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# The base-pairing specificity of cellulose-pdT$_9$*

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## ABSTRACT

The stability of the interaction of oligoadenylates, containing single nucleotide substitutions, with cellulose-pdT$_9$ has been studied by thermal elution. In the case of oligodeoxyriboadenylates, the replacement of an internal dA by dC, dG or dT caused destabilization. In the case of oligoriboadenylates, replacement of an internal A residue by C or U resulted in a similar destabilization. However, replacement of an internal A residue by G resulted in a significantly lesser destabilization. The results indicate that caution should be exercised in extrapolation of data on the specificity of deoxyribopolynucleotide-deoxyribopolynucleotide hybridization to structure of the type deoxyribopolynucleotide-ribopolynucleotide.

## INTRODUCTION

It has been suggested that an oligonucleotide of defined sequence, attached to an insoluble matrix, could provide an appropriate tool for the isolation of natural nucleic acids containing a sequence complementary to the oligonucleotide[1,2,3]. Oligonucleotides of defined sequence, binding to a specific site on a natural polynucleotide are useful primers for sequence determinations. It is important, to the future of both these types of study, to define the specificity and stability of Watson-Crick structures involving oligonucleotides. To this end, the present paper reports studies on the interaction of cellulose-pdT$_9$ and both ribo- and deoxyribo- oligoadenylates where one adenylate residue is replaced by another nucleotide.

Other investigations of paired complementary polynucleotides containing occasional mismatched bases have indicated that these bases are looped out of the double helical regions thereby making the helix unstable[4]. In a system containing mismatched G and U

bases this is not the case. This observation can be interpreted in terms of the formation of a G-U wobble pair[5,6]. Another study indirectly suggests similar pairing, in this case involving the thymine residues of single-stranded natural DNA's and guanine residues of poly $G_n$[7]. The formation of thymine-guanine base pairing was also shown from the observation that RNA polymerase from E. coli catalyses poly $G_n$ synthesis in the presence of defined, repeating DNAs having only thymine and cytosine residues in one strand[8].

With short oligonucleotides of the type $A_n$-$U_n$, self-complementarity occurs in solution to form double helical structures[9]. However when G or C is inserted between adenylic acid-uridylic acid block copolymers, the stability of the bimolecular helical complex is reduced[10]. Cytidylic acid is the more destabilizing, reducing the Tm by an amount equivalent to the removal of two adenylate, uridylate pairs. In contrast to C, a G residue can be accomodated in the structure, through the formation of G-U base pairs resulting in a relatively stable complex.

Self-complementary oligonucleotides are probably the best simple model compunds available for studying the stability of short helical regions in RNA. However, they do not provide information about the interactions between a deoxyribooligonucleotide and a ribooligonucleotide such as would be used in the proposed chromatographic system which we are developing for the isolation of specific messenger RNA[1,2]. Earlier difficulties in the synthesis of oligonucleotides of defined sequence have limited studies on such interactions. However, the development of methods for enzymatic synthesis of deoxyribooligonucleotides[11] and ribooligonucleotides[12,13] has simplified procedures for the preparations of model compounds and made the present studies possible.

Our earlier work has shown that complementary sequences longer than pentanucleotides give stable interactions with a complementary oligonucleotide-cellulose at temperatures conveniently attainable in molar sodium chloride[1]. $Tm^c$, the temperature at which a bound oligomer is eluted from the column increases approximately linearly with helix length provided the hydrogen-bonded oligonucoeotide is not longer than its complementary one covalently bound to the column[3]. This parameter ($Tm^c$) has been

used for the studies reported here on the interactions of model compounds with a cellulose-$pdT_9$ column.

If base non-complementarity disturbs the interaction, one would predict that the $Tm^C$ will be greatly lowered compared to the situation in which all bases exactly paired. On the other hand, one might expect little lowering of $Tm^C$ due to the wobble base-pairing[5-8]. The present studies on the model compounds have provided useful information concerning the stability of helixes containing a single base mispairing. Comparison of the results obtained between deoxyribooligonucleotides or ribooligonucleotides and cellulose-$pdT_9$ suggests that interactions of deoxyribooligo-nucleotides and ribooligonucleotides are not necessarily similar.

