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# High-performance affinity chromatography of DNA

# II☆. Porosity effects

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ABSTRACT

A DNA–silica, $(dT)_{18}$–silica, was prepared and used in a study of the chromatography of the oligonucleotide, $(dA)_{18}$, based upon base pairing. It was shown that hybridization efficiency did not depend upon flow-rates up to 2 ml/min for the small columns (22 × 2 mm) used. As increasing amounts of $(dA)_{18}$ were loaded onto the columns, the columns were found to saturate at a well defined capacity that was always less than the amount that theoretically could have been bound. Maximum capacity was achieved whenever the loading temperature was at least 20–25°C below the temperature at which the loaded oligonucleotide would elute. The effects of porosity on both coupling efficiency and capacity were measured and suggest that pore sizes in the 300–500 Å range are most appropriate for this form of chromatography.

## INTRODUCTION

Previously [1], we synthesized a DNA–silica, $(dT)_{18}$–silica and showed it was able to resolve oligonucleotides differing in length by a single nucleotide. In other experiments, a $(dT)_{50}$–silica was synthesized and used to fractionate polyadenylated messenger RNA [poly(A) mRNA] from *Saccharomyces* by poly(A) tail length [2]. In either case, 300 Å pore silica was used and even small columns (*e.g.*, 30 × 4.6 mm) were capable of binding adequate amounts (*e.g.*, 2.2 units of mRNA, *ca.* 88 μg) of polynucleotides. However, we noticed during these experiments that actual column capacity was always less than the theoretical capacity predicted assuming that all DNA coupled to the silica was capable of hybridization [3]. Here, we further investigate this phenomenon. While we did not learn the cause of the diminished capacity, the experiments do give a great deal of information about how to make and use these columns.

The basis for chromatographic separations on DNA–silica is hybridization of the column attached strand with injected, single stranded DNA or RNA. For this affinity chromatography, the amount of $(dT)_{18}$ linked to the column is the maximum theoretical capacity of the column for binding the same length complementary strand; *i.e.*, $(dA)_{18}$. The fact that this theoretical capacity is much larger than the determined capacity for oligoadenylate binding could be explained if: (1) the flow-rate or other conditions of chromatography were inappropriate for maximal binding, (2) the pore size used was too small to allow the applied samples to interact with DNA inside the pores, or (3) the spacer length between the silica surface and the attached DNA chain was too short to allow productive interactions with applied samples.

The last of these can probably be excluded. From the silica surface to the 5′-phosphate on $(dT)_{18}$–silica is the following chain backbone [1]:–Si–C–C–C–

☆ For Part I, see ref. 1.

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N–C–C–C–C–N–C–C–P, a 13-atom spacer with a fully extended length of more than 15 Å. Furthermore, oligoadenines can hybridize anywhere along the length of the $(dT)_{18}$ chain. A $(dT)_{18}$ chain would have a fully extended length of nearly 130 Å and even in a double helix, would extend nearly 61 Å. Thus, an aligoadenine could base pair anywhere in a region extending from near the silica surface to about 150 Å distant. Given that the spacer chain length is probably adequate, the other possible explanations for diminished capacity were investigated.

## METHODS

### *Oligonucleotide synthesis and end labeling*

$(dA)_{18}$ was synthesized by the standard phosphoramidite chemistry using the departmental DNA synthesis facility and deblocked on the last cycle. 5′-Aminoethyl-$(dT)_{18}$ [amino-$(dT)_{18}$] was synthesized in a similar manner except that a last cycle utilizing the AminoLink reagent (Applied Biosystems) was included. $(dT)_{18}$ was 5′ end labeled with [$\gamma$-$^{32}$P]ATP and T4 polynucleotide kinase using standard procedures [4].

### *Chromatography*

The chromatograph was a Gilson Model 9000 binary gradient chromatograph outfitted with a Jasco variable-wavelength UV detector (260 nm, unless otherwise specified), a Gilson 231/401 autosampler, and a Gilson 203 fraction collector. Data collection and programming of the chromatograph were with an Uniq 386sx computer and the Gilson 714 software. Chromatography was conducted at room temperature unless otherwise stated. When the temperature was varied from room temperature, the column and a preheating coil of 1/16-in. stainless-steel tubing (150 cm × 0.010 in. I.D.) was submerged in a water bath at the desired column temperature. Buffer A was 5 m*M* $Na_2HPO_4$, 0.99 *M* NaCl pH 7.0; buffer B was 5 m*M* $Na_2HPO_4$ pH 7.0; TE500 buffer was 10 m*M* Tris, 0.1 m*M* EDTA, 500 m*M* NaCl, pH 7.5.

