Affinity gel electrophoresis of nucleic acids

Specific base- and shape-selective separation of DNA and RNA on polyacrylamide–nucleobase conjugated gel

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ABSTRACT

Two types of affinity gels consisting of cross-linked polyacrylamide and affinity ligands possessing nucleic acid bases were prepared. One type of gel was polyacrylamide–poly(vinylnucleobase) conjugated gel, where the poly(vinylnucleobase) such as poly(9-vinyladenine) (PVAd) bearing a nucleobase in the side-chain was entrapped in the gel matrix. The other type of gel, in which a nucleobase such as adenine is chemically bonded to polyacrylamide gel, was prepared by copolymerization of acrylamide, cross-linker and 9-vinyladenine. These affinity gels, especially the former, demonstrated characteristic nucleobase- and shape-selective separation of nucleic acids. The gels showed high affinity for single-stranded DNA and both single- and double-stranded polynucleotides and could separate a double-stranded DNA in mixtures of double-stranded DNA and polynucleotides. The electrophoretic mobilities of poly(uridylic acid) and poly(inosinic acid) were selectively retarded even in the presence of 7 M urea. The electrophoretic behaviours of nucleic acids on the polyacrylamide–PVAd conjugated gels were compared with those on the agarose–PVAd conjugated gel. The effects of urea, temperature and concentration of PVAd were also examined. The polyacrylamide–PVAd conjugated gel served to elucidate interactions between PVAd and nucleic acids that could not be detected by usual spectroscopic methods.

INTRODUCTION

Gel electrophoresis, a most commonly used technique for the separation and purification of nucleic acids, has been developed into an essential tool in gene technology and related fields and separates nucleic acids mainly on the basis of size [1]. On the other hand, affinity gel electrophoresis (AGE) [2] may separate nucleic acids with specific base or sequence recognition. However, specific base recognition of nucleic acids has not been realized in gel electrophoresis. Several intercalator dyes [3,4] and phenylboronate [5] have been introduced in a polyacrylamide gel matrix for specific base-sequence or base-content separation and isolation of RNA in electrophoresis, respectively.

One strand of natural or synthetic polynucleotide can recognize the complementary strand in mixtures of polynucleotides but will not be suitable for an affinity ligand in electrophoresis because of electroosmotic flow due to its anionic character. Fully synthetic neutral vinylpolymer analogues of nucleic acids having nucleic acid

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bases in the side-chain should be promising affinity ligands for base-specific separation of nucleic acids in electrophoresis [6–13] and chromatography [14–16]. Poly(9-vinyladenine) (PVAd), a novel water-soluble neutral vinyl polymer analogue [17,18], has been demonstrated to be an excellent affinity ligand for the base-specific separation of oligonucleotides in high-performance liquid chromatography (HPLC) [14,15] and capillary gel electrophoresis [9–13] using PVAd-immobilized silica gel and a capillary filled with cross-linked polyacrylamide gel conjugated with PVAd, respectively. PVAd is also valuable in conventional affinity gel electrophoresis for the base- and shape (single- or double-stranded)-selective separation of DNA and RNA [6–8]. Only single-stranded DNA was selectively adsorbed over double-stranded DNA, and poly(uridylic acid) [poly(U)] and poly-(inosinic acid) [poly(I)] were base-specifically recognized by PVAd entrapped in agarose gel among five polynucleotides having different bases [8].

In this study, we prepared two types of affinity gels consisting of polyacrylamide and affinity ligands having nucleic acid bases as illustrated in Fig. 1. One was a polyacrylamide–poly(vinyl-nucleobase) conjugated gel (type I), in which the vinyl polymer analogues having nucleobases were entrapped within the gel matrix. PVAd, poly[N-(2-uracylethyl)acrylamide] and poly-(vinylhypoxanthine) were used as such a macroligand. In the second affinity gel (type II), nucleobases as affinity ligands were chemically bonded to the polyacrylamide gel matrix by the
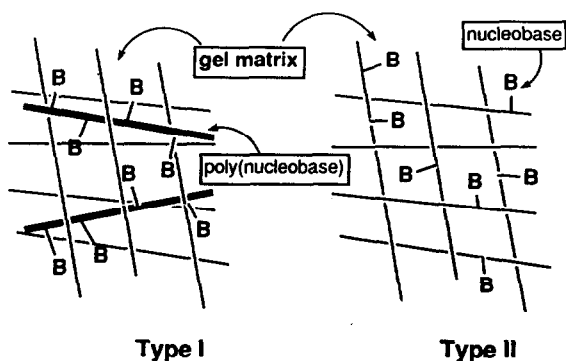
copolymerization of acrylamide, a cross-linker and vinylnucleobases such as vinyladenine and N-(9-adenylethyl)acrylamide. These affinity gels were expected to exhibit different base-recognition abilities for nucleic acids depending on the kinds of immobilized bases, and the electrophoretic behaviours of nucleic acids on the gels were compared with those on the agarose–PVAd conjugated gel [8]. The effects of urea, temperature and concentration of PVAd on the electrophoretic mobility of nucleic acids in affinity gel electrophoresis were also examined. Affinity gel electrophoresis using the polymeric analogues serves to elucidate the interaction of the analogues and nucleic acids, which is too weak to detect by common spectroscopic methods.