## Materials and Methods

Chromatography on cellulose-$pdT_9$. The column chromatography procedures and determination of $Tm^C$ values were as previously described[1,13]. The same batch of cellulose-$pdT_9$ was used in all studies and the solvent was M NaCl, 0.01 M sodium phosphate, pH 7.0.

Oligoadenylates with residue substitution. The synthesis of all these oligomers is described in the preceding paper[13].

Preparation of oligomers of adenylic acid. Poly A was synthesized from ADP using polynucleotide phosphorylase from Micrococcus luteus. The reaction mixture (10 ml) contained 100 mM glycine buffer, pH 9.0, 15 mM $Mg^{2+}$, 10 mM 2-mercaptoethanol, 40 mM ADP, 5% glycerol and 15 mg polynucleotide phosphorylase. After incubation at 25°C for 12 hours, poly A was precipitated by addition of an equal volume of 2 M sodium acetate with stirring and the precipitate was collected by centrifugation. It was dissolved in buffer (0.05 M glycine, pH 8.8 and 0.1 M NaCl) and dialyzed against the same buffer overnight at 4°C.

The enzymatic hydrolysis of poly A was carried out as reported by Coutsogeorgopoulos and Khorana[14]. Poly A (6 ml, 187 $A_{260}$/ml, in 0.05 M glycine, pH 8.8, 0.1 M NaCl and 0.01 M $CaCl_2$) was degraded to a mixture of oligomers by limited hydrolysis with micrococcal nuclease (38µg) at 37° for 60 min. The oligomers were fractionated and terminal phosphates were removed as described earlier[13].

RESULTS and DISCUSSION

Earlier studies have shown that thermal elution of oligonucleotides in Watson-Crick interaction with a complementary deoxyribooligonucleotide-cellulose provides a convenient and specific measure of the stability of such structures[1,3]. The temperature of elution ($Tm^C$) of a particular oligonucleotide is determined by the number of base-pairs, the bases involved and the type of sugar in the nucleosides[1,3]. In addition, the frequent presence of mismatched base-pairs prevented the formation of a double-stranded structure. For example, $pdA_9$ was not adsorbed to cellulose-$p(dC\text{-}dT_2)_3$ and $p(dA_2\text{-}dG)_2$ was not adsorbed to cellulose-$pdT_9$[3]. However, these cases involve mismatching of one out of three base-pairs. If the objective is to form a Watson-Crick structure between an oligonucleotide and a nucleic acid, the effect of a single base-pair mismatching must be determined. Because extensive data are available on the stabilities (i.e. $Tm^C$ values) for the interaction of ribo- and deoxyribooligoadenylates with columns of cellulose-$pdT_9$[1,3], and because ribo- and deoxyribo-oligoadenylates can be obtained readily with single nucleoside substitutions[13], it was convenient to carry out the present study. It should be noted that the basic procedure in all these studies is to adsorb oligonucleotides to a cellulose-$pdT_9$ column at low temperature. The column then is continually washed with buffer whilst the temperature is raised at a linear rate. The eluate is collectd in a fraction collector and the elution of oligonucleotide determined either from U.V. absorption or radioactivity. The temperature at the midpoint of elution of an oligonucleotide is its characteristic $Tm^C$. All experiments reported herein were carried out using a single batch of cellulose-$pdT_9$, since some variation in properties from batch to batch has been observed[1,3].

