

· 实验研究 ·

用凝胶电泳发现肝素与染料之间的相互作用

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摘要 目的:观察肝素与 23 种染料之间的相互作用。方法:利用淀粉琼脂糖凝胶电泳及聚丙烯酰胺凝胶电泳,观察染料加肝素后电泳行为的变化。结果:淀粉琼脂糖凝胶电泳中染料加肝素后多数结果是泳速变慢,有一些留在原点,也有前移、后退者。二氯荧光素、荧光红、荧光素、甲酚红、苯胺蓝、溴酚蓝、茜素红 S、胭脂红、亮绿、氨基黑 10B、丽春红 G、曙红、苯胺黑、氯酚红加肝素后泳速变慢。亚甲蓝、灿烂甲酚蓝、甲基紫加肝素后电泳留在原点。刚果红、洋红加肝素后电泳变化不明显。四氯荧光素、氯化硝基四氮唑蓝加肝素后电泳荧光看不清。聚丙烯酰胺凝胶电泳中溴酚蓝加入肝素后泳速明显变慢,随着肝素量增加溴酚蓝的泳速加快。与淀粉琼脂糖凝胶电泳结果一致,但它的电泳结果更明显。结论:本次所研究的 23 种染料,几乎都能与肝素发生相互作用,给它们的进一步深入研究打下基础。

关键词 电泳;肝素;染料;相互作用

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Using gel Electrophoresis Found the Interaction between Heparin and Dyes

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ABSTRACT Objective: To investigate the relationship between heparin and 23 kinds of dyes. **Methods:** Starch agarose gel electrophoresis and polyacrylamide gel electrophoresis were used to detect the changes of electrophoresis behavior after dye plus heparin. **Results:** Most electrophoresis results were slower than before after plus heparin, there were some stay in origin, also had moved forward, backward by starch agarose gel electrophoresis. The electrophoresis speed of dichlorofluorescein, fluorescent red, fluorescein, cresol red, aniline blue, bromophenol blue, the Sin purple S, carmine, light green, amido black 10B, Ponceau G, eosin, aniline black, chlorophenol red after plus heparin became slower. Methylene blue, brilliant cresyl blue, methyl violet after plus heparin electrophoresis stay at the origin. The electrophoresis of congo red, magenta after plus heparin did not change significantly. There were no electrophoresis fluorescence for tetrachloro-fluorescein, nitro tetrazolium blue after plus heparin. In polyacrylamide gel electrophoresis of bromophenol blue after adding heparin electrophoresis speed slowed down than before obviously, with the amount of heparin increased bromophenol blue electrophoresis speed up. Results of bromophenol blue were consistent with the starch agarose gel electrophoresis but its electrophoresis results are more obvious. **Conclusion:** The study of 23 kinds of dyes, almost can interact with heparin.

Key words: Electrophoresis; Heparindye; Interactions

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前言

肝素是一类结构复杂的糖胺聚糖,它在人体内具有多重生物活性,除了经典的抗凝血作用外,肝素还能与生长因子和细胞因子多种蛋白质发生相互作用。所以,肝素-蛋白质相互作用成为当前的一项重要课题^[1-6]。肝素和硫酸类肝素在体内具有抗凝血、调血脂、抗炎、抗动脉粥样硬化、调节血管生成等多方面的生物活性。这些生物活性是通过与多种蛋白质的相互作用来发挥的。在肝素与染料互作方面,已经有过光谱学研究^[7]和电

化学分析^[8,9],本文则从电泳角度研究肝素与染料的相互作用。我们利用淀粉琼脂糖凝胶电泳及聚丙烯酰胺凝胶电泳,观察染料加肝素后电泳行为的变化。本次所研究的 23 种染料几乎都能与肝素发生相互作用,但是肝素与染料互作时的结构与功能关系还需进一步研究。

1 材料与方法

1.1 试剂

1.1.1 肝素 肝素钠注射液(江苏万邦生化医药股份有限公司),主要成分为硫酸氨基葡聚糖的钠盐,属粘多糖类物质,平均分子量 12000,辅料为氯化钠、注射用水。

1.1.2 染料 共 23 种,1 四氯荧光素、2 二氯荧光素、3 荧光红、4 荧光素、5 亚甲蓝、6 甲基绿、7 甲酚红、8 刚果红、9 苯胺蓝、10 溴酚蓝、11 茜素红 S、12 胭脂红、13 亮绿、14 氨基黑 10B、15 洋红、16 灿烂甲酚蓝、17 丽春红 G、18 溴酚蓝(标签上字