EXPERIMENTAL

Materials

Boric acid, ethylenediaminetetraacetate (EDTA), bromophenol blue, xylene cyanol, ethidium bromide, methylene blue, ammonium peroxodisulphate, potassium peroxodisulphate and urea were of analytical-reagent grade from Nacalai Tesque (Kyoto, Japan). Acrylamide, N,N'-methylenebis(acrylamide) (Bis), and N,N, N',N'-tetramethylethylenediamine (TEMED) were of electrophoretic grade from Nacalai Tesque. Tris(hydroxymethyl)aminomethane (Tris) and ΦX174 Hae III restriction enzyme fragments were obtained from Sigma (St. Louis, MO, USA) and Toyobo (Osaka, Japan), respectively. Poly(adenylic acid) [poly(A)] and poly(U) were obtained from Yamasa (Chiba, Japan) and Sigma. Poly(I), poly(cytidylic acid) [poly(C)], double-stranded poly(deoxyadenylic acid)–poly-(deoxythymidylic acid)] [poly(dA)–poly(dT)], calf thymus DNA, and RNA (Type III from bakers' yeast) were purchased from Sigma, and poly(guanylic acid) [poly(G)] from Pharmacia-LKB (Uppsala, Sweden).

The preparation and polymerization of 9-vinyladenine have been described previously [9,19]. N-(9-Adenylethyl)acrylamide, N-(2-uracylethyl)-acrylamide and vinylhypoxanthine were prepared according to reported methods [20–23]. The polymerization was carried out in water or dimethylformamide with ammonium peroxodi-

Fig. 1. Schematic representation of affinity gels.

sulphate or potassium peroxodisulphate as a radical initiator. Poly(9-vinyladenine) (PVAd) was fractionated using an ultrafiltration technique [24] and PVAd with molecular mass >30 000 was used as an affinity ligand in electrophoresis throughout [8].

Sonication and gel electrophoresis

Sonication and gel electrophoresis were performed in a similar manner to that reported previously [8,24]. RNA from bakers' yeast was small enough and used without sonication. The relative size of the sonicated nucleic acids was estimated against ΦX174 Hae III restriction enzyme fragments by polyacrylamide (5%) gel electrophoresis. The sizes of the sonicated nucleic acids were in the range 60–200 base pairs (bp) except for poly(A) (80–450 bp) and poly(G) (40–300 bp). Double-stranded poly(A)–poly(U), poly(G)–poly(C) and poly(I)–poly(C) were prepared by mixing equimolar amounts of the sonicated single-stranded polynucleotides.

Gel electrophoresis was performed according to the method reported previously [8] using a submarine-type gel apparatus (Bio-Rad DNA SUB CELL) at a constant voltage (100 V) on an ATTO AE-8350 power supply usually for 2 h in TBE buffer (89 mM Tris–89 mM boric acid–2 mM EDTA, pH 8). Polyacrylamide gel (5.0%; acrylamide:Bis = 19:1) was prepared using a Mupid Gel Maker Set (Cosmo-Bio, Tokyo, Japan) under a nitrogen atmosphere and polyacrylamide–PVAd conjugated gel was prepared by copolymerization of acrylamide and Bis (19:1) in the presence of the desired amount of PVAd (0.05–15%, w/w). N-(9-Adenylethyl)-acrylamide and vinyladenine were covalently immobilized to the gel matrix by copolymerization with acrylamide and Bis under a nitrogen atmosphere. Ammonium peroxodisulphate was used as a radical initiator. Unreacted monomers were removed by immersing the gels in TBE buffer for more than 1 h.

After electrophoresis, the gels were first stained with the intercalating dye ethidium bromide to reveal DNA and double-stranded polynucleotides by fluorescence under UV light (312 nm; Cosmo-Bio, CSF-20B transilluminator). As ethidium bromide was not suitable for staining a single-stranded RNA, the gel was then stained with 0.2 wt.% methylene blue. The electrophoretic experiments were repeated several times and the reproducibility was satisfactory.