The effect of base mispairing for deoxyribooligonucleotides. The results for the series of deoxyribooligonucleotides $pdA_5$-dN-$dA_n$ (where N = A,T,G or C and n = 0,1,2 or 3) are recorded in Table I. It is striking that , in all the complexes examined, the replacement of a dA residue by dC, dG or dT results in a reduction of the $Tm^C$ value approaching 15° relative to that of the deoxyriboadenylate of the same length. Thus none of the

Table 1

Effect of substitution of a single nucleoside in a deoxyribooligoadenylate of the type $pdA_5$-dN-$dA_n$ on the $Tm^C$ values with cellulose-$pdT_9$ in M NaCl at pH 7.0

$Tm^C$ (°C)

| | | | |
|---|---|---|---|
| $pdA_6$ 7° | $pdA_5$-dC * | $pdA_5$-dG * | $pdA_5$-dT * |
| $pdA_7$ 18.5° | $pdA_5$-dC-dA 6° | $pdA_5$-dG-dA 4° | $pdA_5$-dT-dA 6.5° |
| $pdA_8$ 26° | $pdA_5$-dC-$dA_2$ 13° | $pdA_5$-dG-$dA_2$ 9.5° | $pdA_5$-dT-$dA_2$ 11.5° |
| $pdA_9$ 31° | $pdA_5$-dC-$dA_3$ 16° | $pdA_5$-dG-$dA_3$ 15.5° | $pdA_5$-dT-$dA_3$ 15.5° |

* These oligonucleotides were not adsorbed at -4°C.

natural deoxyribonucleosides can effectively substitute for dA in its interaction with dT in a deoxyribooligonucleotide of the type $pdA_5$-dN-$dA_n$ (n = 0,1,2 or 3). It is particularly interesting that this also applies in the case of dG since a possibility of pairing with dT might have been predicted[6].

The effect of substitution of a dA residue is not as simple as might be implied from the data in Table 1. This is most conviently illustrated by the data in Table 2 which lists the $Tm^C$

Table 2

Effect of the position of a deoxyriboguanylate residue in a deoxyribooligoadenylate on the $Tm^C$ values with cellulose-$pdT_9$ in M NaCl at pH 7.0

$Tm^C$ (°C)

| | | | | |
|---|---|---|---|---|
| $pdA_6$ 7° | | | $pdA_5$-dG * | $pdA_4$-dG-dA * |
| $pdA_7$ 18.5° | | $pdA_6$-dG 11° | $pdA_5$-dG-dA 4° | $pdA_4$-dG-$dA_2$ * |
| $pdA_8$ 26° | $pdA_7$-dG 22° | $pdA_6$-dG-dA 18° | $pdA_5$-dG-$dA_2$ 9.5° | $pdA_4$-dG-$dA_3$ 5.5° |
| $pdA_9$ 31° | | $pdA_6$-dG-$dA_2$ 23° | $pdA_5$-dG-$dA_3$ 15.5° | |

* These oligonucleotides were not adsorbed by cellulose-$pdT_9$ at -4°.

values from cellulose-$pdT_9$ for the series of deoxyribooctanucleotides $pdA_{7-n}$-dG-$dA_n$ (n = 0,1,2 or 3). The general conclusion is that destabilization is greater for substitutions in the interior of the deoxyribooligonucleotide than for terminal substitutions. It should be noted that even in the most extreme cases of destabilization there is cooperativity between the two deoxyriboadeny-

late tracts. The present experiment does not allow one to determine if this is achieved by looping out of the mispaired base.

Comparison of the data in Tables 1 and 2 for the series $pdA_n$-dN-dA (dN = dC, dG or dT; n = 5 or 6) shows that, in hydrogen-bonding of these deoxyribooligonucleotides to cellulose-$pdT_9$, they have the same stability as the deoxyribooligoadenylate with the same number of dA residues, i.e. in this particular series the effect of the nucleoside substitution is neutral.

