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Comb-like copolymers as self-coating, low-viscosity and high-resolution matrices for DNA sequencing

Comb-like copolymers with a polyacrylamide backbone and poly(*N*,*N*-dimethylacrylamide) grafts were prepared, as a way to combine the superior sieving properties of polyacrylamide with the self-coating properties of polydimethylacrylamide. These matrices function well in the absence of a capillary coating, and achieve separation performances for single-stranded DNA that are comparable to those of state-of-the-art long-chain linear polyacrylamide. Structural parameters such as the grafting density and the polymer molecular mass were varied, and good performance appears to be achieved with a relatively large range of parameters. Surprisingly, excellent separation is achieved even with matrices that have a viscosity as low as 200 mPa/s. A discussion of the physics underlying this behavior is provided.

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1 Introduction

The last few years have seen an explosive development and progress in the area of DNA separations by capillary electrophoresis (CE) [1, 2]. Probably the most spectacular achievement of this technique has been the considerable acceleration of the human genome project permitted by the development and commercialization of automated capillary array sequencers [3–5]. The "read length", *i.e.*, the number of bases read without error in a single run, is the most important parameter for sequencing, since it directly influences the number of clones that must be sequenced for full coverage of a given genome, as well as the ability to read through difficult-to-sequence zones such as long series of repetitive sequences. The read length depends on the distance between different peaks corresponding to DNA fragments differing by one base (called "band spacing" in the following, and directly related to the "selectivity" in chromatography terminology), and the band width (related with the "efficiency" in the chromatography terminology). Depending upon the mode of electrophoresis (measurement of migration time at fixed distance, or of migration distance at fixed time), the band width and band spacing can be expressed in distance or in time units. In this article, we are interested in CE, *i.e.*, in the "fixed distance" mode. Thus, we express the peak spacing (tm_2–tm_1, where tm_1 is the migration time of peak 1 and tm_2 is the migration time of peak 2) and the band width $\sigma_{1/2}$ in time units. From these data, we can derive the resolution R [6–8], defined as:

$$R = \frac{\sqrt{5.5}}{2} \frac{(tm_2 - tm_1)}{(tm_2 + tm_1)} \frac{tm_2}{\sigma} \frac{1}{\Delta N} \quad (1)$$

where ΔN is the number of bases separating peaks 1 and 2. For the best base-callers available, in optimal conditions, sequences can typically be read at an accuracy of 98.5–99%, down to a resolution of 0.3 [8]. Numerous parameters can affect the resolution, in a positive or negative way. Other interesting parameters are the separation time and the robustness to samples from different sources and of different qualities. Optimizing sequencing is thus a multifactorial task, involving the sequencing reaction, the choice of dyes, the prepurification steps, and the base-calling software.

It is widely acknowledged that the quality of the sieving matrix is the first key to success since there is no way to restore resolution that has been lost in the separation process itself. A number of polar (hydrophilic) polymers have been proposed with some success for DNA sequencing (see, *e.g.*, [3, 4, 9] for a review), and considerable efforts have been applied to the improvement of sieving matrices for capillary sequencing. The most spectacular results achieved to date (of the order of 1300 bases, corresponding to a resolution of about 0.25 in less than 2 h) were obtained in linear polyacrylamide (LPA) with an extremely high molecular mass (of the order of 20 MDa) prepared by inverse-emulsion polymerization [8]. A partial clue to this

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Abbreviations: **LPA**, linear polyacrylamide; **M-LPA**, medium molecular mass LPA; **PAM**, polyacrylamide; **PDMA**, poly(*N*,*N*-dimethylacrylamide; **PEO**, polyethylene oxide; **PN/PAM**, poly-*N*-isopropylacrylamide

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success was provided in [10], where the sequencing performances of a series of poly(*N*,*N*-disubstituted acrylamide) copolymers with various hydrophobicities were assessed. Resolution and read length was found to decrease with increased polymer hydrophobicity, and the most hydrophilic polymer of the series, LPA, yielded the best results. Dextran, however, which is even more hydrophilic than LPA, does not perform as well for DNA sequencing, so other structural parameters must play a role.

LPA, unfortunately, has two significant disadvantages for routine applications. First, it imposes the use of precoated capillaries. The state of the capillary wall is a critical issue in sequencing since it can dramatically affect resolution, and thus read length. Uncoated capillaries tend to yield considerable band broadening and irreproducible results. It is believed that even very minute impurities contained in the sample (in particular proteins) can adsorb to bare silica, and induce inhomogeneous charge densities on its surface. Such inhomogeneities yield localized electroosmotic recirculating flows that cause band broadening [11]. Two strategies are currently used for preventing such problems: permanent coating or self-coating matrices.