Photographs were taken with a Polaroid ACMEL CRT camera (M-0851) with Polaroid Type 665 P/N film.

Measurements

UV spectra and "melting temperature" (T_m) corresponding to a 50% transition of complexes were obtained with a Hitachi Model 200-20 spectrophotometer in a 1.0-cm path-length quartz cell equipped with a temperature controller (Tanson TC 3). Hypochromicity and hyperchromicity were obtained by continuous variation mixing curves in 0.1 M phosphate buffer (pH 7.0) containing 50 mM NaCl at 25°C. Melting curves were obtained by monitoring the absorbance change with slow heating at a rate below 0.5°C/min.

RESULTS AND DISCUSSION

Preparation of affinity gel with nucleobase

Some water-soluble synthetic vinyl polymer analogues of nucleic acids can interact with nucleic acids through specific interactions, i.e., complementary hydrogen bonding (base pairing) and hydrophobic interaction or stacking depending on the degree of the polymerization [17–19,24]. Such specific interactions can be applicable to affinity chromatography and affinity electrophoresis in which the analogues serve as an effective affinity ligand for base-specific recognition of nucleic acids having complementary bases.

We prepared two types of affinity gels consisting of polyacrylamide and affinity ligands having nucleic acid bases as illustrated in Fig. 1. One was a polyacrylamide–poly(vinylnucleobase) conjugated gel, in which the vinyl polymer analogues having nucleobases in the side-chain were entrapped within the gel matrix (type I) by polymerization of acrylamide and Bis in the presence of poly(vinylnucleobase). PVAd, poly[N-(2-uracylethyl)acrylamide] and poly(vinylhypoxanthine) were chosen and prepared for use as affinity macroligands because they

possess advantages of ease of preparation and stability against enzyme-catalysed hydrolysis over natural polynucleotides and additionally in electrophoresis they will not undergo electro-osmotic flow because of their neutral character. PVAd was found to show almost no electrophoretic mobility in slab gel electrophoresis [6,7]. Among the analogues, PVAd was fairly soluble in water, but the others were less soluble.

In the second type of affinity gel, nucleobases were chemically bonded to the polyacrylamide gel matrix by the copolymerization of acrylamide, Bis and vinylnucleobases such as vinyladenine and N-(9-adenylethyl)acrylamide (type II). This is a very convenient method of immobilizing nucleobases within the gel matrix by covalent bonding. Another type of affinity gel would be possible using a low molecular mass nucleobase monomer as a mobile carrier. However, the mobile ligand will interact very weakly with nucleic acids and is not suitable as an affinity ligand for the specific separation of nucleic acids. Therefore, the two methods described above were employed for the immobilization of affinity ligands.

We first evaluated the specific base- and shape-recognition ability of the polyacrylamide–PVAd conjugated gel in electrophoresis and the electrophoretic migration behaviour of nucleic acids on the gel was compared with that on agarose–PVAd conjugated gel [8]. Nucleic acids displayed different electrophoretic migrations on the polyacrylamide–PVAd conjugated gel. The effects of the concentrations of PVAd and urea and temperature were also examined.

Electrophoresis of DNA and RNA on polyacrylamide–PVAd conjugated gel

Fig. 2 demonstrates the effect of the polyacrylamide gel matrix on the electrophoretic migration of typical single- and double-stranded DNA and polynucleotides in (b) the presence and (a) the absence of PVAd. The retardation of the mobility of double-stranded DNA was not much affected by PVAd (lane 1). A dramatic decrease in mobility of DNA was observed when the double-stranded DNA was denatured by heating at 95°C for 3 min (lane 2), as seen with
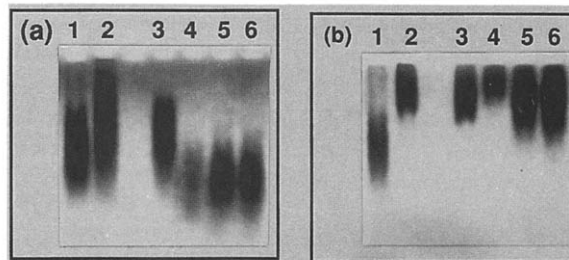

Fig. 2. Electrophoresis of nucleic acids on (a) polyacrylamide gel (5.0%; acrylamide:Bis = 19:1) and (b) polyacrylamide–PVAd (1%, w/w) gel. Gel electrophoresis was performed using TBE buffer (89 mM Tris–89 mM boric acid–2 mM EDTA, pH 8). The gels were stained with methylene blue. Lanes: 1 = calf thymus DNA; 2 = sample 1 heated at 95°C for 3 min; 3 = poly(A); 4 = poly(U); 5 = poly(A)–poly(U); 6 = sample 5 heated at 95°C for 3 min.

the agarose–PVAd conjugated gel [7,8]. This might be due to base pairing of PVAd with the denatured single-stranded DNA, since the electrophoretic migration of single-stranded (+)-M13mp8 DNA was almost completely retarded by PVAd immobilized in agarose gel [8].

