GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION
SPONSORED PROJECT INITIATION

Date: 2/13/80

Project Title: Conformations of Ligand - DNA Complexes and DNA Oligomers

Project No: G-41-A03

Project Director: Roger M. Wartell

Sponsor: DHEW/PHS/NIH - National Institute of General Medical Sciences

Agreement Period: From 1/1/80 Until 12/31/80 (03 year)

Type Agreement: Grant No. 5R01 GM24734-03

Amount: $16,057 New PHS Funds (G-41-A03)
845 GIT Contribution G-41-332
$16,902

Reports Required: Annual Progress Rpts w/Continuation Applications
Terminal Progress Rpt upon Grant expiration

Sponsor Contact Person (s):

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Defense Priority Rating: N/A

Assigned to: Physics (School/Laboratory)

COPIES TO:

Project Director
Division Chief (EES)
School/Laboratory Director
Dean/Director-EES
Accounting Office
Procurement Office
Security Coordinator (OCA)
✓ Reports Coordinator (OCA)

NOTE: Follow-on project to G-41-A02

Library, Technical Reports Section
EES Information Office
EES Reports & Procedures
Project File (OCA)
Project Code (GTRI)
Other

CA-3 (3/76)

GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: 7/31/81

Project Title: Conformations of Ligand - DNA Complexes and DNA Oligomers

Project No: G-41-A03

Project Director: Dr. Wartell

Sponsor: DHEW/NIH/Nat'l Inst. of Gen. Med Science

Effective Termination Date: 12/31/81

Clearance of Accounting Charges: 12/31/81

Grant/Contract Closeout Actions Remaining:

NONE

_ Final Invoice and Closing Documents
_ Final Fiscal Report
_ Final Report of Inventions
_ Govt. Property Inventory & Related Certificate
_ Classified Material Certificate
_ Other _____

Assigned to: Physics (School/~~Laboratory~~)

COPIES TO:

Administrative Coordinator
Research Property Management
Accounting Office
Procurement Office
Research Security Services
~~Reports Coordinator (OCA)~~

Legal Services (OCA)
Library, Technical Reports
EES Research Public Relations (2)
Project File (OCA)
Other: _____

Project Title:	Conformations of Ligand-DNA Complexes and DNA Oligomers
Grant No.:	5R01-GM24734
Project Director:	Roger M. Wartell
Institution:	Georgia Institute of Technology School of Physics Atlanta, Georgia 30329
Period of Grant:	January 1, 1978 - December 31, 1980.

Summary Statement

The overall aim of the research project is to further understanding on how DNA binding molecules discriminate between different base pair sequences. One point of this study examined the interaction of small drug molecules with DNA by laser Raman spectroscopy. Netropsin (Nt) and distamycin (Dt) bind specifically to A·T sequences of duplex DNA. Raman spectra of these drugs were obtained in the presence and absence of DNA (1). A computer subtraction technique was developed to remove DNA and solvent background from the total spectra. Several changes occurred in the Raman spectra of these drugs upon binding DNA. Normal mode calculations and empirical correlation between the spectra of simple molecules with Nt and Dt were used to assign the Raman vibrational bands. Analysis of the differences between bound and unbound drug spectra indicate that pyrrole ring and peptide group vibrations are altered when Dt or Nt binds DNA. Pyrrole ring methyl groups are not altered by the binding process.

A second part of this study was focused on determining the conformational properties of specific nucleotide sequence DNAs. Attempts were made to synthesis oligonucleotide duplexes. These were abandoned in favor of cloning fragments of DNA containing transcription initiation regions or 'promoters'.

A promoter site is the 50-70 base pair region where RNA polymerase specifically binds to initiate transcription. During the past year approximately one milligram of a 144 bp. DNA fragment containing the lactose operon promoter site was isolated (2). Work is currently underway at isolating similar quantities of other promoter containing DNA fragments. Two types of studies have been carried out on the 144 bp. fragment and other DNA molecules containing parts of the lactose operon promoter. The temperature induced transition of duplex DNA to single strands was studied. This work was aimed at characterizing the thermal

stability of different parts of the promoter region. A second set of studies employed Raman spectroscopy to examine the conformation of DNA molecules of known sequence and conformational transitions of these molecules.

