Oligodeoxythymidylate:Polydeoxyadenylate and Oligodeoxyadenylate:Polydeoxythymidylate Interactions*

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ABSTRACT: The melting temperatures of complexes formed between oligodeoxynucleotides and polydeoxynucleotides (adenine–thymine bonding system) were examined in several solvents. When complexes of equivalent stoichiometry are compared, a linear relation between reciprocal melting temperature and reciprocal chain length is observed.

Current concepts of deoxyribonucleic acid structure are based primarily on DNA models (Watson and Crick, 1953) and experimental studies on the interactions between polyribonucleotides (see Steiner and Beers, 1961, Felsenfeld and Miles, 1967, and Michelson *et al.*, 1967, for reviews). Experimental studies on the interaction of clearly defined oligodeoxyribonucleotides are desirable as an aid in interpreting the forces that determine DNA structure. Previous work from this laboratory (Bollum *et al.*, 1964; Bollum, 1966a,b) described the enzymatic synthesis of useful quantities of polydeoxyribonucleotides and the degradative synthesis of oligodeoxyribonucleotides. The methods described have now been used to isolate oligodeoxyribonucleotides varying in chain length from 2 to 25. This paper describes the spectral characteristics of a series of oligodeoxythymidylates, $d(pT)_m$, and oligodeoxyadenylates, $d(pA)_m$,[1] and demonstrates the nature and stoichiometry of some of their interactions with complementary polydeoxyribonucleotides, $d(pA)_n$ and $d(pT)_n$.

Our interest in these interactions was stimulated by the observation that oligodeoxyribonucleotides initiate complementary synthesis on polydeoxyribonucleotide templates (Bollum *et al.*, 1964; Bollum, 1964, 1967). In this reaction, catalyzed by a DNA polymerase from calf thymus gland, oligodeoxyribonucleotides with a chain length of six nucleotides or greater are required for initiation of the replication process on complementary template chains. The interaction studies reported here demonstrate that physical complexes of initiator of that chain length and template are not stable at the temperatures used. We therefore conclude that enzyme plays a part in binding the initiator oligonucleotide to the template. These investigations on replication will be reported separately (Cassani and Bollum, 1966, and unpublished results; Bollum, 1967) and are mentioned because they have influenced our choice of solvents.

The results of the melting studies demonstrate that chemically pure oligodeoxyribonucleotides interact with complementary polydeoxyribonucleotides to produce complexes having abrupt melting transitions. The slope of the transition is practically independent of oligomer chain length and the reciprocal of the temperature at the midpoint is a linear function of the reciprocal of chain length in a given solvent. The double-reciprocal relationship is in accord with the theory described by Magee *et al.* (1963). Complexes of $d(pA)_m$ with $d(pT)_n$ are clearly different from complexes of $d(pT)_m$ with $d(pA)_n$ with regard to the $1/T_{1/2}$ *vs.* $1/m$ function. These observations are related to stoichiometry changes that occur in the $d(pA)_m \cdot d(pT)_n$ interaction, but not in the $d(pT)_m \cdot d(pA)_n$ interaction, under the conditions and with the chain lengths that we have used (Cassani and Bollum, 1967).

Experimental Section

Polymers. Polydeoxyribonucleotides were prepared by the polymerization of deoxyribonucleoside triphosphates in the presence of a *terminal* deoxynucleotidyl transferase isolated from calf thymus gland (Yoneda and Bollum, 1965). The preparation of polydeoxyadenylate has been described in detail previously (Bollum *et al.*, 1964) and the several preparations used in these studies had sedimentation constants of either 3.4 or 4.8. Polydeoxythymidylate was prepared by incubating 51.6 μmoles of dTTP with 1 mg of terminal transferase in the presence of 1 mM $CoCl_2$, 40 mM potassium cacodylate (pH 6.8), and 1 mM mercaptoethanol in a final volume of 25 ml for 72 hr at 35° (*cf.* Kato *et al.*, 1967). The final product isolated by gel filtration on Sephadex G-50 had an optical density of 294 at 260 mμ in 1 mM Tris-Cl (pH 8), corresponding to 33.8 μmoles of product, a yield of about 65%. This poly dT had $s_{20,w} = 6.3$ S.

The homopolymer complex, $d(pA)_n \cdot d(pT)_n$, was prepared using polydeoxyadenylate as a template for the *replicative* deoxynucleotidyl transferase (DNA polymerase) isolated from calf thymus gland (Yoneda and Bollum, 1965) essentially as previously described (Bollum, 1966a). Homopolymer complexes were also prepared by physical mixing of the separately

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[1] All the abbreviations follow the Revised Tentative Rules (1965), IUPAC–IUB Combined Commission on Biochemical Nomenclature, published in *Biochemistry 5*, 1445 (1966), plus the following: *m*, oligonucleotide chain length; *n*, a polymer chain length greater than 100 nucleotides; $T_{1/2}$, temperature at the midpoint of the ultraviolet thermal transition expressed in degrees Kelvin or Centigrade; $d(pA)_m$ and $d(pT)_m$, deoxyadenylate and deoxythymidylate oligonucleotides bearing a 5′-phosphate group and a 3′-hydroxyl group; $d(pA)_n$, polydeoxyadenylate; $d(pT)_n$, polydeoxythymidylate; DNase I, pancreatic deoxyribonuclease; ribonucleotide polymers are prefixed by *r*.

prepared $d(pA)_n$ and $d(pT)_n$. Polyadenylic acid was purchased from Miles Chemical Co., Elkhart, Ind.

Oligomers. Oligodeoxyribonucleotides were prepared by degradation of polydeoxyribonucleotides with electrophoretically purified DNase I purchased from Worthington Biochemical Corp. The procedures for degradation and isolation of the oligodeoxyribonucleotides have been described previously (Bollum, 1966b). All of the oligodeoxyribonucleotides used in this investigation were 5′-phosphate-ended oligomers of the form $d(pX)_m$.