The most different electrophoretic behaviour was found with polynucleotides including poly(A), poly(U) and the double-stranded poly(A)–poly(U), all of which were electrophoretically retarded in spite of the kind of base and shape (single- or double-stranded) (lanes 3–6). Complementary hydrogen bonding between poly(U) and PVAd, established by spectroscopic methods [24,25], reasonably leads to retardation of the migration of poly(U). However, no clear spectroscopic variations in interaction between PVAd and poly(A) were observed from UV and ^{31}P NMR spectroscopy, indicating that the interaction was so weak that the usual spectroscopic methods could not detect it. Hypochromicity and melting between them were not regarded as significant. The same observation was reported by Pitha [6], who claimed that very weak co-stacking between them caused retardation of the mobility of poly(A) in the presence of PVAd in polyacrylamide gel, although the mobility of poly(A) was not affected even in the presence of 10% (w/w) PVAd in agarose gel electrophoresis [7,8]. These results suggest that the mobility retardation of poly(A) in the polyacrylamide–PVAd conjugated gel may be related to the size

of gel porosity of polyacrylamide other than hydrophobic or stacking interaction between PVAd and poly(A). The relatively small pores of the polyacrylamide (5%) compared with those of agarose (0.7%), in which PVAd was entrapped, will make it possible to associate with nucleic acids effectively.

Interestingly, the migration of the double-stranded poly(A)–poly(U) was also strongly retarded without heat treatment in the polyacrylamide–PVAd system (lane 5 in Fig. 2). In the agarose–PVAd conjugated gel, the duplex was separated into two bands; poly(U) migrated slowly and poly(A) migrated rapidly [7,8]. Therefore, in the polyacrylamide–PVAd system, the following complex interaction may be occurring: the duplex first interacts with the immobilized PVAd, and then the PVAd pulls off the poly(U) to complex with it. The resulting single-stranded poly(A) also interacted with PVAd as seen in lane 3. Consequently, both strands were trapped in the gel.

The results clearly indicate that the polyacrylamide–PVAd conjugated gel electrophoresis will be applicable to the separation of double-stranded DNA in mixtures of single- and double-stranded DNA and RNA. An example is demonstrated in Fig. 3. Only double-stranded DNA migrated, but polynucleotides were trapped in the slot.

Next, the effect of the concentration of PVAd on the mobility of single- and double-stranded polynucleotides was examined. The electropherograms are shown in Fig. 4. The detectable retardation of the migration of all polynucleotides occurred only with 0.05% (w/w) PVAd, and the mobility was greatly decreased as the concentration of PVAd increased. A similar concentration effect was also observed in capillary gel electrophoresis of oligo(dT)$_{12-18}$ using polyacrylamide–PVAd conjugated gel [9]. The migration of oligo(dT)$_{12-18}$ in a capillary filled with polyacrylamide gel containing only 0.05% (w/w) PVAd was strongly retarded even in the presence of excess of urea.

From the band broadening in Fig. 4 it was found that poly(U) binds to PVAd more strongly than poly(A).
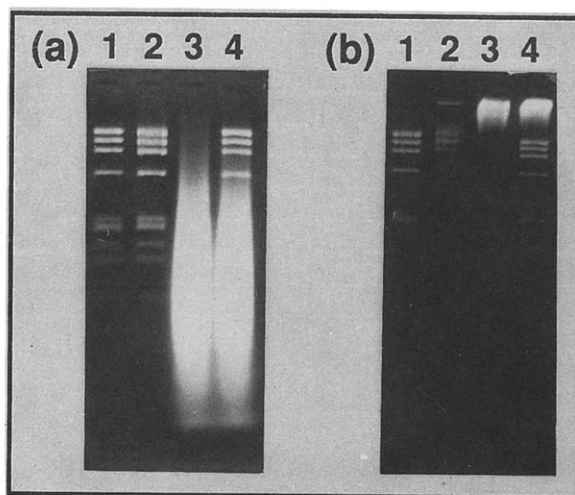
To examine the more detailed base-specific

