Journal of Chromatography, 588 (1991) 157–164
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 652

High-performance affinity chromatography of messenger RNA

Thomas A. Goss and Martin Bard

Department of Biology, Purdue University School of Science, 1125 E. 38th Street, Indianapolis, IN 46205 (USA)

Harry W. Jarrett*

Department of Biochemistry, 800 Madison Avenue, University of Tennessee, Memphis, TN 38168 (USA)

(First received April 3rd, 1991; revised manuscript received July 23rd, 1991)

ABSTRACT

A 50-mer of thymidylic acid, $(dT)_{50}$, was coupled to silica inside prepacked columns using the N-hydroxysuccinimide chemistry. The resulting $(dT)_{50}$-silica columns were used to resolve oligomers of adenylic acid, $(dA)_{19-24}$, and to separate poly(A) mRNA (messenger RNA) from *Saccharomyces*. Oligomers which differed in length by a single nucleotide base were readily resolved. Using either $(dT)_{50}$- or $(dT)_{18}$-silica, poly(A) mRNA could be purified in as little as 8 min. The poly(A) mRNA isolated appeared to be full length and could be used directly for T_4 RNA ligase and RNAse A and T_1 enzymatic reactions. The $(dT)_{50}$-silica column was used to fractionate total poly(A) mRNA by tail length. While the separation was primarily due to poly(A) tail length, most fractions appeared to contain multiple tail lengths. Whether this represents an intrinsic feature of the RNA or a limitation of the method is discussed. These studies show that polynucleotides in the kilobase size range can be separated rapidly and with good resolution on DNA-silica.

INTRODUCTION

Recently, we reported experiments to determine the usefulness of high-performance affinity chromatography (HPAC) of DNA [1]. In the method, an octadecamer of thymidylate, $(dT)_{18}$, tethered by way of the 5'-terminus to macroporous silica was shown to hybridize with and allow the separation of $(dA)_{6-18}$. Differences of a single base pair were sufficient to resolve samples using either temperature or salt gradients. Here, we extend these studies to the fractionation of polydenylated messenger RNA [poly(A) mRNA] from *Saccharomyces cervisiae*.

The first affinity chromatography of polynucleotides used oligothymidylate-cellulose synthesized by Gilham [2]. Today, poly(A) mRNA is routinely isolated by affinity chromatography on polythymidylic acid or polyuridylic acid covalently attached to cellulose, agarose, or Sepharose supports. Most established procedures use abrupt changes from loading buffer to elution buffer and require rechromatography to obtained sufficient purity [3,4]. Higher resolution with high-performance liquid chromatography (HPLC) could improve the purity obtained and increase the speed of isolation. Since degradation of RNA by ubiquitous RNAses is a source of concern [4], rapid isolation which lessens exposure to digestion should produce more intact mRNA. Thus, the higher speed and resolution of HPAC on DNA-silica should prove useful for the preparation of mRNA. There may also be important analytical uses for this support in the analysis of poly(a) tail length polymorphism.

Poly(A) mRNA from the yeast *Saccharomyces cerevisiae* has an average 3'-poly(A) tail length of about fifty [5,6]. Poly(A) tail lengths are thought to be important to mRNA turnover and translational efficiency [7–9]. Other investigators have used poly (U)-Sepharose to crudely fractionate by tail length and have observed that factors such as types of cell

0021-9673/91/$03.50

strain, growth phase, transcription inhibition with actinomycin [10], translation inhibition with cycloheximide and other antibiotics [11], and the osmotic state of the cell [12] affect tail length. After synthesis, some poly(A) tails are progressively shortened with time. The mechanism is unknown but one component may involve the poly(A) binding protein(s) [7,8,13]. There is thus considerable interest in tail length and a facile method for the analysis of tail length could have important potential. Since we have previously shown that $(dT)_{18}$-silica cannot resolve lengths longer than 18, a new silica would be needed for tail length analysis.

Here we report on the synthesis $(dT)_{50}$-silica and its use to fractionate total mRNA by poly(A) tail length. Methods for selective elution using temperature, formamide, and salt gradients are also described.

METHODS

DNA synthesis

5'-Aminoethylphosphoryl-$(dT)_{50}$ was synthesized using standard phosphoramidite chemistry and was purified by reversed-phase chromatography using volatile triethylamine–acetonitrile gradients as previously described [1]. This purified polynucleotide will be referred to as amino-$(dT)_{50}$.

