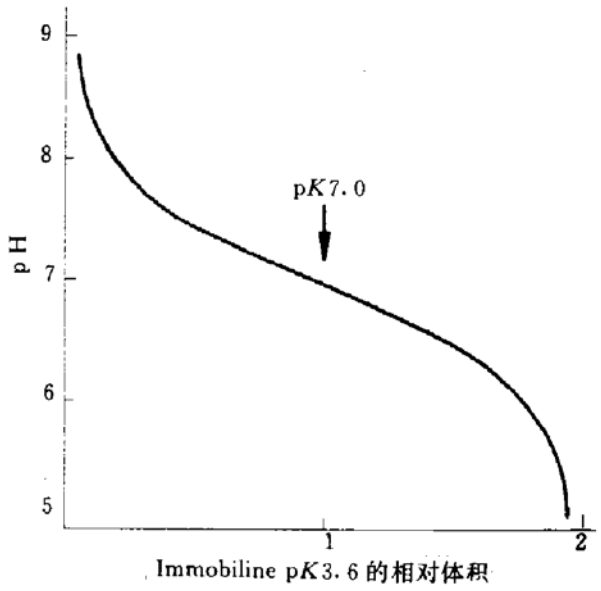


图1 Immobiline滴定曲线

如将缓冲和非缓冲Immobiline配成两种贮液，并使两种贮液内缓冲Immobiline量不同，形成浓度梯度，便可形成偏离该pK的pH梯度，这样在pH3.5—10.5就可任选梯度范围. 每种缓冲组分形成大约1.2个pH范围以内的线性梯度. 对小于1个pH范围的只用一对缓冲和非缓冲Immobiline，而对宽范围pH梯度，则需几种不同pK的Immobiline作为缓冲组分，利用Righetti给出的配制各种pH梯度的Immobiline用量表[8]即可配得pH3.5—

10.5的各pH范围的梯度.

2.2 灌胶

使用瑞典Pharmacia LKB公司多用电泳仪的梯度凝胶灌胶模具如图2组装，把模具立于水平台上.

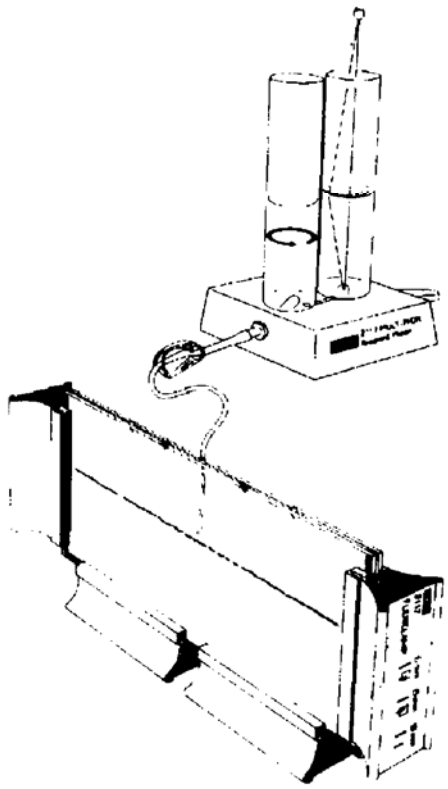


图2 固相pH梯度凝胶灌胶模具组装

查出Immobiline用量，按表1分别将重液（酸性）和轻液（碱性）注入腔中.

表1 重液，轻液配制

缓冲 Immobiline/μl
非缓冲 Immobiline/μl
凝胶贮液 2.5ml （29.1%丙烯酰胺/0.9%甲叉双丙烯酰胺）
Ampholine 200μl
87%甘油 4.2ml（轻液不加甘油）
定容到 15ml

注：配置的量可做二块 125mm×260mm×0.5mm 的胶.

把出液管插在模具上端小孔内，放入同样规格的搅拌子，打开磁力搅拌器. 向两腔中各加20μl新配的10%过硫酸铵和20μl 10% TEMED（宽pH范围应加4%过硫酸铵30μl，10%TEMED 35μl）. 同时打开腔间阀和流出管的夹子，让轻液、重液以线性梯度混合灌入模具中. 在25—35℃下静置15min. 然后放入50℃恒温箱中保温1h.