Fig. 3. Electrophoresis of DNA and polynucleotides on (a) polyacrylamide gel and (b) polyacrylamide–PVAd (10%, w/w). Conditions as in Fig. 2. The gels were stained with ethidium bromide. Lanes: 1 = Hae III restriction enzyme digests of ΦX174 DNA; 2 = sample 1 heated at 95°C for 3 min; 3 = poly(A)–poly(U); 4 = mixtures of Hae III restriction enzyme digests of ΦX174 DNA and poly(A)–poly(U).

recognition ability of PVAd in polyacrylamide gel, RNA from bakers' yeast, five polynucleotides having different bases and double-stranded poly(dT)–poly(dA) were electrophoresed. Among the five polynucleotides, only poly(I) and poly(U) were electrophoretically retarded in the agarose–PVAd gel [8]. The
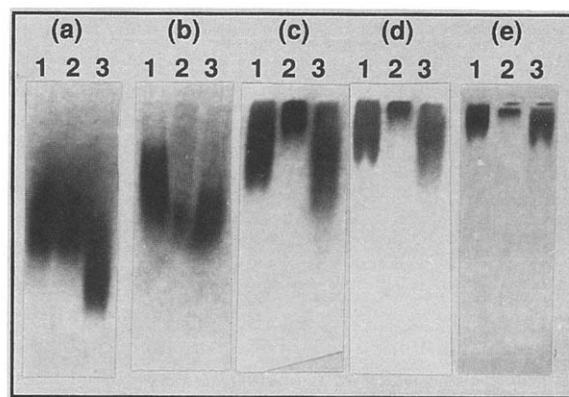

Fig. 4. Effect of the concentration of PVAd on the migration of single- and double-stranded polynucleotides. Concentration of PVAd: (a) 0, (b) 0.05, (c) 0.5, (d) 1 and (e) 5%. Other conditions as in Fig. 2. The gels were stained with methylene blue. Lanes: 1 = poly(A); 2 = poly(U); 3 = poly(A)–poly(U).

electrophoretic patterns of these DNA and RNA are shown in Fig. 5. All single-stranded polynucleotides except poly(C) were strongly adsorbed by PVAd (lanes 4–8). The retardation of the mobilities of poly(U) and poly(I) was due to base pairing, since poly(I) and poly(U) were strongly adsorbed by PVAd in agarose gel and those of poly(A) and poly(G) may be due to a weak hydrophobic interaction. Poly(C), which may not interact with PVAd either by base pairing or by hydrophobic interaction, migrated rapidly in both gels.

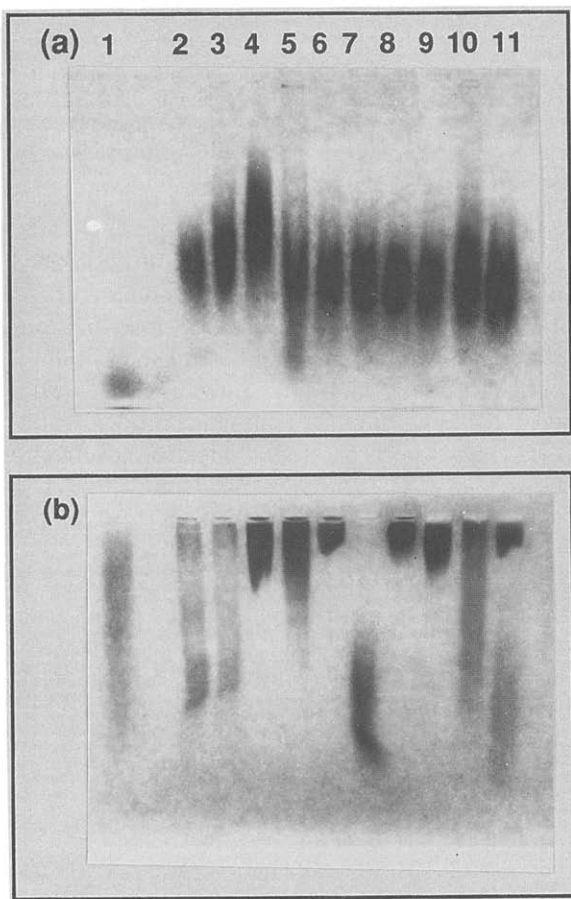The double-stranded poly(G)–poly(C) and

Fig. 5. Electrophoresis of nucleic acids on (a) polyacrylamide gel and (b) polyacrylamide–PVAd (5%, w/w) gel. Other conditions as in Fig. 2. The gels were stained with methylene blue. Lanes: 1 = RNA from bakers' yeast; 2 = poly(dA)–poly(dT); 3 = sample 2 heated at 95°C for 3 min; 4 = poly(A); 5 = poly(G); 6 = poly(I); 7 = poly(C); 8 = poly(U); 9 = poly(A)–poly(U); 10 = poly(G)–poly(C); 11 = poly(I)–poly(C).