### *Column preparation*

All columns were packed from an isopropanol slurry (100 mg silica per ml, 2 ml/min constant flowrate) using a specially made reservoir and Alltech's direct-connect guard column (22 × 2 mm) as the column hardware. The silica was Macrosphere-WCX 7-$\mu$m beads supplied by Alltech (Deerfield, IL, USA) in various pore sizes. The starting material for Macrosphere-WCX was in all cases silicas in the appropriate pore size from Macherey-Nagel derivatized in the same way using the same proprietary process [5]. The columns were activated to the N-hydroxysuccinimidyl ester (NHS) as previously described [6]. Amino-$(dT)_{18}$ was then coupled by recirculation as described by Goss *et al.* [1].

### *Determination of $(dT)_{18}$ coupling*

Unless otherwise stated, the amount of amino-$(dT)_{18}$ coupled was determined by the difference in the absorption (260 nm) units added and the units recovered in column washes. All fractions were titrated to pH 2 to minimize NHS absorption as described by Goss *et al.* [1]. Absorption units were then converted to nmol using a molar absorptivity of 8400 $M^{-1}$ per (dT) base in an oligonucleotide [7]. This gives a conversion factor of 1 unit = 6.61 nmol for $(dT)_{18}$.

For one experiment (Table I), the amount of $(dT)_{18}$ coupled to the silica was also measured using an assay for the inorganic phosphate released when the DNA is oxidatively hydrolyzed. After $(dT)_{18}$ coupling by recirculation as described above, the silica was removed from the 22 × 2 mm columns, washed with water and then acetone and dried in a 110°C oven. The silica was then weighed and the DNA was hydrolyzed to release the inorganic phosphate using a modification of the procedure of Ames and Dubin [8]. Briefly, 200 $\mu$l of 10% Mg $(NO_3)_2$ in 95% aqueous ethanol was added to each sample of DNA–silica (30–33 mg). This was then gently heated to evaporate the liquid. The samples were then strongly heated over a Bunsen burner until the brown fumes had disappeared and the bottom of the Pyrex test tube glowed a dull red. After cooling, 0.3 ml of 1 *M* HCl was added and the samples were heated in a boiling water bath for 15 min with the test tubes capped by glass marbles. A 0.7-ml volume of 0.005 *M* $H_2SO_4$ was then added and 0.1-, 0.2- and 0.4-ml portions were removed and assayed for inorganic phosphate using a modification of the Malachite green method [9]. Briefly, each sample was adjusted to 0.5 ml with 0.005 *M* $H_2SO_4$ and 2 ml of a freshly prepared solution containing

0.0135% Malachite green, 0.84% ammonium molybdate, 0.1 *M* $H_2SO_4$ and 0.04% Tween 20 were added. After 5 min, absorption at 660 nm was determined for each fraction. Phosphate standards prepared from $K_2HPO_4$ were included in each assay. The amount of inorganic phosphate released from the silica is then divided by 18 [the number of phosphates in $(dT)_{18}$] to obtain the $(dT)_{18}$ content of the silica.

*Determination of capacity by temperature-dependent elution (Table III and Fig. 4)*

Columns containing $(dT)_{18}$–silica of each pore size tested were equilibrated at 5°C in a RTE-210 refrigerated, circulating water bath (Neslab Instruments, Newington, NH, USA), and an excess [over the $(dT)_{18}$ content of the column] of $(dA)_{18}$ was injected. Refrigeration was then discontinued and the water bath set temperature was changed to 60°C to elute the column. Throughout, the mobile phase was buffer A, the flow-rate was 0.2 ml/min, and 4-min fractions were collected. The water bath used consistently warms at approximately 1°C/min under these conditions and the $(dA)_{18}$ consistently elutes near 45 min. Thus, the column is always washed with over 100 column volumes before retained $(dA)_{18}$ elutes. Fractions containing eluted $(dA)_{18}$ were pooled and quantified by absorption at 260 nm using a molar absorptivity of 15 200 $M^{-1}$ $cm^{-1}$ per (dA) base [7]. This yields a conversion factor for $(dA)_{18}$ of 1 unit = 3.66 nmol.

