CHEMISTRY LETTERS, pp. 1113-1116, 1990.

Agarose-Poly(9-vinyladenine) Conjugated Gel. Application for Electrophoretic Separation of Nucleic Acids

Eiji YASHIMA,* Natsumi SUEHIRO, Mitsuru AKASHI,*
and Noriyuki MIYAUCHI
Department of Applied Chemistry, Faculty of Engineering,
Kagoshima University, Korimoto, Kagoshima 890

Poly(9-vinyladenine) was conjugated with agarose gel to see the interaction with RNA (polyA and polyU) and DNA for electrophoresis. The conjugated gel showed the nucleobase selective separation for RNA and could discriminate between single-stranded and double-stranded DNA.

The separation and purification of nucleic acids on the basis of size, shape and conformation are of great important in molecular biology and genetics. The chromatographic and electrophoretic techniques have been widely used for them. The attractive approaches to achieve the nucleobase-selective separation of nucleic acids have been carried out; e.g., the use of (1) poly(uridylic acid)(polyU)-agarose[1] for the separation of mRNA containing poly(adenylic acid)(polyA) region, (2) nucleic acid bases substituted on silica gel,[2,3] and (3) intercalators such as phenyl neutral red,[4] acriflavin,[5] and quinacrine[6] for the separation of nucleosides and nucleotides. First successful example for electrophoresis by a using nucleic acid analog was demonstrated by Pitha.[7] He prepared poly(acrylamide)-poly(9-vinyladenine)(PVAd) conjugated gel and investigated the interaction between synthetic polynucleotides and PVAd. Previously, we found that the PVAd-immobilized silica gel showed the nucleobase-selective recognition for the separation of nucleosides and oligonucleotides by means of HPLC.[8]

In the present study, we prepared the agarose-PVAd conjugated gel to see the interaction of PVAd with synthesized and naturally occurring nucleic acids such as RNA (polyA and polyU) and DNA (linear and circular DNA) for electrophoresis.

PVAd was obtained by the polymerization of 9-vinyladenine with ammonium persulfate in H_2O in a similar manner reported previously.[9] The obtained PVAd was fractionated by ultrafiltration technique. PVAd

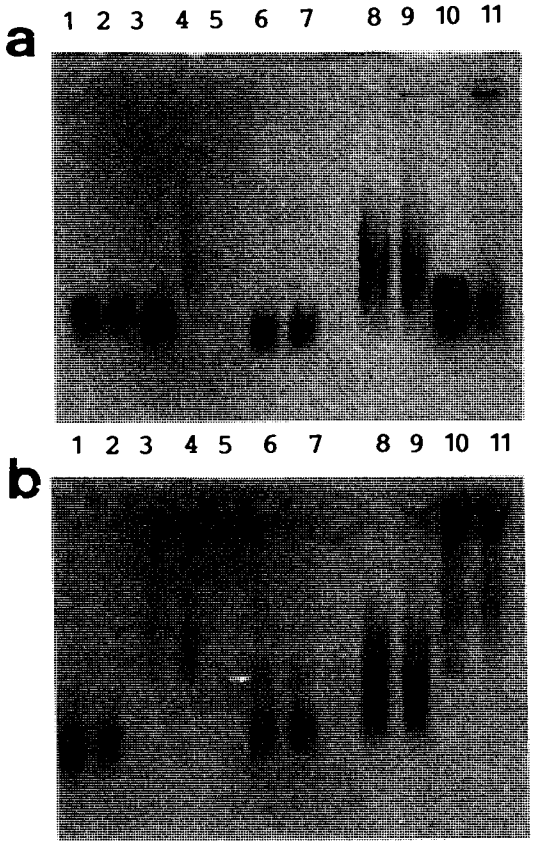
having molecular weight more than 50000 was used for the electrophoretic study, since a higher molecular weight PVAd can interact with RNA strongly by UV spectroscopy.[9]