Table 3

Effect of substitution of a deoxyribonucleoside at the 3'-terminus of deoxyribooligoadenylates on the $Tm^c$ values with cellulose-$pdT_9$ in M NaCl at pH 7.0.

| | $Tm^c$ (°C) | | |
|---|---|---|---|
| $pdA_6$ 7° | | | |
| $pdA_7$ 18.5° | $pdA_6$-dC 6° | $pdA_6$-dG 11° | $pdA_6$-dT 8° |
| $pdA_8$ 26° | $pdA_7$-dC 16° | $pdA_7$-dG 22° | $pdA_7$-dT 16.5° |

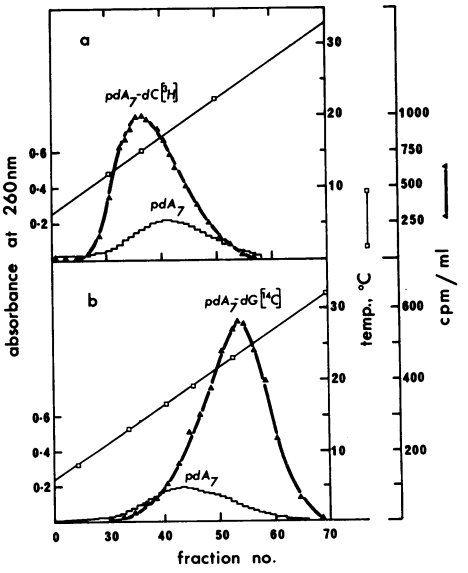


Figure 1. The thermal elution of $pdA_7$-dC[$^3$H] and $pdA_7$-dG[$^{14}$C] from a cellulose-$pdT_9$ column: a, $pdA_7$ (4.0 $A_{260}$ units) was co-chromatographed with $pdA_7$-dC[$^3$H] (3 x $10^4$ cpm); b, $pdA_7$ (4.0 $A_{260}$ units) was co-chromatographed with $pdA_7$-dG[$^{14}$C] (1.2 x $10^4$ cpm). The chromatographic procedures were as described previously[13].

In Table 3 are compiled the data on the $Tm^c$ values for deoxyribooligoadenylates with pdC, pdG or pdT at the 3' -terminus. The differences in the stabilities of $pdA_n$, $pdA_n$-dC and $pdA_n$-dT are relatively small, but significant (figure 1a), i.e., again the effect of the mismatched nucleoside is essentially neutral. However, it is clear that for both $pdA_6$-dG and $pdA_7$-dG there is stabilization relative to $pdA_6$ and $pdA_7$ (Figure 1b). This contrasts with internal dG substitutions. The results suggest that a 3'-terminal dG can pair with dT.

The effect of base mispairing for ribooligonucleotides. A long-term objective of this research is the isolation of mRNA by specific hybridization with a deoxyribooligonucleotide of defined sequence covalently linked to an insoluble matrix[2]. Consequently studies of the mismatching of a substituted ribonucleoside in a ribooligoadenylate in interaction with cellulose-$pdT_9$ were carried out. The results are presented in Table 4 for the series $A_4$-N-$A_n$

Table 4

Effect of substitution of a single nucleoside in a ribooligoadenylate of the type $A_4$-N$A_n$ on the $Tm^c$ values with cellulose-$pdT_9$ in M NaCl at pH 7.0.

$Tm^c$ (°C)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $A_6$ | 3° | $A_4$-C-A | * | $A_4$-G-A | * | $A_4$-U-A | * |
| $A_7$ | 11° | $A_4$-C-$A_2$ | * | $A_4$-G-$A_2$ | 7° | $A_4$-U-$A_2$ | * |
| $A_8$ | 18.5° | $A_4$-C-$A_3$ | * | $A_4$-G-$A_3$ | 15.5° | $A_4$-U-$A_3$ | 1° |
| $A_9$ | 24° | $A_4$-C-$A_4$ | 1° | $A_4$-G-$A_4$ | 19.5° | $A_4$-U-$A_4$ | 7.5° |
| | | $A_4$-C-$A_5$ | 6.5° | | | | |

* These oligonucleotides were not adsorbed at -4°.

