since ^1H–^{31}P cross peaks arising from the four-bond coupling between the phosphorus and the ortho protons have been readily observed by us for the free amino acid.

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Supplementary Material Available: Figures 1 and 2 containing proton projections of 2D ^1H–^{31}P HMQC data for 0.12 M phosphothreonine and 0.1 M phosphoserine in D_2O, respectively (3 pages). Ordering information is given on any current masthead page.

Cationic Oligonucleotides

Robert L. Letsinger,* Charles N. Singman, Gary Histand, and Manikrao Salunkhe[†]

Department of Chemistry, Northwestern University
Evanston, Illinois 60208

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The reversible association of oligonucleotides with complementary sequences is of basic importance in biotechnology. With the objective of gaining further understanding and control of hybridization we have synthesized and examined oligonucleotide analogues **1**, **2**, and **3**, which in neutral or acidic solution carry positive charges along the backbone. We present evidence here that these novel analogues bind to complementary oligonucleotides and show that the extent of interaction can be tuned selectively by changes in salt concentration and pH. Under appropriate conditions the cationic probes bind more effectively than their natural counterparts to single stranded polydeoxyribonucleotide sequences.

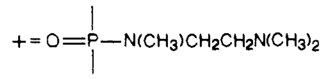
d(T+T+T+T+T+T+T+T+T+T) **1**

d(T-T+T-T+T-T+T-T+T) **2**

d(TxTxCxTxGxAxAxAxAxT) **3**

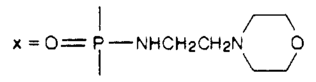
d(TTCTGAAAAT) **3a**

d(ATTTTCAGAATTGGG) **4**



$$+ = O{=}P{-}N(CH_3)CH_2CH_2N(CH_3)_2$$

– = a natural phosphodiester link



The oligomers were synthesized[1] on solid supports in syringes[2] or a Biosearch 8600 DNA synthesizer. Pertinent characterization data are given in Table I. Hydrolysis of **1** and **2** with 88% aqueous formic acid (95° 30 min) afforded dT_{10} and dT_9, respectively. Oligomers **1**, **2**, **3** were resistant to snake venom phosphodiesterase, spleen phosphodiesterase, and P1 nuclease.

Table I. Properties of Oligonucleotides

compd	HPLC, elution time, (min)[a]	PAGE[b] Rm	^{31}P NMR[c]
1	8.2[d]	+1.2	+11.05
2	19.0	0	+10.37; –0.81
3	21.5	+0.90;[b] +0.1[e]	+10.7
dT_{10}	13.6	–0.86	
dT_9	13.6	–0.90	
3a	11.7	–0.87	

[a] Hewlett Packard RP-C18 column (10 cm) (except for **1**, see *d*); 0.1 M Et_3NHOAc (pH 7.0), 1%/min MeCN gradient starting at 0% MeCN; 0.5 mL/min flow rate. [b] Polyacrylamide gel electrophoresis; + indicates migration toward anode in 12% cross-linked gel, pH 5.0; Rm is distance relative to methylene blue; – indicates migration toward cathode in 20% cross-linked gel, pH 8.0, relative to bromophenol blue. [c] ppm relative to 85% H_3PO_4; + is downfield; D_2O solvent. [d] Beckman RPSC C-3 column (5 cm); 0.1 M Et_3NHOAc buffer, pH 5.6; 1%/min MeCN gradient starting at 5% MeCN; 1 mL/min flow rate. [e] pH 7.0.

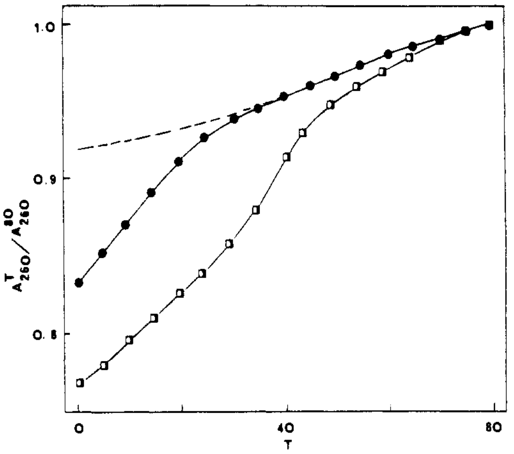


Figure 1. Absorbance profile at pH 7.1 (0.01 M Tris buffer): **1** + polydA (2T/dA) in 1 M NaCl (●), 0.1 M NaCl (◨); calculated for **1** + polydA (2T/dA) from heating curves of separated samples of **1** and polydA, assuming no interaction of the oligomers (- - -).

