GEORGIA INSTITUTE OF TECHNOLOGY — OFFICE OF CONTRACT ADMINISTRATION

PROJECT ADMINISTRATION DATA SHEET

[x] ORIGINAL [ ] REVISION NO. _____

Project No. G-41-B05 GTRI/EES DATE 8/16/84

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

Sponsor: DHHS/PHS/National Institute of General Medical Sciences; Bethesda, Maryland

Type Agreement: Grant No. 5 R01 GM33543-05

Award Period: From 9/1/84 To 8/31/85 (Performance), 11/30/85 (Reports)

Sponsor Amount:	This Change	Total to Date (if last year)
Estimated: $		$ 75,053
Funded: $		$ 75,053

Cost Sharing Amount: $ 4,317 Cost Sharing No: G-41-344

Title: DNA Conformation and Protein DNA Interaction

ADMINISTRATIVE DATA

OCA Contact Lynn Boyd x4820

1) Sponsor Technical Contact:

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Bethesda, Maryland 20205

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Grants Management
Office Associate Director
Program Activities - NIGMS
Bethesda, Maryland 20205
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Defense Priority Rating: n/a Military Security Classification: n/a

(or) Company/Industrial Proprietary: n/a

RESTRICTIONS

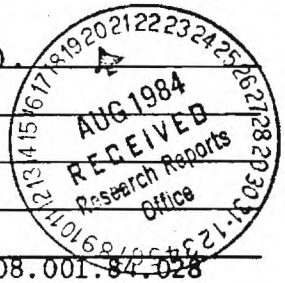
See Attached NIH Supplemental Information Sheet for Additional Requirements.

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 GIT.

COMMENTS:

This is a continuation of G-41-B04, (5th year of continuing grant).



Sponsor I.D. #02.108.001.84.028

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Continues Project No. G-41-B04 Continued by Project No. G-41-B06

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G-41-B05

SECTION IV PROGRESS REPORT SUMMARY	GRANT NUMBER GM 33543-06	
PRINCIPAL INVESTIGATOR OR PROGRAM DIRECTOR Roger M. Wartell	PERIOD COVERED BY THIS REPORT FROM	THROUGH
NAME OF ORGANIZATION School of Physics, Georgia Institute of Technology	Sept. 1, 1984	August 31, 1985

TITLE *(Repeat title shown in item 1 on first page)*
DNA Conformation and Protein-DNA Interaction

(SEE INSTRUCTIONS)
Publications

1. "Physical Characterization of a Kinetoplast DNA Fragment", by J. C. Marini, P. Effron, T. Goodman, R. D. Wells, R. M. Wartell and P. T. England, J. Biol. Chem. 259, 8974, 1984.

2. "Evidence for Mixed Sugar Puckers In B-Type DNAs; Analysis of Raman Spectra from Periodic and Heterogeneous Sequence DNAs", R. M. Wartell, J. T. Harrell, and S. Abhiraman, in preparation.

3. "Thermal Denaturation of DNA Molecules; A Comparison of Theory with Experiment", R. M. Wartell and A. S. Benight, Physics Reports, in press.

4. "Raman Spectroscopic Studies of the Temperature Induced B→Z Transition in poly d(G-meC)·poly d(G-meC)", D. M. Brown and R. M. Wartell, Biophys. J. 47, 14a, 1985.

5. "The Catabolite Activator Protein Stabilizes Its Primary Binding Site in the Lac Promoter", H. DeGrazia, S. Abhiraman, and R. M. Wartell, Biophys. J. 47, 390a, 1985.

1. The general scientific goals of the project have remained the same. Some additional studies were accomplished which were stimulated by a request to write a review on the DNA helix-coil transition.