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看不清)、19 曙红、20 苯胺黑、21 甲基紫、22 氯酚红、23 氯化硝基四氮唑蓝。这些染料,大部分是进口产品,级别为试剂级或生物染料级。

1.2 实验方法和步骤

1.2.1 淀粉-琼脂糖混合凝胶电泳检测肝素与染料之间的相互作用 电泳方法同前^[11,12]。

1.2.2 染料选择及其与肝素与互作实验 对手头的 30 多种染料,做水溶型试验,取 1.5 毫升 EP 管,加入蒸馏水 1 毫升、染料 1 耳勺,振荡后染料全溶解者留下,不溶解者剔除。30 多种染料中有 23 种溶于水。以下用水溶性染料做实验。每种染料用两个 1.5 毫升 EP 管,各加入染料 8 微升,其中之一管加入生理盐水 8 微升,另一管加入肝素氯化钠注射液 8 微升。混匀后上电泳,每种染料占两个泳道,前边是染料加盐水,后边是染料加肝素,然后通电,电势梯度 6V/CM,电泳时间 45 分钟。对于电泳结果,有以下几种处理:用数码相机直接照相、有紫外光灯时照相(透射和反射)。实验分两批进行。

1.2.3 第一批染料 四碘荧光素、二氯荧光素、荧光红、荧光素、亚甲蓝、甲基绿、甲酚红、刚果红、苯胺蓝、溴酚蓝,共 10 种。

1.2.4 第二批染料 茜素红 S、胭脂红、亮绿、氨基黑 10B、洋红、灿烂甲酚兰、丽春红 G、溴酚蓝、曙红、苯胺黑、甲基紫、氯酚红、氯化硝基四氮唑蓝,共 13 种。

1.2.5 PAGE 测肝素与溴酚兰之间的相互作用 PAGE 浓度为 10%。比较溴酚兰与加入不同量肝素后的电泳行为,由电泳仪上直接照相观察。

2 结果

2.1 第一批染料与肝素互作的电泳结果

由图 1-A 可以看出,有一些染料泳向阳极,另一些泳向阴极,加肝素者多数有变化。其中泳道 1 2 四碘荧光素看不清,3 4 为二氯荧光素也不明显,5 荧光红泳向阳极,呈黄绿色,6 荧光红加肝素后稍后退,7 8 荧光素类似荧光红,9 亚甲蓝泳向阴极,呈蓝色,10 亚甲蓝加肝素后变成原点(蓝色)不动,11 为甲基绿泳向阴极更远,呈浅蓝绿色,12 甲基绿加肝素后阴极成分减弱、阳极出现新成分,13 甲酚红泳向阳极,14 加肝素后泳速变慢,15 刚果红留在原点,呈深红色,16 刚果红加肝素后电泳位置和颜色都不变,17 苯胺蓝泳向阳极,呈浅紫色,18 苯胺蓝加肝素后泳速稍变慢,色更浅,19 溴酚蓝泳向阳极,呈蓝色,20 加肝素后泳速变慢、颜色变浅。图 1-B 基本同图 1-A,泳道 5 6(荧光红)7 8(荧光素)结果更明显,17 18(苯胺蓝)显深蓝色。图 1-C 泳道 3 4(二氯荧光素)结果开始明显,5 6(荧光红)7 8(荧光素)结果更明显,泳道 17 18(苯胺蓝)出现多带,差别有三处,相对最明显者位于中间处。

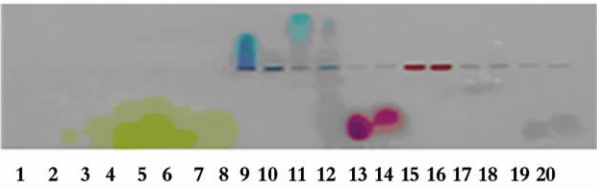


图 1-A 为第一批染料电泳后直接照相结果

Fig. 1-A Directly after the first batch of dye electrophoresis photographic results

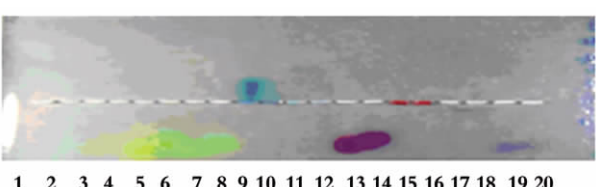


图 1-B 为 UV 灯上(透射)照相结果

Fig. 1-B shows the UV lamp (transmission) camera

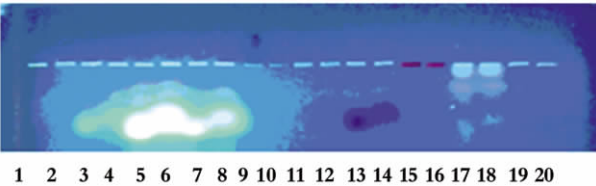


图 1-C 为 UV 灯下(反射)照相结果

Fig. 1-C shows the photographic results of the UV light (reflection)