## RESULTS AND DISCUSSION

DNA–silica columns were prepared by recirculating 5′-aminoethyl-$(dT)_{18}$ over a prepacked column containing an activated, NHS-ester silica. One advantage to this procedure is that prepacked columns available commercially can be used for coupling which require no column packing expertise. From the difference in the amount of $(dT)_{18}$ added to and recovered after coupling, the amount of $(dT)_{18}$ coupled to silica can be calculated [1]. This method of estimating coupling (by difference) may overestimate the amount coupled and this could explain the lower-than-expected capacity found for such columns. Thus, the amount of $(dT)_{18}$ coupled was also determined by second independent procedure in which the DNA-silica is hydrolyzed and the inorganic phosphate released from the DNA is measured. The results are shown in Table I.

TABLE I

COMPARISON OF TWO TECHNIQUES FOR DETERMINING $(dT)_{18}$ COUPLING

| Pore size (Å) | Added (nmol)[a] | Amount coupled | |
|---|---|---|---|
| | | Absorption difference (nmol)[a] | Phosphate assay (nmol)[b] |
| 100 | 27.5 | 21.0 | 29.5 |
| | 25.2 | 24.0 | 25.2 |
| 300 | 30.4 | 23.9 | 29.3 |
| | 16.2 | 12.7 | 15.3 |
| 500 | 16.1 | 12.4 | 10.8 |
| | 15.9 | 11.3 | 14.1 |

[a] nmol $(dT)_{18}$ determined by difference in DNA absorption as described by Goss *et al.* [1].

[b] nmol $(dT)_{18}$ determined by phosphate assay of the $Mg(NO_3)_2$ hydrolyzed silica assuming 18 equivalents of phosphate per $(dT)_{18}$ equivalent.

The results in Table I clearly show that both methods give similar estimates for the amount of $(dT)_{18}$ coupling regardless of the porosity of the silica used. The method based upon absorption difference gives somewhat lower but comparable numbers to those obtained upon hydrolysis and phosphate assay. The agreement between the two methods suggests that either accurately measures $(dT)_{18}$ coupling. Since both methods give a reliable estimate of $(dT)_{18}$ coupling and the method based upon absorption difference is non-destructive to the DNA–silica, this method was used for all further studies.

Since the amount of $(dT)_{18}$ coupled is accurately measured, we next investigated whether low capacity may have resulted from insufficient time for DNA hybridization during chromatography. The rate of hybrid formation between two stands of DNA is a function of the length and complexity of the hybridizing sequences, the concentration of each strand, and the temperature [7]. Since the kinetics of hybrid formation for DNA immobilized to nitrocellulose is similar to that of DNA in solution [10], the rate of hybrid formation inside high-performance liquid chromatography (HPLC) columns also probably follows similar kinetics. When one

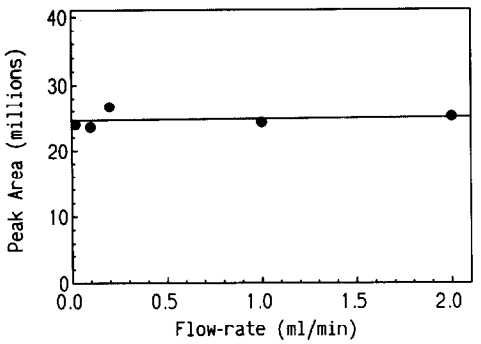


Fig. 1. Column capacity is independent of flow-rate. A 300 Å $(dT)_{18}$ column [containing 35 nmol $(dT)_{18}$ coupled] was loaded with 4 $\mu$l containing 0.47 nmol of $(dA)_{18}$ at flow-rates of 0.02, 0.1, 0.2, 1.0 and 2.0 ml/min in buffer A. The column was washed with 0.4 ml of buffer A (5 m*M* $Na_2HPO_4$, 0.99 *M* NaCl, pH 7). Then the flow-rate was changed to 0.2 ml/min and washing was continued for an additional 3 min. The column was then eluted with buffer B (5 m*M* $Na_2HPO_4$, pH 7) at 0.2 ml/min. Peak areas are reported in integration units of 10 $\mu$V ··milliminute.