Analytical Procedures. Spectra were taken with a Cary-15 recording spectrophotometer at 20–22°. Venom diesterase (Worthington) dissolved in 1 mM Tris·Cl (pH 8) was used to degrade polymers and oligomers to monomers for measurements of hypochromicity as described by Naylor and Gilham (1966). Analysis for total phosphate was carried out using the method of Griswold for colorimetric measurement after wet ashing with H_2SO_4 and HNO_3. More recently an Autoanalyzer method based on wet ashing with $HClO_4$ has been used. We are indebted to Mr. Gerald Wells and Dr. R. L. Lester for the latter analyses.

Mixing Curves. Solutions of oligodeoxyribonucleotides and polydeoxyribonucleotides were mixed in varying mole fractions and allowed to stand at room temperature or at 5° for 1 hr after mixing in the presence of Mg^{2+} and 12 hr after mixing when Mg^{2+} was absent. Absorption measurements were taken in a Zeiss spectrophotometer at room temperature (21–23°) or in a Beckman DU-2 thermostated at 5°. At the low temperature condensation of moisture was prevented by passing dry nitrogen through the sample compartment.

Gel Filtration. Sephadex G-200 beads were swollen in 40 mM potassium phosphate buffer (pH 7.0) alone or containing 8 mM $MgCl_2$ and 1 mM potassium cacodylate buffer (pH 6.8). A 1 cm diameter × 100 cm long column of this material had V_0 = 29.5 ml. Gel filtration experiments were done at room temperature or in a cold room at 5°, depending upon the stability of the complex being examined. Samples obtained from separations carried out at 5° were read in a Beckman DU at 5 and at 23°.

Melting Curves. The thermal transitions were carried out in a Beckman DU-2 spectrophotometer equipped with double thermospacers and heating and cooling facility. Mixtures were routinely equilibrated for 12 hr at 5°, and then transferred to the spectrophotometer equilibrated at the same temperature. Measurements were taken after 15 min of equilibration at each temperature. Checks for time dependence of the optical density at each temperature were made.

When melting curves are expressed in terms of relative absorbance, A_r, this is defined as the ratio of the absorbance of a mixture at the temperature, t, and at the temperature, t_0. The temperature, t_0, is a temperature well below the temperature at which melting begins.

Mixing curves are also expressed in terms of relative absorbance. This is the ratio of the molar absorbance of the oligopolymer mixture to that expected if there were no hypochromic effect (Stevens and Felsenfeld, 1964).

The midpoint, $T_{1/2}$, of the thermal denaturation was measured as the temperature at which the corrected hyperchromicity was one-half of the maximum value. The maximum value of hyperchromicity was obtained by substracting the gradual increment in absorbance due to free oligodeoxyadenylate or polydeoxyadenylate above the melting temperature (see Figure 1). No corrections for oligo- or polydeoxythymidylate melting were necessary.

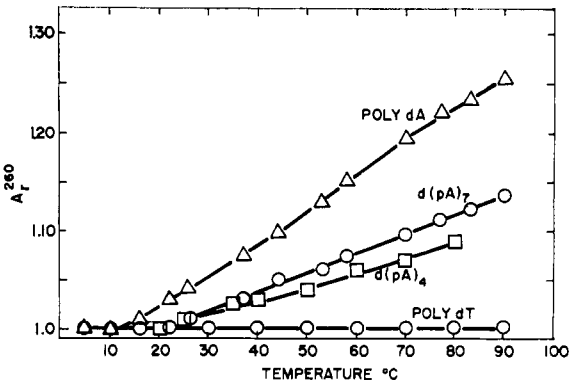

FIGURE 1: Absorbance–temperature curves for oligo- and polydeoxyadenylate in SSC. The absorbance values are not corrected for thermal expansion of the solvent. The concentration was 20 μM in nucleotide residues.

The slope of the transition curves was measured at $T_{1/2}$, where it is a maximum. The maximum slope was calculated geometrically on transition curves corrected for free $d(pA)_m$ or $d(pA)_n$ melting and is defined as the reciprocal of the interval of temperature during which a transition curve with maximum slope would take place completely. The units are $(°C)^{-1}$.

Results

Total Hypochromicity of Oligo- and Polydeoxynucleotides. Extinction coefficients for oligomers and polymers are presented in Table I. The values for the extinction coefficients of this series of oligodeoxyadenylates and oligodeoxythymidylates and poly dA and poly dT show the usual hypochromicity observed upon polymerization of nucleotides. The extinction coefficients presented are useful information for any study on oligodeoxynucleotides. It may be observed that both oligodeoxythymidylates and oligodeoxyadenylates show a decrease in absorbancy with degree of polymerization. The values for polydeoxythymidylate are sensibly constant between 4 and 90° as shown in Figure 1. Thus, the hypochromicity inherent in oligo- and polydeoxythymidylates does not melt out as temperature is increased. This is in contrast to the oligodeoxyadenylates where the extinction coefficient increases with temperature between 20 and 90° (Table I and Figure 1). At 90° $d(pA)_2$ is still hypochromic with respect to the monomer. Chain lengths of 3–300 retain a hypochromicity of about 20% with respect to the monomer (Table I). Thus at 90° there is not much difference in the degree of ordered structure, measured as hypochromicity, between a trimer and a polymer. If the bases are unstacked at 90° the hypochromic value of 20% with respect to the monomer is an intrinsic property of the polymerization of the bases, and this value is already achieved at m = 3. In contrast, poly dT is already in an unstacked configuration at room temperature and shows an hypochromicity of only 12% with respect to the monomer. At the present time we do not know if the larger value for hypochromicity retained by poly dA at 90° is due to an incomplete destruction of the ordered structure or to a characteristic value assumed upon

TABLE I: Millimolar Extinction Coefficients, ϵ_{max}, of the Oligonucleotides.