poly(I)–poly(C) migrated to afford a very broad band and two bands, respectively (lanes 10 and 11). The upper band in lane 11 was assigned to poly(I) and the band broadening of the poly(G)–poly(C) may be due to strong interaction of poly(G) with PVAd in comparison with the migration behaviour of the corresponding single-stranded poly(G) and poly(C) in the presence of PVAd (lanes 5 and 7). The poly(dA)–poly(dT) duplex was also separated into two broad bands, and the upper band should be poly(dT) which binds to PVAd by complementary hydrogen bonding. The migration of oligo(dT)$_{12-18}$ in a capillary filled with polyacrylamide gel containing only 0.05% (w/w) PVAd was strongly retarded even in the presence of excess urea, but the migration of oligo(dA)$_{12-18}$ was not affected by PVAd. The reason for different migration behaviour of single-stranded poly(dA) and poly(A) (lanes 3 and 4) in the presence of PVAd is not clear.

We further examined the effect of urea to elucidate the interaction mechanism of PVAd with polynucleotides in polyacrylamide gel electrophoresis (Fig. 6). Urea is usually used as a denaturing agent to avoid a secondary structure formation of DNA and polynucleotides in gel electrophoresis. Surprisingly, the electrophoretic migration behaviour of most nucleic acids did not change even in the presence of 2 M urea but slightly changed at 7 M urea. RNA from bakers' yeast migrated rapidly using 7 M urea, probably because urea breaks the hydrogen bonds of the base-paired complex of RNA and PVAd. The migrations of poly(A), poly(G) and poly(G)–poly(C) were also affected by urea (lanes 4, 5 and 10 in Fig. 6). We first thought that the mobilities of these polynucleotides would be insensitive to changes in the concentration of urea, because the interaction of poly(A) and poly(G) with PVAd was considered to be caused by weak hydrophobic or stacking interactions. However, the present results indicate the existence of hydrogen bonding interactions between them. Again, the size of the gel porosity of polyacrylamide should be considered in explaining this unusual behaviour, because these polynucleotides migrated rapidly in agarose gel even in the presence of 10% (w/w) PVAd [8].
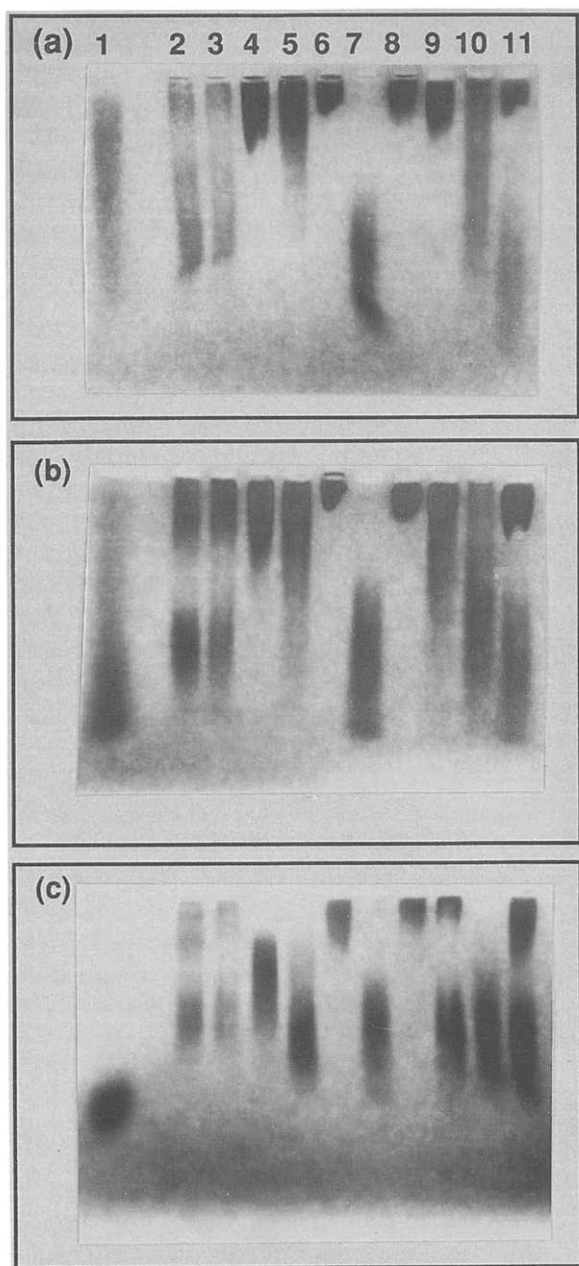
Poly(U) and poly(I) did not migrate even in

