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Capillary electrophoresis of a wide range of DNA fragments in a mixed solution of hydroxyethyl cellulose

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We carried out capillary electrophoresis of 0.1–10.0 kilo base pair DNA fragments in a mixed hydroxyethyl cellulose (HEC) polymer. The mixed HEC polymer was prepared with different molecular weights (M_w) (90k, 250k, 720k and 1300k). The effects of important parameters, including the ratio of the mixture composition and the concentration of the mixing polymer, on the separation performance were investigated. Results show that these parameters can not only shorten the migration time of DNA without great deterioration in resolution, but they can also decrease the viscosity of the polymer, and thus make it easy to fill the capillary. Finally, we separated φ×174-*Hirc* II digest and λ-*Eco*T14 I DNA digest with high resolution in the mixed HEC solution within 18 min.

Introduction

Due to its short separation time, high efficiency, low detection limits, and reduced usage of samples and consumables, capillary electrophoresis (CE) has become a universal technique for the separation and identification of DNA fragments.[1–4] Traditionally, cross-linked gels (*e.g.*, polyacrylamide or agarose) were used as gel matrices in capillary electrophoresis because of their known utility in slab gels for the separation of proteins and DNA. However, due to their instability over time, irreproducibility in polymerization processes, and the fragile nature of the medium, cross-linked gels are not suitable for large-scale DNA separation.[5] Thus, entangled and uncross-linked water soluble polymers are deemed to provide advantages over cross-linked gels, such as easy flushing into the capillaries, longer capillary utilization times and greater speed. These polymers mainly include poly(ethylene oxide) (PEO),[6] polyvinylpyrrolidone (PVP),[7] poly-*N*,*N*-dimethylacrylamide (PDMA),[8] and hydroxyethyl cellulose (HEC).[9,10]

The migration mechanisms of DNA in CE are detailed in ref. 11–14. In this work, we mainly discuss the DNA separation by CE performed in an entangled polymer solution above the threshold concentration c^*. In the entangled polymer sieving matrix, the polymer chains overlap and form networks with dynamic pores. When DNA fragments migrate through, the polymer chains proceed with a constraint release by changing their interacting partners. At the same time, the DNA molecules undergo reptation,[15] and are then resolved by length. It is reported that DNA fragments with radii much larger than the mean pore size of the sieving matrix will disrupt the polymer–polymer entanglements and locally destroy the polymer network.[16–18] Therefore, large DNA molecules are most efficiently separated in relatively dilute solutions of high molecular weight polymers, while small DNA fragments are better resolved in concentrated solutions of homogenous polymer with lower molecular weight.[19–21] In order to resolve DNA fragments within a wide size range, researchers have employed mixtures of polymers with different molecular weights (M_w), and even copolymers of different monomers, as the sieving matrix.[22–26]

The hydrophilic HEC polymer can form highly entangled networks in aqueous solutions and its stiffness is suitable for sieving DNA fragments.[27] The c^* of HEC can be calculated using the empirical formula[28] in eqn (1) and the mean pore size[29] ζ of the sieving matrix can be evaluated using eqn (2):

$$c^* = 3.63[M_n/M_o]^{-1.2} + 1.18 \times 10^{-4} \quad (1)$$

$$\zeta \approx 1.43 R_g (c/c^{*(1+a)/3a}) \quad (2)$$

where M_n is the number average molecular weight, M_o is the average monomer molecular weight of HEC, R_g is the radius of gyration of the polymer and c is the concentration of the polymer. The exponent a varies between 0.5 and 0.8 for different

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polymers. It can be deduced from eqn (1) that the larger the HEC polymer is, the lower c^* it will possess. From eqn (2), it can be determined that the mean pore size of an entangled polymer solution does not depend on the polymer length but mainly on its concentration and on the nature of the polymer.[14] Therefore, if we add some higher M_w HEC molecules into a lower M_w HEC solution, whose c is relatively dilute (slightly above its c^*), the long polymer chains will strengthen the structure of the sieving network. This might help to produce a better degree of entanglement. Moreover, this kind of mixed solution possesses a more ideal viscosity, which is between those of the two single HEC solutions.[27] Alexander P. Bünz and his coworkers reported the separation of DNA restriction fragments in dilute (non-entangled) HEC mixture solutions,[30] A. R. Isenberg, B. R. McCord *et al.* employed a mixture of M_n (number-average molecular weight) 40 000 and M_n 140 000–160 000 HEC and baseline separated DNA fragments in the range 150–1000 base pairs (bp).[31] However, so far there is no detailed report on DNA analysis in an entangled mixed HEC solution.