DNA–silica column preparation

N-Hydroxysuccinylimidyl ester–silica (NHS–silica) was prepared immediately prior to use from Macrosphere-WCX (Alltech) as previously described [14]. The activation chemistry was performed inside prepacked 30 × 4.6 mm I.D. cartridge columns. Amino-$(dT)_{50}$ was coupled to NHS-activated silica using two different protocols. In one, the amino-$(dT)_{50}$ was recirculated through the activated column as previously described [1]; 8.6 units of amino-$(dT)_{50}$ was recirculated for an hour at pH 7.5 and 2.7 units (31%) coupled to the column. Another column was made by a stopped-flow procedure at pH 5.0. In this case, the column was activated and washed with 2-propanol as previously described [1] and then rapidly flushed with 1 ml of water at 10 ml/min. Then 85 μl of 0.2 *M* sodium phosphate, pH 5.0 containing 6 units of amino-$(dT)_{50}$ was injected and the flow stopped with the DNA inside the column. The column was removed, the ends sealed, and the column was then incubated at 50°C for 3 h. This procedure resulted in 4.4 units (73%) coupled to the column.

RNA isolation from Saccharomyces

RNA was prepared essentially as described by Sherman *et al.* [15]. Briefly, yeast (*Saccharomyces cervisiae* strain A184D Mat α) were grown in yeast extract–peptone–dextrose medium at 30°C to between 50 and 115 Klett units. After adding 10 mg cycloheximide per liter, the cultures were mixed for 10 min at 5°C and then centrifuged (Sorvall GLC-2B) for 5 min at 6500 rpm (5400 *g*). The pellet was resuspended in 2.5 ml of LETS buffer [0.1 *M* LiCl, 0.01 *M* EDTA, 0.01 *M* Tris–HCl, pH 7.4, 0.2% sodium dodecyl sulphate (SDS), 0.1% diethylpyrocarbonate]. Phenol equilibrated with LETS buffer (3 ml) was added and the cells were homogenized by vortexing with 1/4 volume glass beads (0.4 mm) for 7.5 min. An aliquot of 5 ml LETS buffer was added and the mixture was centrifuged (8000 rpm, 10 min, 8200 *g*) and the aqeuous layer was recovered and extracted twice with phenol–chloroform–isoamyl alcohol. The final aqeuous layer was made 0.5 *M* LiCl and the RNA precipitated. The RNA was collected by centrifugation and washed with 70% ethanol.

^{32}P-Labeling

RNA fractions were 3'-labeled by the method of Cobianchi and Wilson [16] using 5'-^{32}P-3',5'-cytidine bisphosphate (^{32}P-pCp) and T_4 RNA ligase. $(dA)_{12\text{-}18}$ and $(dA)_{40\text{-}60}$ size markers were 5'-labeled when necessary using γ-^{32}P-ATP and T_4 polynucleotide kinase [4].

Poly(A) tail length analysis

Poly(A) tail length of RNA from various fractions was determined by first removing non-tail RNA by digestion with RNAses T_1 and A and determining the size of the tails on acrylamide gels. The method used was similar to Nichols and Welder [17]. Briefly, mRNA fractions obtained from chromatography were made 8 *M* LiCl and precipitated at −70°C. The precipitated RNA was washed once with 70% ethanol and redissolved in 20 m*M* Tris–HCl, pH 7.5, 2 m*M* EDTA. NaCl was then added to 0.2 *M* and RNAse A and T_1 were added to a final concentration of 0.003 and 0.1

units/μl, respectively. The final reaction volume was typically 10 μl and digestions were for 30 min at 37°C. The samples were then diluted with an equal volume of formamide and applied to 10% acrylamide gels and electrophoresed [4].

Electrophoresis and autoradiography

The gels were 10% acrylamide, 0.3% bisacrylamide, and 8 *M* urea as described by Sambrook *et al.* [4]. Electrophoresis was at 20°C and 50 to 100 V for 4–8 h. $(dA)_{12-18}$ and $(dA)_{40-60}$ mixtures were run as size markers. For autoradiography experiments, 3'-labeled mRNA and 5'-kinased markers were used. After electrophoresis, the unfixed gels were sealed in seal-a-meal bags. Autoradiography was at −70°C using DuPont Chronex film and Chronex intensifying screens.

Chromatography

The chromatograph was a Varian 5000 ternary gradient instrument outfitted with a Varichrome detector. Mobile phase composition and the solvent and temperature gradients used were optimized for each separation and are given in the legends to the figures. Column temperature was maintained by immersion in a Lauda RM-20 refrigerated water bath (Brinkman Instruments); since heating rates were dependent upon the bath volume, this was kept at 15 l of water. For most experiments, the heater and circulator in the Lauda bath were used to raise the bath temperature with refrigeration turned off; this combination allowed a temperature change of about 1.1°C min. For one experiment (Fig. 5) a slower heating rate was obtained by using the circulating pump with both refrigeration and heating turned off and using a small immersion heater and a Variac variable transformer set at 60% of line voltage (120 V). The immersion heater is 200 W and is of the type sold in department stores to heat individual cups of water for tea or coffee.