2. Two research objectives planned for this past year were completed. One concerned the question, how does a bacterial gene activator protein, the catabolite activator protein or CAP, influence the thermal stability of its DNA binding site? This work was reported (publication 5) and is currently being written as a full manuscript. DNA melting curves were obtained from a 61 bp. DNA fragment containing the primary CAP site of the E. coli lactose promoter, and a 234 bp. DNA with no specific CAP sites. Saturating amounts of CAP with cAMP increased the melting temperature of the 61 bp. DNA by 16.4°C. Non-specific binding of CAP resulted in a 5.4°C increase in the Tm of the 234 bp. DNA and the 61 bp. DNA. Gel electrophoresis verified that CAP remained stable for site-specific binding up to the DNA transition region. These results showed that CAP acts as a stabilizing rather than destabilizing protein as had been previously surmised. It's mechanism of transcription enhancement does not involve the unwinding of DNA. A second objective accomplished was a Raman spectroscopy study on the temperature induced B to Z transition of poly d(G-meC)·poly d(G-meC). This work was aimed at determining the conformational pathway of the B to Z transition. Raman spectra were obtained at 2.5°C intervals between 5°C and 50°C. The temperature induced intensity changes of eleven base and backbone vibrational bands were determined. Raman spectra of poly d(I-C)·poly d(I-C), d me CMP and d GMP were obtained in order to assign various uncertain Raman bands. All assignable base vibration bands gave similar transition curves. This implies simultaneous changes of both guanine and methyl cytosine in the transition. Backbone bands did not show identical transition profiles. The 1094 cm^{-1} phosphate stretching band is unaltered until base stacking has progressed through 30-40% of its transition.

(continued)(from part 2.)

Preliminary studies have been carried out on a new phenomena produced by CAP·cAMP when it binds to its specific DNA site. Several experiments in the literature indicated that site-specific binding of CAP.cAMP can bend DNA. We determined that a 144 bp. DNA containing the CAP site could be ligated to a monomer circle in the presence of CAP.cAMP. Circularization by ligase does not occur in the absence of either CAP or cAMP. The circular 144 bp. DNA migrates at a position in an acrylamide gel which differs from linear multimers. Partial restriction endonuclease cleavage and sedimentation equilibrium measurements have verified that a monomer circle is generated.

A 36 bp. DNA containing the site for the lac repressor was made available by P. Lu Univ. of Pennsylvania. We have obtained preliminary Raman spectra of it and will compare its spectra to other short DNA fragments. Shorter fragments of protein binding sites provides an advantage for studying site-specific protein-DNA interactions by Raman spectroscopy. Studies were initiated to develop procedures for examining protein-DNA interactions by Raman spectroscopy. The system being examined is the binding of Ribonuclease A and poly(dA). Commercially obtained RNase A was purified by dialysis to remove fluorescent impurities. Raman spectra were obtained at 20 mg/ml, 50 mg/ml and 80 mg/ml. A Raman difference cell was designed and is being built. The volume of sample in this cell is 6-8 times that of a conventional cell. Experiments are planned to compare the results of this cell with a computer subtraction of individually obtained spectra.

In order to generate large amounts of the 61 bp. DNA restriction fragment for eventual Raman studies of a CAP-DNA comples, a plasmid with multimeric copies of the 61 bp. DNA was constructed. This plasmid has three copies of the 61 bp. DNA which can be cleaved out with Eco RI enzyme.

One additional study made this past year was not part of the original research objective. A detailed comparison was made between theoretically predicted DNA melting curves and experimental transitions. This study (publication 3) indicated that the theory can provide excellent agreement with experiment providing stem-loop structures are not allowed by the DNA sequence. This suggests that stem-loop structures can form in linear DNAs at high temperatures. The study of these structures outside of a closed circular DNA is possible.

3. One objective for the coming year is to finish the Raman study on the 36 bp. lac operator DNA site. This DNA may be useful for studies with the lac repressor protein. The effect of CAP.cAMP on DNA bending will be examined further. We wish to obtain kinetic data on the rate of circularization of the 144 bp. DNA with and without CAP.cAMP and the rate of linear dimer formation. This data determines the probability of DNA closure[1], and can be used to evaluate the amount of curvature induced in DNA by site specific CAP.cAMP binding. The Raman study on the interaction of poly(dA) with Ribonuclease A will be examined. Experimental conditions will be determined to optimize the information one can obtain on protein-DNA complexes. The Raman difference cell will be used. Fermentation scale growths will be made to isolate large amounts of the 61 bp. lac DNA containing the primary CAP site. A Raman study on CAP.cAMP and CAP.cAMP with the 61 bp. DNA or shorter derivatives will hopefully be initiated late in the grant year.

1. Shore and R. L. Baldwin, J. Mol. Biol. 170, 957, 1983.