	Deoxyadenylates				Deoxythymidylates		
m	λ_{max}[c]	ϵ_{max}[a]	ϵ_{max}[b]	ϵ_{max} 90°	λ_{max}[c]	ϵ_{max}[a]	ϵ_{max}[b]
1	259	15.3	15.3	14.85	267	9.65	9.65
2	258	12.45	12.35	12.85	266	9.10	9.10
3	257	11.34	11.35	12.05	266	9.05	8.90
4	257	11.02	10.87	12.23	266	8.78	8.84
5	257	10.89	10.60	12.15	266	8.75	8.78
6	257	10.32	10.40	11.99	266	8.44	8.65
7	257	10.12	10.25	12.10	266	8.60	8.63
8	257	9.98	10.15	11.83	266	8.69	8.62
9	257	10.17	10.05	11.56	266	8.63	8.61
10	257	9.91	10.00	11.61	266	8.66	8.60
>300	257	9.39	9.45	12.04	265	8.54	8.49

[a] The complete spectrum of each sample dissolved in 10^{-3} M Tris-Cl (pH 8.0) was taken. Then 20 μg of venom diesterase was added and another spectrum was taken 24 hr later. All spectra at 21–23°. [b] Extinction coefficients obtained from the least-squares line of a plot of the observed ϵ_{max} *vs.* the reciprocal of the chain length of the oligonucleotide. [c] In mμ.

polymerization of deoxyadenylic acid. It is noteworthy that molar extinction values in Table I for deoxyadenylate polymerization show greater hypochromicity than observed for polymerization of riboadenylate by Singer *et al.* (1962) and Brahms *et al.* (1966). The values of ϵ_{max} for oligodeoxythymidylates are in good agreement (−2%) with the values found by Naylor and Gilham (1966) for chemically synthesized oligomers.

The thermal denaturation of poly dA in 0.1 M Na^+ at neutral pH is identical with the thermal denaturation of poly rA, as was shown by Riley *et al.* (1966). Our calculated value for enthalpy change, ΔH = 13.0 kcal/mole for poly dA, is in agreement with 12.5 kcal/mole found by Riley *et al.* (1966) and of 13.0 kcal/mole found by Leng and Felsenfeld (1966) for poly rA.

Interaction Studies. GENERAL CONSIDERATIONS. The data of the oligopolymer interactions presented here were obtained in three solvents: (1) 40 mM potassium phosphate (pH 7.0), (2) 40 mM potassium phosphate (pH 7.0), containing 8 mM $MgCl_2$, and (3) 0.15 M NaCl–0.015 trisodium citrate. These solvents will be referred to subsequently as phosphate buffer, phosphate–magnesium buffer, and SSC, respectively.

In determining the stoichiometry of the various complexes formed in the several solvents used, we have not placed a great deal of faith in mixing curves. It is clear from a variety of studies that mixing curves in this series, studied only from limited spectral data, can lead to ambiguous interpretations. This is due to the fact that complexes that change the spectrum may be detected by complete spectral studies, but complexes that do not change the spectrum cannot be detected. We have, therefore, examined the stoichiometry of complexes by gel filtration whenever some ambiguity was present. It should be emphasized that no single method is completely dependable.[2] For example, mixing and melting studies on complexes formed between polydeoxyadenylate and polydeoxythymidylate demonstrated the formation of $d(A)_n \cdot d(T)_n$ and $d(A)_n \cdot d(T)_n \cdot d(T)_n$. No $d(A)_n \cdot d(A)_n \cdot d(T)_n$ could be detected in these studies. We have, however, detected $d(A)_n \cdot d(A)_n \cdot d(T)_n$ in cesium chloride gradients (Bollum, 1967). The general problem of the detection and stoichiometry of complexes in polynucleotide interactions remains a major problem in understanding polynucleotide structure.

Kinetics of Helix Formation. INTERACTION OF $d(pT)_m$ WITH $d(pA)_n$. The interactions of $d(pT)_m$ with $d(pA)_n$ in mixtures equimolar with respect to monomer concentration produce double-stranded complexes in a rather rapid and simple way. The changes in optical density that accompany the formation of complexes in a 16.8 μM mixture of $d(pT)_{10}$ and $d(pA)_n$ in SSC are complete 30 sec after mixing (data not presented here). This rapid approach to equilibrium may be due to the fact that the temperature of mixing (15°) is not favorable for the formation of a triple-stranded intermediate of the type $d(pT)_m \cdot d(pT)_m \cdot d(pA)_n$.

INTERACTION OF $d(pT)_n$ WITH $d(pA)_n$. A solution of $d(pT)_n$ interacting with an equimolar amount of $d(pA)_n$ in SSC at 23° monitored at 284 mμ exhibits an increase in optical density for about 1 hr after mixing. This phenomenon is produced by the disappearance of a triple-stranded structure (1A·2T) accounting for about 4% of the polymer concentration at zero time. The slow approach to equilibrium is interpreted as due to the rearrangement of the triple-stranded intermediate into the double-stranded form. This case is analogous to the interaction of $(rA)_n$ with $(rU)_n$ studied by Blake and Fresco (1966).

INTERACTION OF $d(pT)_n$ WITH $d(pA)_m$. The interaction of $d(pA)_8$ with $d(pT)_n$ in a mixture equimolar with respect to monomer concentration and in SSC solvent was observed at 284 mμ over a long period of time. An hyperchromic effect was observed that leveled off at about 2 hr after mixing. The hyperchromic effect at this wavelength is an indication of a decrease (melting) in the triple-stranded structure present in the solution. The final solution that results still contains oligomer complexed in two different structures. These structures can

[2] We note also that gel filtration detects the presence of free oligomer, but fails to resolve the composition of complexes where free polymer is present.

TABLE II: The Effect of Oligonucleotide Concentration on the Slope and $T_{1/2}$ of $d(pT)_8 \cdot d(pA)_n$ Melting in SSC.

Total $d(pA)_n$ μM (Monomer)	Total $d(pT)_m$ μM (Monomer)	Free $d(pT)_m$ μM (Oligomer)	$T_{1/2}$ (°C)	Slope ((°C)$^{-1}$)
17	17	1.0	17.75	0.10
27	27	1.6	18.25	0.10
50	50	3.1	20.00	0.09
75	75	4.6	20.60	0.10
17	50	5.2	20.80	0.16
17	75	8.4	21.80	0.16
17	100	12	23.20	0.16
17	200	25	25.00	0.20

be differentiated by measurements at two different wavelengths during melting. In Figure 2 the melting curves observed at 260 and 284 mμ for such a solution are compared with observations on a solution containing only triple-stranded complex. It is evident that in the equimolar mixture (in monomer) of oligo and polymer both the double-stranded, $d(pA)_8 \cdot d(pT)_n$ (first melting step at 260 mμ or decrease in optical density at 284 mμ), and the triple-stranded complexes, $d(pA)_8 \cdot d(pT)_n \cdot d(pT)_n$, (second melting step at 260 and 284 mμ), are present.

