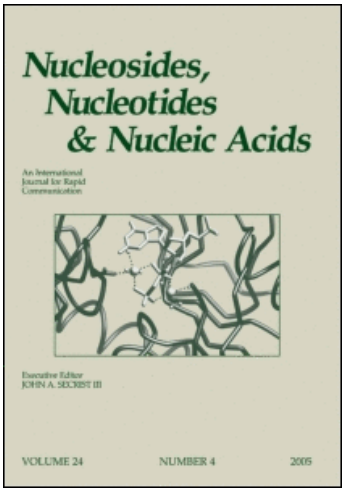

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Stabilization of Deoxyadenosine/polynucleotide Complexes by 3',5'-Di-*O*-Benzoyl Substituents

S. K. Chaturvedi[a]; R. L. Letsinger[a]
[a] Department of Chemistry, Northwestern University, Evanston, IL, USA

To cite this Article Chaturvedi, S. K. and Letsinger, R. L.(1998) 'Stabilization of Deoxyadenosine/polynucleotide Complexes by 3',5'-Di-*O*-Benzoyl Substituents', Nucleosides, Nucleotides and Nucleic Acids, 17: 1, 585 — 592
To link to this Article: DOI: 10.1080/07328319808005201
URL: http://dx.doi.org/10.1080/07328319808005201

NUCLEOSIDES & NUCLEOTIDES, 17(1-3), 585-592 (1998)

STABILIZATION OF DEOXYADENOSINE/POLYNUCLEOTIDE COMPLEXES BY 3', 5'-Di-*O*-BENZOYL SUBSTITUENTS[1#]

S. K. Chaturvedi and R. L. Letsinger*

Department of Chemistry, Northwestern University, Evanston, IL, 60208, USA

ABSTRACT: 3',5'-Di-*O*-benzoyldeoxyadenosine forms stable dT:dA:dT type complexes with poly(dT) and an oligo(dT)-cholesteryl conjugate in dilute aqueous solution. The benzoyl groups play an essential role in stabilizing these"triple stranded"complexes.

INTRODUCTION

The dinucleotide blocks, pApA,[2] ApA,[2] d-ApA,[3] and d-Ap(R)A,[3,4] bind to poly(U) or poly(T) in aqueous salt solutions to give complexes containing two pyrimidine bases per purine unit. These "triple-stranded" complexes can form even in solutions containing low concentrations of nucleotides, e.g. 50 μM total nucleotide units. Adenosine and 2-amino-adenosine afford similar complexes;[5] however, purine concentrations in the millimolar range or higher are require. The enhanced stability of the complexes formed by the dimer blocks may be attributed to the covalent backbones in these compounds, which hold the nucleosides in position for cooperative binding to the polynucleotide.

Recently we showed that a hydrophobic bridge spanning adjacent termini of complementary oligonucleotides can be as effective as a covalent bridge in stabilizing double stranded and triple stranded oligonucleotide complexes.[6] This observation raised the possibility that appropriately positioned hydrophobic groups might also be used to promote alignment of mononucleoside units along a polynucleotide template. One might envision that

---

This paper is dedicated in memory of Professor Tsujiaki Hata - a premier scientist, a creative leader in the field of nucleic acid chemistry, and a warm friend.

hydrophobic interactions could have supplemented base pairing and stacking interactions in assembling monomeric units for prebiotic replication. As an initial test of the role that hydrophobic substituents might play in organizing a nucleoside-polynucleotide system, we have examined the interaction of 3',5'-di-*O*-benzoyldeoxyadenosine (d-bzAbz) with poly(dT) and a dT_{10}-cholesteryl conjugate in dilute aqueous solution. These systems looked promising since molecular models suggested that, in a dT:dA:dT type complex generated by interaction of these components, a 3'-*O*-benzoyl group on a given deoxyadenosine unit would be favorably positioned to overlap the 5'-*O*-substituent on its downstream neighbor, thereby stabilizing the complex by hydrophobic and/or aryl stacking interactions.

RESULTS AND DISCUSSION

Poly(dT) + d-bzAbz. We first monitored the absorbance of deoxyadenosine (17 μM) and poly(dT) (25 μM in dT) in water (0.1M and 1 M in NaCl) as a function of temperature. The absorbance at 260 nm remained constant throughout the temperature range (0 °C to 80 °C), demonstrating that deoxyadenosine does *not* stack on the poly(dT) strands under these conditions. Similar results were obtained with 5'-*O*-benzoyldeoxyadenosine.

