Short Communication

High-performance liquid chromatography of oligoguanylates at high pH

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ABSTRACT

Because of the stable self-structures formed by oligomers of guanosine, standard high-performance liquid chromatography techniques for oligonucleotide fractionation are not applicable. Previously, oligoguanylate separations have been carried out at pH 12 using RPC-5 as the packing material. While RPC-5 provides excellent separations, there are several limitations, including the lack of a commercially available source. This report describes a new anion-exchange high-performance liquid chromatography method using HEMA-IEC BIO Q, which successfully separates different forms of the guanosine monomer as well as longer oligoguanylates. The reproducibility and stability at high pH suggests a versatile role for this material.

INTRODUCTION

It has been shown that polynucleotides can direct the synthesis of complementary oligomers by non-enzymatic mechanisms [1–4]. These reactions follow standard Watson–Crick pairing rules and have been used as models of the earliest replicating systems on the primitive Earth. The most successful synthesis occurs when polycytidylic acid [poly(C)] is used as a template to direct the condensation of the activated monomer, guanosine 5'-phospho-2-methylimidazole (2MeImpG). Oligomerization efficiency exceeds 80% and can produce oligomers up to the 40-mer which are detectable with high-performance liquid chromatography (HPLC) [2].

Since oligomers of guanosine form extremely stable self-structures at neutral pH ranges [5], standard techniques for oligonucleotide fractionation are not applicable. The reactions described above were analyzed by HPLC on an RPC-5 column [6] using a sodium perchlorate gradient at pH 12. At this pH, the oligomers are completely deprotonated, with strand separation driven by charge repulsion.

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While the pH stability of RPC-5 permits its use in oligoguanylate fractionation, it is not widely used in other oligonucleotide separations because the adsorbed quaternary ammonium ion (Andogen 464) is readily stripped off the solid support with resulting loss in resolution. Additionally, the RPC-5 column is not commercially available and the support materials, Kel-F or Plaskon, are not readily available. (See Usher [7] for a more complete discussion.) Because of these limitations, RPC-5 is not ideal for oligonucleotide separation, although no other HPLC option has been available for the analysis of oligomers of guanosine.

More recently, Kanavarioti and Doodokyan [8] have shown that products from the decomposition and oligomerization of 2MeImpG can be analyzed using reversed-phase HPLC on a commercially available, silica-based C_{18} column. This method is superior to RPC-5 when investigating reaction products that do not exceed the guanosine tetramer. However, since the column material is not stable at high pH, it cannot be used to fractionate longer oligomers.

I now report on a new anion-exchange HPLC method that separates different forms of guanosine monomers as well as longer oligomers. The column material is stable at pH 12 and separations are achieved using a sodium perchlorate gradient similar to that used with RPC-5.

EXPERIMENTAL

Materials

Guanosine, guanosine 5'-monophosphate (GMP), poly(C), sodium perchlorate, and bovine pancreatic ribonuclease A were obtained from Sigma (St. Louis, MO, U.S.A.). Poly(G) was obtained from P-L Biochemicals (Milwaukee, WI, U.S.A.). 2MeImpG was a gift from Leslie Orgel of the Salk Institute for Biological Studies, La Jolla, CA, U.S.A. Water was obtained from a NANOpure II system with at least a 17.6 MΩ · cm purity. HPLC solvents were prepared and passed through a 0.2-μm filter under reduced pressure prior to use.

HPLC analysis

Chromatography was performed with a solvent delivery system composed of two Alltex 110A pumps and an Axxiom 710 controller. The eluate was monitored at 254 nm by an Isco V[4] variable-wavelength detector attached to a Linear chart recorder. The analytical (150 × 4.6 mm I.D.) and guard columns were generously provided by Alltech (Deerfield, IL, U.S.A.) and were packed with HEMA-IEC BIO Q, which consists of a quaternary trimethylamino group covalently bound to a pH stable support. Chromatographic conditions were as follows. Mobile phase: solvent A, 2 mM Tris–perchlorate, pH 12; solvent B, 2 mM Tris–perchlorate, pH 12, 0.4 M sodium perchlorate. Gradient elution, 2 to 30% B in 15 min; 30 to 100% B in 87.5 min. The initial steeper gradient is needed to decrease the retention time of the shorter products, while the slower gradient of 0.8% B min^{-1} provides convenient separation of the longer oligomers. A flow-rate of 1 ml min^{-1} was used and produced a system pressure of about 800 p.s.i.