The possibility still exists that this mixture of double- and triple-stranded structures does not represent a true equilibrium state. To test this hypothesis an equimolar mixture of $d(pT)_n$ and $d(pA)_8$ in SSC solvent was stored for 3 months at 5°. During storage a sample of 3 ml was periodically withdrawn from the solution and analyzed by melting at 284 and 260 mμ. No difference was observed in the melting profile obtained from samples at various time of storage, either with respect to $T_{1/2}$ or relative quantities of the two complexes present in the mixture. Moreover, the same composition of double- and triple-stranded structures was obtained when a "melted" solution was cooled and remelted several hours later. This experiment supports the idea that double- and triple-stranded structures coexist in equilibrium in the presence of free oligonucleotide.

In cases where the triple-stranded structure constitutes the major complex present, conditions were selected to favor its formation and the thermal stability of this structure was the only one examined. Thus to obtain $T_{1/2}$ values at a known concentration of free oligomer the stoichiometry at mixing was always 1A·2T. These conditions produce solutions containing triple-stranded complexes only.[3]

Oligomer Concentration. A statistical mechanical treatment of oligopolymer interactions by Magee *et al.* (1963, 1965) has shown that the reciprocal of the temperature at the midpoint of the thermal transition ($1/T_{1/2}$, °K^{-1}) is linearly related to the reciprocal of the oligonucleotide chain length ($1/m$) and to

[3] That is to say we avoided conditions containing triple-stranded complexes plus free oligomer, and mixtures of triple-stranded plus small amounts of double-stranded complexes.

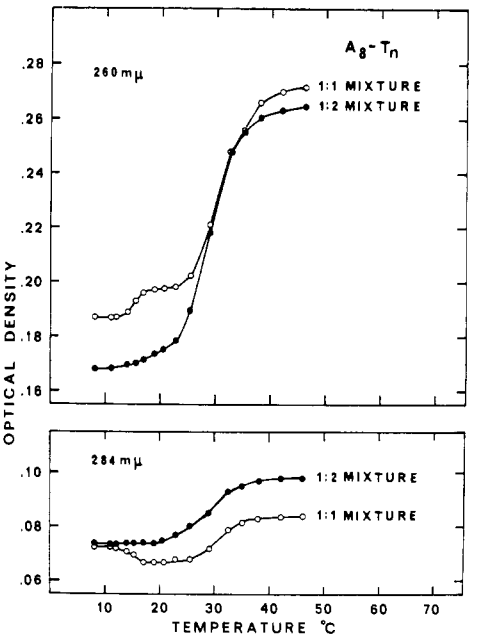

FIGURE 2: Absorbancy–temperature curves at 260 and 284 mμ for $d(pA)_8 \cdot d(pT)_n$ and $d(pA)_8 \cdot 2d(pT)_n$. A mixture containing equimolar amounts of oligomer and polymer nucleotide residues (15 μM) and a mixture containing 2 equiv of polymer/equiv of oligomer (10 μM) are illustrated. The solvent is SSC.

the absolute activity of the oligonucleotide (see eq 19 of Magee *et al.*, 1963). Before undertaking the study of the stability of oligopolymer complexes as a function of oligomer chain length m it is of primary importance to measure the effect of the concentration of free oligomer on the thermal transition at a constant value of m. Moreover at each value of m a particular concentration of oligomer has to be chosen in a way such that the thermodynamic activity is taken constant in the $1/T$ *vs.* $1/m$ relation for all oligomers. This experiment may be carried out two ways: variation of oligomer at fixed polymer concentration or variation of total complex concentration. In Figure 3 the relation of $1/T_{1/2}$ to the logarithm of the concentration of free oligonucleotide at the melting temperature is shown to be linear for the complex $d(pT)_8 \cdot d(pA)_n$ formed by addition of different amounts of oligomer to a constant amount of polymer.[4] In these experiments the slope of the thermal transition is also affected by a change in concentration of free oligomer.

The contribution of the concentration of free oligomer to the stability of a complex was also analyzed in mixtures where the total concentration of the complex was varied. This is useful in the study of those interactions where variations in the concentration of oligonucleotide alone would provoke changes in the stoichiometry of the complex formed. From data shown in Figure 3 it is evident that the dependence of $1/T_{1/2}$ on log C

[4] A referee has called our attention to the relation between log C and ΔH in the form

$$\frac{d \log C}{d\left(\frac{1}{T_{1/2}}\right)} = \frac{-m\Delta H}{R}$$

(see Crothers *et al.* (1965), eq 37). Calculations made on Figures 3 and 4 yield ΔH = 9–9.3 kcal/mole of base pair.

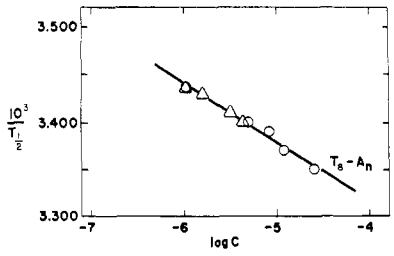

FIGURE 3: Melting temperature as a function of free oligomer concentration at $T_{1/2}$ for d(pT)8·d(pA)$_n$ in SSC solvent. (⊙) Complexes formed by mixing a constant amount of polymer (17 μM calculated on a monomer basis) and variable amounts of oligomer. (△) Complexes formed by mixing oligomer and polymer at constant proportions.

is identical in these two types of experiment for the same oligomer. In this second type of experiment it was noticed that the concentration of free oligomer affects the melting temperature of a thermal transition without affecting the slope. These data are presented in Table II.

The experiments described so far have examined the behavior of double-stranded complexes only. In Figure 4 a plot of $1/T_{1/2}$ *vs.* log C shows that this relationship is linear for triple-stranded complexes also. In these mixtures the ratio of the concentrations of oligomer and polymer was kept constant to avoid changes in the stoichiometry of complexes formed. The concentration of *complexes* was varied.

