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Further Studies on the Properties of Oligonucleotide Cellulose Columns†

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ABSTRACT: Thermal chromatography on columns of deoxyribooligonucleotide cellulose has been used to resolve mixtures of homooligonucleotides differing by one nucleotide residue in length. An extensive examination of the affinity of several series of complementary ribooligonucleotides and deoxyribooligonucleotides for columns of deoxyribooligonucleotide cellulose has confirmed that all the residues in the cellulose-linked oligonucleotide are able to hydrogen bond to their complementary bases. Oligoribouridylates were found to form hybrid structures with deoxyribooligoadenylate cellulose columns having melting temperatures as much as 25° lower than those observed for oligothymidylates of equal length. Comparison of ribooligoadenylates and deoxyribooligoadenylates using columns of oligothymidylate cellulose again showed interactions with ribooligothymidylate to be less stable by 5–10°. A study of the interactions of a series of deoxyribooligonucleotides of mixed repeating base sequence with columns of complementary oligonucleotide cellulose has been made. These results demonstrate that, as expected, the presence of G-C base pairs in the hybrid structure adds considerably to the stability of the hybrid.

The experiments described here extend and amplify the previously reported studies (Astell and Smith, 1972) concerning cellulose-linked deoxyribooligonucleotides of defined length and sequence and the determination of their hydrogen bonding behavior using complementary model oligonucleotides.

Mixtures of deoxyribooligoadenylates have been subjected to thermal chromatography on a column of deoxyribooligothymidylate cellulose and it was found that, in agreement with our earlier conclusions (Astell and Smith, 1972) based on composite elution profiles, consecutive oligomers could be completely resolved.

The affinity of a series of deoxyribooligothymidylates for columns of deoxyribooligoadenylate cellulose has been investigated in a more extensive examination of the number of residues in the cellulose-linked oligonucleotide which are able

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to interact with a complementary sequence. The results have confirmed our earlier observation with deoxyribooligothymidylates linked to cellulose that all the residues participate in the stabilization of the hybrid structure (Astell and Smith, 1972).

A further examination has been made of the interactions of ribooligonucleotides with columns of deoxyribooligonucleotide cellulose. Ribooligouridylates and ribooligoadenylates were compared with deoxyribooligothymidylates and deoxyribooligoadenylates in terms of their affinity for columns of deoxyribooligoadenylate cellulose and deoxyribooligothymidylate cellulose. The ribooligonucleotides behave similarly to their deoxyribo counterparts; however, the hybrid structures involving ribooligonucleotides hydrogen bonded to deoxyribooligonucleotides were less stable than the corresponding hybrid structures containing only deoxyribooligonucleotides. This was especially notable in the case of hybrids containing ribooligouridylates.

We have also examined the specificity and stability of interactions between cellulose-linked deoxyribooligonucleotides of mixed repeating base sequence and a series of complementary deoxyribooligonucleotides. These results have provided useful information concerning the stability of interactions involving both A-T and G-C base pairs.[1]

Materials and Methods

The synthesis of deoxyribooligonucleotides, their attachment to cellulsoe, and the chromatographic procedures have been described (Astell and Smith, 1972).

The preparation of deoxyribooligonucleotides with mixed repeating base sequence was achieved by the synthesis of protected trinucleotides (Narang *et al.*, 1967a) which were then polymerized and the products deprotected and separated on columns of DEAE-cellulose (Narang *et al.*, 1967b).

In addition to the oligonucleotide celluloses listed in Table II of Astell and Smith (1972), we have also prepared cellulose-p(dA)$_8$, cellulose-p(dA)$_9$, and cellulose-p(dA)$_{10}$ using identical preparative procedures. The efficiency of incorporation of nucleotide in these preparations was respectively 40, 73, and 75%.

Oligoribonucleotides of the type $(rXp)_n$ were prepared by micrococcal nuclease digestion of poly(A) and poly(U) (Coutsogeorgopoulos and Khorana, 1964). The digestion products were separated on columns of DEAE-cellulose; in the case of the oligoriboadenylates, the elution was carried out in the presence of 7 M urea (Tomlinson and Tener, 1963).

The 3′-phosphate was removed from these compounds using bacterial alkaline phosphatase.

The interaction between oligonucleotides in solution was studied by following the increase in absorbance at 260 nm of a mixture of two complementary sequences. Approximately 0.025 μmol of each oligomer (based on the mononucleotide unit) were mixed, and made up to 1 ml with molar buffered saline (pH 7.0) and stood at 4° for 16–18 hr. The sample was then transferred to a prechilled (0°) 1-ml, 1-cm light path quartz cuvette in a jacketed sample holder of a recording spectrophotometer. The temperature was reduced to −5° and the sample incubated for 1 hr. The thermal denaturation profile was then observed by slowly raising the temperature (less than 1.5°/3 min).