In marked contrast, a plot of absorbance *versus* temperature for a similar experiment carried out with d-bzAbz and poly(dT) in 0.1 M NaCl exhibited two sharp breaks indicative of highly cooperative dissociation of a complex (FIG. 1). This transition was reversible, as shown by a corresponding decrease in absorbance on cooling the system back to 0 °C. It may be noted that the Tm value for dissociation of the complex (22 °C, 0.1 M NaCl) is close to that for dissociation of the third strand from the triple stranded complex formed from poly(dT) and poly(dA) (Tm 26 °C, 0.1 M NaCl).[7] In the absence of salt, the dissociation temperature was somewhat lower (Tm 16 °C), as expected for a complex formed by assembly of two polyanionic strands with uncharged molecules. For a control carried out under the same conditions with poly(dA) in place of poly(dT) only a gradual rise in absorbance characteristic of the behavior of poly(dA) was observed. This experiment shows that formation of the complex depends on the base groups in the polyanion and is consistent with a model in which the thymine and adenine units are organized by hydrogen bonding as well as base stacking interactions. The stoichiometry for the complex was established by titrating an aqueous solution of poly(dT) with d-bzAbz in 0.1 M NaCl. A sharp break in the plot of

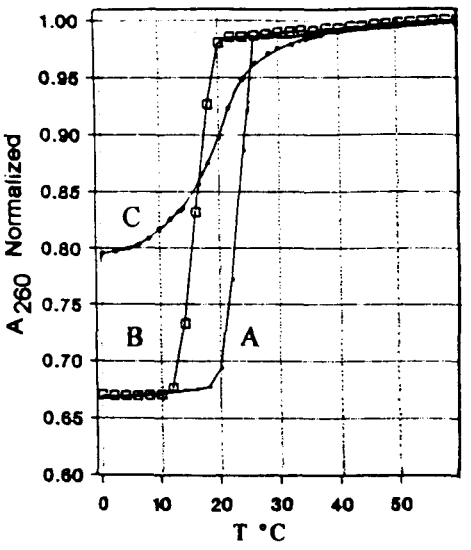

FIG. 1. Thermal dissociation curves for: (A) d-bzAbz + poly(dT), 0.1 M NaCl; (B) Same as in A but without NaCl; (C) d-bzAbz+d-TTTTTTTTT*ch*T, 0.1 M NaCl.

A_{260} *versus* volume of titrant showed the dT/d-bzAbz ratio in the complex to be 2/1 (FIG. 2). Taken together, these data demonstrate that d-bzAbz and poly(dT) form a "triple stranded" dT:dA:dT type complex, and that the 3',5'-di-*O*- benzoyl groups contribute to the stability of this complex.

The CD spectra in FIG. 3 provide further support for an association of d-bzAbz with poly(dT) in dilute solution. The spectrum of a solution containing both components differs substantially from the spectra of individual samples of d-bzAbz and poly(dT) at the same concentrations. In particular, the curve for the mixture lies well below those for the individual components in the 260-300 nm region, a feature clearly indicative of an interaction between the two absorbing species (d-bzAbz and poly(dT)).[8]

Finally, fluorescence spectra obtained for ethidium bromide in the presence and absence of d-bzAbz and poly(dT) are pertinent (FIG. 4). Although neither d-bzAbz nor poly(dT) significantly influenced the fluorescence of ethidium at these concentrations, we found that the fluorescence of ethidium is strongly enhanced when both are present in the solution. This pattern parallels that for the enhancement of fluorescence of ethidium on

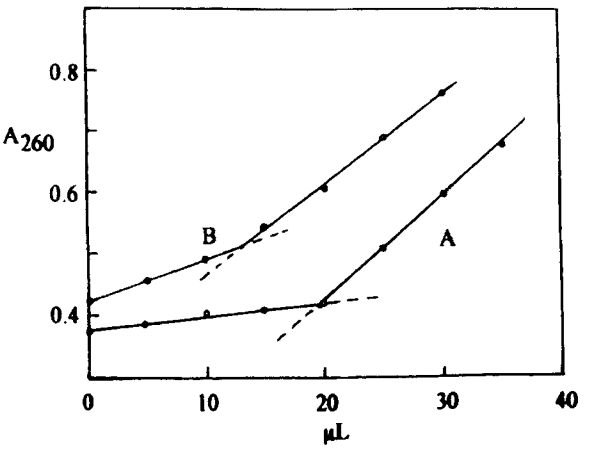

FIG. 2. Titrations at 0 °C of: (A) poly(dT) (46.3 nmol in dT), and (B) d-TTTTTTTTT*ch*T (51.4 nmol in dT), with d-bzAbz (5.77 nmol/5 μL).