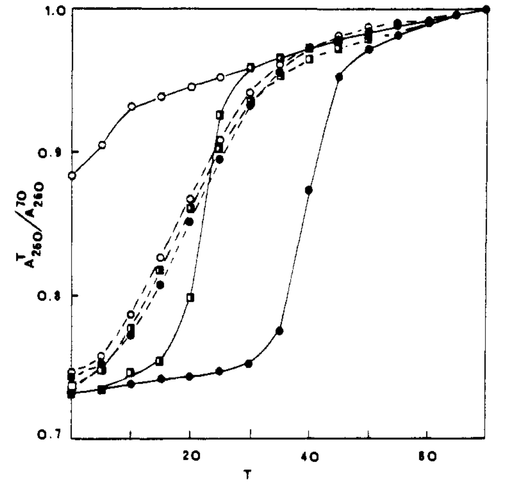


Figure 2. Absorbance profile at pH 7.0 (0.01 M Tris buffer): **2** + polydA (2T/dA) in 1.0 M NaCl (- - ● - -), 0.1 M NaCl (- - ◨ - -), no NaCl (- - ○ - -); dT_9 + polydA (2T/dA) in 1.0 M NaCl (–●–), 0.1 M NaCl (–◨–), no NaCl (–○–).

Hybridization was assayed by changes in A_{260} as a function of temperature. As shown in Figure 1, the absorbance of a solution of **1** and polydA in 0.1 M NaCl at 0 °C is strongly reduced relative to that calculated for non-interacting oligomers. This hypochromic effect and the "melting out" on heating are indicative of a complex with ordered stacking of the bases. In 1.0 M NaCl the complex is relatively unstable. This response to changes in salt concentration is the reverse of that found for conventional nucleotide duplexes and is consistent with expectation for a complex stabilized by attraction of oppositely charged ions. Comparable experiments with **1** + polydT and **1** + polyrA served as useful negative controls. Essentially no hypochromic effect was observed, suggesting that specific hydrogen bonding is needed as well as ionic attraction and that bulky substituents at phosphorus interfere in formation

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[†] On study leave from the Department of Chemistry, Shivaji University, Kolhapur 416004, India.

[1] Methyl phosphoramidite chemistry[3] was used in generating phosphodiester links and hydrogen phosphonate chemistry[4] followed by oxidative coupling[5] with the appropriate diamine was used in generating the aminoalkylphosphoramidate links. Standard deprotection conditions were employed.

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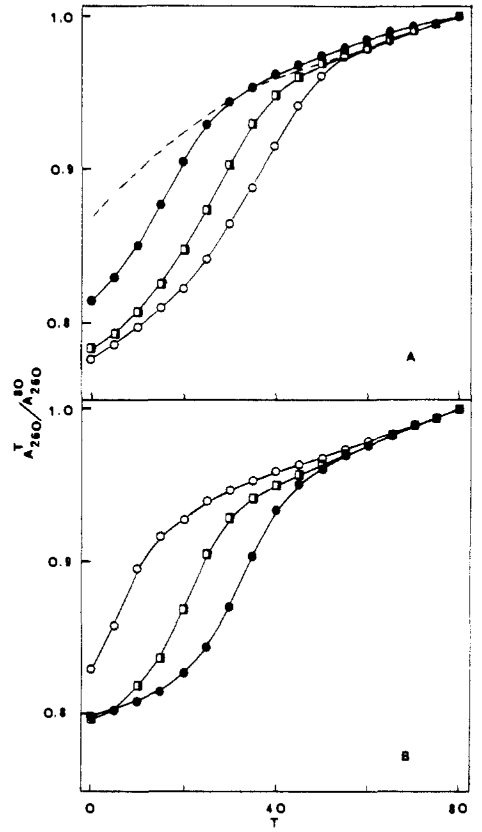

Figure 3. Absorbance profile at pH 5.6 (0.01 M phosphate buffer): A, **3** + **4** (1:1 mole equivalents) in 1 M NaCl (●), 0.1 M NaCl (◨), no NaCl (○); B, **3a** + **4** (1:1 mole equivalents) in 1 M NaCl (●), 0.1 M NaCl (◨), no NaCl (○). The dashed line in A gives absorbance data for **3** + **3a** (1:1 mole equivalents) at pH 5.6, no NaCl.

of hybrids involving ribonucleotide polymers.