[1] The abbreviations and definitions used are the same as those described in Astell and Smith (1972). The T_m is the melting temperature of a hybrid structure in solution. T_m^e is the temperature at the midpoint of a peak of oligonucleotide eluted from an oligonucleotide cellulose column.

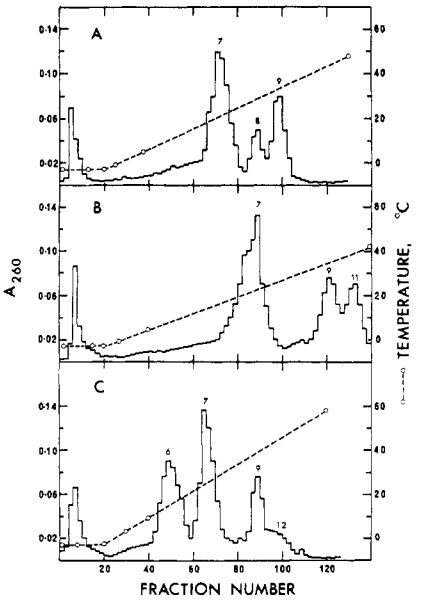


FIGURE 1: Thermal chromatography of mixtures of different length oligodeoxyadenylates on cellulose-p(dT)$_9$ (column 0.6 × 12 cm, 179 A.U. of p(dT)$_9$). The column was eluted with 1 M sodium chloride–0.01 M sodium phosphate (pH 7.0), flow rate 1 ml/10 min. The mixtures applied (A.U. in brackets) were: (A) p (dA) (2.25), p(dA)$_8$ (0.56), p(dA)$_9$ (1.20); (B) p(dA)$_7$ (2.25), p(dA)$_9$ (1.20), p(dA)$_{11}$ (1.94); (C) p(dA)$_6$ (2.50), p(dA)$_7$ (2.26), p(dA)$_9$ (1.20), p(dA)$_{12}$ (1.26). The number above each peak identifies the length of the oligodeoxyadenylate.

Results

Resolution of Mixtures of Deoxyribooligoadenylates on Columns of Cellulose-p(dT)$_9$. Previous work has indicated that with a sufficiently high ratio of cellulose-linked oligomer to oligomer in solution, members of a series of complementary homooligomers should be completely resolved by thermal elution (Astell and Smith, 1972).

We have studied the thermal chromatography of mixtures of deoxyribooligoadenylates on columns of cellulose-p(dT)$_9$ having a large theoretical capacity. In agreement with our expectations, a mixture of oligomers of length <9 were completely resolved (Figure 1a), while oligomers of chain length >9 were only partially resolved (Figures 1b and c). In all the cases studied, the T_m^e of a particular oligomer was identical either when eluted alone or when eluted in a mixture from the same column indicating that the interaction of each oligomer is completely independent of any other oligomers in the mixture.

Availability of the Nucleotides Attached to the Cellulose for Hydrogen Bond Formation. Preliminary work with cellulose-p(dT)$_9$ has indicated that possibly all the nucleotides in the oligonucleotide attached to cellulose are free to bind to complementary bases (Astell and Smith, 1972). This observation is of importance in the design of oligonucleotide celluloses for the fractionation of complementary sequences. The thermal stability of the interaction of short complementary sequences is dependent on the number of hydrogen bonded base pairs (Astell and Smith, 1972; Naylor and Gilham, 1966; Laird *et al.*, 1969; Gillespie and Spiegelman, 1966; Niyogi and Thomas, 1967, 1969). Therefore it must be known whether a nucleotide adjacent to cellulose is capable of forming hydrogen bonds with a complementary nucleotide, or if it is prevented from doing so, possibly by steric factors. To investigate this problem several series of deoxyribooligonucleotides (Table I) and ribooligonucleotides (Table II) were

TABLE I: Elution of Oligodeoxyadenylates and Oligothymidylates from Columns of Cellulose-p(dT)$_n$ and Cellulose-p(dA)$_n$.