Two precautions were taken to prevent degradation of RNA during chromatography: (1) The chromatograph was cleaned monthly by pumping 10% nitric acid through the system (without a column) for 30 min. (2) All mobile phases and samples were prepared using water that had been treated with 0.1% diethylpyrocarbonate and autoclaved.

Sample preparation is also important. Before injection onto a column, ethanol precipitated RNA (either crude or previously purified Saccharomyces mRNA) is redissolved in either diethylpyrocarbonate treated water or TE buffer (10 m*M* Tris, 1 m*M* EDTA, pH 7.5), heated to 80°C, and rapidly cooled in an ice-water bath. Immediately prior to injection, sufficient 2 *M* or 5 *M* NaCl is added to the sample to give a final concentration of 1 *M* NaCl.

RESULTS

When $(dT)_{50}$ was coupled by either the pH 7.5 recycling protocol or the pH 5 stopped-flow procedure, columns with good chromatographic performance were obtained. At room temperature in mobile phases containing 1 *M* Na^+, either column quantitatively bound 0.5 unit injections of either an oligoadenylic acid mixture (lengths 19–24) or polyadenylic acid (average length, 5000) that had been sonicated. Neither was bound when the column was equilibrated at 40°C in water or 0.005 *M* Na^+ containing mobile phases, demonstrating that low ionic strengths and moderate temepratures can be used to completely elute the columns. Since the length of poly(A) tails (average length is 50 bases for *Saccharomyces* [5,6]) is small relative to the length of mRNA (greater than one kilobase), a column that binds 0.5 units of $(dA)_{24}$ would be expected to bind at least 10 units ($\approx$0.3 mg) of poly(A) mRNA.

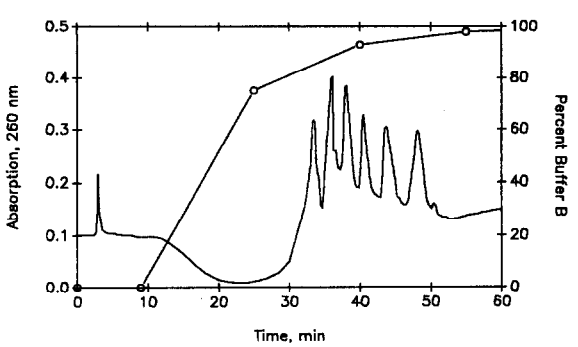

Fig. 1. Formamide gradient separation of adenylic acid oligomers on DNA-silica. An aliquot of 20 μl of 0.025 unit/μl of the $(dA)_{19-24}$ mixture was injected at zero time. Buffer A was 1 *M* NaCl, 57 μ*M* thymidylic acid (TMP), 50 m*M* Tris/phosphate, pH 7 and buffer B was 50% (v/v) formamide, 0.5 *M* Tris/phosphate, pH 7. The flow-rate was 0.2 ml/min and the temperature was 27°C throughout, and the gradient (open circles) was 0 min, 0% B; 9 min, 0% B; 25 min, 75% B; 40 min, 93% B; 55 min, 98% B; 70 min, 99% B. From left to right, the peaks eluting after 30 min are $(dA)_{19}$ to $(dA)_{24}$, respectively.

Since the experiments here required capacities of less than ≈ 3 units of mRNA, these columns had adequate capacity.

More selective methods for eluting the columns using salt, temperature, (data not shown) or formamide gradients were developed using oligoadenylate mixtures. Since we have previously demonstrated the use of salt and temperature gradients for separating oligomers [1], here we show only the formamide based separation. The separation of a $(dA)_{19-24}$ mixture using a formamide gradient is illustrated in Fig. 1. All six components of the oligoadenylic acid mixture, $(dA)_{19-24}$, were readily resolved (Fig. 1). The most challenging aspect of this separation was to develop a gradient in which the high UV absorption of formamide in buffer B would not interfere. This was accomplished by adding a small amount of thymidylic acid (TMP) to buffer A just sufficient to obtain the same absorption at 260 nm for both mobile phase buffers as specified in the figure legend. Even with this refinement, the baseline was still not ideal (Fig. 1). Since equivalent or better separations were also obtained with either salt or temperature gradients similar to those previously described with considerably less baseline problems [1], we used salt and temperature gradients to carry out all other separations presented here.