2.3 洗胶

聚合后撬开模具，取下胶片并立即称重. 然后放入去离子水中1—4h，期间换两次水，以洗去过硫酸铵，TEMED和未聚合的单体. 用冷风吹至其重量与洗胶前重量相差5%以内，此时胶可立即使用或放入保湿盒中（pH<8的胶可保存一周）. 如欲制成干胶长期保存则把胶泡在2%—2.5%的甘油中1h，并放置过夜使胶完全干燥. 干胶可封入塑料袋中，在−20℃保存1—2个月. 用Pharmacia公司的重泡胀模具泡胀，便可重新使用.

2.4 电泳

IPG IEF与CA IEF的样品处理方法相似，只是前者pH梯度已在凝胶中固定，所以样品中的盐对梯度基本上没有影响，一般不必脱盐，但过高的盐浓度也会造成凝胶的电导降低而烧胶和降低分辨率. 电泳时的电参数参见表2.

表2 电参数

	电流/mA	电压/V	功率/W	时间/h
预聚焦	15	2500—5000	20	0.5—1
聚焦	5	2500—5000	5	14—16 （pH范围小于1） 2—5 （pH范围大于1）

注：如在制胶时用CA作为添加剂，因为增加了凝胶的导电性可以大大缩短电泳时间. 如在1个pH的梯度范围中，聚焦时间可减少到3—5h.

2.5 染色

电泳后的染色方法与CA IEF基本相同，可根据样品量的多少选择考玛斯亮蓝或银染色，也可根据实验的需要进行同工酶染色，免疫固定或进行电泳转移后再染色.

3 IPG IEF 和 CA IEF 的比较

CA IEF 技术虽然已被广泛应用，但它还有一些弱点尚待解决. 如：a. pH 梯度不稳定，易产生阴极漂移. b. 在凝胶中导电和缓冲能力不均一. c. 离子强度低. d. 由于等电点沉淀加样能力有限，盐的干扰大. 但 IPG IEF 却克服了这些缺点：(1) pH 梯度范围可任意选择并可做得很窄 (0.01pH/cm). 而且由于 Immobiline 电导低，可加高电压，所以分辨率可比 CA IEF 提高 10—50 倍. 见图 3. (2) 由于缓冲基团共价结合到凝胶介质中，克服了阴极漂移，pH 梯度非常稳定，由此保证了实验的重复性. (3) 由于 pH 梯度已在凝胶中固定，所以样品中盐的干扰很小，且上样量比 CA IEF 大，可用于制备. (4) 由于 Immobiline 凝胶中没有第一氨基和第二氨基，故对多肽样品可使用经典的多肽染色方法. 但 IPG IEF 也有制胶需要技术，以及电泳时间较长这两个问题需要解决.

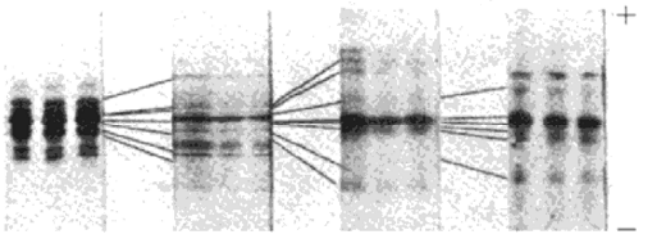


图 3 卵白蛋白的等电聚焦分析

(a) CA pH 梯度；(b)，(c) 和 (d) IPG pH 梯度.

4 CA/IPG 混合等电聚焦技术及进展

等电聚焦技术由于它的高分辨率在整个电泳技术中占有极其重要的地位而且得到了不断的发展. 它的发展经历了四个阶段. 1954 年和 1955 年[9 11]由 Kolin 建立了在连续 pH 梯度下聚焦离子的概念并设计了 U-型电泳槽，这是等电聚焦的开始. 60 年代由 Svensson-Rilbe[1,2]和 Vesterberg[3]建立了系统的等电聚焦理论并合成了 CA，使 IEF 技术得以广泛应用于各个领域，这是第二阶段. 接着 1975 年由 Gasparic 等人[5]建立的 IPG IEF 技术大大提高了分辨率和克服了 CA IEF 的一些缺点，但它本身也还有一些问题. 如在加样处样品的纹理 (streaking) 以至沉淀现象. 为此 1985 年 Rimpilainen 和 Righetti[12]提出了在 IPG 凝胶中用 CA 作为添加剂的 CA/IPG 混合等电聚焦技术. 由于 CA 起了屏蔽分子的作用，遮挡了蛋白分子表面的疏水基团，明显地增加了蛋白质的溶解度，同时由于 CA 的导电性高于 IPG，使 CA/IPG 凝胶的电泳时间也缩短了. 但有关的理论和实验仍在不断的探索之中. 目前这个技术已由 Görg[13 15]成功地应用于作为双向电泳的第一向，以克服纹理现象和提高分辨率.