Oligonucleotide Cellulose (A.U. Incorporated)	Oligonucleotide (A.U. Loaded)	T_m^c (°C)
Cellulose-p(dT)$_8$ (27)	p(dA)$_6$ (7.2)	3.5
	p(dA)$_7$ (6.3)	13.5
	p(dA)$_8$ (6.2)	19.0
	p(dA)$_9$ (6.2)	25.5
	p(dA)$_{10}$ (6.0)	28.5
Cellulose-p(dT)$_9$ (39)	p(dA)$_6$ (5.4)	8.5
	p(dA)$_7$ (5.5)	18.0
	p(dA)$_8$ (5.5)	26.0
	p(dA)$_9$ (5.6)	32.0
	p(dA)$_{10}$ (5.7)	35.0
	p(dA)$_{11}$ (5.6)	37.0
Cellulose-p(dT)$_{12}$ (30)	p(dA)$_6$ (4.0)	8.5
	p(dA)$_7$ (4.8)	14.5
	p(dA)$_8$ (4.2)	20.5
	p(dA)$_9$ (3.0)	27.5
	p(dA)$_{10}$ (4.4)	34.0
	p(dA)$_{11}$ (4.8)	37.5
Cellulose-p(dA)$_8$ (22)	p(dT)$_6$ (1.4)	3.0
	p(dT)$_7$ (1.2)	12.5
	p(dT)$_8$ (1.5)	18.0
	p(dT)$_9$ (1.5)	20.0
	p(dT)$_{10}$ (1.2)	23.0
	p(dT)$_{11}$ (1.8)	25.5
Cellulose-p(dA)$_9$ (44)	p(dT)$_6$ (4.9)	4.5
	p(dT)$_7$ (5.4)	14.0
	p(dT)$_8$ (5.7)	21.0
	p(dT)$_9$ (5.4)	26.0
	p(dT)$_{10}$ (5.5)	28.5
	p(dT)$_{11}$ (5.8)	31.0
	p(dT)$_{12}$ (5.3)	33.5
Cellulose-p(dA)$_{10}$ (49)	p(dT)$_6$ (2.0)	4.0
	p(dT)$_7$ (2.0)	17.0
	p(dT)$_8$ (2.0)	24.0
	p(dT)$_9$ (2.0)	29.5
	p(dT)$_{10}$ (2.0)	33.5
	p(dT)$_{11}$ (2.0)	34.5
	p(dT)$_{12}$ (2.0)	36.5
	p(dT)$_{13}$ (2.0)	38.5

TABLE II: Elution of Oligoribouridylates and Oligoriboadenylates from Columns of Cellulose-p(dA)$_n$ and Cellulose-p(dT)$_n$.

Oligonucleotide Cellulose (A.U. Incorporated)	Oligonucleotide (A.U. Loaded)	T_m^c (°C)
Cellulose-p(dA)$_{10}$ (49)	(rUp)$_7$ (4.0)	−4.0
	(rUp)$_8$ (4.0)	−3.0
	(rUp)$_9$ (3.3)	3.5
	(rUp)$_{10}$ (5.0)	8.5
	(rUp)$_{11}$ (4.1)	12.0
	(rUp)$_{12}$ (5.7)	15.5
	(rUp)$_{13}$ (4.8)	18.5
	(rUp)$_{14}$ (4.1)	20.0
Cellulose-p(dA)$_{10}$ (49)	rU(pU)$_7$ (1.1)	−2.0
	rU(pU)$_8$ (0.9)	6.0
	rU(pU)$_9$ (1.0)	10.0
	rU(pU)$_{10}$ (1.2)	14.0
	rU(pU)$_{11}$ (0.9)	15.0
	rU(pU)$_{12}$ (1.0)	16.0
	rU(pU)$_{13}$ (1.0)	22.0
Cellulose-p(dT)$_9$ (39)	rA(pA)$_5$ (2.4)	4.5
	rA(pA)$_6$ (2.5)	12.5
	rA(pA)$_7$ (2.0)	18.0
	rA(pA)$_8$ (2.0)	25.0
	rA(pA)$_9$ (4.5)	25.5
	rA(pA)$_{10}$ (2.5)	26.0
	rA(pA)$_{11}$ (2.7)	28.5
	rA(pA)$_{12}$ (2.0)	29.9
Cellulose-p(dT)$_{12}$ (30)	rA(pA)$_6$ (2.5)	9.0
	rA(pA)$_7$ (3.0)	15.5
	rA(pA)$_8$ (4.0)	22.0
	rA(pA)$_9$ (3.0)	27.5
	rA(pA)$_{10}$ (3.0)	30.0
	rA(pA)$_{11}$ (3.0)	30.5
	rA(pA)$_{12}$ (1.4)	30.0

chromatographed on cellulose-p(dT)$_n$ (n = 8, 9, and 12) and cellulose-p(dA)$_n$ (n = 8, 9, and 10).