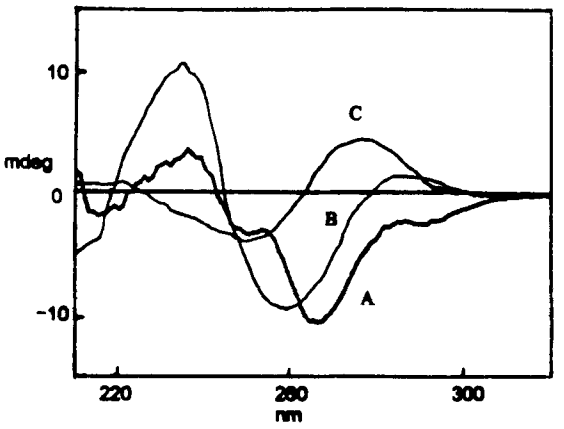

FIG 3. CD spectrum at ~5 °C: (A) d-bzAbz + poly(dT); (B) d-bzAbz; and (C) poly(dT). The d-bzAbz and dT concentrations were 17 μM and 25 μM, respectively.

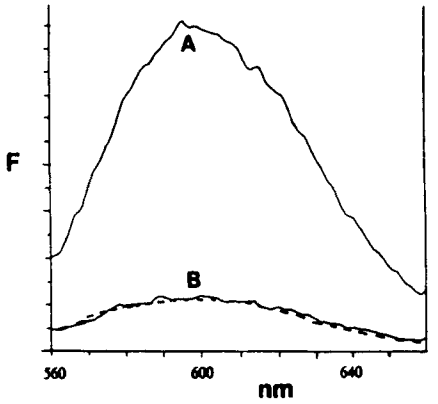

FIG 4. Fluorescence spectra at 0 °C in standard buffer (see Exp. Sect.): (A) ethidium bromide (EB) (8 μM) + d-bzAbz (17 μM) + poly(dT) (25 μM in dT); (B) EB + poly(dT) (------), and EB alone (– —). The concentrations were the same as in A.

intercalation into triple stranded 2dT/dA complexes,[9] and strongly suggests that ethidium intercalates into a "triple stranded" complex formed by alignment of the neutral d-bzAbz molecules along the poly(dT) strands.

d-TTTTTTTTTchT + d-bzAbz. No complex was observed when d-bzAbz was mixed with dT_{10}. Evidently this oligonucleotide is too short to form a stable complex either by aligning d-bzAbz along two different dT_{10} molecules or along two segments of one molecule folded into a hairpin structure. Since double and triple stranded complexes can be stabilized significantly by cholesteryl groups at the termini of the strands,[6] it appeared that 5' d-TTTTTTTTT*ch*T might function in place of poly(dT) in binding d-bzAbz. We found that d-bzAbz did indeed interact with this oligonucleotide conjugate, albeit, somewhat less efficiently (FIG. 1). A titration of the conjugate with d-bzAbz indicated a stoichiometry of ~3 dT/1dA, which corresponds to ~7 d-bzAbz molecules per two strands of the decanucleotide conjugate. The low dA/dT ratio is not surprising since the cholesteryl groups could interfere with binding of d-bzAbz at the 3' end of the conjugate and fraying of the strands could reduce binding at the 5' end. Interestingly, this complex led to even greater enhancement (18 fold) in the fluorescence of ethidium bromide than did 2poly(dT):dbzAbz (6 fold) under the same conditions.

Although the orientation of the dT strands in this complex has not been independently established, one may infer that they are held in a parallel arrangement by the hydrophobic interaction of the terminal cholesteryl groups.[6] In this case the orientation would differ from that for the conventional dT.dA.dT motif where the pyrimidine strands are antiparallel relative to each other, and likely differ from that in the d-bzAbz/2poly(dT) complex where the dT strands can readily adopt an antiparallel arrangement. A triple stranded complex containing d-bzAbz and parallel oligo(dT) segments could form with one dT strand held by Watson-Crick base pairing and the other by reverse Hoogsteen base pairing. Hudson et al. have reported formation of an analogous complex from dA_{10} and a compound containing two dT_{10} segments linked at their 5'-termini to the 2' and 3' oxygen atoms of adenosine.[10]

Attempts to observe formation of discrete complexes from polynucleotides and the 3',5'-di-*O*-benzoyl derivatives of the other common deoxyribonucleosides in water have not been successful, possibly in part because of low solubility of the derivatives. However, the di-*O*-nicotinyl derivatives look to be promising compounds for further study of stabilization of nucleoside/polynucleotide hybrids. Preliminary studies show that 3',5'-di-*O*-nicotinyl-deoxyadenosine is readily soluble in water and in aqueous salt solutions, yet forms a relatively stable complex with poly(dT) (Tm 11 °C in 0.1 M NaCl, and 14 °C in 1 M NaCl) at the same concentrations employed for d-bzAbz.

