CHEMISTRY LETTERS, pp. 1831-1834, 1989.

Gel Electrophoretic Demonstration of Pseudo-Grafting of Polyacrylamide onto λ Phage DNA

Mizuo MAEDA,* Akira HIRAI, and Makoto TAKAGI*
Department of Organic Synthesis, Faculty of Engineering,
Kyushu University, 6-10-1, Hakozaki, Higashi-ku, Fukuoka 812

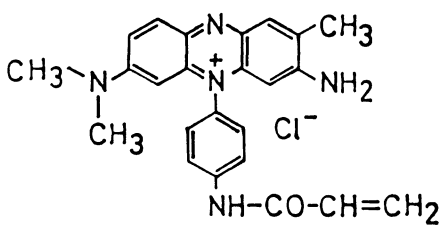
5-(4-Acryloylaminophenyl)-3-amino-7-dimethylamino-2-methylphenazinium chloride (1) which is a DNA-binding (intercalative) molecule was radically copolymerized with acrylamide in the presence of λ phage DNA. A gel electrophoretic examination of the reaction product has strongly suggested that the DNA was grafted with polyacrylamide chains through 1 ("pseudo-grafting").

A combination of different types of polymers may give rise to new features. Such heterogeneous macromolecular conjugates occur widely in biological system. One of the typical examples is nucleoprotein including DNA-histone complexes where DNA is a polynucleotide and histone is a polypeptide. DNA molecules carry genetic information, whereas the basic proteins, histones, appear to be an integral part of the eukaryotic chromosome and to play some roles in the control of chromosome structure and function.[1)]

On the other hand, semi-synthetic conjugates are of much interest since the modification of biological macromolecules with synthetic polymers may allow us to regulate their function.

One of the successful works was demonstrated by Matsushima et al. on an enzyme/polyether conjugate.[2)] Non-immunoreactive asparaginase was obtained by modification with polyethylene glycol chains. A lipid vesicle/vinyl polymer conjugate was prepared in order to control the permeability of liposomal membranes.[3)]

In the present paper will be described a novel macromolecular assembly consisting of naturally occurring polynucleotide and synthetic vinyl polymer. A DNA-binding (intercalative) molecule having polymerizable vinyl group (1) was radically copolymerized with acrylamide in the presence of λ phage DNA. The result of gel electrophoresis of the reaction product has strongly suggested that λ DNA was grafted with polyacrylamide chains by means of DNA-binding molecules 1. Since the conjugation is based on non-covalent bonding, we term the phenomenon as "pseudo-grafting".



1

5-(4-Acryloylaminophenyl)-3-amino-7-dimethylamino-2-methylphenazinium chloride (1) was synthesized according to the method described by Bünemann et al.[4] DNA was purchased from Nippon Gene Co. Ltd. To a micro test tube (Eppendorf, 1.5 cm^3) was introduced TE (10 mM(1 M = 1 mol dm^{-3}) Tris-HCl, 1 mM EDTA, pH 8.0) solution of λ DNA, BPE (8 mM Na_2HPO_4, 2 mM NaH_2PO_4, 1 mM EDTA, pH 7.4) solution of 1, and BPE solution of acrylamide (AAm). After 20 min incubation at 20 °C, BPE solution of N,N,N',N'-tetramethylethylenediamine (TEMED) and aqueous ammonium peroxodisulfate were added to the reaction mixture. The final concentration of each constituent was as follows: λ DNA, 0.02 mM (expressed in nucleotide); 1, 0-0.02 mM; AAm, 1% (w/v); ammonium peroxodisulfate, 0.3 mg cm^{-3}; TEMED, 1 mg cm^{-3}. The total volume of reaction mixture was 0.2 cm^3 (TE:BPE = 1:19, by volume).

The reaction was carried out for 40 min at 20 °C. Then, 0.01 cm^3 portion of the mixture was combined with 0.01 cm^3 of gel-loading solution consisting of glycerol and water (1:1, by volume), and was immediately subjected to agarose gel electrophoresis run in TBE buffer (89 mM Tris-borate, 25 mM EDTA, pH 8.0). After the electrophoresis, DNA in the gel was visualized by using ethidium bromide.