For permanent coating, a polymer (generally neutral and hydrophilic) is bonded to the capillary wall prior to the introduction of the sieving matrix. Numerous coating protocols have been proposed, and several ones using polymers such as covalently coupled polyvinyl alcohol (PVA) [12], or polyacryloylaminoethanol (PAAE) [13] are now able to yield stable results for several hundred electrophoresis runs in routine applications. In the "self coating" or "dynamic coating" approach, the matrix is introduced in a bare capillary, to achieve the double goal of separating DNA by size and preventing adsorption of impurities on the capillary wall and electroosmosis. This requires a rather delicate balance, because very hydrophilic polymers do not adhere to bare silica, and hydrophobicity of the sieving matrix is detrimental to resolution in sequencing applications [9] (and probably in many other applications involving biological molecules). In spite of this difficulty, several matrices with dynamic coating properties have been proposed with success, such as polyethylene oxide (PEO) [14], poly(*N*,*N*,-dimethylacrylamide) (PDMA) [15–17] and a proprietary acrylic polymer called "Duramide" [18]. Random copolymers of PDMA and allyl glycidyl ether have also been thought initially to achieve dynamic coating [19], but it seems most probable at present that the epoxy group actually binds covalently to the capillary wall (Chiari, personal communication). Copolymers of PEO and polypropylene oxide (PPO), called "pluronic" also achieve dynamic coating [20, 21], but only at a very high concentration (typically 20%), yielding slow migration and poor sequencing performances. To date, the resolution achieved with self-coating matrices is significantly worse than achieved with the best noncoating matrix, LPA. In spite of this disadvantage, dynamic coating matrices are widely used in practical applications, because of the reduced cost and improved convenience associated with the use of bare capillaries.

A second significant disadvantage of LPA for practical sequencing applications is the viscosity of the polymer matrices. For optimal read length, it is recommended to use a capillary diameter no larger than 75 μm, and capillary lengths of 50–65 cm. Even if one takes advantage of the shear-thinning properties of entangled polymers, introducing solutions with a zero-shear viscosity larger than 10 000 mPa/s requires a high pressure and a long loading time which becomes more and more significant with the reduction of separation time (now typically less than 90 min). It is expected that reasonably low matrix viscosities will also be beneficial for operation in the new generation of analytical methods involving microfluidics and "laboratories on chips". Polymer solutions comprising LPA at sequencing concentration are extremely viscous. The injection of such matrices in a capillary requires a pressure of the order of 7 MPa, and a filling time of 10 min in a 75 μm ID capillary. Interestingly, the other most common commercial matrices, POP5 and POP6 ("performance optimized polymer"), used in the capillary sequencers made by ABI, are based on PDMA with a much lower molecular mass and viscosity when compared to LPA solutions [16]. The read length is significantly shorter than with LPA in coated capillaries, but better than that obtained with LPA of comparable viscosity.

To combine high sieving performance with low viscosity, we recently [23] proposed a new family of thermoviscosifying polymers, with the following properties: (i) a low viscosity at room temperature for easy loading and flushing of the capillary; (ii) a high viscosity and optimal sieving properties (*i.e.*, in particular good resistance to deformation) at and above 50°C, with absence of turbidity and absorption in the visible range, minimal specific interactions with DNA and sequencing dyes/labels, compatibility with denaturating buffers, and charge-neutrality at pH 7–8. These polymers present a polyacrylamide (PAM) backbone, and side-chains made of poly *N*-isopropylacrylamide (PNIPAM). The polymer matrices yield resolutions significantly better than that of PDMA, but somewhat lower than that of LPA, with an extremely low injection viscosity at room temperature (around 100 mPa/s). A limitation of such matrices, however, is that they require a fast cycling between sequencing temperature and room

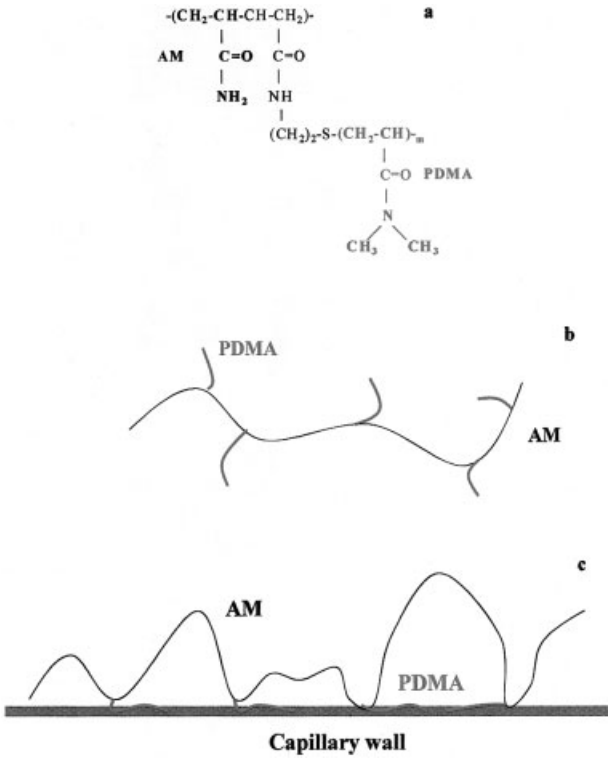

Figure 1. Description of the comb-like copolymers, P(AM-PDMA). (a) Chemical formula, (b) structure and (c) illustration of grafts adsorption on the capillary wall.

temperature for taking full advantage of their thermosensitivity without compromising the cycle time in routine applications (the cycle time is the effective time necessary for a complete separation, including capillary washing, filling and conditioning, injection and separation). It is also worth mentioning that, in contrast with LPA for which resolution continuously improves with increasing temperature up to about 70°C [7, 8] (beyond this temperature, the components of the buffer itself probably start to degrade significantly during a single run), the resolution in PAM-PNIPAM copolymers starts to deteriorate between 55 and 60°C (Barbier *et al.*, in preparation). We attribute this to the increasing hydrophobicity of PNIPAM domains.