图 1 注释:电泳图中上方为负极,下方为正极。由左向右 20 个泳道,单数为染料,双数为染料加肝素。泳道 1 2 为四碘荧光素,3 4 为二氯荧光素,5 6 为荧光红,7 8 为荧光素,9 10 为亚甲蓝,11 12 为甲基绿,13 14 为甲酚红,15 16 为刚果红,17 18 为苯胺蓝,19 20 为溴酚蓝
Fig. 1 Note: Electrophoresis of the top is negative, below the positive. 20 lane, from left to right singular dyes, double the number of dye plus heparin. Lane 12 is four iodine fluorescein, dichlorofluorescein in 34, fluorescent red in 56, 78 for fluorescein, 910 methylene blue, methyl green in 1112, cresol red in 1314, Congo red in 1516, 1718, aniline blue, bromophenol blue in 1920

2.2 第二批染料与肝素互作的电泳结果(见图 2)

由图 2-A 可以看出,多数染料泳向阳极,少数泳向阴极,加肝素者多数有变化。图 2-B 基本同图 2-A。图 2-C 泳道 17 18 显示明显荧光。

2.3 淀粉琼脂糖凝胶电泳综合结果

淀粉琼脂糖凝胶电泳中染料加肝素后多数结果是泳速变慢,有一些留在原点,也有前移、后退者。二氯荧光素、荧光红、荧光素、甲酚红、苯胺蓝、溴酚蓝、茜素红 S、胭脂红、亮绿、氨基



图 2-A 为第二批染料电泳后直接照相结果

Fig. 2-A Direct photographic results for the second batch of dye after electrophoresis

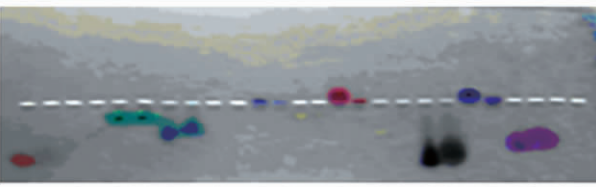


图 2-B 为 UV 灯上(透射)照相结果

Fig. 2-B UV lamp (transmission) photographic results

黑 10B、丽春红 G、曙红、苯胺黑、氯酚红淀琼电泳原染料泳向阳极 加肝素后泳速变慢。亚甲蓝、灿烂甲酚蓝、甲基紫淀琼电泳原染料退向阴极 加肝素后电泳留在原点。刚果红、洋红淀琼电泳加肝素后电泳变化不明显。四氯荧光素、氯化硝基四氮唑蓝淀琼电泳加肝素后电泳荧光看不清。

2.4 PAGE 检测肝素与溴酚兰互作结果

由图 3-B 可以看出，溴酚蓝加入肝素后泳速明显变慢 随着肝素量增加溴酚兰的泳速加快（见图 3-A 图 3-B）。

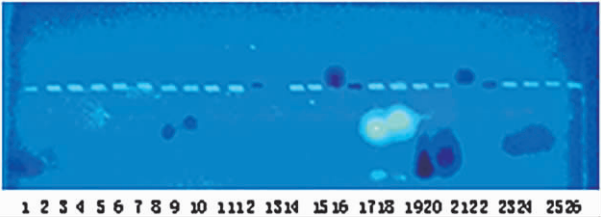


图 2-C 为 UV 灯下(反射)照相结果

Fig. 2-C The UV light (reflection) photographic results

图 2 注释 电泳图中上方为负极,下方为正极。由左向右 26 个泳道,单数为染料,双数为染料加肝素。泳道 1 2 为茜素红 S,3 4 胭脂红,5 6 亮绿,7 8 氨基黑 10B,9 10 洋红,11 12 灿烂甲酚蓝,13 14 丽春红 G,15 16 溴酚蓝,17 18 曙红,19 20 苯胺黑,21 22 甲基紫,23 24 氯酚红,25 26 氯化硝基四氮唑蓝,共 13 种。

Fig. 2 Note: Electrophoresis of the top is negative, below the positive. Left to right 26 lanes, a single number for the dye, double the number of dye plus heparin. Alizarin red S Lane 1 2 3 4 Carmine, 5 6 bright green, 7 8 Amido Black 10B, 9 10 magenta, 11 12 Brilliant Cresyl Blue, 13 14 Ponceau G 15 16 bromophenol blue?, 17 18 eosin, 19 20 aniline black, 21 22 Methyl Violet, 23 24 Chlorophenol Red, 25 26 nitro blue tetrazolium, a total of 13 kinds