参 考 文 献

1 Svensson H. Acta Chem Scand, 1961; **15**: 325—341
2 Svensson H. Acta Chem Scand, 1962; **16**: 456—466
3 Vesterberg O. Acta Chem Scand, 1969; **23**: 2653—2666
4 Righetti P G. Isoelectric focusing: Theory, methodology and applications. Amsterdam: Elsevier Biom Press, 1983: 314—334
5 Gasparic V, Bjellqvist B, Rosengren Å. Swidish patent, 1975: 140, 49-1 w5 patent, 1978: 4, 130, 470. French patent, 1980: 76, 37, 402
6 Righetti P G. Immobilized pH gradients and methodology. Amsterdom: Elsevier Biomedical Press, 1990: 53—115
7 Righetti P G. Immobilized pH gradients and methodology. Amsterdom: Elsevier Biomedical Press, 1990: 119—139
8 Righetti P G. Immobilized pH gradients and methodology. Amsterdom: Elsevier Biomedical Press, 1990: 64—67
9 Kolin A J. Chem Phys, 1954; **22**: 1628—1629
10 Kolin A J. Chem Phys, 1955; **23**: 407—410
11 Kolin A. Proc Natl Acad Sci USA, 1955; **41**: 101—110
12 Rimpilainen M A, Righetti P G. Electrophoresis, 1985; **6**: 419—422
13 Görg A. Nature, 1991; **349**: 545—546
14 Görg A, Postel W. Electrophoresis, 1988; **9**: 531—546
15 Görg A. Biochemical Society Transactions, 1993; **21**: 130—132

DNA double helix. antisense inhibit translation or transcription. Both strategies can be applied to control the expression of oncogenes and growth factors in tumor cells. Here. the application of antisense was reviewed in cancer research briefly. a. Inhibition of oncogene and growth factor expression to suggest their function in tumor cells. b. Discrimination between proto-oncogene and activated oncogene. c. Problems and approaches in antisense gene therapy.

Key words antisense. oligodeoxynucleotide. tumor

Thermogenesis in Brown Adipose Tissue and Its Regulation. Ye Zucheng. Cai Yipeng. *(Dept. of Biology. Peking University. Beijing* 100871*). Prog. Biochem. Biophys.* (China). 1994; **21** (2): 135

Brown adipose tissue (BAT) is a kind of facultative-thermogenic organ. especially important in small mammals. The key element in BAT heat production is the uncoupling protein (UCP). a unique protein located in the inner membrane of BAT mitochondria. Thermogenic stimulation of this tissue opens the UCP's proton channel. results in a proton short-circuit. thus bypasses the relatively small amount of ATP synthetase present in BAT mitochondria resulting in a severalfold accelerated oxidative metabolism. The structral and functional state of BAT is regulated by many factors. such as norepinephrine. thyroid hormone. insulin. pH. and food. enviormental temperature etc.

Key words brown adipose tissue. uncoupling protein. proton channel. facultative thermogenesis. norepinephrine

A New Era in Production of Monoclonal Antibodies. Zhang Zhiwen. Zou Changjiang. *(Department of physiology. Beijing Medical University. Beijing* 100083*). Prog. Biochem. Biophys.* (China). 1994; **21** (2): 139

Construction of complex antibody libraries that expressed soluble antibody fragments on the surface of fd-phagemid with high screening efficiency subjected to rounds of *in vitro* mutations. The individual antibody gene can then be affinity matured by emulating the process that occurs in B-cells *in vivo* . The affinity matured antibody fragments are selected for their ability to bind antigen after phage recovery. This novel recombinant DNA methods may replace the technology of using mice and hybridoma for the selection and production of antibodies.