Plots of T_m^c against chain length n, for interactions of the type p(dA)$_n$ with celluloses-p(dT)$_n$, and p(dT)$_n$ with celluloses-p(dA)$_n$, are shown in Figure 2. The experimental T_m^c values appear to fall along two straight lines which intersect about $^1/_2$ a nucleotide to the left of where the lengths of the cellulose-linked and free oligomers are equal. The fact that the T_m^c values increase with increasing chain length up to the point where the length of the oligonucleotide in solution equals the length of the oligonucleotide linked to cellulose is good evidence that all the nucleotide residues linked to cellulose are able to hydrogen bond with complementary nucleotides. In fact, this relationship may not be a linear one, particularly when the oligonucleotide in solution is much longer than the covalently bound oligonucleotide. In this case, the results can also be fitted by a shallow curve. None of the previously postulated relationships between T_m and chain length for solution hybridizations (Michelson and Monny, 1967; Niyogi and Thomas, 1967; Cassani and Bollum, 1967) give a good fit to these experimental data.

If the affinity of the oligonucleotide in solution for cellulose-linked oligomer is solely due to hydrogen bonding between complementary oligonucleotides, we cannot explain the observation that T_m^c values continue to rise, albeit with a significantly slower rate, with each additional residue, beyond where the lengths of the oligomer in solution and cellulose-linked oligomers are equal. Possibly this observation may prove to be connected to the nonspecific absorption on the cellulose matrix. It has been known for some time that poly(A) and some natural nucleic acids bind to cellulose (C. R. Astell, M. T. Doel, and M. Smith, unpublished results). There have recently been several reports concerning the binding of polyadenylate containing mRNA molecules to cellulose (Kitos *et al.*, 1972; DeLarco and Guroff, 1973). In fact, we have evidence in this paper that there is some affinity of the cellulose for oligoadenylates as small as six nucleotides long. In Table I, the p(dA)$_n$ series is eluted from cellulose-p(dT)$_9$ at a T_m^c some 4–6° higher than is the equivalent length p(dT)$_n$ from cellulose-(dA)$_9$. Because of this secondary stabilization of oligonucleotides and polynucleotides, it is reasonable to suggest that some affinity of the matrix for the unpaired portions of the long

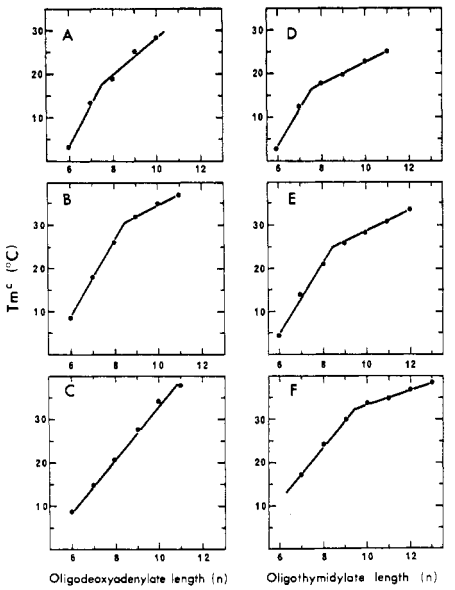


FIGURE 2: T_m^c *vs.* oligomer length n for the elution of complementary oligonucleotides $d(pA)_n$ or $d(pT)_n$ (n = 6–13) on six preparations of oligothymidylate- or oligodeoxyadenylate-linked cellulose: (A) cellulose-$d(pT)_8$ (27 A.U.), (B) cellulose-$d(pT)_9$ (39 A.U.), (C) cellulose-$d(pT)_{12}$ (30 A.U.), (D) cellulose-$d(pA)_8$ (22 A.U.), (E) cellulose-$d(pA)_9$ (44 A.U.), (F) cellulose-$d(pA)_{10}$ (49 A.U.). Drawn from the data in Table I.

oligonucleotides may contribute to the increased stability of the interaction.

It is instructive to compare the behavior of ribooligonucleotides and deoxyribooligonucleotides in terms of their elution characteristics on deoxyribooligonucleotide celluloses (Figure 3). Ribooligouridylates are eluted from columns of cellulose-$p(dA)_n$ with T_m^c values 20–25° lower than the corresponding oligothymidylates. It has been shown that for homopolymers in solution the T_m values of poly(dA)·poly(rU) hybrids are 20° or more lower than those of poly(dA)·poly(dT) hybrids (Chamberlin, 1965). Removal of the 3′-phosphate from the ribooligonucleotides caused a slight stabilization (1–2°) of their interaction with complementary oligonucleotides. As previously noted (Astell and Smith, 1971), the ribooligoadenylates elute from columns of cellulose-$p(dT)_n$ at temperatures only 2–5° lower than the corresponding deoxyribooligoadenylates (Figure 3b). Again this correlates well with the work of Chamberlin on homopolymer duplexes in solution (Chamberlin, 1965).