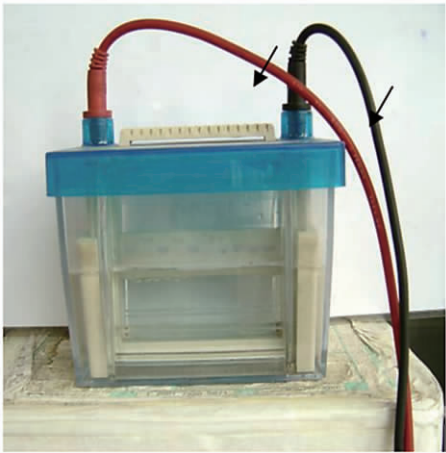


图 3-A 为聚丙烯酰胺电泳仪 电泳在进行中

Fig. 3-A Polyacrylamide gel electrophoresis, electrophoresis in progress

3 讨论

肝素与染料互作的研究种类不多,每一项目只研究一两种染料,而且用的是光谱学和电化学分析方法^[7-9],本文用电泳方法可同时研究多种染料与肝素的相互作用,效率较高。本文所研究多种染料大多数都与肝素有相互作用,从电泳行为角度可分成两类:泳向正极成分和泳向负极成分。泳向正极成分中

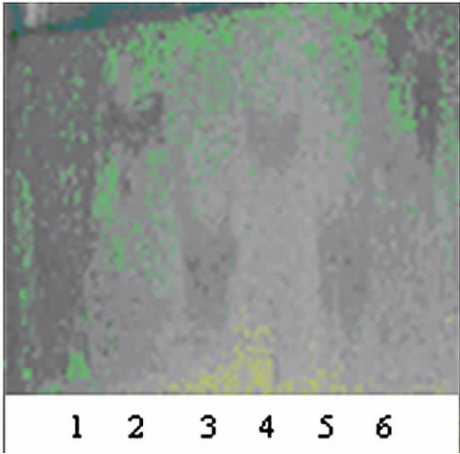


图 3-B 来自对电泳仪的直接照相结果 泳道由左向右,1 3 5 为溴酚蓝,2 4 6 为溴酚蓝加肝素(第 2 泳道肝素量与溴酚蓝相等,第 4 泳道肝素量增加一倍,第 6 泳道肝素量增加两倍)

Fig. 3-B From the direct photographic results of electrophoresis: lane from left to right, bromophenol blue, 135, 246, bromophenol Langa heparin (lane 2 heparin amount of bromophenol blue, equal, lane 4 heparin doubling, lane 6 heparin increased twice as much)

有一部分属于荧光染料,泳向负极成分则几乎不显荧光。荧光染料中苯胺蓝的情况比较特殊:电泳后直接照相时,只有一个白点被暗蓝色区带包围,加肝素者也差不多,稍靠后一点,UV 灯上透射照相时,见到两个蓝带,苯胺蓝色深,加肝素者色浅,也是稍靠后一点,UV 灯上反射照相时,苯胺蓝出现 6-7 条浅蓝绿色荧光区带,加肝素者有三条带泳速变慢,也就是说有一部分成分与肝素发生相互作用。荧光红与荧光素的情况与苯胺蓝有一些类似,UV 灯上透射照相时,出现 3-4 条浅蓝绿色荧光区带,加肝素者有一条带泳速变慢。二氯荧光素的荧光区带比荧光素弱、区带减少,四碘荧光素的荧光区带更弱、甚至消失,可见氯化或碘化处理对荧光素的影响较大。第二批染料中曙红的荧光明显,快泳的次要成分不受肝素影响,慢泳的主要成分与肝素互作而变得更慢。PAGE 检测肝素与溴酚蓝互作结果,也是溴酚蓝加肝素时泳速变慢,与淀琼电泳结果一致,但它比淀琼电泳结果更明显。淀琼电泳可以观察泳向正极和负极的两类染料,PAGE 无法检测泳向负极的染料。总之,我们用两种电泳方法都证明了多种染料能与肝素发生互作,给它们的进一步深入研究打下基础,特别是结构分析。肝素的结构与功能关系的文章不少^[12-20],都是肝素与各种蛋白互作时的结构与功能关系,没有涉及肝素与染料互作时的结构与功能关系。肝素是一类结构复杂的糖胺聚糖,它的哪个部位与染料结合,有多少个结合部位,正负电荷的分布情况如何等都是需要深入研究的内容。

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