In the present article, we propose a novel use of the comb-polymer architecture (Fig. 1), to combine in a single polymer the high hydrophilicity and unprecedented sieving performances of a PAM backbone, with the good dynamic coating properties of PDMA. For this, we synthetized comb-like copolymers with a PAM backbone of intermediate molecular mass (M_w) (typically from one to a few MDa), bearing a multiplicity of PDMA side chains, and investigated their properties as sieving matrices for DNA in denaturating conditions.

2 Materials and methods

2.1 Synthesis of PAM-PDMA in three steps

2.1.1 Step 1: preparation of PDMA side-chains

First, *N*,*N*-dimethylacrylamide (DMA) is polymerized by radical polymerization in water, using the redox couple comprising potassium persulfate ($K_2S_2O_8$) and the hydrochloride salt of aminoethanethiol, also called cysteamine hydrochloride ($C_2H_7NS{\cdot}HCl$), as an initator, in order to prepare short-chain PDMA (called "macromonomer" in the following). The reaction scheme is:

Initating reaction:

$$K_2S_2O_8 + 2Cl^-,NH_3^+\text{-}CH_2\text{-}CH_2\text{-}SH \rightarrow 2KHSO_4 + 2Cl^-,NH_3^+\text{-}CH_2\text{-}CH_2\text{-}S \quad (1)$$

$$CH_2 = CH\text{-}CO\text{-}N(NH_3)_2 + Cl^-,NH_3^+\text{-}CH_2\text{-}CH_2\text{-}S \rightarrow Cl^-,NH_3^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH\text{-}CO\text{-}N(CH_3)_2 \quad (1')$$

Chlorhydrate

The reaction extent and speed can be controlled by varying the ratios $R_0 = [C_2H_7NS{\cdot}HCl]/[DMA]$ and $A_0 = [K_2S_2O_8]/[DMA]$. In particular, $C_2H_7NS{\cdot}HCl$, used in excess, plays the role of a transfer agent, which leads to chain polymerization termination. Thus, the length of the monoaminated PDMA can be controlled by varying the ratio R_0.

At this point, each macromolecule bears a chlorhydrate at one end. After neutralization with potassium hydroxyde, one obtains amino PDMA:

$$Cl^-,NH_3^+\text{-}CH_2\text{-}CH_2\text{-}S\text{-}PDMA + KOH \rightarrow NH_2\text{-}CH_2\text{-}CH_2\text{-}S\text{-}PDMA + H_2O + KCl \quad (2)$$

The protocol proceeds as follows: 10 g of DMA (Aldrich, Milwaukee, WI, USA) are dissolved in 100 mL of Milli-Q water and carefully degassed by strong bubbling of argon gas with magnetic stirring, at a controlled temperature of 25°C. $C_2H_7NS{\cdot}HCl$ and $K_2S_2O_8$, both received from Sigma (St. Louis, MO, USA), were dissolved immediately before use in 10 mL Milli-Q water, and added to the DMA solution to reach a final ratio $[K_2S_2O_8]/[DMA] = 0.01$, and ratios R_0 varying between 0.015 and 0.03. The reaction was carried out under permanent stirring and argon bubbling, and completed after 3 h. The solution was lyophilized and then resuspended in 50 mL of methanol. The chlorhydrate was neutralized by adding KOH, drop by drop, in stoichiometric quantity with regards to the initial amount of $C_2H_7NS{\cdot}HCl$. The solution was filtered to remove solid KCl and then precipitated in 2 L of diethyloxide (Aldrich, St. Quentin Fallavier, France). Monoaminated PDMA chains, with molecular weights depending on R_0, were recovered as a white solid, with a yield around 35%.

2.1.2 Step 2: formation of PDMA acrylamido macromonomer

A double bond is grafted at the amino end of the monoaminated PDMA, by reacting the macromonomer with acrylic acid in the presence of dicyclohexylcarbodiimide (DCCI). The resulted product is an acrylamido PDMA.

$$\text{PDMA -S-CH}_2\text{-CH}_2\text{-NH}_2 + \text{OH-CO-CH = CH}_2 \rightarrow \text{PDMA-S-CH}_2\text{-CH}_2\text{-NH-CO-CH = CH}_2 \quad (3)$$

Nine g of monoaminated PDMA, 1.5 g of acrylic acid (Fluka, Buchs, Switzerland) and 4.3 g of DCCI (Aldrich) are reacted in 50 mL methylene chloride at room temperature. After 1 h reaction, the solid side-product (DCCI precipitate) is filtered out. The solution is precipitated in 200 mL of diethyloxide and washed 3 times with 100 mL of precipitation solvent before being dried under vacuum. The product obtained is a white fine solid. The yield is $\sim$ 50%.

2.1.3 Step 3: copolymerization

In a third and final step, the macromonomer is copolymerized with acrylamide by radical polymerization [22], using ammonium persulfate ($(NH_4)_2S_2O_8$) and sodium bisulfite ($Na_2S_2O_5$) to initiate the reaction. The molecular mass of the comb-block copolymer is controlled by varying the concentation of $Na_2S_2O_5$, which is involved in the termination process.