strand of a potential DNA duplex is in excess, the rate of hybrid formation is pseudo-first order and the rate constant, $k$, may be calculated as:

$$k = 3 \cdot 10^5 \, L^{1/2}/N \, (M^{-1} \, s^{-1})$$

where $L$ is the length of the strands and $N$ is the hybrid complexity in base pairs [7]. For $(dT)_{18}$ hybridizing with $(dA)_{18}$, $L$ and $N$ are both 18 and $k = 7 \cdot 10^4 \, M^{-1} \, s^{-1}$.

For this experiment, the column-attached DNA is usually in excess and allows for rapid hybridization as shown by the experiment in Fig. 1. When flow-rate during sample injection was varied over a two order of magnitude range (0.02 to 2 ml/min), the amount of DNA retained by hybridization to the column and subsequently eluted did not change significantly. The small variations found are probably due more to inaccuracies in injecting small samples (4 $\mu$l) and in integrating the peaks obtained than to any real effect of flow-rate. The column used in this experiment contains 35 nmol of $(dT)_{18}$ in a column volume of 69 $\mu$l or about 0.51 m*M* $(dT)_{18}$. From the equation above, hybridization should occur with a half-time of about 20 ms. Even at 2 ml/min, the highest flow-rate used in Fig. 1, the transit time through the column would be about 2 s or about 100 half-times. At 2 ml/min, backpressures in excess of 150 bar were encountered, thus flow-rates much more than twice this would not be possible with these silica supports and this column size. Thus, even at the highest flow-rate possible for these columns, the amounts of DNA coupled to silica give such rapid hybrid formation that injected DNA is retained regardless of flow-rate. Thus, the lower-than-theoretical capacities under study are not due to slow hybridization under less than optimal conditions.

The lower than expected capacities could also arise if only some fraction of applied DNA hybridizes because of poor mass transfer during chromatography. This is apparently also not the case as the experiment in Fig. 2 shows. As the amount of $(dA)_{18}$ injected onto a column is increased, the amount bound increases up to the point at which the column capacity is reached. The column capacity found in this experiment is the same as that found by simply loading a large excess of $(dA)_{18}$ sample, washing the column, and measuring the amount which elutes. Thus, sample binding by the column is a saturable phenomenon, saturating at a well-defined capacity.

Fig. 2 also demonstrates a strategy that can be used to increase recovery of precious DNA samples: the greatest recovery of injected DNA occurs at sample loads which are only a fraction off the column's actual capacity. The three smallest DNA samples injected in Fig. 2 (leftmost data points,

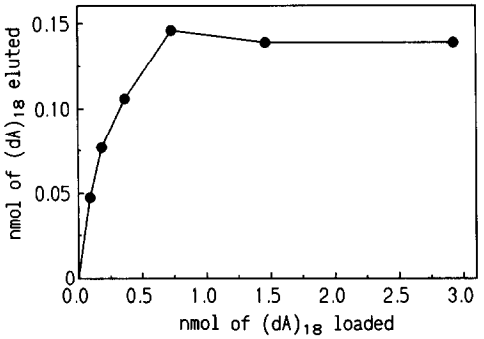


Fig. 2. Column capacity determined with increasing sample load. A 100 Å $(dT)_{18}$ column in buffer A and 30°C was loaded with different amounts of $(dA)_{18}$ (0.092, 0.18, 0.37, 0.73, 1.46 and 2.93 nmol) in 4 $\mu$l buffer A and washed for 10 min. Each load was eluted by abruptly changing the mobile phase to water. The flow-rate was 0.5 ml/min and 1.5-min fractions were collected. The $(dA)_{18}$ eluted was determined by absorption of pooled fractions.