Sample preparation

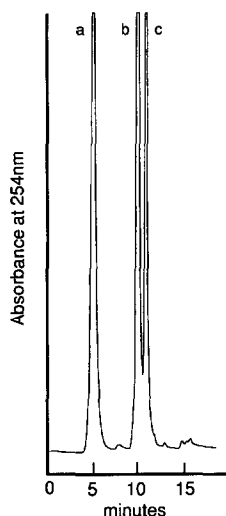*Separation of guanosine, GMP and 2MeImpG.* Approximately 0.5 absorption

Fig. 1. HPLC elution profile of guanosine (a), 2MeImpG (b) and GMP (c). Column, HEMA-IEC BIO Q; mobile phase: solvent A, 2 mM Tris–perchlorate (pH 12); solvent B, 2 mM Tris–perchlorate (pH 12), 0.4 M sodium perchlorate; gradient elution, 2 to 30% B in 15 min, 30 to 100% B in 87.5 min. The flow-rate was 1 ml min^{-1}, with the chart speed set at 30 cm h^{-1} and the detector scaled to 0.2 a.u.f.s.

units of each were mixed with solvent A and directly injected. Individual peaks were identified by comparison of retention times with authentic samples. The chromatogram shown in Fig. 1 was included for direct comparison with the other elution profiles, but better resolution could be obtained with a slower gradient (not shown).

Poly(G) hydrolysis products. A 3 mM solution of poly(G) was adjusted to pH 12 with KOH and heated at 70°C for 40 min. A 50-μl volume was diluted to 200 μl with solvent A and directly injected.

2MeImpG oligomerization reactions. Reaction mixtures were prepared which contained 1.0 M NaCl, 0.2 M MgCl$_2$, 0.1 M Tris–perchlorate, pH 8.5, 0.04 M 2MeImpG, and, where necessary, 0.02 M poly(C). After mixing, the samples were kept at 4°C. At predetermined times, aliquots were withdrawn and the reaction quenched by mixing with an excess of EDTA, pH 9. Prior to analysis, surviving imidazolides were hydrolyzed (pH 5) for 12 h at room temperature. The poly(C) template was degraded by incubation at 37°C with bovine pancreatic ribonuclease A, which hydrolyzes internucleotide linkages on the 3' side of pyrimidine residues. The aliquots were diluted with solvent A prior to injection.

RESULTS AND DISCUSSION

Although excellent separations of long oligomers of guanosine can be achieved with RPC-5, the difficulties described above limit its general use. The HEMA-IEC BIO Q material used in the present study overcomes many of those problems. Fig. 1 shows that different monomers of guanosine can be efficiently separated with this technique. The separation of 2MeImpG from GMP is particularly useful since GMP is the major product of 2MeImpG decomposition [8]. The main focus of this study was to
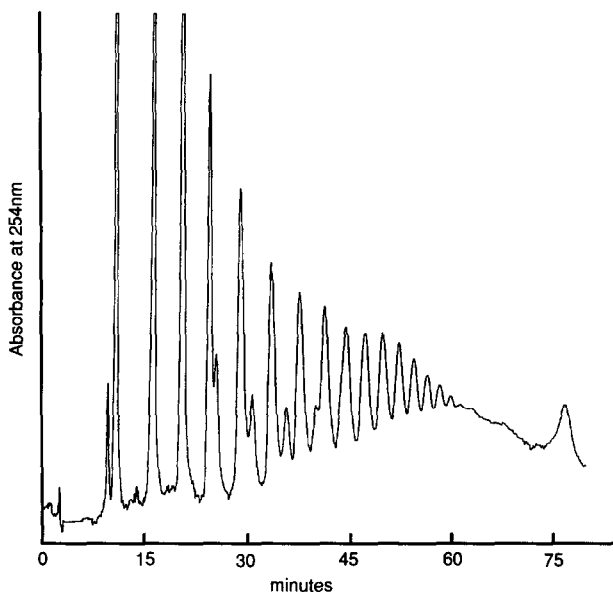
Fig. 2. HPLC elution profile of poly(G) hydrolysis products. The chromatographic conditions are the same as in Fig. 1 except with a chart speed of 20 cm h^{-1} and 0.1 a.u.f.s.
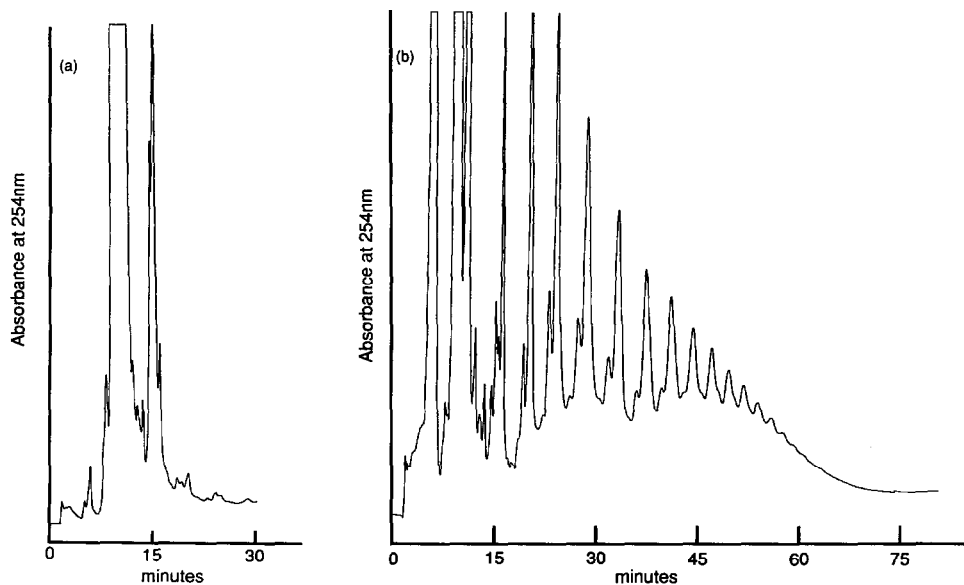