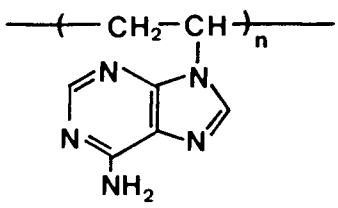
PolyA and polyU were purchased from Sigma Chemical Co. Because the molecular weight of those samples was very high (more than 100000) and the distribution of the molecular weight was very broad, both polynucleotides were sonicated in TE buffer (10 mM Tris and 1 mM EDTA, pH 8.0) with 0.5 M NaCl. The sizes of sonicated (s)-polyA, polyU, and double stranded (ds)-(polyA-polyU) were 530 ± 200, 470 ± 200, and 310 ± 100 bp, respectively, against ϕX174 Hae III and λ Hind III restriction enzyme fragments[10] from Toyobo. Agarose gel electrophoresis was performed on a submaline type gel apparatus (Bio-Rad DNA SUB CELL) at a constant voltage (100 V; ATTO AE-8350 power supply) for 2h, using TBE buffer (89 mM Tris base, 89 mM boric acid, and 2 mM EDTA, pH 8). 10 wt% of PVAd was dissolved with agarose. In order to visualize the presence of nucleic acids, gels were stained with aqueous ethidium bromide for DNA and methylene blue[11] for polyA, polyU, and ds(polyA-polyU).

Figure 1 shows the electrophoretic patterns of polyA, polyU, and ds(polyA-polyU) on agarose (0.7%) (a) and agarose-10%PVAd (b) gels. It should be noted that the elution of PVAd from gel is negligibly small. The mobility of the sonicated polyA was not affected by PVAd (lane 1), although a slight retardation of unsonicated (u)-polyA was observed (lane 8). On the other hand, polyU was strongly adsorbed on the gel containing 10% PVAd (lanes 3 and 10) and the mobility of higher molecular weight polyU was much decreased and a large amount of polyU was trapped in the slot. This should be ascribed to the complementary hydrogen bonding formation of PVAd with polyU. The hypochromic effect of PVAd to polyA and polyU also supports this. The hypochromicities of u-polyA-PVAd (MW>50000) and u-polyU-PVAd (MW>50000) are nearly 0% and 23.4%, respectively.[12] Similar observation was reported by Pitha who used poly-(acrylamide)-PVAd conjugated gel to see the interaction between PVAd and unsonicated radioactive polyA and polyU.[7]

Complex formation of PVAd with polyU was confirmed in the electrophoretic profiles of lanes 4 and 11 (Fig. 1 (a)), where an equimolar amount of polyU and PVAd was injected. The significant decrease of the mobility was observed, while the mobility of polyA and PVAd mixture was not changed (Fig. 1 (a);lanes 2 and 9).

The mobility of a sonicated ds(polyA-polyU) was examined on both gels (lanes 6 and 7). Two spots were observed on the agarose-PVAd gel, whereas a single spot was detected on agarose gel. It is well known that polyA and polyU form double (polyA-polyU) and triple (polyA-polyU-polyU)





PVAd

Fig. 1. Electrophoresis of RNA on agarose gel (0.7%) (a) and agarose-10%PVAd gel (b). Lane 1; sonicated (s)-polyA, 2; s-polyA-PVAd, 3; s-polyU, 4; s-polyU-PVAd, 5; PVAd, 6; s-(polyA-polyU), 7; s-(polyA-polyU)-PVAd, 8; unsonicated (u)-polyA, 9; u-polyA-PVAd, 10; u-polyU, 11; u-polyU-PVAd. Concentration was 13 - 19 mM.