Fig. 1 shows that all six of the different length oligoadenylic acids present in the mixture could be separated. Previously, we had shown that a $(dT)_{18}$ column could separate a $(dA)_{12-18}$ mixture but could not fractionate oligomers longer than 18 from one another [1]. Fig. 1 shows that by increasing the length of the column bound DNA to $(dT)_{50}$, longer oligomers such as the $(dA)_{19-24}$ mixture can be resolved.

The separation of crude, LiCl-precipitated mRNA from *Saccharomyces* was then investigated as shown in Fig. 2. For these experiments, a $(dT)_{18}$-silica column previously described was used [1]. Similar separations were found with the $(dT)_{50}$-silica columns. The individual peaks and the starting material were also investigated using polyacrylamide gel electrophoresis (data not shown). LiCl-precipitated RNA is known to contain other RNAs (*e.g.*, ribosomal RNA) in addition to mRNA. These other RNAs and nonpolyadenylated mRNA elute in the flow-through peak (peak 1). mRNAs that contain only short stretches of oligoadenylic acid elute next (peak 2) when the column temperature was abruptly increased by immersion in a 40°C water bath. This was confirmed by determining the poly(A) tail length using RNAses A and T_1. Peak 3, representing 28% of the LiCl precipitated cellular RNA, represents the bulk of the poly(A) mRNA. It elutes in this experiment when the salt in the mobile phase was rapidly decreased to 0.005 *M*. Notice that this fraction was obtained in about 7 min and peak 3 in Fig. 2 represents about 2.2 units of mRNA collected in a total volume of 2 ml. Thus, the $(dT)_{18}$-silica is capable of very rapid isolation of mRNA in amounts adequate for most experiments and in a more concentrated form than is typically obtained by low-pressure chromatography. Peak 4 (which elutes when the column is placed in a 55°C bath) represents the longest poly(A) tail mRNAs (lengths of about 80).

The poly(A) mRNA (peak 3) obtained from the rapid chromatography in Fig. 2 showed no evidence of degradation. Degradation could occur by either

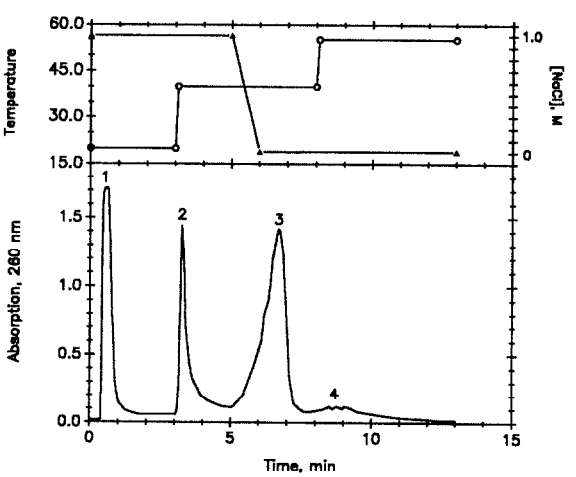

Fig. 2. Rapid isolation of messenger RNA. An aliquot of 100 μl of 1 *M* NaCl containing 6.7 units of LiCl-precipitated Saccharomyces RNA was injected onto a $(dT)_{18}$-silica column (30 × 4.6 mm I.D.) prepared as previously described [1]. Buffer A was 1 *M* NaCl, 10 m*M* sodium phosphate, pH 7, buffer B was 5 m*M* sodium phosphate, pH 7, and the flow-rate was 1 ml/min throughout. Initially, the column was equilibrated in buffer A at 20°C. Temperature (circles) was that of the water bath and was changed abruptly at 3 min and at 8 min by immersing the column in 40 and 55°C water baths, respectively. The salt gradient (triangles) was 0 min, 0% B; 5 min, 0% B; 6 min, 100% B; 15 min, 100% B.

the shear forces expected during chromatography or by RNAses contaminating the apparatus. That the RNA was not degraded was shown in two ways (data not shown): (1) the poly(A) mRNA obtained by HPLC (Fig. 2) was shown to be the expected length by comparison on acrylamide gels to λ phage Hind III digest and ϕX174 DNA length markers and (2) the size distribution of HPLC purified poly (A) mRNA does not change upon rechromatography. Degradation by RNAses was of special concern since RNAses are frequently found to contaminate laboratory apparatus and a chromatograph is a particularly complex piece of apparatus. Evidently, monthly cleaning of the chromatograph, the treatment of water with diethylpyrocarbonate, and the rapid separation performed are adequate precautions to ensure that the RNA is isolated intact. On autoradiograms of gels, the mRNA appeared as a diffuse band in the 1–2 kilobase size range. Previous studies [1] with DNA-silica had used only short oligonucleotides. Fig. 2 demonstrates that 300 Å pore DNA silica can be used to fractionate polynucleotides in the kilobase size range.