Chromatography of Complementary Oligomers on Oligonucleotide Celluloses of Mixed Repeating Base Sequence. In order to learn something of the effect of GC base pairs on the stability of interactions between oligomers and oligonucleotide celluloses, we have studied the thermal chromatography of $p(dA\text{-}A\text{-}G)_n$ (n = 2,3) on celluloses-$p(dT\text{-}T\text{-}C)_n$ and -$p(dC\text{-}T\text{-}T)_n$ (n = 2,3). Because of the isomeric relationship of these two types of pyrimidinic celluloses, a number of different interactions are possible with $p(dA\text{-}A\text{-}G)_n$, and from these studies several useful observations have been made. A summary of these data is given in Table III.

In general, oligonucleotides containing mixed base sequences elute from complementary oligonucleotide celluloses as do complementary oligomers from homooligonucleotide celluloses. The retained peaks are released by a thermal gradient, and can be characterized by the T_m^c for a particular interaction. In one case, a complementary nucleotide, $p(dA\text{-}A\text{-}G)_2$, was not retained by cellulose-$p(dT\text{-}T\text{-}C)_2$. This interaction permits a possible 4 AT and 1 GC or 2 AT and 2 GC base pairs (Table III, 1A and 1B), which are apparently not sufficient to form a stable hybrid in molar salt and −4°. However, this hexanucleotide was definitely retarded by the column (Figure 4a), as it is eluted 10–20 ml after a noncomplementary nucleotide, $p(dA)_9$ (dotted line, Figure 4a).

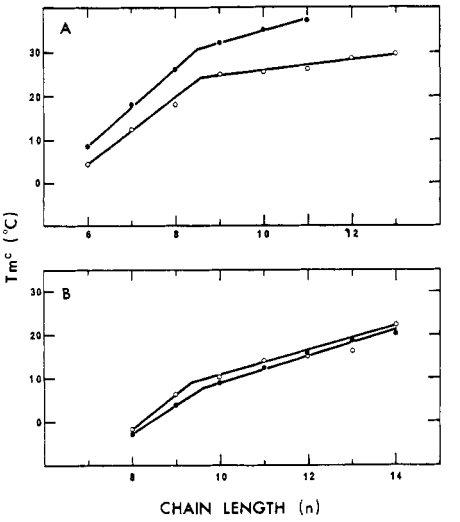
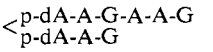


FIGURE 3: (A) Comparison of the elution of ribooligoadenylates $rA(pA)_{n-1}$ (○), and deoxyribooligoadenylates, $p(A)_n$ (●), from cellulose-$p(dT)_9$. (B) The effect of a 3′-terminal phosphate. Elution of ribooligouridylates with (●) and without (○) 3′-terminal phosphates from cellulose-$p(dA)_{10}$. Drawn from the data in Tables I and II.

With a larger number of potential base pairs (for example, 6 AT and 3 GC base pairs), the nucleotide in solution is tightly bound to a complementary oligonucleotide cellulose (Figure 4B). Thus, the nucleotide $p(dA\text{-}A\text{-}G)_3$ is eluted from cellulose-$p(dC\text{-}T\text{-}T)_3$ with a T_m^c of 40.5°. The uv absorbing material eluted as a broad peak between tubes 30 and 80 (Figure 4b) is probably a contaminating oligonucleotide pyrophosphate