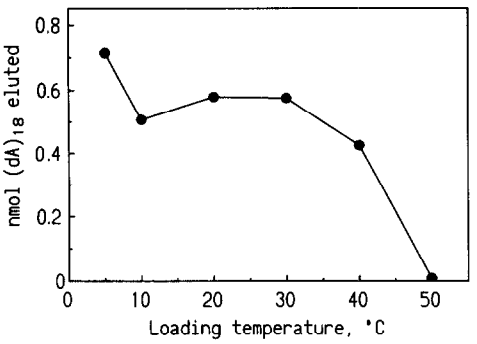


Fig. 3. Effect of loading temperature on column capacity. A 100 Å $(dT)_{18}$ column (containing 11.8 nmol $(dT)_{18}$ and listed on line 3 of Table III) equilibrated in TE500 buffer (10 m*M* Tris, 0.1 m*M* EDTA, 500 m*M* NaCl, pH 7.5) in a water bath at the various temperatures shown was loaded with 0.99 nmol of 5′ end labeled $(dA)_{18}$ (23.1 cpm/pmol) in 10 μl of TE500. The column was then washed for 15 min under the loading conditions and eluted by transferring the column to a 65°C water bath. The flow-rate was 0.5 ml/min and 1-min fractions were collected. The fractions were counted for Cerenkov radiation without scintillation fluid.

0.09, 0.18, 0.37 nmol $(dA)_{18}$) demonstrate this trend. As injected DNA was increased the proportion of it bound by the column decreased (52, 42 and 29%, respectively). This trend has not been rigorously explored but in some experiments where the sample load was approximately 1% of column capacity, 90% of the injected DNA was bound by and eluted from the column (data not shown).

In other experiments (data not shown), 5′ end labeled DNA was either injected as described in Fig. 2 onto a complementary DNA–silica column or recirculated through the column for 1 h. In either case, the amount of labeled DNA retained by the column and subsequently eluted was the same. Thus, recirculation does not improve sample retention and is thus unnecessary.

The effect of loading temperature on column capacity was also investigated as shown in Fig. 3. For this experiment, an amount of $(dA)_{18}$ approximately equal to the column's capacity was injected and the column was washed while maintaining the temperatures shown in the figure. The column was then eluted by abruptly changing the column temperature to 65°C. Under the conditions used, the temperature for elution ($T_e$) of $(dA)_{18}$ has been measured [1] to be 52°C. While it is clear that loading a sample onto a column at temperatures too close (*i.e.*, within about 25°C) to $T_e$ adversely affects capacity and should be avoided, capacity is reasonably constant from 5–30°C, a fairly wide range of loading temperature.

TABLE II

THE EFFECT OF PORE SIZE ON THE EFFICIENCY OF $(dT)_{18}$ COUPLING

| Pore size (Å)[a] | Area ($m^2/g$)[a] | Added (nmol)[b] | Coupled (nmol)[b] | Coupled (%) |
|---|---|---|---|---|
| 50 | 450 | 56.2 | 18.9 | 34 |
| | | 19.2 | 8.3 | 43 |
| 100 | 350 | 72.7 | 8.9 | 12 |
| | | 75.3 | 11.8 | 16 |
| 300 | 100 | 41.9 | 34.8 | 83 |
| | | 66.1 | 57.3 | 87 |
| 500 | 35 | 66.1 | 47.8 | 72 |
| 1000 | 25 | 109.7 | 40.3 | 37 |
| 4000 | 10 | 77.1 | 35.0 | 45 |

[a] Values supplied by the manufacturer.
[b] nmol $(dT)_{18}$ determined by absorption, 260 nm.

The above experiments were carried out on two pore sizes (*i.e.*, 100 and 300 Å). Other pore sizes were also investigated. The effect of pore size on the efficiency of $(dT)_{18}$ coupling was investigated; the results are shown in Table II. Silicas of all porosities tested coupled $(dT)_{18}$ effectively. The most efficient coupling was found for silicas with pores in the 300–500 Å range but the dependence of coupling efficiency on porosity is clearly complex. The lower

TABLE III

EFFECT OF PORE SIZE ON COLUMN CAPACITY FOR $(dA)_{18}$ BINDING

| Pore size (Å) | Coupled $(dT)_{18}$ (nmol) | $(dA)_{18}$ Eluted (nmol) | Ratio (%) |
|---|---|---|---|
| 50 | 18.9 | 0.57 | 3.1 |
| 100 | 8.9 | 0.40 | 4.5 |
| | 11.8 | 0.61 | 5.2 |
| 300 | 34.8 | 0.90 | 2.6 |
| 500 | 47.8 | 1.18 | 2.5 |
| 1000 | 40.3 | 0.57 | 1.4 |
| 4000 | 35.0 | 0.01 | 0.0 |