The poly(A) mRNA purified as in peak 3 of Fig. 2 was used for all subsequent experiments. This mRNA was 3'-labeled with ^{32}P-pCp and the complete labeling reaction mixture was applied to the $(dT)_{50}$-silica column for further fractionation as shown in Fig. 3.

As stated above most of the length of mRNA is not contained in the poly(A) tail; an average mRNA is 1–2 kilobase while an average tail length is 50 [5,6]. The previous fractionation [1] of $(dA)_{12-18}$ and $(dA)_{19-24}$ (Fig. 1) leaves little doubt that isolated tails could be readily fractionated. However, except for the poly(A) tail, most of the polynucleotide sequence of mRNA will not hybridize to the column and represent a heterogeneous mixture of sequences which test the selectivity of the column. This challenging separation should test polynucleotide HPAC resolution. Such a separation was carried out in Fig. 3 using increasing temperature and decreasing salt gradients.

Most of the radioactivity and absorption contained in the flow through peak (Fig. 3) comes from unincorporated pCp and other components of the labeling reaction; unlabeled poly(A) or poly(A) mRNA was shown to be quantitatively retained by the column (data not shown). The fractionation ap-

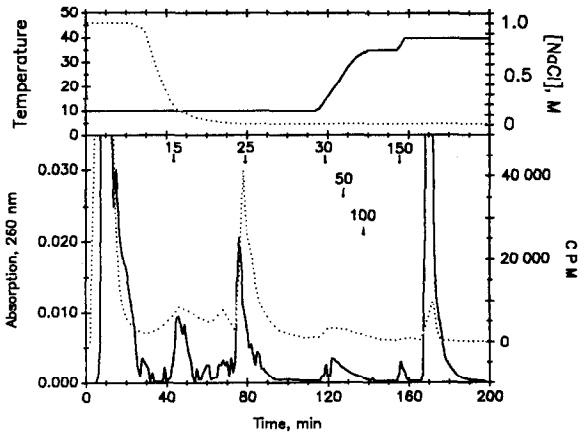

Fig. 3. Fractionation of polyadenylated messenger RNA on DNA-silica. The mRNA was purified as shown in Fig. 2 (peak 3), 3'-labeled with 5'-^{32}P-pCp, and 500 μl (0.025 units, $1.5 \cdot 10^6$ cpm) was applied to the $(dT)_{50}$-silica column [30 × 4.6 mm I.D., containing 4.4 units $(dT)_{50}$]. The flow-rate was 0.085 ml/min and 1-min fractions were collected for further analysis. The lower part of the figure shows the absorption (solid line, from the flow detector) or the counts per min ("C.P.M.", dotted line, measured for the fractions). The arrows show where the indicated length of poly(A) was calculated to elute using the gradient conditions and an equation [18] for predicting hybrid melting behavior. Gradient: buffer A was the same as in Fig. 2, buffer B was 0.1 *M* NaCl, 10 m*M* sodium phosphate, pH 7, and buffer C was water titrated to pH 7. The solvent gradient was 0 min, 100% A; 5 min, 100% A; 20 min, 10% A, 90% B; 40 min, 100% B; 80 min, 50% B, 50% C; 120 min, 10% B, 90% C; 130 min, 100% C. At 165 min, 500 μl formamide was injected to further elute the column. The upper part of the figure shows the measured NaCl concentration (dotted line, measured by conductivity of the fractions) or temperature (solid line, measured using a J-type thermocouple) of the water bath during the separation. The temperature gradient shown was obtained by immersing the column in the Lauda RM-water bath cooled to 10°C. At 114 min, the refrigeration was turned off and the bath set temperature was changed to 35°C. At 154 min, the set temperature was changed to 40°C. The temperature gradient shown simply represents the intrinsic heating rate of the water bath used.

pears to show an unequal distribution of tail lengths in *Saccharomyces*. For DNA-RNA hybrids [such as between the column and the poly(A) mRNA], an empirical equation describing the influence of length, temperature, salt and formamide concentration on hybrid melting temperature has been described [18]. From this equation and the elution conditions, the main peak eluting at 78 min corresponds to a hybrid with a predicted $(A)_{25}$ length tail. The presence of large amounts of mRNA with $(A)_{21}$ tails has been previously reported for *Saccha-*

romyces [6] and perhaps this is the peak at 78 min. The conditions used to elute the column would have eluted tail lengths up to about length 200 and yet clearly some possible lengths are not observed. For example, calculated tail lengths in the 15–25 range are quite common and abundant and another somewhat less abundant cluster of tail lengths between 30 and about 80 is also seen, but tail lengths between 25–30 and greater than 80 appear to be uncommon.

