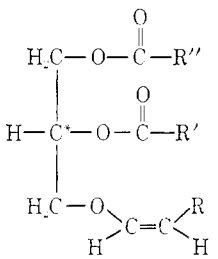
ppm which is considered to be characteristic of the acylated H_2CO group of glycerol.[14] Additional signals are: a triplet at 0.89 ppm, accounting for the terminal methyl groups; a single peak at 1.28 ppm, associated with the internal methylene groups of the aliphatic chains; a triplet at 5.37 ppm, representing the isolated olefinic groups; and an apparent doublet corresponding to the CH_2 groups in the position α to the carbon double bonds centered at 2.03 ppm. The doublet which appears at 2.27 ppm can be correlated with an α-CH_2 group of the acyl functions. The apparent doublet occurring at 3.83 ppm can be assigned to the CH_2O group of glycerol connected to the ether linkage. The signal of the acylated HCO group of glycerol near 5.17 ppm is partially obscured by the triplet representing the isolated carbon double bonds. A signal near 2.9 ppm, characteristic of a methylene group between two double bonds,[14] is absent. Approximately two out of three aliphatic chains per molecule are monounsaturated.



Chemical reactions, specific optical rotations, and spectroscopic data prove that the fraction (1) isolated from the liver of *Hydrolagus colliei* consisted of D(+)-1-O-*cis*-alk-1′-enyldiglycerides.

(14) C. Y. Hopkins in "Progress in the Chemistry of Fats and Other Lipids," Vol. 8, R. T. Holman, Ed., Pergamon Press, New York, N. Y., p 213.

H. H. O. Schmid, W. J. Baumann, H. K. Mangold
University of Minnesota, The Hormel Institute
Austin, Minnesota 55912
Received June 30, 1967

Oligodeoxynucleotide–Polydeoxynucleotide Interactions. Adenine–Thymine Base Pairs[1]

Sir:

Quantitative data on the interaction of structurally defined oligonucleotides with complementary polynucleotides are of value in practical and theoretical studies on nucleic acid structure. An investigation of oligodeoxynucleotide–polydeoxynucleotide interactions, covering a broad range of chain lengths in both members of the interacting pairs, displays partial confirmation of theory and some anomalous behavior.

A series of oligodeoxynucleotides, $d(pA)_m$ and $d(pT)_m$[2] with m = 2–25, was prepared by DNase I degradation of $d(pA)_n$ and $d(pT)_n$ followed by chromatography on diethylaminoethylcellulose.[3] The melting behavior of the complexes $d(pA)_m + d(pT)_n$, $d(pA)_n + d(pT)_m$, and $d(pA)_n + d(pT)_n$, formed by mixing the constituents in a suitable solvent, was then observed by ultraviolet absorbance changes. The stoichiometry of the interactions was determined from mixing curves or (more directly) by gel filtration on Sephadex G-200.[4]

The interaction of $d(pA)_n$ with $d(pT)_m$ follows the relationship $1000/T_{1/2} = A + B/m$ and the stoichiometry is 1:1 in equimolar mixtures in all solvents used (Table I). This interaction is a multimolecular process

Table I. Melting Transitions for Oligodeoxythymidylate–Polydeoxyadenylate Complexes[a]

Solvent	Oligo-P concn, $M \times 10^5$	A	B
NaCl, 0.15 M; sodium citrate, 0.015 M (SSC)	14.0	2.88	4.57
	55.0	2.88	4.13
Potassium phosphate, 40 mM, pH 7	13.5	2.97	4.78
Potassium phosphate, 40 mM, pH 7, with 8 mM $MgCl_2$	14.5	2.88	4.26

[a] The relationship, $1000\,T_{1/2} = A + B/m$ is observed. The values in the table are A and B for this equation, in different solvents and at different oligodeoxynucleotide concentrations (expressed in nucleotide residues). Polymer is present in an equivalent amount.

and thus $T_{1/2}$ values[5] (Table I) and the slope of the transition are quite sensitive to oligonucleotide concentration. These results are in accord with statistical thermodynamic treatment of oligo and polymer interactions.[6]

The interaction of equimolar amounts of $d(pA)_m$ with $d(pT)_n$ in SSC is complicated by a change in the stoichiometry of the *major* complex present to 1A–2T when $m < 16$. The double-stranded (1:1) complex is not detected in the interaction involving $d(pA)_7$, but it is clearly evident as an early rise in absorbance at 260 mμ (or a decrease at 284 mμ) when $d(pA)_m$, m = 8–12, complexes with $d(pT)_n$ are melted. The presence of complexes of different stoichiometry in the mixture could result from a slow approach to equilibrium. Repeated analysis on an equimolar mixture of $d(pA)_8$ and $d(pT)_n$ over a period of 3 months demonstrated that the amounts of double helix and triple helix, detected by melting, remain unchanged. We conclude that an equilibrium mixture of double and triple helices are present in this case. The triple-stranded complexes $d(pA)_m$–$2d(pT)_n$ (with $m < 16$), obtained by mixing the

(1) Supported by Grant CA-08487 from the U. S. Public Health Service.