In this paper, we have separated DNA fragments ranging in size from 0.1 to 10.0 kilo base pairs (kbp) in mixed solutions of HEC by CE, and investigated the influence of the concentration of the HEC mixture and the ratio of the mixture composition on the separation performance. Such a study may provide new insight on the fast separation of DNA by CE.

Experimental

Chemicals

0.1 kbp, 1.0 kbp DNA ladder, φ×174-*Hirc* II digest and λ-*Eco*T14 I digest were purchased from Takara (Shiga, Japan). SYBR Green I was purchased from Invitrogen (Carlsbad, CA, USA). HEC with M_w values of 1300k, 720k, 250k and 90k was purchased from Sigma (St Louis, MO, USA). 10× Tris–borate–EDTA (TBE) buffer was purchased from Bio-Rad (Hercules, CA, USA). HEC polymer solution containing 1× SYBR Green I was prepared by dissolving it in the 0.5× TBE buffer. DNA samples were dissolved in 0.5× TBE buffer and mixed to make each DNA ladder concentration 16 μg ml^{-1}. Prepared DNA samples were kept frozen at −20 °C before use.

Apparatus

The CE system used in this study has been described in detail elsewhere.[32,33] Briefly, it consisted of a microscope with epi-illumination (IX71, Olympus, Tokyo, Japan) and a high-voltage power supply (MODEL 610E, TREK, Medina, NY, USA). The power supply was controlled by the locally programmed LabVIEW software (National Instrument). A mercury lamp produced the excitation light with a wavelength range of 460–495 nm, which matches the wavelength of the excitation maximum of the conjugate of SYBR Green I and the nucleic acid, by using an optical filter (U-MWIB-3, Olympus, Tokyo, Japan). The fluorescence emission was collected using a 100× objective (PlanApo/IR, Olympus). The fluorescence signal was detected using a photo multiplier tube (R928, Hamamatsu Photonics, Hamamatsu, Japan), and the signal was digitized using a National Instrument PCI-6024E (Austin, TX, USA). Fused-silica capillaries (PolymicroTehchnologies, Phoenix, AZ, USA) with ID/OD = 75/365 μm were covalently coated with polyacrylamide.[34,35] DNA samples were electrokinetically injected at 100 V cm^{-1} for 2.0 s. The entire detection system was enclosed in a black box.

Results and discussion

Separation of DNA fragments in HEC with different M_w values

In order to review the role of polymer M_w in the DNA separation performance of CE, we first resolved DNA fragments (0.1–10.0 kbp) in mixed HEC solutions in a 100 V cm^{-1} electric field. The mixed solutions were prepared from equal amounts of HEC with different M_w values (1300k, 720k, 250k, and 90k) in 0.5× TBE buffer. Fig. 1A–C show a typical trend in the change in the DNA separation with the molecular weight of the mixed HEC solutions. They show that DNA fragments from 0.1 to 7.0 kbp were well resolved in the three mixed HEC solutions, however the separation time was different, implying that the two solutions of the same type of polymer and with the same concentration but different M_w may have the same "pore size" if they are entangled. Fig. 1D depicts the effect of mixed polymers on the migration times of DNA. It shows that in terms of speed, the migration time corresponding to the 0.1 kbp DNA fragment was nearly the same in each of the different mixed solutions, but the other DNA fragments (0.2–10.0 kbp) move faster when the 1300k HEC is mixed with the lower M_w HEC. Furthermore, the slope of the short DNA fragments (0.1–1.0 kbp) decreased with the decrease in the M_w of HEC added to the background electrolyte, but the slope of the longer DNA fragments was very stable, emphasizing that the successful separation of short DNA fragments was mainly attributed to the low M_w HEC, while the separation of longer DNA fragments was dependent on the high M_w HEC. Moreover, we found that the DNA separation process