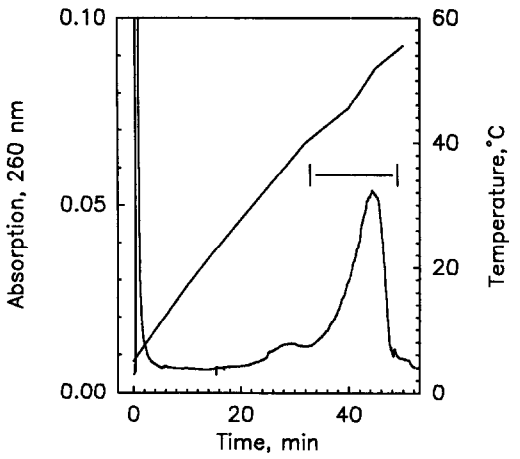


Fig. 4. Chromatography by temperature-dependent ellution. A 100 Å $(dT)_{18}$ column (11.8 nmol coupled) was equilibrated at 5°C in buffer A. A 30-μl volume of buffer A containing 11.6 nmol of $(dA)_{18}$ was then loaded. The temperature gradient was produced as described in the Methods section using a water bath. The temperature of the water bath was measured every 4 min during the separation with a mercury thermometer. The bar shows the position of the fractions pooled to determine column capacity.

amounts coupled to the largest pore sizes are probably due to the lower surface area of these silicas. The lower coupling to the smaller pore sizes may result from restricted access of $(dT)_{18}$ because of its large size. The highest coupling efficiencies for the intermediate pore sizes probably results from a trade-off of adequate pore size with adequate surface area.

The various pore size columns were also tested for the amount of $(dA)_{18}$ they would retain. The results are shown in Table III. For each pore size, an excess of $(dA)_{18}$ [*i.e.*, an amount greater than the $(dT)_{18}$ content of the column] was loaded onto each column at 5°C in 1 *M* $Na^+$ (*i.e.*, buffer A), and eluted by raising temperature to 60°C. Before elution, over 100 column volumes of buffer A passes through the column (see Fig. 4) ensuring that any non-specifically retained DNA is washed away before the eluted DNA is measured. The data in Table III show that under these conditions never is more than about 5% of the theoretical capacity of the columns actually realized in practice. In other experiments (data not shown), column capacity was also measured using different loading temperatures and eluting by lowering salt concentration; the results are in qualitative agreement with those shown. Perhaps the most remarkable feature of these data is that while $(dT)_{18}$ apparently couples well to 4000 Å pore silica (Table II), the coupled DNA does not retain injected $(dA)_{18}$ (Table III). The reason for this phenomenon is not known.

Considering the data in both Tables II and III, it can be seen that 300 and 500 Å pore silicas give both high coupling efficiencies and relatively high capacities and would be best suited for chromatography of oligonucleotides. Since 300 Å pore columns have also been used to separate mRNA [2], this pore size would also be appropriate for kilobase size polynucleotides.

Fig. 4 shows a typical chromatogram obtained with the temperature-dependent elution protocol used to measure capacities in Table III. It shows that injected DNA not bound by the column appear as a sharp peak; in less than 3 min following injection, the absorption at 260 nm falls to baseline values and remains there until about 25 min into the separation. Thus, diminished capacity is not due to DNA slowly bleeding off the column during the extensive washing procedure used. After about 25 min, the water bath has reached 33°C and some DNA begins to elute in a broad hump extending to about 32 min. This hump contains 12% of the total eluted DNA and was not included in the pooled fractions or used to calculate the capacities in Table III. Capacity was calculated from the major peak containing 88% of the total eluted DNA which reaches a maximum at 44 min when the temperature was 51°C. This melting temperature agrees closely with the 52°C elution temperature previously reported for $(dT)_{18}$:$(dA)_{18}$ hybrids [1]. What the broad hump represents was not investigated other than to demonstrate that it is not a baseline artifact and occurs to about 12% of the total area in all the chromatograms used for Table III. It is likely that this represents oligomers of dA shorter than 18 in length present in the synthetic $(dA)_{18}$ sample since average efficiency of this DNA synthesis was 99% and thus only 83% ($= 0.99^{18}$) of the total synthesized should be an 18-mer and this agrees reasonably well with the 88% peak area for the major peak. Thus, the early eluting broad hump probably represents short oligomers which should elute at lower temperatures as observed. Fig. 4 demon-