Thermal Stability of Oligopolymer Complexes. INTERACTION OF d(pT)$_m$ WITH d(pA)$_n$. Information obtained on the stability of complexes formed by mixing d(pT)$_m$ with d(pA)$_n$ in the 1:1 proportion, calculated on the monomer content, is presented as a function of m in Figure 5a–c and Table III. The data in Table III are not analyzed further. They are presented as "typical" data obtained at concentrations easily accessible for optical measurements and provide a body of useful empirical information. The analysis presented in Figure 5a–c is restricted to a more limited set of data in which sufficient concentration studies were carried out so that a comparison of $T_{1/2}$ data could be made at constant oligomer activity, as indicated in the previous section. A variation of ±5% in the value of oligomer concentration was tolerated in this comparison.

As predicted by theory the $1/T_{1/2}$ *vs.* $1/m$ relation in Figure 5 is observed to be linear in the three solvents and at all values of m, and the slope of the line is a function of the thermodynamic activity of oligomers. The following equations are obtained for the three solvents used: (1) phosphate, $10^3/T_{1/2} = 5.25/m + 2.94$; (2) phosphate–$Mg^{2+}$, $10^3/T_{1/2} = 4.65/m + 2.86$; and (3) SSC, $10^3/T_{1/2} = 4.85/m + 2.87$. It should be pointed out that small differences in the values of slopes are not really dependent upon the solvent used, but are probably due to experimental error in the evaluation of oligomer concentrations. These differences are probably not significant with respect to the general theory.

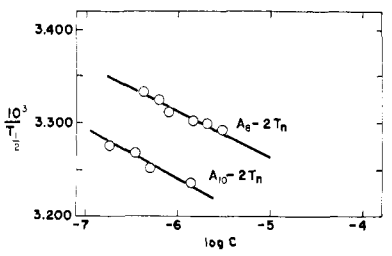

FIGURE 4: Melting temperature as a function of the free oligomer concentration. The values of $T_{1/2}$ for triple-stranded complexes formed by mixing oligomer and polymer in constant proportions but increasing total concentration. Solvent is SSC.

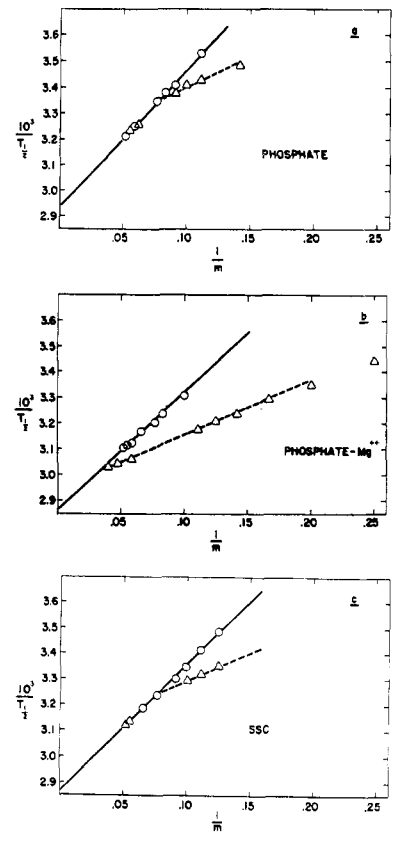

FIGURE 5: Melting temperature *vs.* chain length for oligodeoxyribonucleotide–polydeoxyribonucleotide complexes. Plot of the reciprocal midpoint of the thermal transition ($°K^{-1}$) of complexes formed by mixing oligonucleotides with complementary polymers as a function of the reciprocal of the chain length of the oligonucleotide in three solvents. The solid lines were determined by least squares. (⊙) d(pA)$_m$·d(pT)$_n$ mixtures, (△) d(pA)$_n$·d(pT)$_m$ mixtures.

The stoichiometry of the complexes formed in equimolar mixtures of d(pT)$_m$ with d(pA)$_n$, as examined by mixing curve and by gel filtration, indicated that 1:1 complexes were formed. A mixing curve for d(pT)$_{11}$ and d(pA)$_n$ in phosphate–Mg^{2+} buffer at 10° below the $T_{1/2}$ of the complex is shown in Figure 6. The minimum absorbance at 260 mμ is observed at 50 mole % of polymer and indicates the formation of a double-stranded complex. This stoichiometry was also confirmed by gel filtration and the results are shown in Figure 7. An equimolar mixture of oligomer and polymer is eluted as a single peak from Sephadex G-200, thus showing that when a 1:1 complex is formed the complex is stable to gel filtration (top of Figure 7). When twice as much oligomer as polymer is present in the mixture the optical density elution profile shows two peaks. The first peak represents the complex formed by oligomer and polymer in equal proportion, and the second peak is free

TABLE III: Parameters Characteristic of Oligopolymer Interactions.[a]

		$d(pT)_m + d(pA)_n$									$d(pA)_m + d(pT)_n$								
		Phosphate			Phosphate–Mg^{2+}			SSC			Phosphate			Phosphate Mg^{2+}			SSC		
m	$1/m$	R	$T_{1/2}$	S	R	$T_{1/2}$	S	R	$T_{1/2}$	S	R	$T_{1/2}$	S	R	$T_{1/2}$	S	R	$T_{1/2}$	S
4	0.250													1:2	16.8	0.14			
5	0.200													1:2	25.5	0.14			
6	0.166										1:2	9.5	0.10	1:2	30.4	0.14	1:2	19.5	0.11
7	0.142				1:1	14.5	0.12	1:1	9.0	0.14	1:2	14.0	0.10	1:2	35.7	0.20	1:2	24.0	0.16
8	0.125				1:1	21.0	0.12							1:2	38.6	0.20	1:2	27.5	0.16
9	0.111	1:1	14.0	0.14				1:1	20.8	0.14				1:2	41.5	0.20	1:2	30.2	0.14
10	0.100	1:1	17.0	0.15	1:1	29.2	0.10	1:1	25.8	0.14	1:2	20.0	0.11				1:2	34.0	0.15
11	0.091	1:1	20.5	0.14	1:1	33.6	0.12	1:1	30.0	0.10							1:2	35.4	0.16
12	0.083	1:1	23.0	0.14	1:1	35.7	0.10												
13	0.077	1:1	25.5	0.14	1:1	38.5	0.10	1:1	36.0	0.11									
14	0.071																		
15	0.066	1:1	28.5	0.14	1:1	42.2	0.10	1:1	40.5	0.09									
16	0.062										1:1	33.8	0.10						
17	0.059	1:1	34.3	0.09	1:1	46.8	0.12				1:1	34.3	0.10	1:2	53.5	0.30			
18	0.055	1:1	34.8	0.10	1:1	47.5	0.12				1:1	35.8	0.10				1:1	46.0	0.08
19	0.052	1:1	38.0	0.10	1:1	48.8	0.12	1:1	44.5	0.09							1:1	46.6	0.09
20	0.050										1:1	38.0	0.10				1:1	47.5	0.08
21	0.047										1:1	38.3	0.10	1:2	55.0	0.30			
22	0.045										1:1	40.0	0.10						
23	0.043																		
24	0.041																		
25	0.040										1:1	41.0	0.10	1:2	56.6	0.30			
∞		1:1	63.0	2.25	1:1	73.0	0.25	1:1	71.5	0.25									