Initating reaction:

$$S_2O_8^{2-} + S_2O_5^{2-} \rightarrow SO_4^{-} + SO_4^{2-} + S_2O_5^{-} \quad (4)$$

2.8 g of electrophoresis-grade acrylamide (Sigma) and the desired amount of PDMA macromonomer are dissolved in 50 mL Milli-Q water and degassed 2 h by argon bubbling. Temperature is controlled at 25°C, and the reaction is started by addition of $(NH_4)_2S_2O_8$ (ratio $[(NH_4)_2S_2O_8]/[AM] = 0.1\%$) and $Na_2S_2O_5$ (ratio $[Na_2S_2O_5]/[AM] = R_1$ varying between 0.015 and 0.03%). The polymerization reaction is carried out under permanent stirring and degassing for 4 h. The final copolymer is recovered with a yield around 90% as a white, fluffy solid, by precipitation in 2.5 L of acetone (SDS). For the sake of comparison, we also prepared a pure LPA polymer, by following the same step 3 with no macromonomer added and with R_1 = 0.03% (this polymer is called in the following M-LPA, for "medium molecular mass linear polyacrylamide").

2.2 Characterization of polymers

To characterize the molar mass averages and the corresponding distributions of the macromonomers and copolymers, samples were fractionated by size-exclusion chromatography (SEC) prior to analysis by on-line multi-angle laser light scattering (MALLS) detection, using a Waters 2690 Alliance Separations Module (Milford, MA, USA) with Shodex (New York, NY, USA) OHpak columns SB-806 HQ, SB-805 HQ, and SB-804 HQ connected in series. In this tandem SEC-MALLS mode, the effluent from the SEC system flows into the DAWN DSP Laser Photometer and Optilab DSP Interferometric Refractometer (both from Wyatt Technology, Santa Barbara, CA, USA). Sample aliquots of 100 μL (sample concentration: 0.5 mg/mL,) were injected into the aqueous mobile phase of the SEC system (0.1 M NaCl, 50 mM NaH_2PO_4, and 200 ppm NaN_3) at a flow rate of 0.3 mL/min). The tandem SEC-MALLS data was processed using ASTRA for Windows software from Wyatt Technology. The graft density of each copolymer was determined by ^{1}H NMR (Bruker 400 MHz) and is represented by the molar fraction of NIPAM units incorporated into the copolymer. Combining this ratio with the weight-average molecular mass of both copolymer and macromonomer, the average number of grafts per chain can be estimated. Viscosities of polymer solutions were determined in water, at 3% concentration, in a computer-controlled cone-plate rheometer (LDV III; Brookfield). Measurements were performed with a temperature increase rate of 2°C per minute and at a shear rate of 10 s^{-1}.

2.3 Capillary electrophoresis

Separations were studied in an ABI 310 instrument and performed with a 100–1500 bases, single-stranded DNA ladder, in which each fragment is singly labeled with fluorescein (Bioventures, Murfreesboro, USA). The bands are 100, 200, 300, 400, 500, 600, 700, 800, 1000, 1250 and 1500 bases. Before injection, DNA was mixed with formamide (50:50 v:v), heated at 95°C for 5 min for denaturation, and then cooled on ice. Capillaries used were standard, uncoated 75 μm ID fixsed silica (Polymicro Technologies, Phoenix, AZ, USA), with 50 cm to the detector and a full length of 61 cm, except for experiments in which the electric field was varied, which were carried out in a 50 cm ID capillary. Polymer solutions were prepared in a 0.05 M Tris, 0.05 M TAPS, 2 mM EDTA buffer (pH 8.2), in the presence of 7 M urea. Each solution was mixed overnight for dissolution. DNA injection was performed for 30 s at 40 V/cm. Prior to separation with M-LPA (3%) and CEQ separation gel I (Beckman Coulter, Fullerton, CA, USA), the capillary was coated with a 3% solution of poly(dimethylacrylamide-allylglycidyl ether) in water [19]. In the case of separations with P(AM-PNIPAM), electroosmosis was controlled with a different dynamic coating (0.2% long PDMA added to the sieving matrix). For separations with CEQ polymer and POP5 (ABI, Courtaboeuf,

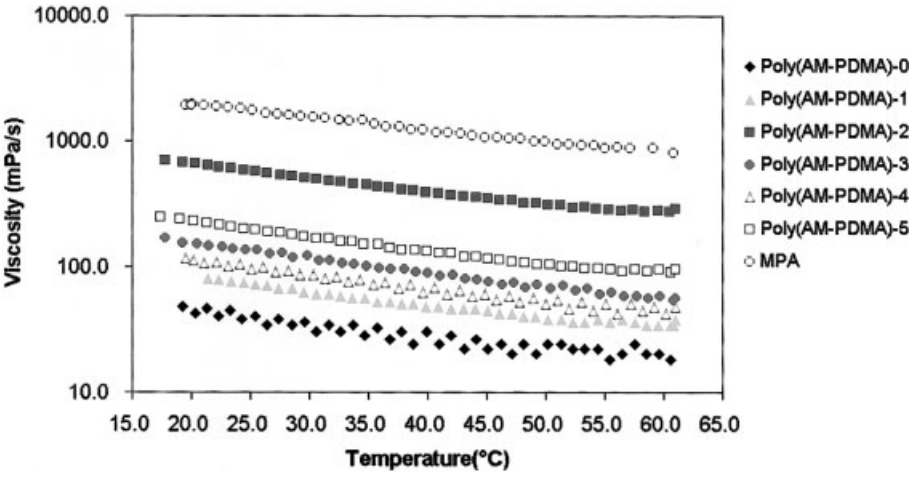

Figure 2. Viscosity *versus* temperature for different P(AM-PDMA) synthetized copolymers. Comparison with M-LPA, prepared in the laboratory under the same conditions.