Fig. 3. HPLC elution profiles of 2MeImpG oligomerization reactions using HEMA-IEC BIO Q. Reaction conditions are described in the text. These profiles are of aliquots taken after incubation at 4°C for 6 days. The chromatographic conditions are the same as in Fig. 1 except with a chart speed of 20 cm h^{-1} and 0.05 a.u.f.s. (a) 2MeImpG alone; (b) 2MeImpG in the presence of poly(C).
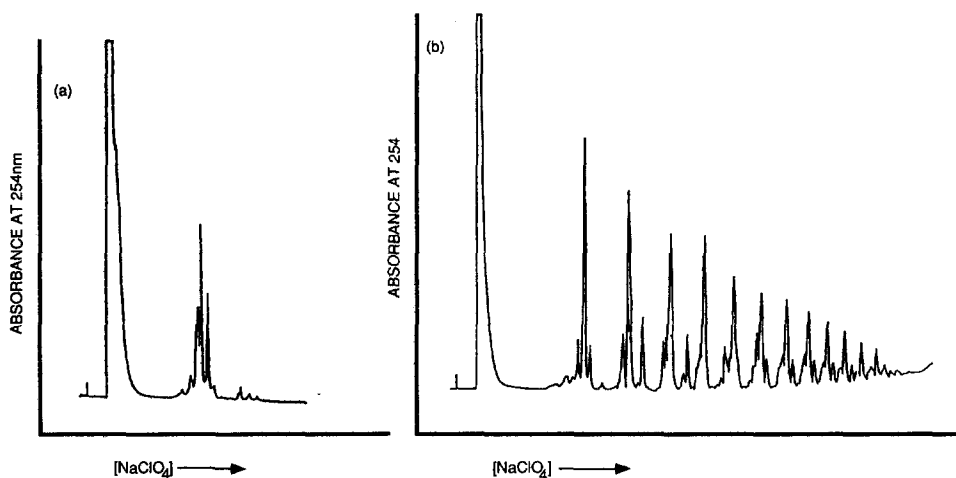
Fig. 4. HPLC elution profiles of 2MeImpG oligomerization reactions using RPC-5. The reaction and sample preparation conditions used here are from previous work [9], and are similar to those described in the text except that the incubation was at −18°C for 4 weeks. The chromatography was performed using a Beckmann solvent delivery system composed of two 110B pumps and an NEC 8300 gradient controller at pH 12 using a linear gradient of sodium perchlorate (0 to 0.06 M in 90 min) and a flow-rate of 1 ml min^{-1}. The eluate was monitored at 254 nm by a Kratos spectroflow 757 variable-wavelength detector attached to a Soltec Model 1242 recorder. (a) 2MeImpG alone; (b) 2MeImpG in the presence of poly(C).

find ways to separate longer oligomers, and it is likely that better resolution can be obtained with investigations of other mobile phase conditions.

Fig. 2 shows the elution profile produced when poly(G) is subjected to partial base-catalyzed hydrolysis. At least some of the peak broadening seen in the longer oligomers is due to the preparative flow cell used in the UV detector. This result can be directly compared with the 2MeImpG oligomerizations shown in Fig. 3. Finally, similar oligomerizations analyzed by RPC-5 are shown in Fig. 4. It should be noted that the profiles in Fig. 4 are from previously published work [9] which employed reaction conditions different from those described in this study. More efficient reaction conditions can be found elsewhere [2].

It is clear from the results reported here that HEMA-IEC BIO Q is useful for separating oligonucleotides at high pH. Its stability and reproducibility, as well at its ability to separate both long and short oligonucleotides suggest a versatile workhorse role for this type of column.

ACKNOWLEDGEMENTS

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