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研究论文

低pH毛细管区带电泳法分离寡核苷酸和硫代反义寡核苷酸

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摘要 在低pH条件下采用毛细管区带电泳法对寡核苷酸(PO-ODNs)及具有药用价值的硫代反义寡核苷酸(PS-ODNs)药物“癌泰得”系列样品(18~20 mers)进行分离,并系统考察优化了缓冲溶液的pH、缓冲溶液的种类及浓度、添加剂的种类及浓度、电压、温度等因素对样品单碱基分离的影响。其中缓冲溶液的pH对分离起决定性的作用,而添加剂尿素的加入显著提高了PS-ODNs样品的分离度。结果表明,采用未涂层弹性石英毛细管(50 μm, 总长49.0 cm,有效长度40.7 cm),以50 mmol/L磷酸二氢钠-磷酸(pH 2.24)-7 mol/L尿素为缓冲溶液,压力进样(2 kPa×10 s),负极进样,正极检测,在分离电压20 kV、柱温25 ℃、检测波长260 nm条件下,可实现寡核苷酸及硫代反义寡核苷酸混合样品的单碱基分离。PO-ODNs的18~19 mers及19~20 mers样品的平均分离度分别为4.68,3.20;PS-ODNs的18~19 mers及19~20 mers样品的平均分离度分别为1.23及0.81。该法操作简单,重现性好,可为反义寡核苷酸药物的分析提供借鉴。

关键词 低pH 毛细管区带电泳法 寡核苷酸 硫代反义寡核苷酸

分类号

Separation of Phosphodiester Oligodeoxynucleotides and Phosphorothioate Antisense Oligodeoxynucleotides by Capillary Zone Electrophoresis at Low pH

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Abstract

Oligodeoxynucleotides (ODNs) may possess biological activity in vivo, and are used for the cancer therapeusis. Synthesized ODNs contains many by-products, and so their purity check and resolution of single-base, i.e., the separation of ODNs differing by one nucleotide in length, become necessary. In this study, capillary zone electrophoresis (CZE) method was developed for the separation of two sets of model compounds of single-stranded oligodeoxynucleotide mixtures (1820 mers), phosphodiester oligodeoxynucleotides (PO-ODNs) and their phosphorothioate modifications (PS-ODNs), with equal sequences differing in a single base. The effects of the CZE operating parameters on the separation were investigated and optimized to further improve the resolution, such as the pH values and the concentrations of running buffer, the varieties and concentrations of additives, the separation voltage as well as the temperature. It was confirmed that the pH value of the buffer played the most important role in the separation, and the urea used as the additive in the system improved significantly the resolution of PS-ODNs. Consequently, the PO-ODNs and PS-ODNs mixtures could be single-based separated on a fused-silica capillary of 50 μm×49.0 cm (40.7 cm of effective length) under the optimum conditions: the running buffer system of 50 mmol/L NaH2PO4-H3PO4(pH 2.24)-7 mol/L urea, the pressure injection of 2 kPa×10 s, the separation voltage of 20 kV, the column temperature of 25 ℃, and the ultraviolet (UV) detection at 260 nm. The average resolutions for the separation of 1819 mers and 1920 mers of PO-ODNs were 4.68 and 3.20, respectively; and the average resolutions for the separation of 1819 mers and 1920 mers of PS-ODNs were 1.23 and 0.81, respectively. The relative standard deviations of the migration time and the resolution were all less than 5%. This method will be useful for the qualification of PO-ODNs and PS-ODNs samples as they are used in antisense drug development due to the relatively easy operation and good reproducibility of the method in comparing with the capillary gel electrophoresis.

Key words low pH capillary zone electrophoresis (CZE) phosphodiester oligodeoxynucleotides (PO-ODNs) phosphorothioate antisense oligodeoxynucleotides (PS-ODNs)

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