Since the above analysis is based on lengths calculated from an equation rather than actual lengths, we further investigated the individual fractions using RNAse A and T_1 to recover the poly(A) tails which were sized on acrylamide gels. The results are shown in Fig. 4. Before RNAse digestion, the fractions contain kilobase size RNA (Fig. 4A). After RNAse A and T_1 digestion, the densitometry scans of the gel autoradiographs (Fig. 4B) show that only poly(A) tails between about 15 and 60 in length are recovered. These densitometry scans are of long exposure (3 days) autoradiographic films which increases the apparent broadness of bands and thus underestimates the resolution obtained.

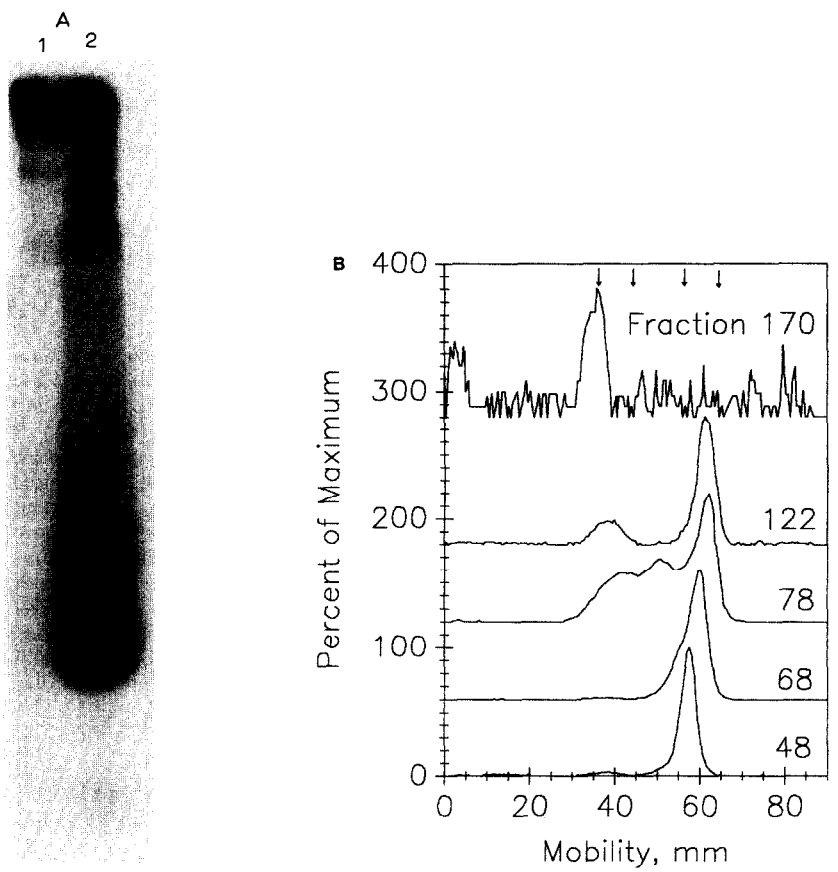

Fig. 4. Analysis of fractionated messenger RNA and its tail lengths. The autoradiography is of a 10% acrylamide, 8 *M* urea gel. (A) Lane 1: the sample was from the fraction eluting at 78 min, Fig. 5, showing intact mRNA (*i.e.*, before RNAse digestion). Lane 2: mRNA isolated as in peak 3 of Fig. 2 and 3′-labeled with pCp. The entire, unfractionated labeling reaction was applied to the gel. (B) Fractions (1 min, 0.085 ml) from the experiment in Fig. 3 were precipitated, digested with RNA A and T_1, and electrophoresed. The gel was then sealed in a plastic bag and used to expose X-ray film for 3 days at −70°C and the film was then scanned for densitometry. The results of the densitometry scans for fractions (Fig. 3) eluting at 48, 68, 78, 122, and 170 min, are shown as indicated in the figure. The tracings were all scaled so that the largest peak represents 100% which results in larger amounts of noise in weakly detected fractions (*e.g.*, fraction 170). The arrows at the top of the figure shows (from right to left) the position of $(dA)_{12}$, $(dA)_{18}$, $(dA)_{40}$, and $(dA)_{60}$ size markers, respectively.