Figure 1 shows the profiles of migration behavior of λ DNA in the reaction mixture. Retarded migration as compared with the native DNA was observed in 0.5% agarose gel (Fig. 1A) when the initial concentration of 1 in the polymerization mixture was 3 μM (lane 4) or more (lanes 5-8). Moreover, DNA trapped in the slot was seen in lanes 5-8. Especially in lanes 7 and 8 was observed no migration of DNA.

Such a pronounced retardation was not seen when 1 was absent in the polymerization mixture, although a small deviation observed in lane 2 of Fig. 1A could suggest the occurrence of some side reaction other than polymerization.[5] The presence of AAm and the initiator was also essential to the retardation; 1 itself did not significantly affect the migration behavior of λ DNA in the concentration range examined here (not shown). One percent agarose gel, which has a tighter network compared with 0.5% gel, was sufficient for 3 μM of 1 to trap DNA strands fully in the sample well (lanes 4-8, Fig. 1B).

Since the mobility of a certain molecule in gel electrophoresis is primarily a function of molecular size and net charge, the retarded migration of λ DNA is considered to be due to the "fattening" of DNA brought about by the modification of DNA molecules with nonionic polyacrylamide chains.

Now the next question is how the DNA-polyacrylamide conjugate formed. Two possibilities were considered: (a) 1 as intercalated into DNA was copolymerized with AAm and (b) copolymer of 1 and AAm first formed and then became bound to DNA. In order to distinguish between the two, 1 and AAm were copolymerized for 40 min at 20 °C in the absence of λ DNA. The DNA was then added, and the mixture was incubated for 40 min at 20 °C. The gel electrophoretic profiles of the mixture showed neither DNA band-retardation nor -broadening under the present experimental conditions (Fig. 2). This result strongly suggests that the "fattening" was caused by copolymerization of AAm with the DNA-bound monomer 1; the conjugate formed in the course of polymerization.

In addition to the mechanism of the conjugate formation, the result presented in Fig. 2 has shed light on a unique feature of the conjugate; the difference in

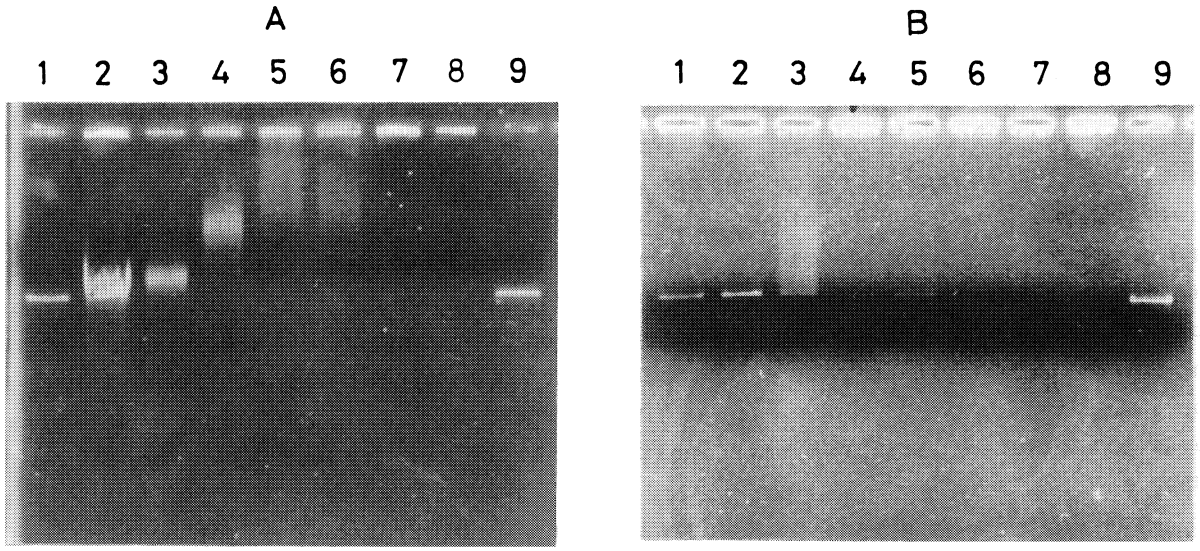


Fig. 1. Gel electrophoresis of λ DNA co-incubated in the copolymerization reaction of 1 and acrylamide. A, 0.5% agarose gel; B, 1% agarose gel. Lanes 1 and 9, control (λ DNA only); lane 2, [1]= 0 M; lane 3, [1]= 2 μM; lane 4, [1]= 3 μM; lane 5, [1]= 4 μM; lane 6, [1]= 5 μM; lane 7, [1]= 10 μM; lane 8, [1]= 20 μM. For concentration of other constituents, see the text.