(2) Abbreviations used: m, oligonucleotide chain length; n, a polymer chain length greater than 300 nucleotides; $T_{1/2}$, temperature at the midpoint of the ultraviolet thermal transition in degrees Kelvin; $d(pA)_m$ 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.

(3) F. J. Bollum, "Procedures in Nucleic Acid Research," G. Cantoni and D. Davies, Ed., Harper and Row, Inc., New York, N. Y., 1966, pp 577–592.

(4) Separations were made on a 1 × 100 cm column on Sephadex G-200 equilibrated with the appropriate solvent and developed at a temperature about 15° below the $T_{1/2}$ of the complex to be tested. Detailed results will be presented in a future publication.

(5) M. N. Lippsett, L. A. Heppel, and D. F. Bradley, *Biochim. Biophys. Acta* **41**, 175 (1960), have observed the dependence upon the total nucleotide concentration in a study of the interactions of $r(pA)_m$ with poly rU.

(6) (a) W. S. Magee, Jr., J. H. Gibbs, and B. H. Zimm, *Biopolymers*, 1, 133 (1963). Note that the authors have called attention to the dependence on absolute activity of the oligonucleotide in eq 19, and in accordance with their treatment our $B = B'$ ln CD, where B' = slope at unit activity, C = absolute activity of the m-mer, and D = the ratio of two internal partition functions. We assume that the absolute activity is essentially constant for all m-mers at the nucleotide residue concentration used (15×10^{-6} M). (b) W. S. Magee, Jr., J. H. Gibbs, and G. F. Newell, *J. Chem. Phys.*, **43**, 2115 (1965).

constituents in 1:2 proportion, undergo a single-step thermal transition observed at 260 and 284 mμ. The latter wavelength is specific for the triple strand melting.[7] Values of $1/T_{1/2}$, when $m = 6$–9, for an oligonucleotide residue concentration of 10 μM do follow a linear relationship, but $A = 3.05$ and $B = 2.20$ (Figure 1). The 1:1 complex for equimolar mixtures of $d(pA)_m$ and $d(pT)_n$ is completely formed only when $m > 16$ in SSC, and these double-stranded complexes have $T_{1/2}$ similar (within the range of our experimental error, ±0.5°) to those formed by $d(pA)_n$ and $d(pT)_m$ at equal values of m.[8] $1/T_{1/2}$ values now fit on the same line (Figure 1). The anomalous stoichiometry described above for a Na^+ concentration of 0.19 M (SSC) is also exhibited in 40 mM phosphate buffer, pH 7.0, with or without the presence of 8 mM $MgCl_2$ (data not presented here).[9]

The over-all reactions that may be postulated to occur in our studies are given in eq 1–3 (major complex at equilibrium is underlined) when equimolar amounts of

$$d(pA)_n + d(pT)_n \rightleftharpoons \underline{d(pA)_n\text{-}d(pT)_n} \rightleftharpoons d(pA)_n\text{-}2d(pT)_n + d(pA)_n \quad (1)$$

$$d(pA)_n + d(pT)_m \rightleftharpoons \underline{d(pA)_n\text{-}d(pT)_m} \rightleftharpoons d(pA)_n\text{-}2d(pT)_m + d(pA)_n \quad (2)$$

$$d(pA)_m + d(pT)_n \rightleftharpoons \underline{d(pA)_m\text{-}d(pT)_n} \rightleftharpoons \underline{d(pA)_m\text{-}2d(pT)_n} + d(pA)_m \quad (3)$$

reactant residues are mixed. Reaction 1 is analogous to the interaction of rA with rU because stable 1:1 and 1:2 complexes are formed at equilibrium when the two polymers are mixed in the 1:1 and 1:2 proportion. The kinetics of the approach to the equilibrium in the equimolar mixture of the two polymers are also similar because the complex $d(pA)_n$-$2d(pT)_n$, detected by differential spectroscopy at 284 mμ, is a transient form as in the case of rA–rU formation.[10] In reaction 2 the triple-stranded complex is not formed in equimolar mixture at $m < 11$ (probably because the conditions are not permissive), and we find that equilibrium is reached a few seconds after mixing. We anticipate that as m increases 1A–2T complexes will be observed in 1:1 and 1:2 mixtures. In reaction 3 we notice that the ability of $d(pA)_m$ to compete with $d(pT)_n$ held in the three-stranded configuration increases with increasing value of m. The equilibrium point for this reaction at $m < 16$ (SSC) is shifted toward the triple-stranded helix.