Key words antibody fragment. surface display vector. affinity maturation

A Current Break Through Electrophoresis Technique with Supermost Resolution: Immobilized pH Gradients Isoelectrofocusing. Guo Yaojun. Guo Qiang. *(Institute of Biophysics. CAS. Beijing* 100101*). Prog. Biochem. Biophys.* (China). 1994; **21** (2): 143

The mentioned immobilized pH gradients isoelectrofocusing is a electrophoresis technique developed in 80′ s. An approximate linear pH gradient is genarated by titrating weak acidic and basic acrylamide derivaties which then covalent bound into the polyacrylamide matrix. The pH gradient is stable and independent of electric field fluctuation. The present method provides higher resolution and larger loading capacity comparing with conventional carrier ampholyte isoeletrofocusing. It can analyse and purify protein with only minor p*I* difference.

Key words isoelectrofocusing (IEF). immobilized pH gradient (IPG). carrier amphelyte

(CA)

Study on the Mechanism of Protective Effet of Zinc to Cells. Zhang Jingxia. Huang Ping. Xu Shiwen. An Lizhi. Yao Huiying. Xiao Yanan. Pan Juxiang. Chao Zhiyu. Zhu Jieqing. Wu Xiankang. *(Beijing Medical University. Beijing* 100083*). Prog. Biochem. Biophys.* (China). 1994; **21** (2): 147

On analyzing the distribution and composition of element in single cell with scanning proton microprobe (SPM) and synchrotron radiation X-Ray fluoroscence microprobe (SR-XMF) it shows that zinc is a constituent of cell plasma. The levels of cellular zinc and iron were detectecd by above nuclear technique and the contents of MDA, SH group were estimated respectively by biochemical method for both normal and injured cell induced an peroxidation damage by hydroxyl-free radical. It was found that MDA content increased, SH group decreased, as well as Fe/Zn ratio raised during lipoperoxidation. By supplementation of zinc to culture medium, the inhibitory effect of zinc on lipoperoxidation was obvious from following experiment results that MDA content decreased. SH group content increased as well as Fe/Zn ratio reduced. The results suggest that zinc plays a role in maintaining the integrity of the cell and protects SH group of membrane protein thus preventing catalytic peroxidation reaction of iron.

Key words scanning proton microprobe (SPM), synchrotron radiation X-ray fluoroscence microprobe (SR-XME), lipoperoxidation, zinc, protect

Studies on the Photoelectric and Kinetic Spectroscopic Properties of Acetylation Bacteriorhodopsin. Hu Kunsheng. Shi Hua. Huang Ying. Yu Bi. *(Institute of Biophysics, Academia Sinica, Beijing* 100101*). Prog. Biochem. Biophys.* (China). 1994; **21** (2): 150

The role of lysine residues in the structure and function of bacteriorhodopsin (bR) was studied by the chemical modification method——acetylation. After acetylation, the photoresponse signals and the decay of photocycle intermediate M412 were slowed down while the yields of M412 were decreased. But UV/VIS absorption spectra did not show that the conformation around retinal chromophore was disturbed by acetylation. The effect of acetylation was weakened by high pH or salt media. The results imply that lysine residues do not directly participate in the proton translocation, instead, they affect this process by their contribution to the surface protentials.

Key words bacteriorhodopsin, photoresponse, acetylation, lipid bilayer

Studies on Preparation and Properties of Lauric Acid-Modified Superoxide Dismutase. Yan Jiaqi. Xie Wenzheng. *(Zhengzhou College of Animal Husbandry Engineering, Zhengzhou* 450045*). Prog. Biochem. Biophys.* (China), 1994; **21** (2): 154

Superoxide dismutase is modified with lauric acid to improve its stabillity. Activated lauric acid was reacted with bovine Cu, Zn-superoxide dismutase at 40℃ for 1h and it was further purified by a Sephacryl S-200 colum. There are 93% recovery of enzyme activity from the resulting lauric acid-superoxide dismutase conjugate. The specific activity of this enzyme product was 6000U/mg. The modified enzyme showed enhanced stability, substantially free from immunogenicity and prolonged its half-life in blood. As a consequence, it can beneficially be used as an enzyme for cosmetics and