

GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION  
SPONSORED PROJECT INITIATION

*act*

Date: February 16, 1978

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

Project No: G-41-A01

Project Director: Dr. Roger M. Wartell

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

Agreement Period: From 1/1/78 Until 12/31/78 (01 year)

Type Agreement: Grant No. 1 R01 GM24734-01

Amount: \$51,133 New PHS Funds (G-41-A01)  
4,691 GIT Contribution (G-41-313)  
\$55,824 Total

Reports Required: Annual Progress Reports with Continuation Applications  
Terminal Progress Report upon Grant expiration

Sponsor Contact Person (s):

Technical Matters

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Contractual Matters

(thru OCA)  
Evelyn W. Carlin  
Grants Management Officer  
Office of Assoc. Director for Program  
Activities  
National Institute of General Medical  
Sciences  
Bethesda, MD 20014

Defense Priority Rating: None

Assigned to: Physics (School/Laboratory)

COPIES TO:

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- Other \_\_\_\_\_

GEORGIA INSTITUTE OF TECHNOLOGY  
OFFICE OF CONTRACT ADMINISTRATION  
SPONSORED PROJECT TERMINATION

*Handwritten initials and signature*

Date: 2/1/79

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

Project No: G-41-A01

Project Director: Dr. R. M. Wartell (Dr. R. Salvo, Acting)

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

Effective Termination Date: 12/31/78 (end of 01 year)

Clearance of Accounting Charges: by 12/31/78 for 01 year

Grant/Contract Closeout Actions Remaining:

**TERMINATED**

- Final Invoice and Closing Documents
- Final Fiscal Report
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
- Classified Material Certificate
- Other Annual Report of Expenditures due by 3/31/79.

NOTE: Follow-on Project (02 year) is G-41-A02.

Assigned to: Physics (School/Laboratory)

COPIES TO:

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| Security Coordinator (OCA) | Other _____                        |
| Reports Coordinator (OCA)  |                                    |

APPLICANT. REPEAT GRANT NUMBER SHOWN ON PAGE 1 ———>	GRANT NUMBER	
<b>SECTION IV—SUMMARY PROGRESS REPORT</b>	GM24734-02	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR (Last, First, Initial)	PERIOD COVERED BY THIS REPORT	
Wartell, Roger M.	FROM	THROUGH
NAME OF ORGANIZATION	January 1, 1978	December 31, 1978
Georgia Institute of Technology, Atlanta, Georgia		
TITLE (Repeat title shown in Item 1 on first page)		
<b>Conformations of Ligand-DNA Complexes and DNA Oligomers</b>		

- List publications: (a) published and not previously reported; (b) in press. Provide five reprints if not previously submitted.
- List all additions and deletions in professional personnel and any changes in effort.
- Progress Report. (See Instructions)

- "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. In press.

### Progress Report

The overall aims of the research project are to gain an understanding of how DNA binding ligands discriminate between different base pair sequences, and how one region of DNA influences the conformation of adjacent regions. There are two specific objectives of the project. One objective is to determine detailed information about the complexes which netropsin (Nt) and distamycin (Dt) form with A·T sequences of duplex DNA. These two oligopeptide molecules are of interest as antibiotics and as simplified models of proteins which bind to specific DNA sites. They have been shown to specifically bind to A-T regions of duplex DNAs. They do not bind single stranded DNA, RNA, or GC DNA polymers under conditions (0.1M sodium ion) in which they bind to AT DNA polymers. The second objective is to examine the conformational properties of block DNA oligomers d(C<sub>15</sub>A<sub>15</sub>)·d(T<sub>15</sub>G<sub>15</sub>) and d(C<sub>15</sub>A<sub>5</sub>)·d(T<sub>5</sub>G<sub>15</sub>). These oligomers have a block of A·T pairs joined to a block of G·C base pairs. These two sequences, d(A)<sub>x</sub>·d(T)<sub>x</sub> and d(G)<sub>y</sub>·d(C)<sub>y</sub> (where x and y are the number of base pairs), show different conformational properties at polymer lengths. X-ray diffraction studies on fibers of poly d(A)·poly d(T) show it to be in the "B" type conformation. Similar studies show that poly d(G)·poly d(C) under similar conditions is in the "A" type conformation. We wish to examine the conformation of this DNA by Raman spectroscopy. This technique is also being employed in the drug-DNA binding experiments.