(N = C,G or U; n = 1,2,3,4 or 5). As has been reported previously ribooligoadenylates form less stable structures with cellulose-$pdT_9$ that do the corresponding deoxyribooligoadenylates[1,3]. However, qualitatively, the results are similar to those presented in Table 1. There are some quantitatively significant differences, however. The most notable is that internal G residues have a significantly greater stabilizing effect than do dG residues (Figure 2). This suggests that these G residues have the ability

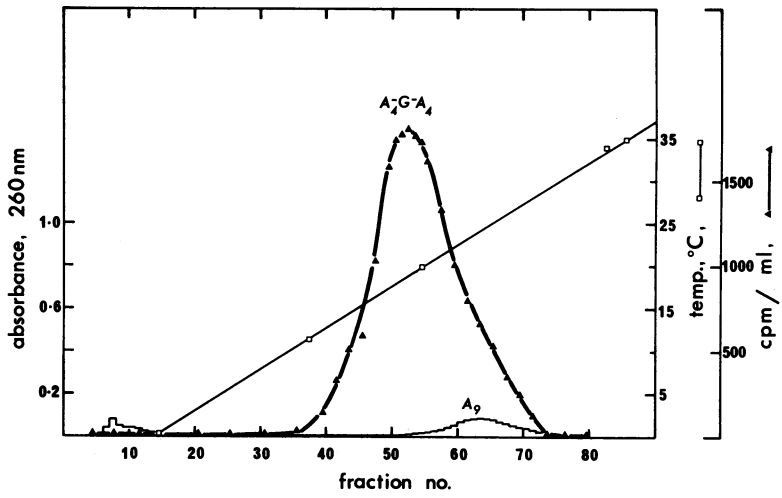


Figure 2. The thermal elution of $A_4$-G-$A_4$[$^3$H] and $A_9$ from a cellulose-pd$T_9$ column. $A_9$ (2 $A_{260}$ units) was co-chromatographed with $A_4$-G-$A_4$[$^3$H] (4.6 x $10^4$ cpm)[13]. The chromatographic procedures were as described previously[13].

to form hydrogen bonds with dT residues. The formation of hydrogen bonds between G and dT residues has been suggested by other studies as have hydrogen bonds between G and U residues[4-10]. It seems possible that the non-formation of a dG, dT pairing (except when the dG residue is terminal) may result from differences in the shape of the double helix in the three series of double stranded polymers. As might be anticipated, a G residue at the 3'-terminus of a ribooligoadenylate can pair with a dT as illustrated by the data in Table 5.

The differences in stabilities of structures involving C and U substitutions (Table 4) are not understood.

General comments. A number of conclusions can be drawn from the present study which are of practical and theoretical value. Thus, it seems that a deoxyribooligonucleotide is less likely to form a mismatched Watson-Crick structure with a DNA than with an RNA molecule. It seems clear that dT, dG structures are much less favored than dT, G or U, G pairings.

It is evident that there is cooperativity in the hydrogen bonding of oligonucleotides on either side of a mismatched nucleoside, contrary to the conclusions of Schott[15].

Table 5

Effect of a G residue at the 3-terminus of a ribooligoadenylate on the $Tm^C$ values with cellulose-$pdT_9$ in M NaCl at pH 7.0.

| | $Tm^C$ (°C) | | $Tm^C$ (°C) |
|---|---|---|---|
| $A_6$ | 3° | $A_5$-G | * |
| $A_7$ | 11° | $A_6$-G | 7.5° |
| $A_8$ | 18.5° | $A_7$-G | 16° |
| $A_9$ | 24° | $A_8$-G | 19.5° |

* Not adsorbed at -4°C.

Thermal elution from oligonucleotide-celluloses gives data which is generally compatible with solution thermal denaturation studies[9,10,16]. Because the thermal elution method can use both optical absorbance or radioactivity in its measurements it is applicable to a wider range of oligo- and poly-nucleotides which are only available in trace amounts, but which are readily labelled with a radioactive isotope. Of course, the present conditions or the particular matrix may not be optimal, but the basic principle of thermal elution from an oligonucleotide covalently linked to an insoluble matrix has considerable potential in both model studies and nucleic acid isolation.

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