[a] R: ratio in which oligomer and polymer are present in the mixture. This ratio also represents the composition of the major complex formed in an equimolar mixture of oligomer and polymer. $T_{1/2}$ is in degrees centigrade. S is the slope of the thermal transition at $T_{1/2}$ $(°C)^{-1}$.

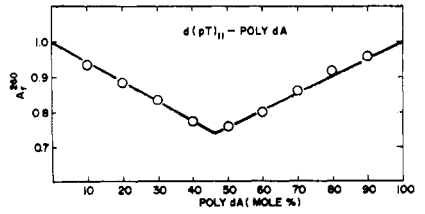

FIGURE 6: Mixing curve for $d(pT)_{11} \cdot d(pA)_n$ complex in phosphate–magnesium buffer at 23°. The total oligomer and polymer concentration expressed as mononucleotide was 26 μM.

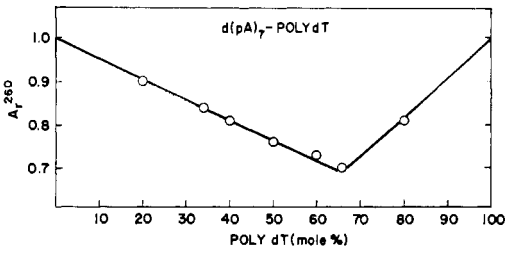

FIGURE 8: Mixing curve of $d(pA)_7 \cdot d(pT)n \cdot d(pT)_n$ in phosphate buffer at 5°. The total nucleotide concentration was 60 μM.

$d(pT)_{11}$. This demonstrates that $d(pT)_{11}$ can only interact with an equimolar amount of polymer.

INTERACTION OF $d(pA)_m$ WITH $d(pT)_n$. The interaction of oligodeoxyadenylate with polydeoxythymidylate is complicated by a change in the stoichiometry of the major complex present in *equimolar* mixtures to 1A:2T when m is smaller than a certain value. Above this critical value of m, double-stranded complexes are formed in *equimolar* mixtures of oligomer and polymer. The critical value of m for the stoichiometry change is characteristic of the solvent used. The interaction of $d(pA)_m$ with $d(pT)_n$ is therefore described separately for each solvent.

Phosphate Buffer. The data obtained in this solvent are plotted in Figure 5a. In the double-reciprocal plot (Figure 5a) a change in slope is observed for $1/T_{1/2}$ of $d(pA)_m + d(pT)_n$ when $m < 12$. These complexes show a stability considerably higher than that of the complexes formed from $d(pT)_m + d(pA)_n$ at equal values of m and at the same concentration of free oligomer. An analysis of the stoichiometry of the complex formed when $m \leq 12$ was carried out by mixing curves and gel filtration. Figure 8 shows a mixing curve, at about 10° below $T_{1/2}$, of the complex formed by $d(pA)_7$ with poly dT. A minimum absorbance at 66 mole % polymer in the mixture is observed at 260 mμ denoting the formation of a triple-stranded structure, $d(pA)_7 \cdot d(pT)_n \cdot d(pT)_n$. At 50 mole % polymer the mixture may contain a pure double-stranded complex or a triple-stranded complex plus free oligomer. An analysis by gel filtration of the mixtures at 50 and 66 mole % of polymer is shown in Figure 9. The mixture at 66 mole % polymer is eluted as a single peak and the melting curve of the complex shows no free oligomer. The mixture at 50 mole % polymer is clearly resolved into two peaks. The complex is present in the first peak and the second peak is free $d(pA)_7$. We thus conclude that the major complex present when $m \leq 12$ is in the triple-stranded form regardless of the proportion at mixing. Complexes formed by mixing $d(pA)_m$ with $d(pT)_n$ in the 1:2 proportion show a single-step melting transition at 260 and 284 mμ. Riley *et al.* (1966) have shown that the double helix–coil transition for A–T base pairs is not detectable at 284 mμ.

When $m > 12$, $d(pA)_m$ interacts with $d(pT)_n$ forming a double-stranded complex in *equimolar* mixture. The identification of these complexes was also achieved by gel filtration, and by mixing and melting at 260 and 284 mμ. These double-stranded complexes are indistinguishable, by melting, from the complexes formed by $d(pT)_m$ with $d(pA)_n$ at the same value of m, and the $T_{1/2}$ values obtained from $d(pA)_m$ and $d(pT)_n$ interactions with the complementary polymer fit on the same line (Figure 5a). Figure 10 shows thermal transition curves for this analogous set of double-stranded complexes, $d(pA)_{18} \cdot d(pT)_n$ and $d(pT)_{18} \cdot d(pA)_n$, formed under identical conditions.

