GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

# PROJECT ADMINISTRATION DATA SHEET

[X] ORIGINAL [ ] REVISION NO. _____

Project No. G-41-B03 DATE 9/16/82

Project Director: R.M. Wartell School/~~Lab~~ Physics

Sponsor: NIH DHHS/PHS - National Institute of Allergy & Infectious Diseases

Type Agreement: Grant No. 1R01AI16874-03 (03 year)

Award Period: From 9/1/82 To 8/31/83 (Performance) 11/30/83 (if last year) (Reports)

Sponsor Amount: $49,733* Contracted through:

Cost Sharing: $2,618 (G-41-365) ~~EXEC~~/GIT

Title: Interaction of RNA Polymerase with DNA

ADMINISTRATIVE DATA OCA Contact William F. Brown, Ext. 4820

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Defense Priority Rating: N/A Security Classification: N/A

RESTRICTIONS

See Attached NIH Supplemental Information Sheet for Additional Requirements.

Travel: Foreign travel must have prior approval – Contact OCA in each case. Domestic travel requires sponsor approval where total will exceed greater of $500 or 125% of approved proposal budget category.

Equipment: Title vests with None proposed.

COMMENTS:

Third year follow-on to G-41-B02

*Includes $1,174 of unexpended overhead funds from year 01 which is rebudgeted into direct cost.

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FORM OCA 4:781

GEORGIA INSTITUTE OF TECHNOLOGY OFFICE OF CONTRACT ADMINISTRATION

## SPONSORED PROJECT TERMINATION/CLOSEOUT SHEET

Date May 9, 1984

Project No. G-41-B03 School/~~XXX~~ Physics

Includes Subproject No.(s) _____

Project Director(s) Dr. R. M. Wartell ~~XXXX~~ / GIT

Sponsor DHHS/PHS - National Institute of Allergy & Infectious Diseases

Title Interaction of RNA Polymerase with DNA

Effective Completion Date: 8/31/83 (Performance) 11/30/83 (Reports)

Grant/Contract Closeout Actions Remaining:

- [ ] None
- [x] Final Invoice or Final Fiscal Report
- [x] Closing Documents
- [ ] Final Report of Inventions sent 4/27/84
- [ ] Govt. Property Inventory & Related Certificate
- [ ] Classified Material Certificate
- [ ] Other _____

Continues Project No. _____ Continued by Project No. _____

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Form OCA 60:1028

Final Progress Report

for National Institutes of Health Grant

| | |
|---|---|
| Grant Number: | AI16874 |
| Principal Investigator: | Roger M. Wartell |
| Grantee Institution: | Georgia Institute of Technology |
| Project Title: | Interaction of RNA Polymerase with DNA |
| Entire Period of Grant: | 9/01/80 to 8/31/83 |

Progress Report

The main research objectives for the grant period were to obtain milligram quantities of each of two lac promoter DNAs (61 bp. and 144 bp.) and conduct studies on their conformational and thermodynamic properties. The first question we wished to ask is does the lac promoter region possess physical properties significantly different than non-promoter regions? A considerable portion of these objectives have been achieved. Another objective was to carry out studies on the interactions of RNA polymerase and the catabolite activator protein plus cyclic adenosine monophasphate (CAP-cAMP) with the two DNA fragments containing their binding sites. Some progress has been achieved on these objectives and work is continuing.

An opportunity developing from this work resulted in a study of the junction between $\bar{Z}$ and $\bar{B}$ type DNA conformations in a 157 bp. DNA fragment containing the lac promoter. Although this study was not a planned objective of the project, it is within the general scientific goals of the work, and has led to some interesting results.

An 18 liter fermentor system was built in order to establish a practical means of growing large quantities of plasmid DNA and obtaining cloned DNA fragments. Approximately 3 mgs of plasmid DNA per liter of cell growth is now reproducably achieved. Methods wer established for cell lysis, DNA extraction and plasmid DNA purification. The plasmid DNA pRMW27 contains the 144 bp. sequence of the lac control region cloned into the single Eco RI site of the plasmid DNA pVH51. Methods were developed to separate the 144 bp. fragment from the residual linear pVH51 vector DNA. Approximately 5 mgs. of the 144 bp DNA fragment was isolated. Half of this material was digested by the restriction enzyme Hpa II to generate a 61 bp. DNA containing a CAP°cAMP site and an 80 bp. DNA.

In addition to the isolation of the above fragments, seven fragments have been isolated from Hae III digested pBR322 plasmid DNA. The pBR322 DNA sequence is known. This digest provided a number of non-promoter containing fragments.

Raman spectroscopy and circular dichroism were employed to characterize the conformation of the lac DNA fragments and non-promoter DNA fragments. Initial Raman studies indicated that the lac control region has a $\bar{B}$ characteristic spectra in 10mm NaCl and 4.5M NaCl. This was reported in publications (1) and (2). A backbone vibration peaking around 832 $cm^{-1}$ is observed. This

band and other characteristics of the spectra have been correlated with the $\bar{B}$ type structure of fibrous calf thymus DNA. These initial studies also indicated the need for careful data analysis procedures in order to make a quantitative comparison of various DNA Raman spectra.

Quantitative analysis of DNA Raman peak heights and widths have not been generally made because of two main difficulties; overlapping Raman bands of uncertain number in portions of the spectra, and non-flat slowly varying backgrounds in solution spectra. We have made progress at overcoming these difficulties and have developed procedures to allow for quantitative analysis.