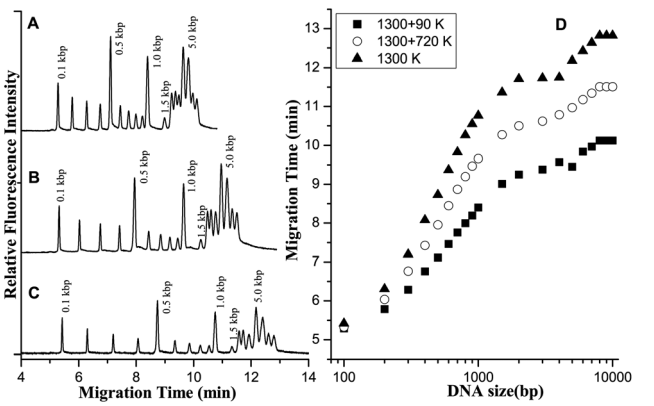

Fig. 1 Electrophoretic separation of DNA in 0.4% mixed solutions of (A) HEC (1300k + 90k); (B) HEC (1300k + 720k); (C) 1300k HEC. (D) Migration times of DNA *versus* the DNA size corresponding to (A–C). Electrophoretic conditions: the ratio of the two HEC polymers was 1 : 1; the samples were loaded at 100 V cm^{-1} (2.0 s), the electric field strength was 100 V cm^{-1} and the sample was diluted in 0.5× TBE buffer. The total length (l_t) and the effective length (l_e) of the capillary are 14.0 cm and 8.0 cm, respectively.

could be finished within 11.0 min, while the resolution for the larger DNA fragments (>1.5 kbp) did not deteriorate, thus we chose the 250k/1300k mixed HEC solution for our research in the following sections.

Effect of the ratio of HEC with different M_w values on the separation performance

From Fig. 1, we have found that the composition of the mixed polymer solution will influence the migration time of DNA, and thus it is necessary to research the effect of the ratio of the mixed polymer composition on the separation performance. The separation performance was evaluated based on the migration times of DNA and the resolution between adjacent peaks in the electropherogram. The resolution (R)[36] is calculated using the following equation:

$$R = \Delta t/1/2(W_1 + W_2) \quad (3)$$

where Δt is the difference between two adjacent peaks and W_1 and W_2 are the peak widths measured at the baseline.

The mixed HEC polymer solutions were prepared with different ratios: 0/10, 2/8, 4/6, 6/4, 8/2, and 10/0. Then we separated the DNA samples in the various mixed HEC polymers by CE at 100 V cm^{-1}. Fig. 2 shows an example of the separation of DNA in 0.4% HEC (250k/1300k) solutions with ratios of 10/0, 6/4 and 2/8. Fig. 2 and 3 display the separation performance in the mixed HEC solutions with ratios from 0/10 to 10/0. This shows that even though the ratio of the composition of the mixed solution changes, the trend in DNA fragment movement remains the same (Fig. 3A). Moreover, because the viscosity is positively related to the M_w of the polymer,[14] the migration time of the DNA fragments increases in solutions with excessive amounts of high M_w HEC (1300k). Another interesting phenomenon is that the resolution (Fig. 3B) between the short adjacent DNA fragments (0.1–1.0 kbp) degrades with an increase in the proportion of 250k HEC in the mixed solution, while the resolution of the larger DNA fragments (1.0–10.0 kbp) seems very stable. Furthermore, it is worth noting that the resolution of longer DNA fragments (>1.0 kbp) was almost the worst when they were resolved in single M_w HEC solutions of 250k. This was mostly because the pore size of the matrices is too small to have sieving power for those longer DNA fragments.