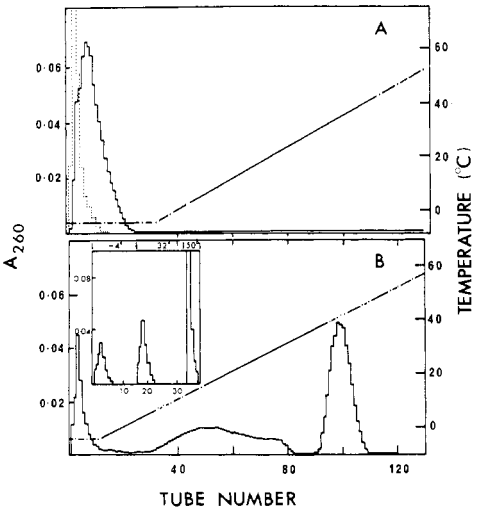


FIGURE 4: Thermal elution profiles for two mixed repeating base sequence oligonucleotide celluloses. Conditions were as described in the legend to Figure 1. (A) Elution of $p(dA\text{-}A\text{-}G)_2$ from cellulose-$p(dT\text{-}T\text{-}C)_2$. The dotted line is the elution profile of the noncomplementary oligonucleotide $p(dA)_9$. (B) Elution of $p(dA\text{-}A\text{-}G)_3$ from cellulose-$p(dC\text{-}T\text{-}T)_3$. The uv absorbing material between tubes 30 and 80 is probably a contaminant and is discussed in the text. Insert, stepwise elution of $p(dA\text{-}A\text{-}G)_3$ on cellulose-$p(dC\text{-}C\text{-}T)_3$.

TABLE III: Chromatography of Deoxyribooligonucleotides of Mixed Repeating Base Sequence on Columns of Complementary Deoxyribooligonucleotide Cellulose (5 cm × 0.9 cm).[a]

Interaction	A-T pairs	G-C pairs	T_m[c] (°C)
5′ ⟶ 3′			
1a. Cellulose-pd T-T-C-T-T-C	4	1	Retarded but not retained at −5°
dG-A-A-G-A-Ap			
⟵			
or 3′ 5′			
1b. Cellulose-pdT-T-C-T-T-C	2	2	
dG-A-A-G-A-Ap			
2. Cellulose-pdT-T-C-T-T-C	4	2	15.5
dG-A-A-G-A-A-G-A-Ap			
3. Cellulose-pdC-T-T-C-T-T	4	2	14.0
dG-A-A-G-A-Ap			
4. Cellulose-pdC-T-T-C-T-T	4	2	19.0
dG-A-A-G-A-A-G-A-Ap			
5. Cellulose-pdT-T-C-T-T-C-T-T-C	4	2	4.5
dG-A-A-G-A-Ap			
6a. Cellulose-pdC-T-T-C-T-T-C-T-T	4	2	16.0
dG-A-A-G-A-Ap			
or			
6b. Cellulose-pdC-T-T-C-T-T-C-T-T	4	2	
dG-A-A-G-A-Ap			
7. Cellulose-pdT-T-C-T-T-C-T-T-C	6	2	33.5
dG-A-A-G-A-A-G-A-Ap			
8. Cellulose-pdC-T-T-C-T-T-C-T-T	6	3	40.5
dG-A-A-G-A-A-G-A-Ap			

[a] The columns contained the following amounts of covalently linked oligonucleotide: p(dT-T-C)$_2$, 92 A_{260} units; p(dC-T-T)$_2$, 97 A_{260} units; p(dT-T-C$_3$), 77 A_{260} units; p(dC-T-T)$_3$, 103 A_{260} units. Samples of the complementary oligonucleotides (2.5–3.0 A_{260} units) were loaded at −5° in 1 ml of 1 M sodium chloride–0.01 M sodium phosphate (pH 7.0) and eluted in the same buffer using a linear temperature gradient. The interactions have been drawn out to illustrate possible hybrid structures.

The method used to prepare p(dA-A-G)$_3$ is believed to result in the production of very little pyrophosphate (Moon and Khorana, 1966), and the purification procedures used have been reported to separate the desired linear product from any nucleotide pyrophosphate (Narang *et al.*, 1967b). It seems that some contamination remains, however, and it is interesting to note the discriminating power of the oligonucleotide celluloses. We have previously observed heterogeneity particularly in purine oligonucleotide preparations and have noted that chromatography on a complementary oligonucleotide cellulose resulted in a more homogeneous preparation, reflected by elution of the nucleotide as a sharper, symmetrical peak on rechromatography (Astell and Smith, 1972). In the present experiments, we were also able to further purify the p(dA-A-G)$_3$, on cellulose-p(dC-T-T)$_3$, using a stepwise thermal gradient (inset, Figure 4B). The sample of nucleotide eluted at 50° was dialyzed against water, lyophilized, and hybridized with p(dC-T-T)$_3$. The thermal denaturation curve (Figure 5) has a much steeper transition slope and higher T_m (31°) compared with that of the "nonpurified" p(dA-A-G)$_3$ (T_m, 25°).