An ability to modulate sensitivity to changes in ionic strength by controlling the ratio of plus and minus charges in the probe is demonstrated by the experiments with **2** (Figure 2). In this case, in which the charges in the probe are balanced, binding to polydA is essentially independent of the salt concentration.

The most revealing data are those for interaction of the mixed-base probe, **3**, and the natural counterpart, **3a**, with target **4**. Use of the less hindered anchoring moiety (–NH– in place of $-N(CH_3)-$) favors binding and the weaker basic group (morpholino in place of dimethylamino) allows greater control over protonation. Thermal dissociation curves (pH 5.6) are sigmoidal and clearly demonstrate reversal in the effect of salt concentration on binding affinity (Figure 3). With an increase in concentration of NaCl from 0 to 0.1 to 1.0 M, T_m[6] values for the complex of cationic analogue **3** decreased from 32.5° to 27.5° to 15° whereas the T_m values for the complex of oligonucleotide **3a** increased from <10° to 22.5° to 38°. The difference in affinities of the cationic and natural probes at low salt concentration is especially striking. Lack of significant interaction in a control experiment with an equimolar mixture of **3** and **3a** (non-complementary oligomers) confirmed that proper base pairing as well as electrostatic attraction is necessary for formation of a stable hypochromic complex from the cationic and anionic oligomers.

In addition, we found that the binding properties of the morpholino probe could be selectively influenced by pH changes. Thus, an increase in pH to 7.0 had little effect on the affinity of **3a** for **4** (T_m < 10° in 0 M NaCl, T_m 20° in 0.1 M NaCl) but strongly destabilized the complex between **3** and **4** (T_m 17° in 0 M NaCl, T_m 12° in 0.1 M NaCl). This effect reflects a low extent of protonation of the morpholino groups at pH 7 (see electrophoresis data in Table I).

These findings open new possibilities for designing oligonucleotide probes and may have relevance in controlling processes in biochemical and biological systems.

Acknowledgment. This research was supported by Grants GM10265 and AI24846 from the National Institutes of Health.

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[6] T_m specifies the temperature corresponding to the midpoint of the region of maximum slope in the plot of A_{260} vs temperature.

Tautomerization Involving M-H-M and C-H-M Interactions in Capped Trimetal Clusters. Promotion of the C-H-M Interaction in a Mixed-Metal Cluster

Reynaldo D. Barreto and Thomas P. Fehlner*

Department of Chemistry, University of Notre Dame
Notre Dame, Indiana 46556

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Agostic hydrogens, hydrogens bridging carbon and transition-metal centers, are now well-known structural features of mono- and polynuclear transition-metal compounds.[1,2] Although the factors promoting an E–H–M interaction (E = main group atom) in the capped trimetal cluster system are complex,[3] it is self-evident from a comparison of $Fe_3(CO)_9EH_n$, E = C, n = 4, and E = B, n = 5, that the effective nuclear charge difference between the metals and the capping atom is one factor that plays a large role in the formation E–H–M interactions.[4] Hence, we have sought isoelectronic clusters with endo hydrogens, which differ only in the identity of the metal atoms.

The $Fe_3(CO)_9CH_3R$ (I), R = H, cluster exists as three tautomers in solution: $(\mu\text{-H})_3Fe_3(CO)_9CR$ (Ia); $(\mu\text{-H})_2Fe_3(CO)_9(\mu_3\text{-HCR})$ (Ib); and $(\mu\text{-H})Fe_3(CO)_9(\mu_3\text{-H}_2CR)$ (Ic) in the relative abundances 16:3:1 at 20 °C.[5] On the basis of a comparison of system I with the isoelectronic ferraborane, we would expect that replacing one or two FeH units with Co would favor tautomer Ib over Ia.[6] Indeed neutral mixed metal clusters $FeCo_2(CO)_9CHR$ (II), R = Me, Et, and Ph, are known.[7] In contrast to our prediction, the single endo hydrogen has been assigned a position associated with the trimetal face on the basis of ^1H NMR

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