Absorption spectroscopy was used to measure the helix-coil transition of eight short DNA restriction fragments 80-301 bp. in length. These DNAs form different parts of the E. coli lactose operon transcription initiation region. Since the base pair sequences of these DNAs were known, a comparison of the experimental transitions with theoretical models of the transitions was possible. An accurate theoretical model was developed which predicted the experimental curves in solvents of 0.1 M NaCl or higher (3,4). Theoretical analysis shows that thermal stability boundary exists about 50 base pairs behind the transcription start point. The theoretical model is being applied to the question of base pair opening under conditions relevant to the binding of RNA polymerase (5).

Raman spectroscopy was used to examine the $\overline{B}$ to $\overline{A}$ transition of calf thymus DNA induced by increasing the percent of ethanol in an ethanol/water solution. We quantified the intensity changes of 17 Raman bands during the transition (6). Most bands show sharp intensity changes between 70-74% ethanol (v/v). Two bands undergo a pretransition intensity change. These changes suggest that a deoxribose-phasphate vibration is effected first in the $\overline{B}$ to $\overline{A}$ transition induced by ethanol dehydration.

Raman spectroscopy was also used to examine the vibrational spectra of purified DNA restriction fragments. X-ray structures of the DNAs have been previously correlated with characteristic vibrational frequencies and intensity ratios in the Raman spectra. Studies have been made on 95 bp. and 144 bp. DNA fragments containing the lactose operon promoter site. In 0.01 Na^+, 0.1 M Na^+ and 4 M Na^+ solvents, these DNAs are observed to maintain the $\overline{B}$ type conformation.

A collaborative study was carried out with R. D. Wells and associates (Univ. of Wisconsin, Madison, Wisconsin) on a 157 bp. DNA containing $(dG\text{-}dC)_n$ $(dG\text{-}dC)_n$ sequences at both ends of the 95 bp. lac fragment. Raman spectra of this molecule provides conclusive evidence for a junction between left handed and right handed duplex helices in 4.0 M NaCl solutions. Analysis of this Raman data is underway (5). Recent work has focused on developing methods to quantify the peak heights and widths of overlapping Raman bands. This information will provide added information on the Raman spectra of complex molecules such as DNA.

Publications:

1. "Conformational Features of distamycin-DNA and netropsin-DNA Complexes by Raman Spectroscopy", by J. C. Martin, R. M. Wartell and D. C. O'Shea, Proc. Nat. Acad. Sci. 75, 5483-5487 (1978).
2. "Cloning DNA Restriction Endonuclease Fragments with Protruding Single Stranded Ends", by R. M. Wartell and W. S. Reznikoff, Gene 9, 307-319 (1980).
3. "Theory Agrees with Experimental Thermal Denaturation of Short DNA Restriction Fragments", by A. S. Benight, R. M. Wartell and D. K. Howell, Nature 289, 203-205 (1981).
4. "High Resolution Experimental and Theoretical Thermal Denaturation Studies on Restriction Fragments Containing the E. coli Lactose Control Region", by W. Hillen, T. Goodman, A. S. Benight, R. M. Wartell and R. D. Wells, J. Biol. Chem., in press.
5. "Fluctuation Base Pair Opening in DNA at Temperatures Below the Transition Region", by R. M. Wartell and A. S. Benight, submitted for publication.
6. "Changes in Raman Vibrational Bands in Calf Thymus DNA During the $\overline{B}$ to $\overline{A}$ Transition", by J. C. Martin and R. M. Wartell, submitted for publication.
7. "The Junction Between $\overline{Z}$ and $\overline{B}$ Conformations in a DNA Restriction Fragment: Evaluation by Raman Spectroscopy", by R. M. Wartell, J. Klysik, W. Hillen and R. D. Wells, in preparation.