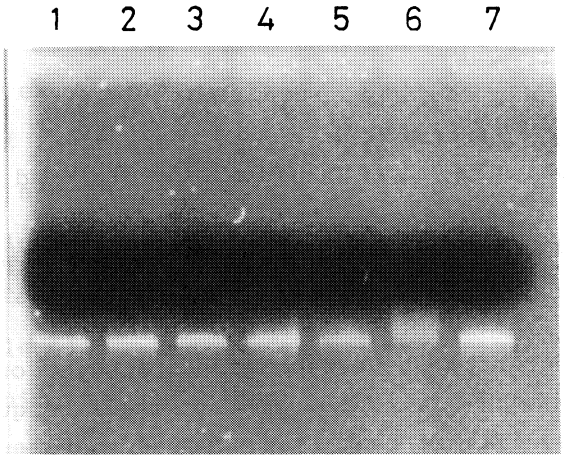


Fig. 2. Gel electrophoresis (0.5% agarose) of λ DNA incubated with the reaction mixture after the copolymerization of 1 and acrylamide; lanes 1 and 7, control (λ DNA only); lane 2, [1]= 0 M; lane 3, [1]= 2 μM; lane 4, [1]= 5 μM; lane 5, [1]= 10 μM; lane 6, [1]= 20 μM.

the DNA-binding ability of the copolymer prepared in the presence of DNA from that prepared without DNA. Since the copolymer contains 1 as an intrinsically intercalative structural unit, both polymers are considered to have an ability to interact with DNA. In fact, Bünemann et al. have described that polyacrylamide containing 1 residues ("pre-formed" copolymer) forms a complex with DNA;[4,6] however, the interaction between the pre-formed copolymer and DNA is very weak as is exemplified by the use of the polymer as chromatographic support for separating DNA fragments. The results in Fig. 2 also indicate that the interaction of pre-formed 1-AAm copolymer with DNA is none; the copolymer never combines with DNA under ambient conditions to form such a stable conjugate as was formed by 1-AAm copolymerization in the presence of DNA. The latter conjugate, on the contrary, never dissociates under ambient conditions to the constituent 1-AAm copolymer and DNA.

There would be a desirable distribution, configuration, and/or orientation of intercalative moieties for the copolymer to interact with DNA. Such steric demands might be met by the copolymer prepared in the presence of DNA but not by that prepared without DNA. There can also be a kinetic factor which affects the combination or dissociation behavior of copolymer-DNA conjugate. These points should be clarified by studying the recombination behavior with λ DNA of 1-containing copolymer dissociated from the conjugate. The experiment is now under way.

The pseudo-grafting presented here of vinyl polymer chains onto DNA may be applicable to the detection of trace amount of DNA, when a certain vinyl monomer having a reporter group is used. This method hopefully provides us a new concept for chemical amplification.

References

1) S. Weisbrod, Nature, 297, 289 (1982).
2) A. Matsushima, H. Nishimura, Y. Ashihara, Y. Yokota, and Y. Inada, Chem. Lett., 1980, 773.
3) M. Maeda, A. Kumano, and D. A. Tirrell, J. Am. Chem. Soc., 110, 7445 (1988).
4) H. Bünemann, N. Dattagupta, H. J. Schuetz, and W. Müller, Biochemistry, 20, 2864 (1981).
5) R. C. Moscel and E. J. Behrman have reported intensive works on the oxidation of nucleic acid bases with peroxodisulfate in alkaline aqueous solution (in 1 M KOH, at 40 °C). No significant reaction, however, between peroxodisulfate and any nucleic acid base was observed under neutral or acidic conditions at 40 °C: J. Org. Chem., 39, 1983 (1974) ; ibid., 39, 2699 (1974). Since our conditions were rather mild in terms of pH and reaction temperature (at pH 7.4, at 20 °C), the direct reaction of the initiator with bases, if any, is considered to be small. In fact, the result in lane 2 of Fig. 1A was not always reproducible as seen in lane 2 of Fig. 1B, although other results presented here were fully reproducible.
6) H. Bünemann and W. Müller, Nucleic Acids Res., 5, 1059 (1978).

(Received July 20, 1989)