The digests of all the column fractions contained radiotracer at a position corresponding to a tail length in the $(dA)_{12-18}$ range (the most rapidly migrating band in each sample) What this represents will be discussed further below. Also, salt carried over from the early eluting HPAC fractions is retarding their mobility on the gel (notice how the most rapidly migrating peak is retarded in fractions 48 and 68). In spite of these factors, the result is still readily seen: fractions eluting early during chromatography contain short poly(A) tails while those eluting later contain longer tail lengths. Thus, fraction 48 contains only the rapidly migrating $(A)_{12-18}$ peak. Since the average size determined was a length of 15, we will refer to this band as $(A)_{15}$. Fraction 68 shows an additional band represented as a shoulder on the $(A)_{15}$ peak, fraction 78 contains additional tail lengths in the $(A)_{20-50}$ size range, and fraction 122 contains primarily $(A)_{50}$. Fraction 170, eluted by a formamide injection (which should elute even very long tails) in fact elutes very little (notice the low cpm in Fig. 3 and the baseline noise in Fig. 4B resulting from densitometry scanning of a faint band) and that is of length $\approx(A)_{60}$. However, it is also clear that more than one tail length is present in some of the fractions. Whether this is due to a limitation of the chromatography or due to an intrinsic feature of the mRNA will be discussed below.

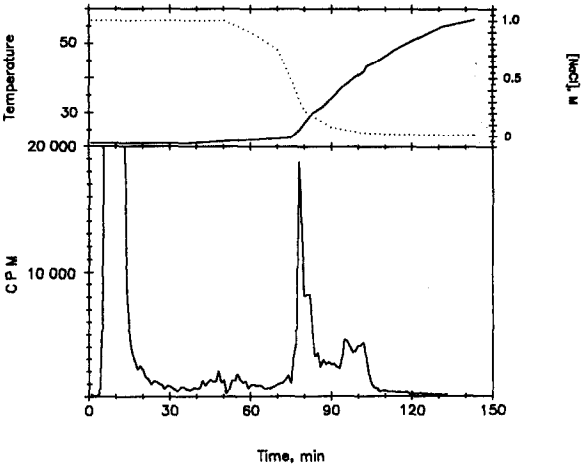

Fig. 5. Alternative gradients for fractionating mRNA by tail length gave similar results. The experiment is similar to that in Fig. 3 except that an alternative gradient was used (shown in the upper part of the figure) in which temperature and salt were varied simultaneously. In this case, the Lauda water bath was used with only the circulating pump running (initial temperature, 21°C). The temperature increase up to 75 min (to 22.8°C) was due to heating by the pump alone. At 75 min, a 200 W immersion heater and a variable transformer set at 60% was turned on and used to obtain a slow rate of heating. The sample load ($1.5 \cdot 10^6$ cpm) and the mobile phase buffers were the same as in Fig. 3.

A somewhat different elution protocol in which salt and temperature were varied simultaneously gave similar results as shown in Fig. 5. The largest peak (at 81 min, Fig. 5) has a calculated poly(A) tail length of about 25. Again the length of the poly(A) tails present in various column fractions was investigated using the RNAse method (gel not shown) with results similar to those already discussed for Fig. 4.

DISCUSSION

Here, we were primilarly interested in DNA-silica chromatography and used mRNA fractionation to test the usefulness of these columns. The experiment in Fig. 2 leaves little doubt that DNA-silica can be used for the rapid purification of poly(A) mRNA. This alone would be a powerful use of DNA-silica, but for that $(dT)_{18}$-silica would be sufficient (Fig. 2) and perhaps preferred because of its lower cost of preparation. For the analysis of poly(A) tail length polymorphism though, columns such as $(dT)_{50}$-silica would be needed. Here we show that such columns can be made and are capable of high resolution of oligomers (Fig. 1); however, their usefulness in the study of polymorphism requires further discussion.

Fractionating mRNA by tail length using low-pressure supports [6,10,19] shows lower resolution than our HPLC fractionation (Figs. 3 and 4B); HPLC thus represents a significant improvement over the previously used techniques. However, Fig. 4B also shows that after fractionation, most fractions contain at least two different lengths of poly (A). One poly(A) stretch is about 15 in length and is present in almost every fraction examined (except for fraction 170). The other poly(A) stretches are longer and the length increases in later column fractions. This may result from mRNA containing more than one stretch of poly(A) of different origins [6,19]. In *Dictyostelium*, two different stretches of poly(A) are present in the mRNA; one of about $(A)_{25}$ in length is apparently coded in the genome,

while another of variable length is added post-transcriptionally [19]. Both tail lengths are observed in poly(U)-Sepharose fractionated mRNA, even those eluted at high stringency [10]. *Saccharomyces* mRNA is similar is some respects [6]. Here, the genome coded, constant length stretch is apparently $(A)_{21}$ [6] which is somewhat longer than that estimated from Fig. 4B ($\approx$15 long), but this could be accounted for by differences in the procedures used for RNAse A and T_1 digestion and the ways length was determined in the two experiments. This short, constant tail length was found in most fractions from fractionated mRNA (in this case using oligo (dT)-cellulose) and is apparently at the 3'-terminus of some of the mRNA [6]. Thus, our results are consistent with previous studies [6,10,19] with two different organisms in that we also find a short, constant length poly(A) as well as a longer, variable length poly(A) in fractionated mRNA. However, the presence of two kind of poly(A) tail indicated by this (Fig. 4) and previous studies [6,10,19] complicates the use of chromatography to estimate tail length. Thus, while DNA-silica can be used to improve upon the fractionation of mRNA by tail length, the analytical uses of such separation should be approached with caution.