France), reservoirs were filled with their respective buffer. In all cases, the separation temperature was 50°C. The electropherograms were analyzed by fitting a Gaussian shape to the individual peak profiles using Peakfit Software (Release 4.0).

3 Results

3.1 Rheological behavior and molecular mass

Molecular mass and graft density results are presented in Tables 1 and 2 and the viscosities of solutions of the different polymers synthesized are presented in Fig. 2. All copolymers show a rather low viscosity at 30 g/L, consistent with their intermediate molecular mass. The viscosity increases with molecular mass, as expected, except for P(AM-PDMA)-4, the only polymer with a larger fraction of PDMA (20% *versus* 7–8%). This specific behavior suggests that the PDMA lateral grafts do not contribute as efficiently to entanglement of the matrix, as does the PAM backbone. For all polymers with a similar PDMA content and graft length, viscosities are also consistent with the concentration of metabisulfite during the polymerization. It is worth to notice, however, that P(AM-PDMA)-2, which was polymerized with the same amount of metabisulfite as P(AM-PDMA)-3 and P(AM-PDMA)-5, and has a grafting density between those of the latter polymers, has a significantly higher molecular mass. This suggests that the presence of long grafts tend to reduce the molecular mass of the final copolymer. A comparable effect was observed for P(AM-PNIPAM) copolymers. Our present interpretation is that the attachment of a more bulky side chain to the "living" end of the polymer during the polymerization process may reduce the reaction rate, and thus favor chain termination. In contrast with the P(AM-PNIPAM) [*22*], none of the P(AM-PDMA) showed

Table 1. Different PDMA macromonomers: initiator/monomer ratios used and weight-average molecular mass

Macromonomer	A_0	R_0	Molecular mass (Da)
macroPDMA-1	0.01	0.03	18 000–20 000
macroPDMA-2	0.01	0.015	40 000–45 000

Table 2. Different P(AM-PDMA): initiator/monomer ratios used, weight-average molecular mass, polydispersity indices (PDI) and graft density

Copolymer	Macromonomer	PDMA initial weight fraction	Graft density	R_1	Molecular mass (Da)	Polydispersity
P(AM-PDMA)-0	macroPDMA-1	12.5%	8.5%	0.03%	791 000	1.81
P(AM-PDMA)-1	macroFDMA-1	12.5%	8.2%	0.0225%	1 110 000	1.92
P(AM-PDMA)-2	macroPDMA-1	12.5%	7.9%	0.15%	2 730 000	1.59
P(AM-PDMA)-3	macroPDMA-2	12.5%	8.6%	0.015%	1 740 000	2.29
P(AM-PDMA)-4	macroPDMA-2	30.0%	20.2%	0.015%	2 040 000	2.45
P(AM-PDMA)-5	macroPDMA-2	12.5%	7.0%	0.015%	1 990 000	2.13