There are four goals this year for the drug-DNA binding studies. The first is to obtain the Raman spectra of Nt and Dt in the free state and bound to calf thymus DNA. The second objective is to obtain the Raman spectra of molecules which are molecular subunits of Dt and Nt (described below). The third goal is to formulate and apply a normal mode calculation to the molecular subunits as well as the drugs. By combining the results of the second and third goals it should be possible to assign the frequencies of the drugs' Raman bands to vibrational motions of specific regions of the drugs. Many of these goals have been achieved. (see below).

The major goal for the study on the block oligomer DNAs is to synthesize 1-2 mgs of d(C<sub>15</sub>A<sub>5</sub>)·d(T<sub>5</sub>G<sub>15</sub>) and carry out characterization and preliminary Raman spectroscopy studies. Several steps in the synthesis have been accomplished so far.

**RESULTS:** The enclosed manuscript describes the results to date on Nt and Dt binding to DNA. Raman spectra have been obtained of the drugs in the presence and absence of calf thymus DNA. Methods were devised to determine a bound drug's Raman spectra in the presence of DNA and solvent. A computer subtraction technique was developed to remove DNA and solvent background from the total spectra. Several changes occur in the Raman spectra of these drugs upon binding DNA. To analyze these changes, it was necessary to assign specific motions to the observed Raman bands. The first approach to this problem was to examine the Raman spectra of pyrrole, N-methyl pyrrole and N-methyl pyrrole carboxaldehyde,

R.H. Wartell

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and correlate these spectra to the drug spectra. Some empirical correlations of bands were possible, however a more fundamental understanding of the vibrational characteristics of the molecules was necessary. A normal mode calculation was formulated for asymmetric molecules and applied to pyrrole, its derivatives and the central portion of both drugs. A very good correlation was obtained between many observed band frequencies of Nt and Dt and the predicted frequencies. This allowed confident assignments for these bands. The analysis of the differences between bound and unbound distamycin spectra indicated that pyrrole ring and peptide group vibrations are altered when Dt binds to DNA. The environment of the pyrrole ring methyl groups are not affected by the binding. The results suggest that the pyrrole ring N1 position (where the methyl groups are) are logical sites for chemical modifications aimed at creating cell uptake selectively while maintaining DNA binding activity. Analysis of the spectra of Nt gave similar results. However, due to the lower solubility limit of Nt in aqueous solution (and thus reduced Raman intensities), it was not possible to quantify changes in the peptide group vibration.

The synthesis of the block DNA polymers has made some progress, however, it is uncertain if the original goal for this year will be met. Part of the delay has been necessitated by the building of a gel scanner, and the isolation of M.luteus DNA polymerase. 25 mg. of poly(dI)·poly(dC) was synthesized, and about 6-8 mg of poly(dC) is now digested to (dC)<sub>x</sub> oligomers. The techniques of analytical gel electrophoresis and RPC-5 (Reverse Phase Chromatography) have been developed.

Research goals for the coming year are to complete the synthesis of d(C<sub>15</sub>A<sub>5</sub>)·d(T<sub>5</sub>G<sub>15</sub>) if it is not yet completed and construct d(C<sub>15</sub>A<sub>15</sub>)·d(T<sub>15</sub>G<sub>15</sub>). Raman studies on these block oligomers in aqueous solution will be carried out. Further studies on the binding of Nt and Dt to DNA will focus on one or two bands of their Raman spectra involved in peptide vibrations. By quantitatively examining these bands at different molar ratios of drug to DNA it may be possible to determine the number of hydrogen bonds involved in the drug-DNA complexes. Theoretical low energy calculations of the drug complexed to DNA will also be made.

The undersigned agrees to accept responsibility for the scientific and technical conduct of the project and for provision of required progress reports if a grant is awarded as the result of this application.

September 29, 1973

Date

Roger M. Wartell

Principal Investigator or Program  
Director