Effect of G-C Base Pairs on the Stability of Oligonucleotide Cellulose. Oligomer Interactions. In Table IV we have listed for comparison the interactions between homooligodeoxyribonucleotides (A-T base pairs only) and interactions which include both A-T and G-C base pairs. In each case where an equal number of nucleotides is involved, those which included G-C pairs are qualitatively considerably more stable. From these limited data, it is not possible to give a quantitative estimate of the increase in stability due to the introduction of a single G-C pair, and certainly other factors must also affect the stability of the interactions. In Table IV, one interaction involving four A-T and two G-C base pairs appears to be significantly less stable than the others in this group. This interaction is illustrated in Table III (number 5). Due to the nature of the two complementary oligonucleotides, in order to permit four A-T and two G-C base pairs, the free oligomer is displaced from the cellulose by two residues. On the isomeric

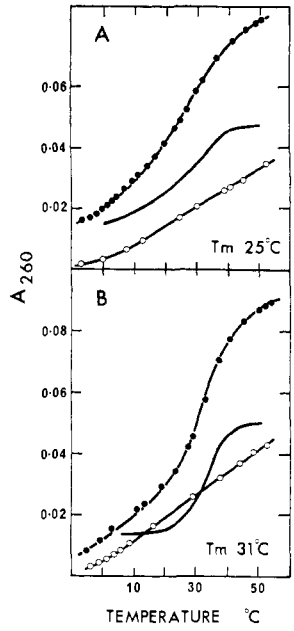


FIGURE 5: Thermal denaturation profiles of the interaction p(dA-A-G)$_3$· p(dC-C-T)$_3$. The melting profiles were obtained as described in Methods: (A) the profile for "nonpurified" p(dA-A-G)$_3$; (B) "purified" p(dA-A-G)$_3$ (see text) (●), denaturation profile of the oligonucleotide mixture, (○) denaturation profile of purine oligomer alone, (—) true thermal denaturation profile for the mixture.

TABLE IV: Effect of GC Base Pairs on the Stability of Oligonucleotide Cellulose–Oligomer Interactions. A Comparison of T_m^c Values for Homooligodeoxynucleotide Interactions p(dT)$_n$·p(dA)$_n$ and Complementary Oligomers of Mixed, Repeating Base Sequence Resulting in GC Base Pairs As Well As AT Base Pairs.[a]

AT Base Pairs Only	T_m^c Values (°C)	AT and GC Base Pairs	T_m^c Values (°C)
6	3.5, 8.5, 8.5, 3.0, 4.5, 4.0 ($\bar{x}$ = 5.3)	4, 2	14.0, 4.5,[b] 16.0, 19.0, 15.5 ($\bar{x}$ = 16.1)
8	19.0, 26.0, 20.5, 18.0, 21.0, 24.0 ($\bar{x}$ = 21.4)	6, 2	33.5
9	32.0, 27.5, 25.0, 29.5 ($\bar{x}$ = 28.5)	6, 3	40.5

[a] The data for interactions of only AT pairs are taken from Table I, and the data for AT and GC interactions are taken from Table III. $\bar{x}$ is the mean value of T_m^c for a given number of base pairs. [b] This value appears to be significantly lower than the rest in this group. A possible explanation is discussed in the text. This value was excluded from the calculation of $\bar{x}$ for this group.

cellulose-p(dC-T-T)$_3$ (number 6A, Table III), the p(dA-A-G)$_2$ hexanucleotide can hybridize immediately adjacent to the cellulose. If there is some stabilizing effect associated with this latter configuration, then this may (at least partially) explain the difference in T_m^c values for these interactions. A possible stabilizing effect might result from interaction between nucleotides at the 3′ end of the purine oligomer and the cellulose. Secondary binding forces between nucleotides (particularly purine derivatives) and cellulose have been observed, and the elution of different oligonucleotide isopliths on DEAE-cellulose is greatly improved when the chromatography is carried out in the presence of 7 M urea (Tomlinson and Tener, 1963).

A limited examination of the specificity of binding of oligomers to oligonucleotide celluloses has shown that noncomplementary molecules are excluded. For example, the series p(dA)$_n$ was excluded from celluloses-p(dC-T-T)$_n$ (n = 2, 3) (see Figure 4A), while p(dA-A-G)$_2$ was excluded from cellulose-p(dT)$_9$.