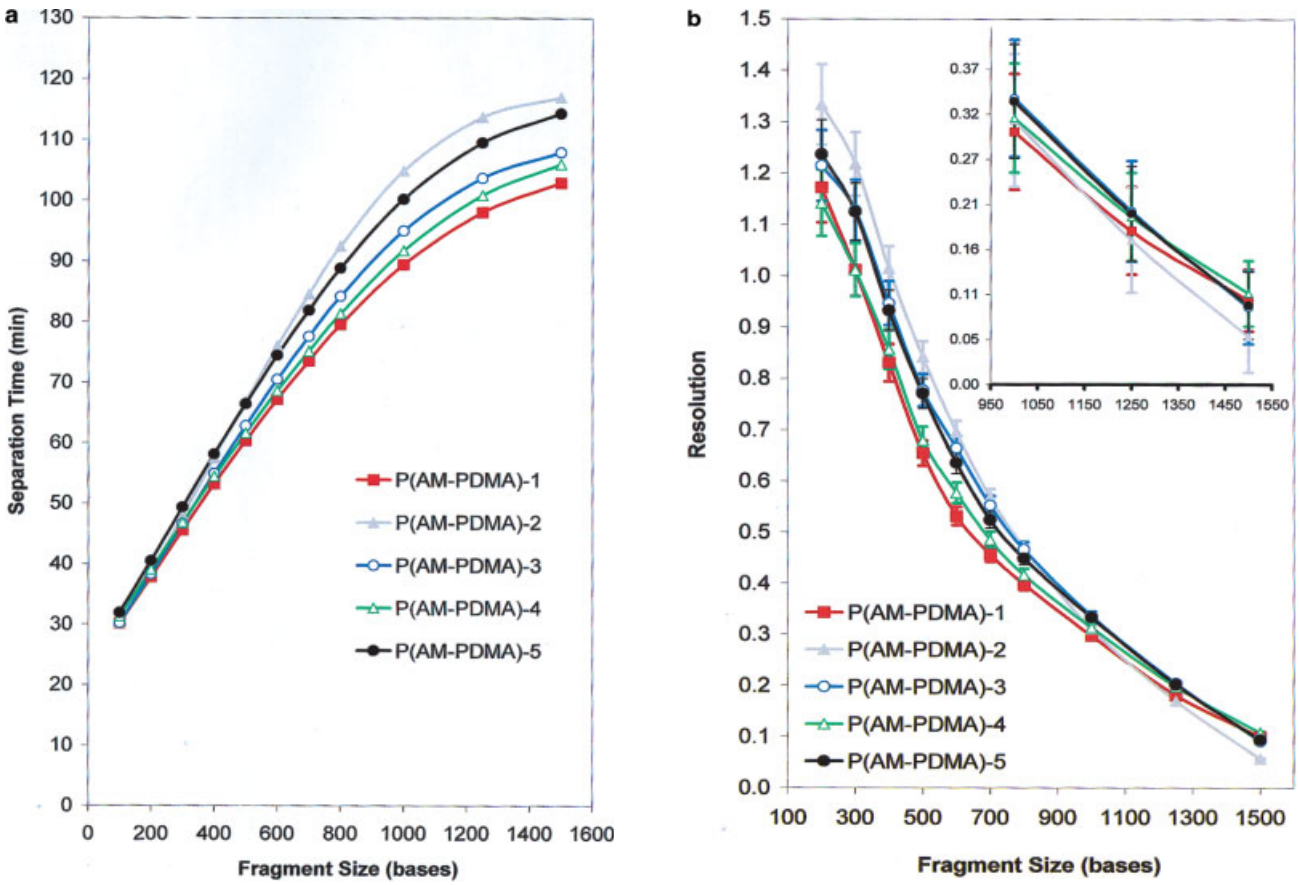

Figure 3. Comparison of (a) separation time and (b) resolution for different P(AM-PDMA) (3%) copolymers. Field strength, 140 V/cm; temperature, 50°C; for other conditions see Section 2.

thermosensitive behavior, which is consistent with the absence of a lower critical solution temperature (LCST) for PDMA.

3.2 Dependence of performance on chemical structure

Separations were performed in the different polymers at 30 g/L and under a 140 V/cm field (these conditions correspond to a good compromise between sieving performances and separation time). For all polymer solutions, from 5 to 10 consecutive runs were performed sequentially within the same capillary, replacing the sieving solution between runs, and the resolution and separation times remained unaffected, except for P(AM-PDMA)-0, which yielded a significant degradation from run to run. Since this polymer is also the shortest, and it has been well demonstrated that dynamic coating properties increase with molecular mass, we suspect that this poor performance is due to an insufficient molecular mass. This polymer was discarded for the following study. The data for the other polymers are diplayed in Figs. 3a (separation time) and 3b (resolution). The separation time only weakly depends on the molecular structure, suggesting that all the matrices studied are entangled strongly enough to deform only weakly under the migration of DNA, and that the mesh size depends only weakly on chemical structure. This latter point is not surprising, since chains are mainly based on acrylamide. The weak, residual variation observed is in the same order as that of the viscosity; it probably reflects a weak deformation of the matrix [24], which is more pronounced for lower molecular masses.

The resolution also seems to increase with increasing molecular mass. This is clear when comparing, *e.g.*, P(AM-PDMA)-1 and P(AM-PDMA)-2, which differ only by their molecular mass. The poor results obtained with P(AM-PDMA)-0, which has the same graft length and grafting density as P(AM-PDMA)-1 and P(AM-PDMA)-2, but the lowest molecular mass of all polymers, also confirms this trend. This is consistent with the results previously reported for PAM. The comparison between