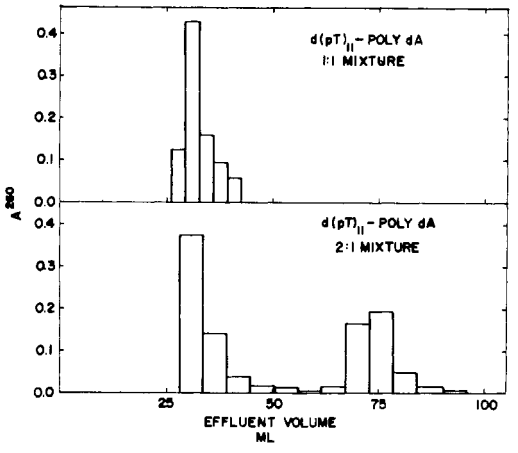

FIGURE 7: Gel filtration of $d(pT)_{11} \cdot d(pA)_n$ complex on Sephadex G-200. The equimolar mixture contained 0.3 μmole of oligomer and 0.3 μmole of polymer. The mixture of 66 mole % of oligomer contained 0.6 μmole of $d(pT)_{11}$ and 0.3 μmole of $d(pA)_n$. The column was developed with phosphate–magnesium buffer at room temperature.

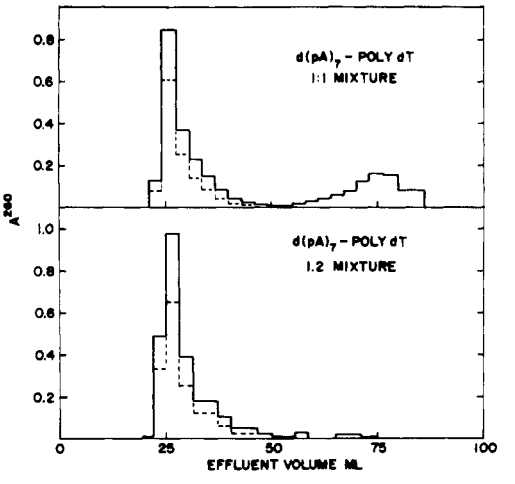

FIGURE 9: Gel filtration of the $d(pA)_7 \cdot d(pT)_n \cdot d(pT)_n$ complex on Sephadex G-200. The equimolar mixture contained 0.55 μmole of $d(pA)_7$ and 0.55 μmole of $d(pT)_n$. The mixture at 66 mole % polymer contained 0.37 μmole of $d(pA)_7$ and 0.74 μmole of $d(pT)_n$. The column was developed with phosphate buffer at 5°. Broken and solid lines represent the absorbance at 260 mμ at 5 and 23°, respectively.

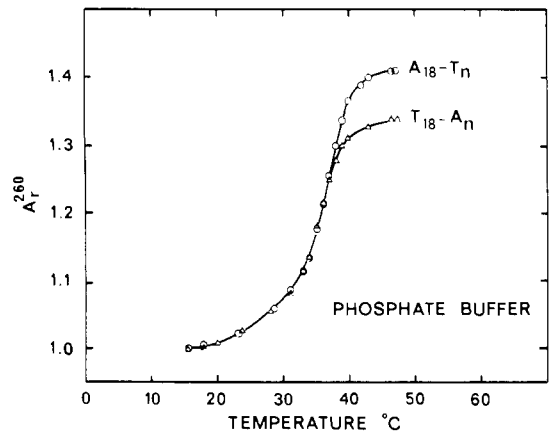

FIGURE 10: Relative absorbancy–temperature curves for $d(pA)_{18} \cdot d(pT)_n$ and $d(pT)_{18} \cdot d(pA)_n$ in phosphate buffers. The free oligomer concentration was 0.43 μM at $T_{1/2}$.

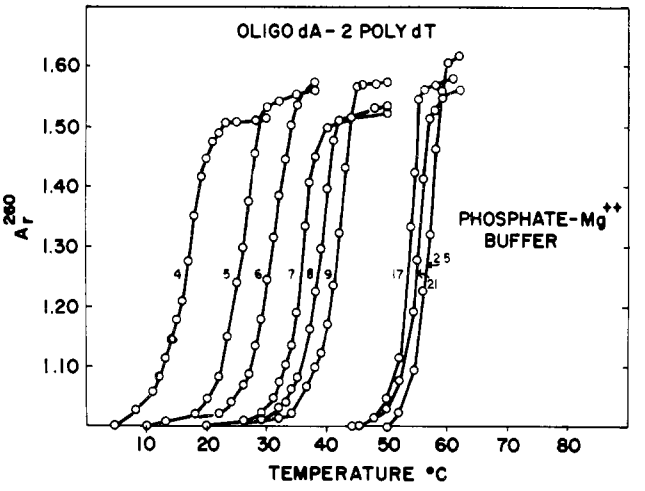

FIGURE 11: Relative absorbancy–temperature curves for $d(pA)_m \cdot d(pT)_n \cdot d(pT)_n$. Complexes were formed at 66 mole % polymer in 40 mM potassium phosphate (pH 7) containing 8 mM $MgCl_2$ and melted in the same solvent. The value of m for each complex is indicated by the small number on the side of every thermal profile. The mean oligonucleotide concentration was 8 μM expressed as mononucleotide.

Phosphate–Magnesium Buffer. The addition of 8 mM $MgCl_2$ to the phosphate buffer stabilizes the formation of triple-stranded complexes. All of the oligonucleotides studied with $m = 4$–25 interact with polydeoxythymidylate, forming $d(pA)_m \cdot d(pT)_n \cdot d(pT)_n$ even in equimolar mixtures. None of the $d(pA)_m + d(pT)_n$ interactions show a value of $T_{1/2}$ identical with the $d(pT)_m$, $d(pA)_n$ interactions (Figure 5b). The stoichiometry of the complexes examined by mixing curve or by gel filtration was found to be 1A:2T. A series of melting curves for mixtures at 66 mole % polymer are shown in Figure 11. An increase in the slope of the thermal transition is seen as m increases.

0.15 M NaCl–0.015 M Trisodium Citrate (SSC). In this solvent, at moderate sodium ion concentration (0.195) M), the interactions of $d(pA)_m$ with $d(pT)_n$ present the same general picture already observed in the phosphate buffers (0.065 M K^+). At $m \leq 16$ the major complex formed in *equimolar* mixtures is of the 1A:2T type and at $m > 16$ a double-stranded complex is formed. The results obtained for this interaction are plotted in Figure 5c.