Fig. 6. Effect of the concentration of urea on the migration of nucleic acids on polyacrylamide–PVAd (5%, w/w) gel. Concentration of urea: (a) 0, (b) 2.0 and (c) 7.0 M. Other conditions as in Fig. 2. The gels were stained with methylene blue. Lanes: 1 = RNA from bakers' yeast; 2 = poly(dA)–poly(dT); 3 = sample 2 heated at 95°C for 3 min; 4 = poly(A); 5 = poly(G); 6 = poly(I); 7 = poly(C); 8 = poly(U); 9 = poly(A)–poly(U); 10 = poly(G)–poly(C); 11 = poly(I)–poly(C).

the presence of 7 M urea (lanes 6 and 8) but migrated at 10 M urea in polyacrylamide–PVAd (0.5%, w/w) gel electrophoresis. Base pairing of poly(U)–PVAd and poly(I)–PVAd was so strong that it could not be broken even in the presence of excess urea. To evaluate the base-pairing ability of poly(I) and poly(U) with PVAd, the polynucleotides were electrophoresed on polyacrylamide–PVAd (0.5%, w/w) gel at different temperatures (29 ± 1, 55 ± 1 and 77 ± 1°C). Poly(U) migrated at 55 ± 1°C but poly(I) did not and the band was broadened considerably even at 77 ± 1°C, probably owing to very strong binding to PVAd. The results suggest that the binding ability of poly(I) to PVAd is superior to that of poly(U).

From these results, the order of the binding ability of polynucleotides with PVAd was assumed to be poly(I) > poly(U) > poly(G) ≈ poly(A) ≫ poly(C).

Poly[N-(2-uracylethyl)acrylamide] and poly(vinylhypoxanthine) were also used as macroligands for the base-specific separation of nucleic acids. However, significant nucleobase recognition could not be observed in AGE, because the polymers were not soluble in a running buffer; therefore, the conjugated gel became turbid, which gave rise to normal electrophoretic migration of nucleic acids even in the presence of the macroligands.

Electrophoresis of polynucleotides on polyacrylamide–nucleobase immobilized gel

A type II affinity gel was prepared by copolymerization of acrylamide, Bis and a vinyl monomer bearing a nucleobase in the side-chain such as 9-vinyladenine and N-(9-adenylethyl)acrylamide. The electrophoreses of poly(A), poly(U) and the poly(A)–poly(U) duplex using the affinity gel are shown in Fig. 7. The concentration of the vinyl monomers was held constant at 10% (w/w). Fig. 7 demonstrates that the base-specific separation of poly(U) was achieved only by using 9-vinyladenine as a comonomer; the migration of poly(U) was retarded, but that of poly(A) was not changed in the electrophoresis. In contrast, the electrophoretic migrations of polynucleotides were not influenced by introduction of N-(9-adenylethyl)acrylamide (Fig. 7c). The copolymerizability of the monomers
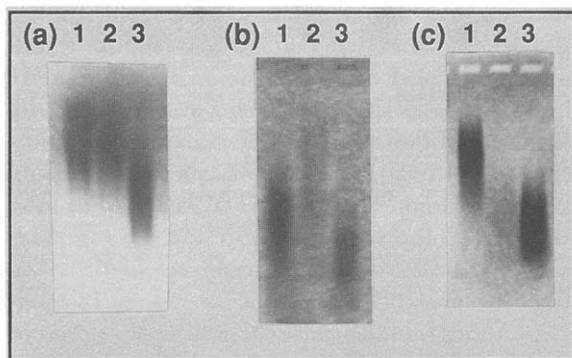
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Fig. 7. Electrophoresis of polynucleotides on (a) poly-acrylamide, (b) poly[acrylamide–co-10% (w/w) vinyl-adenine] and (c) poly[acrylamide–co-10% (w/w) adenylethyl-acrylamide]. Other conditions as in Fig. 2. The gels were stained with methylene blue. Lanes: 1 = poly(A); 2 = poly(U); 3 = poly(A)–poly(U).