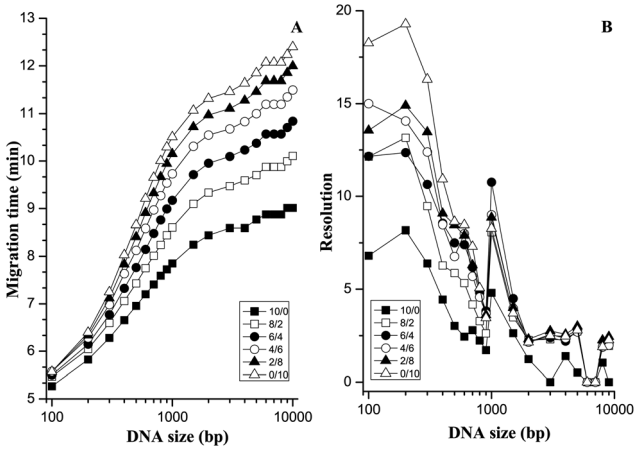

Fig. 3 The effect of the ratio of the HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig. 1.

Effect of the mixed polymer concentration on the separation performance

In Fig. 3, we find that if there is too much low M_w HEC in the mixed polymer, the sieving solution offers poor resolution for the DNA fragments, and when the ratio of higher M_w HEC is larger, the migration of DNA is prolonged because of the high viscosity of the polymer matrices. Furthermore, it is obvious that it will be harder to fill the capillary with a polymer solution with a higher viscosity. Therefore, we choose the mixed HEC polymer comprised of polymers of different M_w values with a

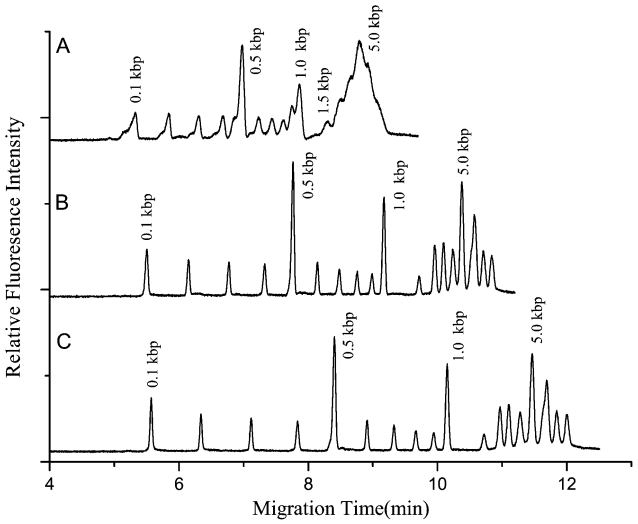

Fig. 2 Electrophoretic separation of DNA in 0.4% mixed solutions of HEC (250k/1300k) with different ratios: (A) 10/0 (B) 6/4; (C) 2/8. The other electrophoretic conditions are the same as in Fig. 1.

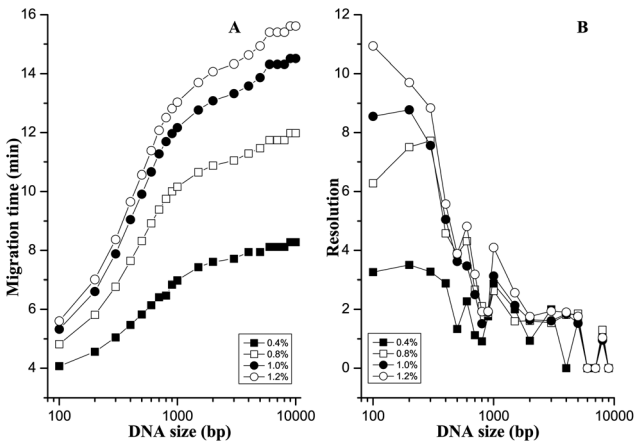

Fig. 4 The effect of concentration of the HEC mixture on the separation performance of DNA by CE. The other electrophoretic conditions are the same as in Fig. 1.