Discussion

Several additional properties of oligonucleotide celluloses have been examined. It has been shown that a mixture of oligomers of a series differing by one nucleotide in length may be chromatographically resolved provided that the length of the free oligomer is less than or equal to the length of the complementary matrix-linked oligomer. An extensive study of a number of homodeoxyribooligonucleotide celluloses (cellulose-p(dT)$_n$ and cellulose-p(dA)$_n$) has indicated that all the nucleotides attached to a cellulose matrix are able to interact with complementary oligonucleotides containing ribo or deoxyribo residues. The possibility remains that steric repulsion may destabilize the interaction when larger oligonucleotides complementary over only part of their length are examined. If this is found to be so then the construction of a hydrogen bonding exchanger must be modified to include an "extension arm" to keep the bound oligonucleotide away from the cellulose matrix. This kind of extension has been found to be necessary in the construction of some affinity chromatography matrixes (Cuatrecasas, 1970; Steers and Cuatrecasas, 1971).

Ribooligonucleotides were found to have a lower T_m^c on complementary oligonucleotide celluloses than their deoxy counterparts. While (rA)$_n$ is slightly less stable than p(dA)$_n$ on cellulose-p(dT)$_n$, the oligoribouridylate series was considerable less stable on cellulose-p(dA)$_n$. These observations correlate well with reports on the stability of the corresponding polymers in solution (Chamberlin, 1965). Apart from this difference their behavior is similar to that of the deoxy series. The presence of a 3′-terminal phosphate group on the ribooligomer was found to have a small destabilizing influence on the hybrids, as is the case for a 5′-terminal phosphate (Astell and Smith, 1972).

Studies with oligomers containing guanylate and cytidylate residues demonstrate that introduction of GC base pairs stabilize the interaction of complementary oligonucleotide. However, the data presented are not sufficient to give a quantitative estimate of this increase in stability per GC residues.

We have not yet commented on the observation that hybrid interactions in solution are several degrees less stable than the same interaction when one of the oligomers is linked to cellulose. For example, for the T_m of p(dC-C-T)$_3$, the p(dA-A-G)$_3$ is 31°, while for the T_m^c of cellulose-p(dC-C-T)$_3$, the p(dA-A-G)$_3$ is 40.5°. Few studies have been reported on the interaction between complementary oligonucleotides in solution. Naylor and Gilham (1966) studied the thermal stability of p(dA)$_n$ and p(dT)$_n$ (n = 3–9). These workers reported that mixtures of oligothymidylates and oligoadenylates as short as pentanucleotides demonstrate hypochromicity when the

temperature is reduced to 0°. The thermal denaturation profiles for most of the interactions described here and in Astell and Smith (1972) have been determined (Astell, 1970). However, because of the observation that the T_m for a particular interaction increases several degrees when the purine oligomer is further purified on an oligonucleotide cellulose column, it is necessary to first purify all our synthetic purine oligomers using this procedure, and then reexamine the thermal stability of the interactions between complementary oligonucleotides. From preliminary results (Astell, 1970), it appears that oligomer–oligomer hybrid structures are more stable when one of the oligomers is linked to cellulose. While these data suggest that there is some stabilization due to interaction of the nucleotides with the cellulose (see above), it may also be explained largely by the difference in concentration. The solution denaturation curves were done at total nucleotide concentrations of 0.05 mM, while the total nucleotide concentration on an oligonucleotide cellulose column is approximately 2 mM (largely due to the bound oligomer).

It should also be pointed out that the T_m and T_m^c values measure slightly different phenomena. The T_m is calculated from a measure of the actual number of hydrogen bonded nucleotides. Therefore the T_m is the temperature at which one-half of the nucleotides of complementary oligomers exist in a hydrogen bonded form. However, the T_m^c is the temperature at which the molecules are sufficiently weakly bonded to their complement that they are eluted from the column. This predicts that as the lengths of the complementary oligomers increase the discrepancy between the T_m and T_m^c values should increase. Such a comparison must await the T_m determination on "purified" purine oligomers.

In all the interactions studied so far the complementary oligonucleotide strands have consisted either entirely of purine bases or entirely of pyrimidine bases. Since the vertical stacking of oligopurine tracts is known to contribute additional stabilization to hybrid interactions (Szybalski, 1967), it would be of interest to study the stability of oligomer–oligomer interactions in which each strand contains purines and pyrimidines.

In the application of oligonucleotide celluloses to the isolation of large natural polynucleotides a number of experiments suggest themselves. They are, briefly, to determine whether the many nucleotide residues at the 3′ end of the polynucleotide in solution will destabilize or prevent the interaction with the cellulose-bound oligonucleotide. Using appropriate synthetic and naturally occurring polynucleotides, experiments are in progress to determine whether the oligonucleotide celluloses should have the oligonucleotide attached to a spacer molecule to lessen interactions between the oligonucleotide hydrogen bonded to the covalently linked oligonucleotide and the matrix. Also other matrix materials are being examined for comparison with cellulose.

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