High Resolution Capillary Electrophoresis Determined by Triblock Copolymer Matrix

by

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ABSTRACT

Although capillary electrophoresis single-strand conformation polymorphism (CE-SSCP) analysis combined with a polyacrylamide matrix had enormous potential as a simple and versatile pathogen detection technique, low resolution of CE-SSCP caused a limited application. In this study, DNA samples were separated by CE-SSCP using different polymer matrices. The polymer matrix was considered the most critical variable capable of improving resolution. The experiment focused on the polyacrylamide gel and the poly(ethyleneoxide)-poly(propyleneoxide)-poly(ethyleneoxide) (PEO-PPO-PEO) triblock copolymer (a conventional polymer matrix and a triblock copolymer matrix).

The purpose was to determine which polymer matrix separated the DNA samples with the highest resolution, focusing upon the clarity and sharpness of the peaks. Using four same-sized DNA fragments of similar sequence content, samples based on the nucleic sequence, as well as Hi-Di formamide and standard solution, were placed in the capillary electrophoresis apparatus for high resolution analysis to assess various times and concentrations. The resulting diagrams were studied to examine if the DNA samples could be determined based on the molecular weight.

Peak broadening developed because of the interaction between hydrophobic moiety of polymer matrices and DNA. This supported hypotheses of a conventional polymer matrix (polyacrylamide) not producing ideal results for CE-SSCP analysis. However, due to enhanced dynamic coating and sieving ability, the PEO-PPO-PEO triblock coplymyer displayed a fourfold enhancement of resolving power in the CE-SSCP to separate same size DNA molecules. Specifically, the Pluronic F127

$PEO_{99}PPO_{69}PEO_{99}$ and the Pluronic F108 $PEO_{137}PPO_{43}PEO_{137}$ had the potential to be used as an effective separation medium. Although both suppressed the electroosmotic flow induced by ionization, the Pluronic F108 displayed the greatest separation of DNA molecules.

An optimized polymer matrix was established by assessing the effect of polymer composition and concentration on the resolution of CE-SSCP separation. Along with the polyacrylamide gel, three other matrices of the PEO-PPO-PEO triblock coplymyer family were selected due to their transparency in the operable range of viscosity. A significant difference in the resolution between the matrices studied discriminated polymers of a similar sequence, as well as a conventional polymer, and indicated that the PEO-PPO-PEO triblock copolymer may serve as an ideal polymer matrix for high resolution CE-SSCP analysis.

Keywords: CE-SSCP / High-resolution / Polyacrylamide / Poly(ethyleneoxide)-poly(propyleneoxide)-poly(ethyleneoxide) (PEO-PPO-PEO) tri-block copolymer / Pluronic F127