EXPERIMENTAL SECTION

Thermal dissociation experiments were carried out with a Perkin Elmer Lambda 2 UV-Visible spectrophotometer equipped with a Peltier temperature programmer for increasing the temperature at the rate of 1 °C/min. CD spectra were obtained on an Hitachi Joel 500 instrument using a refrigerated water bath and continuous circulation of nitrogen through the cuvette compartment, and fluorescence spectra (λ_{exc} 540 nm) were obtained on a Perkin Elmer LS 50B spectrofluorimeter equipped with a Lauda circulating bath for controlling the temperature . The extinction coefficients used were: 8.2 A_{260} units/μmole for dT in poly(dT) and d-TTTTTTTT*ch*T and 16.8 A_{260} units/μmole for d-bzAbz.

Synthesis of the of the oligonucleotide cholesteryl conjugate and isolation by HPLC was carried out as previously described.[6] 3',5'-Di-*O*-benzoyldeoxyadenosine was prepared by reaction of benzoyl chloride with deoxyadenosine in pyridine. The products were isolated by silica gel chromatography and characterized by uv, proton-NMR, and mass spectroscopy.

Except where noted, measurements were made on solutions 0.1 M in NaCl, 10 mM in Tris.HCl (pH 7.0), 17 μM in d-bzAbz, and 25 μM in dT units. Solutions for thermal dissociation and fluorescence studies were prepared by adding d-bzAbz (10 μL of a solution in 95% ethanol) to the poly or oligo-dT compound in one mL of the aqueous buffer. The reactions were followed at 260 nm, 280 nm, and 310 nm. The curves at 280 nm paralleled those measured at 260 nm, but showed less hyperchromicity. The absorbance values at 310 nm were obtained to monitor for any turbidity or precipitation; no absorbance developed at this wavelength. Titrations were carried out by stepwise addition of 5 μL aliquots of d-bzAbz in 95% alcohol to the dT substrate in 1 mL of the buffer solution at 0 °C.

ACKNOWLEDGMENT

This research was support by a grant (GM 10265) from the National Institute of General Medical Sciences.

REFERENCES AND NOTES

1. Abbreviations: d-bzAbz, 3',5'-di-*O*-benzoyldeoxyadenosine; d-TTTTTTTTT*ch*T, a thymidine oligonucleotide containing a -OP(O)($NHCH_2CH_2NHCOO$-cholesteryl)-substituent at a terminal internucleoside linkage; CD, circular dichromism;
2. (a) Lipsett, M. N.; Heppel, L. A.; Bradley, D. F. *J. Biol Chem.* **1961**, *236*, 857-863. (b) Cantor, C. R.; Chin, W. W. *Biopolymers* **1968**, *6*, 1745-1752. (c) Kreishman, G. P.; Chan, S. J. *Biopolymers*, **1971**, *10*, 150-174.
3. Miller, P. S.; Yano, J.; Yano, E.; Carroll, C.; Jayaraman, K. *Biochem.* **1979**, *18*, 5134-5143.
4. Letsinger, R. L.; Bach, S. A.; Eadie, J. S. *Nucleic Acids Res.* **1986**, *14*, 3487-3499.
5. (a) Howard, F. B.; Frazier, J.; Singer, M. F.; Miles, H. T. *J. Mol. Biol.* **1966**, *16*, 415-439. (b) Huang, W. M.; Ts'o, P. O. P. *J. Mol. Biol.* **1966**, *16*, 523-543. (c) Davis, R. J. H. *J. Mol. Biol.* **1972**, *63*, 117-137. (d) Damle, V. N. *Biopolymers* **1972**, *11*, 1789-1816.
6. Letsinger, R. L.; Chaturvedi, S. K.; Farooqui, F.; Salunkhe, M. *J. Am. Chem. Soc.* **1993**, *115*, 7535-7526.
7. Riley, M.; Maling, B.; Chamberlin, M. J. *J. Mol. Biol.* **1966**, *20*, 359-389.
8. The large red shift (to 260 nm) in the strong negative band in the CD spectrum is unusual. Little change in the principal negative band is noted on forming oligonucleotide dT:dA and 2dT:dA complexes; see Pilch, D. S.; Levenson, C.; Shafer, R. H. *Proc. Natl. Acad. Sci. USA* **1990**, *87*, 1942-1946. The shift may reflect an induced CD band arising from overlap of neighboring benzoyl groups in the complex.

9. (a) Salunkhe, M.; Wu, T.; Letsinger, R. L. *J. Am. Chem. Soc.* **1992**, *114*, 8768-8772. (b) Mergny, J.-L.; Collier, D.; Rougee, M.; Chassingnol, M.; Thuong, N. E.; Helene, C. *Nucleic Acids Res.* **1991**, *19*, 7775-7777. (c) Letsinger, R. L.; Wu, T. *J. Am. Chem. Soc.* **1995**, *117*, 7323-7328.
10. Hudson, R. H. E.; Uddin, A. H.; Damha, M. J. *J. Am. Chem. Soc.* **1995**, *117*, 17470-12477.