strates that low capacity does not arise from slow bleeding of DNA from the column but rather suggests that only a fraction (about 5%, Table III) of the $(dT)_{18}$ coupled to silica ever binds injected DNA at all and that this binding is, for the most part, a normal hybridization between 18-mers. Thus, while capacity is lower than expected, coupled DNA which does not participate in the affinity chromatography appears to be inert and does not interfere with the separations obtained.

The experiments show that high flow-rates can be used with DNA–silica columns since hybridization is quite rapid (Fig. 1). The columns become saturated by high sample loads and bind only a well defined capacity (Fig. 2). The capacity of a column is also relatively unaffected by loading temperatures well below $T_e$ (Fig. 3). However, the capacity of the column is a function of the porosity of the silica support, and the amount of DNA coupled to the silica (Table III). While all pore size silicas couple DNA efficiently (Table II), porosities of 300 and 500 Å give the best combination of coupling efficiency (Table II) and column capacity (Table III). However, even a column optimized for porosity will typically bind less than 5% of the amount it should be capable of binding (Table III). We did not discover the cause for this diminished capacity, but a lower than expected capacity has also been observed for other kinds of affinity chromatography. For example, melittin forms a 1:1 complex with calmodulin [11] but melittin–silica containing 1.4 mg of melittin (0.49 μmol) bound only 1.6 mg (0.096 μmol) or about 20% of the theoretical capacity [12]. In other cases, actual and theoretical capacity were found to be nearly identical [13].

Hybridization was rapid at the levels of $(dT)_{18}$ coupled in the experiments here. The highest flow-rates permissible with these columns allowed 100 half-times for hybrid formation (Fig. 1). Since hybridization would be *ca.* 97% complete in only 5 half-times, the amount of DNA coupled could be decreased about 20-fold and still allow rapid chromatography. However, the results in Fig. 2 show that when sample load is well below column capacity, a larger fraction of the sample is retained by the column. When sample load is only a small fraction of capacity, nearly quantitative recovery would be expected. Thus, column capacity should be kept high to improve recovery and detection of injected samples; an aditional consequence of this high capacity will be that chromatography can be performed rapidly. Highest capcity was found at temperatures less than about 25°C below $T_e$ (Fig. 3). It is interesting to note that in using DNA probes for complementary sequences, the most rapid hybrid formation occurs at 25°C below the hybrid melting temperature [7]. In previous studies, we have also found a reasonably good agreement between the temperature and salt dependence of DNA–silica elution and what would be predicted from other studies of DNA hybridization [1]. Thus, chromatography follows similar rules to those already discovered in other DNA hybridization experiments and thus is a very predictable form of chromatography. This is not surprising since all DNA hybridization, whether in an HPLC column or not, depends ultimately upon the chemistry of the specific base pairing in double-stranded DNA.

The observation that no more than about 5% of theoretical capacity could be obtained with DNA–silica would be important if adequate capacities could not be obtained and this is clearly not the case. This is not a significant limitation since the capacities measured are already quite large for many practical experiments. For example, DNA–silica columns have been used to fractionate poly (A) mRNA, from yeast [2]. For these uses, very little of the mass of a mRNA sample is actually polyadenylate and so fairly large capacities are obtained. For example, for a 2-kilobase mRNA with a poly(A) tail 50 long (such as is typical in *Saccharomyces*), only about 2.5% of the length is actually poly(A). For such an mRNA, a column which binds 0.7 nmol of $(dA)_{18}$ (such as was encountered in Fig. 3) should bind about 8 units of mRNA (about 400 μg). Since we obtain capacities over 0.7 nmol $(dA)_{18}$ routinely on some pore sizes in 23 × 2 mm columns (Table III) containing only 33 mg of silica, capacities are already quite high and larger columns or coupling larger amounts of $(dT)_{18}$ should allow columns of adequate capacity for most experiments.

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