The studies presented here show that polynucleotides of kilobase lengths can be fractionated by HPAC. The mRNA recovered is apparently full length and shows no evidence of short fragments being produced by either shear forces or RNAse digestion during the chromatography experiment. The mRNA recovered is equivalent to mRNA isolated by more traditional techniques in size and that it can be ligated and specifically digested by enzymes (*e.g.*, T_4 RNA ligase, RNAse A and T_1). It could presumably also be used directly for the various other enzymatic manipulations commonly used in molecular biology. When kilobase size mRNA was fractionated (Fig. 3 and 5), the fractionation is again clearly based on the length of the poly(A) tail (Fig. 4) and is of higher resolution than that obtained in similar low-pressure applications. Using DNA-silica preparatively, poly(A) mRNA can be isolated from crude RNA in as little as 8 min (Fig. 2). DNA-silica can separate oligonucleotides which differ by a single base in length (Fig. 1 and ref. 1).

Thus, DNA-silica, because it is a fast, high-resolution method for fractionating polynucleotides, represents a significant improvement over existing low-pressure chromatographic supports.

ACKNOWLEDGEMENTS

This work was supported by grants from the NIH (GM 43609) and NSF (DMB 8996229). We thank William Foster of Alltech/Applied Science for the kind gift of the Macrosphere-WCX cartridges, Ms. Jo Akhavan for RNA isolation, and Dr. Steve Larsen for synthesizing the 5'-aminoethyl-$(dT)_{50}$. T.A.G. is a recipient of the Alltech Fellowship in Chromatography whose support is gratefully acknowledged.

REFERENCES

1 T. A. Goss, M. Bard and H. W. Jarrett, *J. Chromatogr.*, 508 (1990) 279–287.
2 P. T. Gilham, *J. Am. Chem. Soc.*, 86 (1964) 4982–4989.
3 N. Tanner, *Methods Enzymol.*, 180 (1989) 25–41.
4 J. Sambrook, E. Fritch and T. Maniatis, *Molecular Cloning—A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2nd ed., 1989 pp. 7.1–7.77.
5 C. S. McLaughlin, J. R. Warner, M. Edmonds, H. Nakazato and M. H. Vaughan, *J. Biol. Chem.*, 248 (1973) 1466–1471.
6 S. L. Phillips, C. Tse, I. Serventi, and N. Hynes, *J. Bacteriol.*, 138 (1979) 542–551.
7 A. J. P. Brown, *Yeast*, 5 (1989) 239–257.
8 P. Bernstein, S. W. Pelz and J. Ross, *Mol. Cell. Biol.*, 9 (1989) 659–670.
9 D. Monroe and A. Jacobson, *Gene*, 91 (1990) 151–158.
10 C. Palatnic, R. Storti, A. Capone and A. Jacobson, *J. Mol. Biol.*, 141 (1980) 99–118.
11 H. Rubin and M. Halim, *Biochem. Biophys. Res. Commun.*, 144 (1987) 269–271.
12 D. Carter and D. Murphy, *Neurosci. Lett.*, 109 (1990) 180–185.
13 J. M. Kelly and R. A. Cox, *Nucleic Acids Res.*, 10 (1982) 4173–4179.
14 H. W. Jarrett, *J. Chromatogr.*, 405 (1987) 179–189.
15 F. Sherman, G. R. Fink and J. B. Hicks, *Laboratory Course Manual for Methods in Yeast Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1986, pp. 143–144.
16 F. Cobianchi and S. Wilson, *Methods. Enzymol.*, 152 (1987) 103–110.
17 J. L. Nichols and L. Welder, *Biochim. Biophys. Acta*, 652 (1981) 99–108.
18 G. M. Wahl, S. L. Berger and A. R. Kimmel, *Methods Enzymol.*, 152 (1987) 399–407.
19 A. Jacobson, R. A. Firtel, and H. L. Lodish, *Proc. Natl. Acad. Sci. USA*, 71 (1974) 1607–1611.