with acrylamide in water might affect the electro-phoretic migration behaviour. N-(9-Adenylethyl)acrylamide, which has an ethylene group as a spacer between the adenine and vinyl groups, will be easily copolymerized with acryl-amide in a random fashion. However, 9-vin-yladenine will copolymerize with acrylamide to afford a block-like copolymer, because 9-vin-yladenine aggregates in water as a result of hydrophobic interactions [19]. Therefore, the 9-vinyladenine gel, in which nucleic acid bases localized in the gel matrix, can weakly interact with poly(U) to result in the retardation of electrophoretic migration. To achieve effective nucleobase recognition, at least three or more continuous 9-vinyladenine sequences should be necessary in the gel matrix [15].

In conclusion, we have found that poly(9-vin-yladenine) was a useful affinity ligand for affinity polyacrylamide gel electrophoresis to achieve the shape-selective separation of DNA and RNA and the nucleobase-selective separation of poly-nucleotides could be possible using urea gels. The polyacrylamide–PVAd gel served to eluci-date interactions between PVAd and nucleic acids that could not be detected by the usual spectroscopic methods.

REFERENCES

1 D. Rickwood and B.D. Hames (Editors), Gel Electro-phoresis of Nucleic Acids —a Practical Approach, IPL Press, Oxford, 2nd ed., 1990.
2 K. Takeo, Adv. Electrophoresis, 1 (1987) 229.
3 H. Bunemann and W. Muller, Nucleic Acids Res., 5 (1978) 1059.
4 S. Takenaka, K. Dohtsu, N. Nakashima and M. Takagi, Anal. Sci., 3 (1987) 557.
5 G.L. Igloi and H. Kossel, Nucleic Acids Res., 19 (1985) 6881.
6 J. Pitha, Anal. Biochem., 65 (1975) 422.
7 E. Yashima, N. Suehiro, M. Akashi and N. Miyauchi, Chem. Lett., (1990) 1113.
8 E. Yashima, N. Suehiro, N. Miyauchi and M. Akashi, J. Chromatogr. A, 654 (1993) 151.
9 Y. Baba, M. Tsuhako, T. Sawa, E. Yashima and M. Akashi, Anal. Chem., 64 (1992) 1920.
10 M. Akashi, T. Sawa, Y. Baba and M. Tsuhako, J. High Resolut. Chromatogr., 15 (1992) 625.
11 T. Sawa, M. Akashi, Y. Baba and M. Tsuhako, Nucleic Acids Symp. Ser., 27 (1992) 51.
12 T. Sawa, E. Yashima, M. Akashi, Y. Baba and M. Tsuhako, J. High. Resolut. Chromatogr., submitted for publication.
13 Y. Baba, M. Tsuhako, T. Sawa and M. Akashi, J. Chromatogr., 632 (1993) 137.
14 M. Akashi, M. Yamaguchi, H. Miyata, M. Hayashi, E. Yashima and N. Miyauchi, Chem. Lett., (1988) 1093.
15 E. Yashima, T. Shiiba, T. Sawa, N. Miyauchi and M. Akashi, J. Chromatogr., 603 (1992) 111.
16 S. Nagae, Y. Suda, Y. Inaki and K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., 27 (1989) 2593.
17 J. Pitha, Polymers, 18 (1977) 425.
18 J. Pitha, M. Akashi and M. Draminski, in E.P. Goldberg and A. Nakajima (Editors), Biomedical Polymers: Poly-meric Materials and Pharmaceuticals for Biomedical Use, Academic Press, New York, 1980, pp. 271–297.
19 M. Akashi, H. Iwasaki, N. Miyauchi, T. Sato, J. Sunamoto and K. Takemoto, J. Bioact. Compatible Polym., 4 (1989) 124.
20 N.N. Leonard and R.F. Lambert, J. Org. Chem., 34 (1969) 3240.
21 Y. Inaki, S. Sugita, T. Takahara and K. Takemoto, J. Polym. Sci., Polym. Chem. Ed., 24 (1986) 3201.
22 K. Kondo, S. Tanioku and K. Takemoto, Makromol. Chem. Rapid Commun., 1 (1980) 303.
23 K. Kondo, H. Iwasaki, N. Ueda, K. Takemoto and M. Imoto, Makromol. Chem., 125 (1969) 42.
24 E. Yashima, T. Tajima, N. Miyauchi and M. Akashi, Biopolymers, 32 (1992) 811.
25 H. Kaye, J. Am. Chem. Soc., 92 (1970) 5777.