INTERACTION OF $d(pT)_{10}$ WITH $r(pA)_n$. In our study of the interaction of $d(pT)_m$ with polydeoxyadenylate at low or medium salt concentrations, or in the presence of Mg^{2+}, we have always observed formation of double-stranded complexes in equimolar mixtures of oligomer and polymer. The interaction of $d(pT)_m$ with polyriboadenylate results in formation of triple-stranded complexes according to observations with $d(pT)_{11-13}$ by Rich (1960) and with $d(pT)_{10}$ by Naylor and Gilham (1966). In Figure 12 polydeoxyadenylate and polyriboadenylate are shown to form different complexes with $d(pT)_{10}$ under identical conditions at 66 mole % of decathymidylate. A triple-stranded complex is formed with the ribopolymer and a double-stranded complex is formed with the deoxypolymer. This result adds another aspect to the different physicochemical properties of polydeoxyadenylate and polyriboadenylate (*cf.* Ts'o *et al.*, 1966).

Discussion

Oligopolymer complexes in the deoxy series have been studied with regard to the following aspects: (a) stoichiometry of complexes formed by mixing constituents in various proportions; (b) kinetics of helix formation for different complexes; and (c) thermal stability of complexes formed.

The Stoichiometry of Complexes Formed by Mixing Solutions of Oligomer and Polymer Are Not Immediately Predictable from the Proportion of Constituents at Mixing. The temperature, the salt concentration, the presence or absence of divalent ions, the chain length and concentration of free oligomer, the chemical nature of the base pair involved, and the proportions at mixing are factors that affect the composition of complexes present at equilibrium in oligopolymer mixtures. The interaction of $d(pT)_m$ with poly dA is the least complex of the pair of interactions in the three solvents studied. When the constituents are mixed in equal proportions stable double-stranded complexes are rapidly formed. When the polymer is mixed with a double amount of oligomer, triple-stranded complexes containing one polymeric chain and two equivalent oligomeric chains are also formed at a permissive temperature. These structures are less stable than the double stranded.

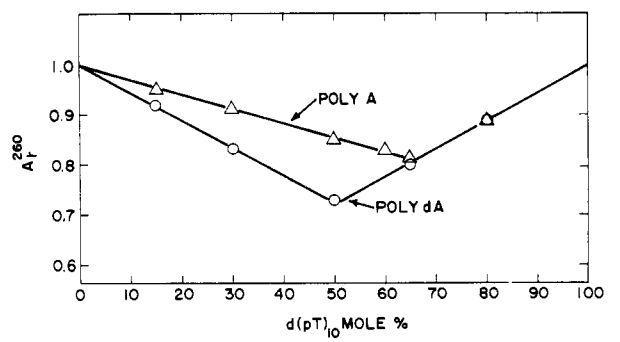

FIGURE 12: Complexes of $d(pT)_{10}$ with polydeoxyriboadenylate and polyriboadenylate. Samples were mixed in 1.0 M LiCl–0.01 M cacodylate buffer, pH 6.8, at 23°. The total nucleotide concentration was 60 μM.

The following reactions occur at melting: $d(pA)_n \cdot d(pT)_m \cdot d(pT)_m \rightarrow d(pA)_n \cdot d(pT)_m + d(pT)_m \rightarrow d(pA)_n + 2\, d(pT)_m$.

The second case examined, the interaction of $d(pA)_m$ with poly dT, is very complicated. The difference in behavior between oligoadenylates and the oligothymidylates is primarily due to the chemical differences between adenine and thymine with respect to the ability to form hydrogen-bonded structures with each other. Under our experimental conditions structures formed by hydrogen bonding of two thymines with one adenine are formed but structures formed by two adenine with one thymine are not. Thus the structure formed by two strands of polydeoxythymidylate and one chain of oligodeoxyadenylate does not have correspondence in the oligodeoxythymidylate series. Triple-stranded structures of this kind are more stable than double-stranded structures formed by oligodeoxyadenylates with poly dT. These anomalous properties may provide explanation for the fact that displacement reactions in which a triple-stranded complex rearranges into double stranded in the presence of free oligomer occurs to a limited extent only. This was shown, as an example, in Figure 2 under conditions that are very likely to represent an equilibrium state. More detailed studies on the kinetics of formation of oligopolymer complexes are necessary to understand the details of the approach to equilibrium. In the anomalous case, oligodeoxyadenylates mixed with a double amount of poly dT produce triple-stranded complexes. Depending upon the oligomer chain length this structure will undergo a single- or a double-step melting. The critical value of m at which one form is converted into the other is defined by the choice of solvent.

The Thermal Stability of an Oligopolymer Complex Depends Mainly upon Two Parameters: the Concentration of Free Oligomer and the Oligomer Chain Length. In Figures 3 and 4 the reciprocal of the $T_{1/2}$ for oligopolymer complexes is expressed as a function of the concentration of free oligomer at a constant value of m. The slope of the line is characteristic of the magnitude of m. As the value of m increases the slope of the line becomes negligible. For small values of m the effect of the concentration of free oligomer on the $T_{1/2}$ of the complex is such that inaccuracy in the determination of the oligomer concentration is a serious limitation to the validity of the results of the study.

The concentration of free oligomer also has a detectable effect on the slope of thermal transition. A very high concentration of *free* oligomer is required for a perfectly sharp transition. It should be obvious that this imposes certain experimental limitations on the present kind of study.

The relationship between melting temperature and oligomer chain length has been shown to be linear for double- and triple-stranded complexes at a constant value of the thermodynamic activity of oligomers in the double-reciprocal plots of Figure 5. The slope of the line relating stability to oligomer chain length exclusively depends upon the activity of the oligomer at the melting temperatures of complexes and does not depend upon the solvent used. The stoichiometry of complexes formed is, however, dependent upon the chain length of the oligomer *and* upon the solvent. In the double-reciprocal plot this change in stoichiometry is represented by a line for triple-stranded complexes having a slope different from that of the line for double-stranded complexes.

The results presented above describe some rather important aspects of oligopolymer interaction that have not always received adequate attention. The results and discussion here could also undergo further experimental refinement. But within these limitations, and the further restriction to only one kind of base pair, the data recorded here should allow some calculations of the changes in free energies of stacking and hydrogen bonding involved in the process of helix–coil formation.[4]

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