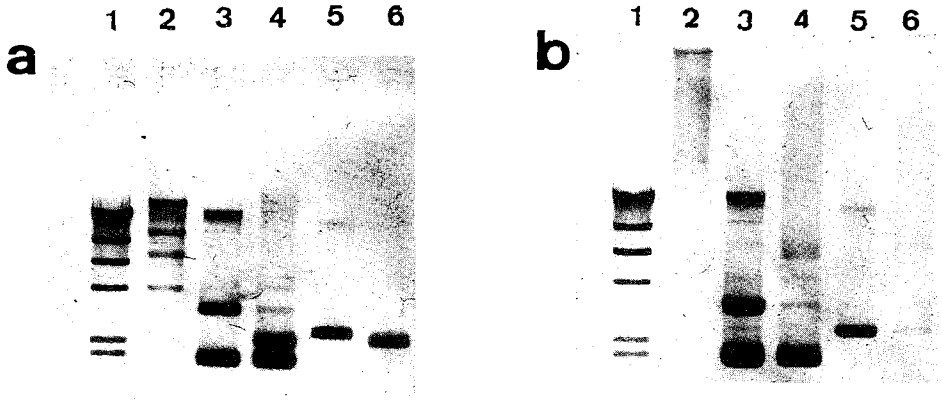


Fig. 2. Electrophoresis of DNA on agarose gel (a) and agarose-10%PVAd gel (b). Lane 1; Hind III digests of λ DNA, 2; sample 1 heated to 95 °C for 3 min, 3; pUC19, 4; sample 3 heated to 95 °C for 3 min, 5; linear pUC19, 6; sample 5 heated to 95 °C for 3 min. Concentration of DNA was 1.0 g l^{-1}. The DNA bands in lane 3 are arranged from bottom to top in the order ccc- and oc-plasmid DNA and linear chromosomal DNA.

stranded helices in an equilibrium.13) Therefore, the retarded spot on the agarose-PVAd gel may be the triple helical complex or extra polyU.

The interaction of PVAd with linear and circular DNA was investigated by using the same gel (Fig. 2). The mobility of linear ds-DNA (lanes 1 and 5) and circular DNA (lane 3) on the agarose-10%PVAd gel was little decreased compared to that on agarose only. The dramatic decrease of the mobility was observed in lanes 2, 4, and 6 (Fig. 2 (b)), where all samples were denatured by heating to 95 °C for 3 min. In cases of lanes 2 and 6 most of DNA were strongly retarded. This suggests that the denatured, probably a single stranded DNA was trapped by PVAd.[14] In a similar manner circular DNA was denatured and injected. The bands of open circular (oc) and chromosomal DNA were almost disappeared on the agarose-PVAd gel. There have been reported several methods to separate covalently closed circular (ccc) plasmid.[15] Our method may be applicable for separating ccc plasmid.

Since a number of synthetic nucleic acid analogs have been prepared so far,[16] most of them may be applicable for electrophoresis to see the interaction with nucleic acids and to separate them by an affinity of the analog.

The authors are grateful to Professor Higashi and Dr. Uchiumi of Department of Science, Kagoshima University, for supplying pUC19 plasmid and for valuable discussion and encouragement.

References

1) U. Lindberg and T. Persson, Eur. J. Biochem., **31**, 246 (1972).
2) S. Nagae, T. Miyamoto, Y. Inaki, and K. Takemoto, Polym. J., **21**, 19 (1989) and references cited therein.
3) M. Akashi, T. Tokiyoshi, N. Miyauchi, and K. Mosbach, Nucleic Acids Res. Symp. Ser., **16**, 41 (1985).
4) H. Bunemann and W. Müller, Nucleic Acids Res., **5**, 1059 (1978).
5) J. M. Egly, J. Chromatogr., **215**, 243 (1981).
6) S. Takenaka, K. Dohtsu, and M. Takagi, Anal. Sci., **6**, 139 (1990).
7) J. Pitha, Anal. Biochem., **64**, 422 (1975).
8) M. Akashi, M. Yamaguchi, H. Miyata, M. Hayashi, E. Yashima, and N. Miyauchi, Chem. Lett., **1988**, 1093.
9) M. Akashi, H. Iwasaki, N. Miyauchi, T. Sato, J. Sunamoto, and K. Takemoto, J. Bioactive and Compatible Polym., **4**, 124 (1989).
10) W. D. Wilson and R. A. Keel, J. Am. Chem. Soc., **103**, 6267 (1981).
11) A. C. Peacock and C. W. Dingman, Biochemistry, **6**, 1818 (1967).
12) M. Akashi, E. Yashima, and N. Miyauchi, unpublished results.
13) W. Saenger, "Principles of Nucleic Acid Structure," Springer-Verlag, New York (1988), p.242.
14) This was confirmed by the electrophoresis of single stranded (+)-M13mp8 DNA, which was completely adsorbed on the agarose-10%PVAd gel.
15) W. S. Vincent III and E. S. Goldstein, Anal. Biochem., **110**, 123 (1981).
16) Y. Inaki and K. Takemoto, "Functional Monomers and Polymers," ed by K. Takemoto, Y. Inaki, and R. M. Ottenbrite, Marcel Dekker, Inc., New York (1987), p.413.

(Received March 31, 1990)