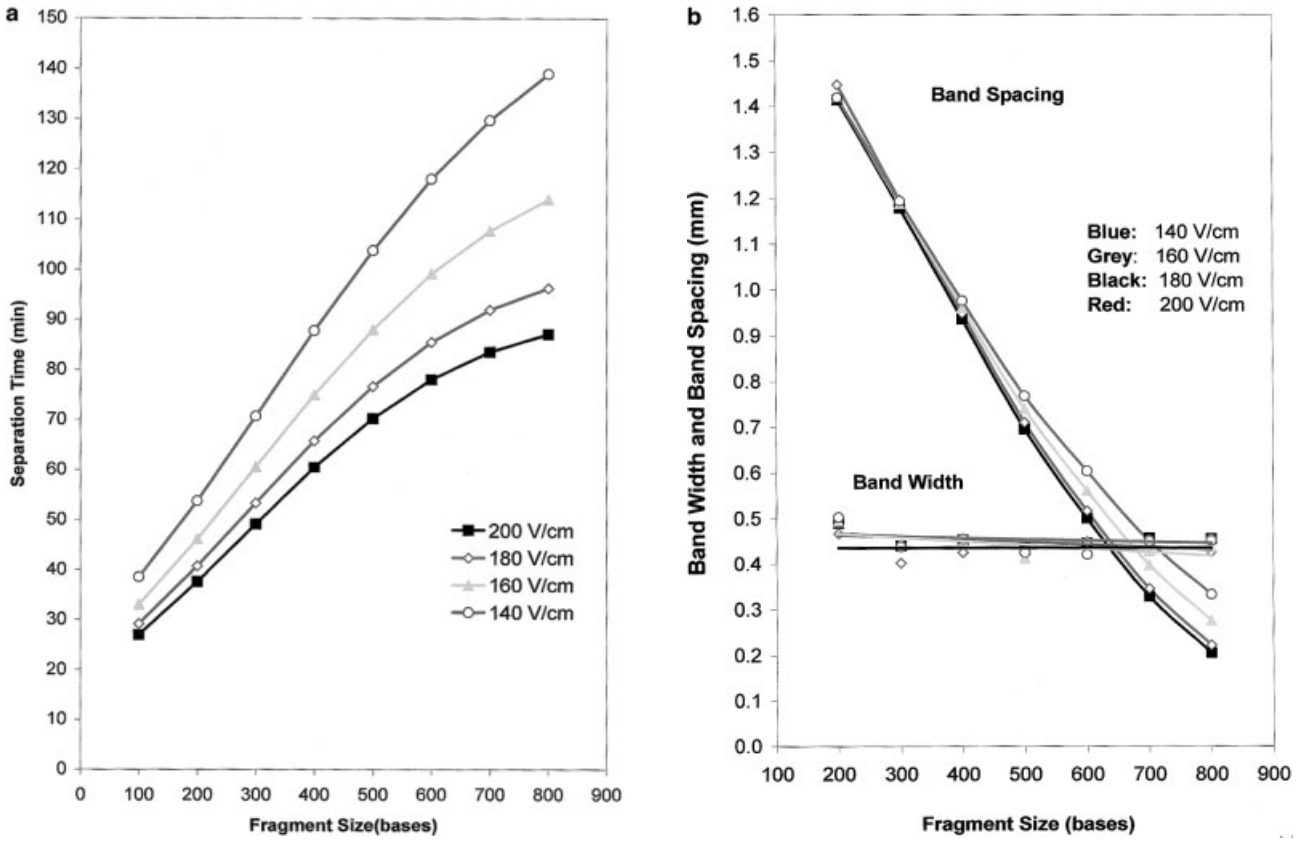

Figure 4. Influence of field strength on (a) separation time, (b) cross-point and (c) resolution using a 4% P(AM-PDMA)-2 solution in 0.05 M Tris, 0.05 M TAPS, 2 mM EDTA buffer (pH 8.2), in the presence of 7 M urea. See Section 2 for other conditions.

P(AM-PDMA)-3 (graft density 8.6%) and P(AMPDMA)-4 (graft density 20.2%) suggests that increasing the graft density beyond 10% (in mass of PDMA relative to total mass) is detrimental to resolution. Following the work of Albarghouthi *et al.* [9], this could be due to the increased hydrophobicity. However, one cannot rule out the possibility that this effect, or at least part of it, could be due to the slightly higher molecular mass of P(AM-PDMA)-3. Comparing P(AM-PDMA)-2 with P(AM-PDMA)-3 and P(AMPDMA)-5, which were polymerized under the same conditions except for the graft length, it appears that increasing the graft length seems to decrease resolution. With the polymers available for this study, it was not possible to discriminate if this is arises to a direct effect of the graft length on the sieving mechanism, or simply is an indirect effect associated to the change in molecular mass with graft length. Adapting polymerization conditions to achieve equivalent molecular mass with different graft length, will be necessary to answer this question.