volume ratio of 1 : 1 as the separation buffer. Fig. 4 depicts an example of the DNA separation performance in the mixed polymer (250k and 1300k) with concentrations ranging from 0.4% to 1.2% in an electric field of 100 V cm^{-1}. The plot is also derived from the electropherogram similar to Fig. 1. We find that when the concentration of the mixed solution is lower than 0.4%, DNA fragments larger than 1.5 kbp almost migrate together (data not shown). When the concentration of the mixed HEC solution is above 1.2%, the situation contributes more to increasing the DNA fragment migration time rather than the resolution, especially for the larger fragments (>5.0 kbp). Fig. 4A shows that the migration time of DNA increases with an increase in the mixed polymer concentration because of the increase in the viscosity of the polymer. Data shown in Fig. 4B demonstrate that with an increase in the mixed polymer concentration, the resolution of the short DNA fragments (<1.0 kbp) improves. When the concentration is higher than 0.8%, it seems that there is no great improvement in the resolution of the longer DNA fragments (>0.3 kbp). Furthermore, 1.2% mixed HEC solution offers the most ideal resolution over a wide range, but it is at the cost of longer separation times because of its high viscosity.

Separation of φ×174-*Hirc* II digest and λ-*Eco*T14 I DNA digest in mixed HEC polymer

Based on the results obtained above, we separated φ×174-*Hirc* II and λ-*Eco*T14 I DNA digests in a mixed solution of 0.8% HEC (250k and 1300k) by CE. The electric field strength is 100 V cm^{-1}, and the ratio of two mixed HEC solutions with different M_w values is 1 : 1. The DNA digests mainly contain 24 DNA fragments, and the sizes of the gene fragments are 74, 79, 162, 210, 291, 297, 335, 341, 345, 392, 421, 495, 612, 770, 925, 1057, 1489, 1882, 2690, 3472, 4254, 6223, 7743, and 19 329 bp. As shown in Fig. 5, DNA samples were successfully resolved over a wide range within 18 min.

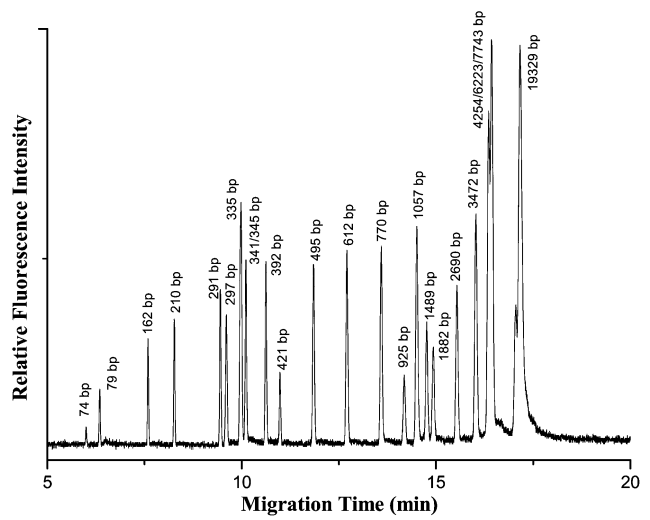

Fig. 5 Separation of φ×174-*Hirc* II and λ-*Eco*T14 I DNA digests in a mixed solution of 0.8% HEC (250k and 1300k) by CE. The other electrophoretic conditions are the same as in Fig. 1.

Concluding remarks

This paper reports the separation of DNA fragments (0.1–10.0 kbp) in mixed polymers of different M_w HEC by CE. We have mainly investigated the factors (*i.e.* the ratio of the mixed polymer composition and the concentration of the mixed solution) on the separation performance. Results show that the mixed HEC solution can provide a comparative DNA separation performance at a lower viscosity. Also, a mixed HEC polymer (250k and 1300k) at 0.8% with a ratio of 1 : 1 offers high resolution for DNA ranging from 74 to 19 329 bp within 18 min.

Conflict of interest

The authors have declared no conflict of interest.

Abbreviations

HEC	Hydroxyethyl cellulose
kbp	Kilo base pairs
M_w	Weight average molecular weight
CE	Capillary electrophoresis
TBE	Tris–borate–EDTA

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