All of this discussion can be summed up by noting that an extrapolation of the $T_{1/2}$ line for triple-stranded complex to the $T_{1/2}$ axis would cross the line for two-stranded complexes (Figure 1). This means that there will be a region of m where three-stranded complexes are more stable than two-stranded complexes. The melting transitions here will be three → one. There is also a region where three-stranded complexes are less stable than two-stranded complexes. Here melting transitions will be three → two → one.

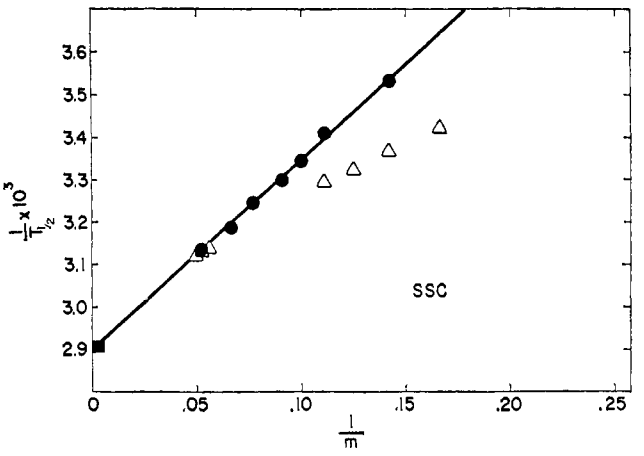


Figure 1. Melting temperature *vs.* chain length for oligodeoxynucleotide-polydeoxynucleotide complexes. Plot of the reciprocal midpoint of the thermal transition (in degrees Kelvin) of complexes formed by mixing oligodeoxynucleotides with complementary deoxypolymers as a function of the reciprocal chain length of the oligodeoxynucleotide. The total nucleotide residue concentration of the mixtures was 30 μM. The line was determined by least squares: ●, $d(pA)_n$-$d(pT)_m$ mixtures; △, $d(pA)_m$-$d(pT)_n$ mixtures; ■, $d(pA)_n$-$d(pT)_n$ mixture.

The value of m at the cross point is also related to the solvent used. We anticipate that there should be a low salt concentration where all $d(pA)_m$ form a double-stranded complex with $d(pT)_n$ in equimolar mixture, and a salt concentration (*ca.* >1.0 M) where all the $d(pA_m)$ or $d(pA)_n$ form a triple-stranded complex with $d(dT)_n$.

The complexities observed in the present case require that the contribution of the chain length to the stability of oligo–polymer interactions can be evaluated only after careful determination of the stoichiometry of the complexes formed. In the "critical region," where stoichiometry values are changing, the exact molecular stoichiometry will be indeterminate.

(11) Department of Genetics, University of Pavia, Pavia, Italy.

G. Cassani,[11] F. J. Bollum
Department of Biochemistry
University of Kentucky, Lexington, Kentucky
Received May 4, 1967

(7) M. Riley, B. Maling, and M. J. Chamberlin, *J. Mol. Biol.*, **20**, 359 (1966); F. J. Bollum, unpublished data, 1965.

(8) A. M. Michelson, *Boll. Soc. Chem. Biol.*, **47**, 1553 (1965), has shown that complexes of different stability are formed when $r(A)_m$ or $r(U)_m$ of the same chain length are mixed with the complementary ribopolymer, but the stoichiometry of the complexes formed was not discussed.

(9) The choice of solvents may be considered arbitrary, but in fact the Mg^{2+} phosphate buffer is used for the enzymatic replication of homopolydeoxynucleotides. This investigation was a result of problems encountered in the replication study.

(10) R. D. Blake and J. R. Fresco, *J. Mol. Biol.*, **19**, 145 (1966).

Di-*t*-butyl Trioxide and Di-*t*-butyl Tetroxide

Sir:

Kinetic, tracer, and product studies of autoxidation[1-4] support the sequence of reactions 1–4 as controlling chain termination. Experimental observations

$$2RO_2\cdot \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} RO_4R \quad (1)$$

$$RO_4R \xrightarrow{k_2} [RO\cdot O_2\cdot OR] \text{ (in solvent cage)} \quad (2)$$

$$[RO\cdot O_2\cdot OR] \xrightarrow{k_3} O_2 + ROOR \quad (3)$$

$$[RO\cdot O_2\cdot OR] \xrightarrow{k_{diff}} O_2 + 2RO\cdot \quad (4)$$

(1) H. S. Blanchard, *J. Am. Chem. Soc.*, **81**, 4548 (1959).
(2) P. D. Bartlett and T. G. Traylor, *ibid.*, **85**, 2407 (1963).
(3) J. R. Thomas, *ibid.*, **87**, 3935 (1965).
(4) G. A. Russell, *ibid.*, **79**, 3871 (1957).