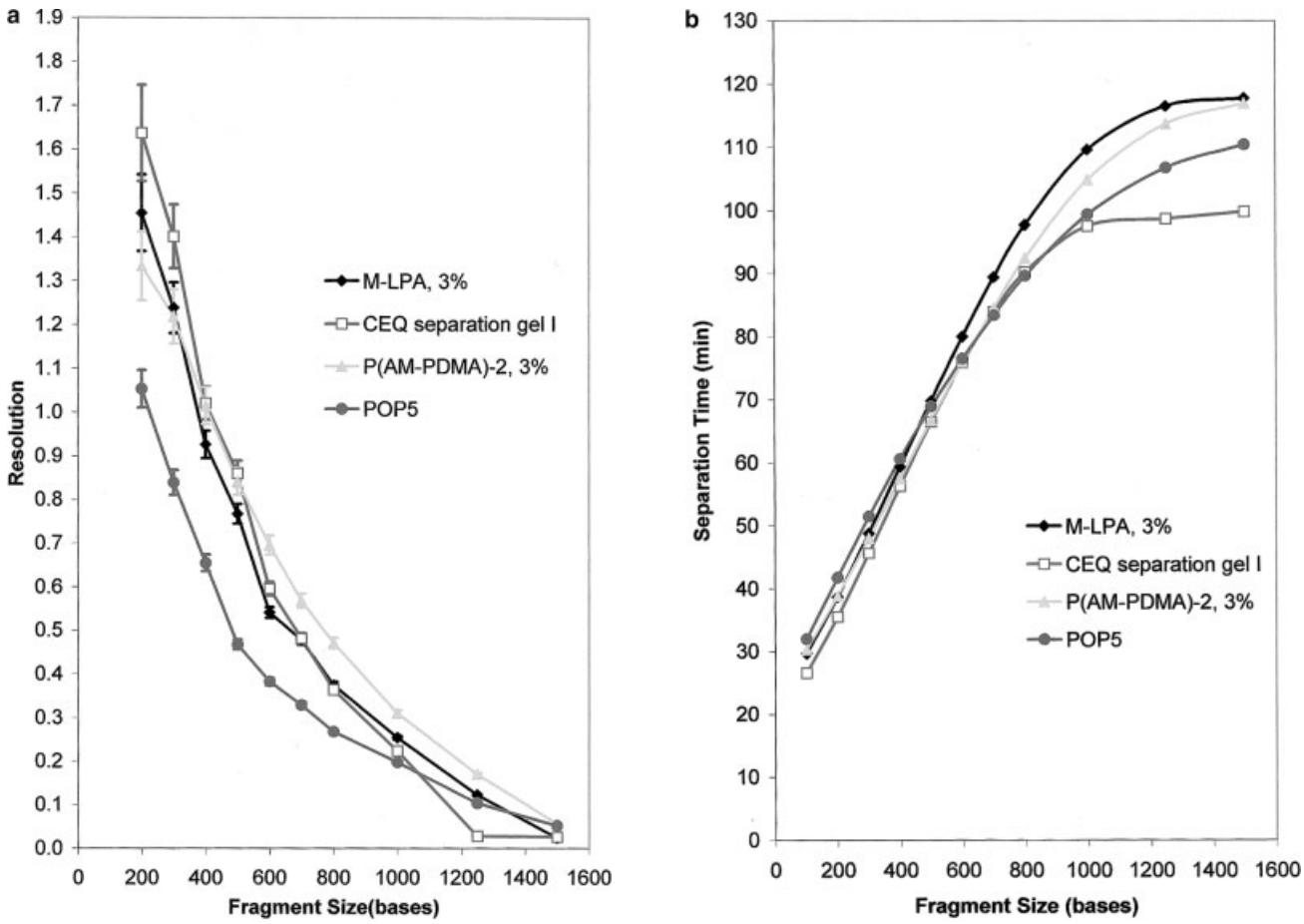

Figure 5. Comparison of P(AM-PDMA)-2 at a concentration of 3% with other long-read matrices (MPA 3% in 0.05 M Tris, 0.05 M TAPS, 2 mM EDTA buffer (pH 8.2), 7 M urea, POP5 and CEQ separation gel 1). (a) Resolution at equal separation time; (b) separation time. See Section 2 for other conditions.

3.3 Effect of field strength

The effect of field strength on performance was essentially the same for all polymers in the series. Expectedly, separation time is strongly affected by field strength (Fig. 4a), and the effect is more dramatic for the largest chains, due to the nonlinear mobility of large fragments in the reptation regime. This is also reflected in the band spacing (Fig. 4b), which degrades more rapidly at large sizes for the largest fields. Data for the band width are more scattered, but no systematic trend can be observed, and band widths seem to be essentially independent of field strength and DNA size. Resolution (Fig. 4c), which is a combination of band spacing and band width, is as a matter of consequence decreasing with DNA size, and more rapidly so for the largest fields. The improvement of the resolution of large fragments with decreasing field, however, tends to saturate below 140 V/cm, so that it is probably counterproductive to use lower fields.

3.4 Comparison with other sieving matrices

Finally, we compared the resolution (Fig. 5a) obtained with one of our polymers, P(AM-PDMA)-2, with that obtained with other polymers available (POP5 and CEQ separation gel I) at approximatively equal separation time for the 100 bases fragment (Fig. 5b). All polymers were used in the conditions suggested by the authors or manufacturers for optimizing real length. We also plot in the figures the results obtained with a pure LPA of medium molecular mass (average M_w 4.4 MDa) (called M-LPA in the figure), synthesized in our laboratories in conditions identical with those of the P(AM-PDMA) copolymers R_1= 0.03%). The resolution obtained with P(AM-PDMA) is, within experimental error, slightly better than that obtained with LPA in a coated capillary, and significantly better than that obtained with POP5 . The resolution is also much improved with regard to M-LPA, even when the latter is used in a coated capillary. This surprising finding is discussed in the next section.

4 Discussion

The results demonstrate that comb-like copolymers, with an acrylamide skeleton and PDMA grafts, seem to provide "the best of both world", *i.e.*, to conserve the superior sieving performance of acrylamide, and to add to it the dynamic coating capability of PDMA. Obviously, a lot of work remains to be done before the use and mechanism of action of this novel family of polymers can be fully assessed and understood. On the practical side, an extensive evaluation of the capillary lifetime in routine sequencing conditions should be performed: present commercial systems have a typical lifetime of 500 runs, and comparable performances should be achieved for use in capillary array sequencers. Also, a more complete series of microstructures should be synthesized, in order to fully unravel contributions to the performances arising from molecular mass and from other structural parameters.

In spite of the still incomplete character of the present study, this family of copolymers seems very promising. First, it is worthwhile to notice that in spite of some differences in performance, all the copolymers studied, with the exception of P(AM-PDMA)-0, perform quite well for sequencing (*e.g.*, significantly better than POP5 and comparable to LPA). This shows that the performances are, from a practical point of view, rather robust to moderate changes in polymer microstructure. The best polymer in the series, P(AM-PDMA)-2, even appears better than LPA above 600 bases.

The copolymers follow the general trend, already observed for LPA or PEO, of improving resolution with increasing molecular mass. It is very striking, however, that excellent performances are achieved with a much lower molecular mass (and thus a much lower viscosity) than with these previously studied homopolymers. Notably, the resolution is significantly better than that achieved with solutions of LPA with a comparable molecular mass, even if the latter is used in a coated capillary. One hypothesis to explain this striking observation would be that noncoating polymers actually require a much higher entanglement level than self coating ones to prevent any wall-slippage effect. In other words, the hydrodynamic/molecular coupling of noncoating polymers with chemically grafted coating layers, might be less efficient than that of dynamically coating ones. This hypothesis will require further verification. In any case, observing superior resolution at relatively low molecular weights is, of course, very good news for applications, since the polymers seem able to combine optimal sieving with dynamic coating and with moderate viscosity. This should make these matrices particularly interesting for separations in microfluidic systems, in which high-pressure loading of sieving matrices raises more difficulties than in conventional capillaries.

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