Computer analysis procedures were developed to evaluate the intensities of Raman bands. After removing the background the experimental spectra was compared to a theoretical spectra calculated from trial parameters of band positions, intensities and widths. The theoretical spectra are based on Lorentzian band shapes convoluted with the instrument function of the spectroscopy system. The parameters were iteratively refined until good matches with unsmoothed experimental data were achieved. This analysis was first applied to a Raman study of the B to A transition of calf thymus DNA. The results of this study were described in publication (3). This work provided an important precursor for a similar study on other DNA fragments. The computer analysis procedure was employed to quantify Raman band location, intensities and widths for the lac DNA fragments and six non-promoter DNAs of varying % G·C. This work verified the conclusion of the initial studies and provided information on assignment of a number of Raman bands. This work is being completed as the dissertation thesis of Juan T. Harrell. A preprint of this work will be forwarded when completed. The analysis procedure is described in publication (7).

In addition to the CD and Raman studies, the thermal stability properties of DNA fragments were studied. UV-Absorption spectroscopy was employed to obtain high resolution differential melting curves. Theoretical analysis of DNA thermal stability was also carried out. Good agreement was obtained between theoretical and experimental melting curves of four lac DNA fragments in 0.1 M NaCl (4).

This study determined the location of two thermal stability boundaries during denaturation. One occurs 80 bp. upstream from the transcription startpoint, and the other is 14 bp. downstream from the startpoint. The location of these boundaries is not apparent from the DNA sequence alone. Experimental melting curves have been recently determined for the seven Hae III DNA fragments

of pBR322 DNA. Two of these fragments 192 bp. and 587 bp., are known to have promoter regions for the tetracycline gene and ampicillin gene, respectively. Theoretical analysis of the pBR322 DNA fragments do not show a correlation between thermal stability boundaries and specific promoter locations (5,6). The boundaries appear to depend on the detailed base sequence. These melting studies have improved the understanding of DNA unwinding and how base pair changes influence DNA melting.

During the initial Raman spectroscopy studies on the lac DNA fragments an opportunity developed which resulted in a study of a 157 bp. DNA. This DNA has the 95 bp. lac region sandwiched between 26 and 32 base pairs (dC-dG) sequences at each end. It was provided by R. D. Wells (University of Alabama, Birmingham, Ala.). It was previously shown that in 4.5 M NaCl this DNA contained junctions between a left-handed Z-type DNA and a B-type duplex. Raman spectra were obtained from the 95 bp. DNA, the DNA polymer $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$, and the 157 bp. DNA in 0.01 and 4.5 M NaCl. In 0.01 M NaCl all three DNAs have Raman spectra characteristic of a B-type conformation. The high salt spectra of the 95 bp. is also characteristic of a B conformation although some changes were observed. The 832 $cm^{-1}$ band characteristic of the B conformation is reduced in intensity. The spectrum of the 157 bp. DNA in 4.5 M NaCl shows several major changes from the 0.01 M NaCl spectrum. These changes are observed in the high salt spectrum of $(dG\text{-}dC)_n \cdot (dG\text{-}dC)_n$ and are correlated with the presence of Z conformation. Comparisons were made between the high salt spectra of the 157 bp. DNA and spectra calculated from the other two DNAs. Analysis of several base ring vibrational bands indicate that the 95 bp. segment is $\bar{B}$ like whereas the dC-dG regions are in the $\bar{Z}$ conformation, i.e., the junction region is short. Yet a deoxyribose-phosphate band, characteristic of B conformation has an intensity 50% $\pm$ 15% less than expected if the 95 bp. region was in a B backbone conformation. This indicates that the Z regions distort the backbone conformation of the middle segment but does not appear to effect base pair stacking. These results are described in publication (2) and (7).

Some preliminary progress has been made on the studies of the interaction of CAP·cAMP with its site on the 61 bp. lac DNA. 10 mgs of CAP protein has been isolated. The ability of CAP·cAMP to bind to its specific site have been verified using gel electrophoresis. Raman spectroscopy studies on CAP have been carried out. Further work on the effect of cAMP on the CAP spectra and the interaction of CAP·cAMP with DNA is continuing. Raman spectroscopy studies of CAP·cAMP binding to its lac DNA site are planned.

(3). List of Publications

In Print


1. J. T. Harrell, S. Abhiraman, and R. M. Wartell "Conformational Properties of two Restriction Fragments from the lac Promoter Region" Biophysical J. 37, 295a, 1982.
2. R. M. Wartell, J. Klysik, W. Hillen, R. D. Wells "Junction Between Z and B Conformations in a DNA Restriction Fragment" Proc. Nat. Acad. Sci. (USA) 79, 2549, 1982.
3. J. C. Martin & R. M. Wartell "Changes in Raman Bands of Calf Thymus DNA During the $\bar{B}$ to $\bar{A}$ Transition". Biopolymers 21, 499, 1982.
4. A. S. Benight, Ph.D. thesis, Georgia Institute of Technology 1983.
5. R. M. Wartell and A. S. Benight, "Fluctuational Base-Pair Opening in DNA at Temperatures Below the H-C Transition". Biopolymers 21, 2069, 1982.
6. A. S. Benight and R. M. Wartell, "Influence of Base Pair Changes and Cooperativity Parameters on Melting Curves of Short DNAs". Biopolymers 27, 1409, 1983.
7. R. M. Wartell, J. T. Harrell, W. Zacharias & R. D. Wells J. Biomolecular Struct. & Dynamics 1, 83, 1983.


Publication Planned


8. J. T. Harrell, R. M. Wartell "The Conformation of DNA Promoter Fragments Probed by Raman Spectroscopy".