GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF RESEARCH ADMINISTRATION
RESEARCH PROJECT INITIATION

Date: 27 March 1974

Project Title: Kinetic Study of the Binding Between the Regulatory Repressor Protein and the DNA Operator Region of the Lactose Genes.

Project No: G-41-633

Principal Investigator: Dr. E. M. Wartell

Sponsor: Research Corporation

Agreement Period: From March 1, 1974 Until Open

Type Agreement: Grant No. 7188

Amount: $14,120

Reports Required: Annual/Final

Sponsor Contact Person(s):

Mr. Jack W. Powers
Regional Director of Grants
Research Corporation
6075 Roswell Road, N. E.
Atlanta, Georgia 30328

Assigned to: Physics

COPIES TO:

Principal Investigator
School Director
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Library
Rich Electronic Computer Center
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Project File

RA-3 (6-71)
Date: 8/16/78

Project Title: Kinetic Study of the Binding Between the Regulatory Repressor Protein and DNA Operator Region of the Lactose Genes

Project No.: G-41-633

Project Director: Dr. Roger M. Wartell

Sponsor: Research Corporation

Effective Termination Date: 7/24/78 (Final Report Subm.)

Clearance of Accounting Charges: by 7/31/78

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:
- Project Director
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Project File (OCA)
Project Code (GTRI)
Other

CA-4 (3/76)
The aim of the research has been to study the interaction between the lactose repressor protein and DNA. The original goal was to (1) obtain purified repressor protein, (2) obtain DNA fragments containing the base sequence to which the repressor binds, (3) determine a spectroscopic assay of the interaction between the protein and DNA, and (4) make kinetic measurements of the interaction. Progress has been made in goals (1) and (3) and some change has been made in the original plans due to the findings obtained.

The first several months of the past grant year (March 1, 1974-March 1, 1975) were concerned with isolation of the repressor protein. Some pure material has been obtained and characterized. Absorbance difference spectroscopy examined the change in the absorption spectra of repressor protein when bound to the synthetic DNA polymer (dA-dT)_n · (dA-dT)_n. A very small change was observed. This change was too small to provide quantitative measurements.

Several drugs were examined to determine if a probe of the repressor-DNA binding could be found. The idea of this study was to find a drug which exhibits a large spectral change when it binds DNA. Providing this change is at a wavelength outside of the region where repressor and DNA absorb, one can monitor repressor displacing the drug without absorption interference. Several potential candidates were examined; proflavine, ethidium bromide, netropsin, and distamycin. My findings indicate netropsin and distamycin can be used to probe protein-DNA binding. Netropsin and distamycin show absorbance changes between 3200-3600 nm upon binding DNA. Titrating the repressor protein displaces the drugs from DNA. The synthetic DNA with only adenine-thymine base pairs, (dA-dT)_n · (dA-dT)_n was used.

Theoretical studies employing the equations of McGhee and Von Hippel (J. Mol. Biol, 86, 469, 1974) indicate one can evaluate the number of base pairs covered by the repressor and the binding constant of repressor-DNA interaction. The significance of this is that it
provides a general physical method to examine many different DNA binding proteins. Current plans are to quantitate the repressor-DNA interaction for the synthetic DNA (dA-dT)_n · (dA-dT)_n , a natural DNA without the specific repressor site, and with DNA fragments containing the specific repressor site. Work is then planned on examining other DNA binding proteins; RNA polymerase and the arabinose repressor protein. Kinetic studies are still contemplated but not until the equilibrium studies are completed.
STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.)

None as of yet. Two students may participate starting this summer.

PAPERS AND SCIENTIFIC TALKS (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.)

OTHER SUPPORT (List amounts and sources—including institutional—of other contributions received or expected for this work.)

EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

Supplies and Equipment for growing bactertia & isolating protein $492.00
Platinum resistance thermometer and bridge circuit $317.35

b. Stipends (Academic status, rates, periods of appointment)

c. Other expenditures (Itemize and give purpose)

Signature of principal investigator

February 27, 1975

Signature of authorized officer of institution (required for terminal report only)

Date

Name and position of authorized officer of institution
Investigations have been carried out on the range of cooperative interactions along DNA, and the binding of site specific molecules to DNA. These studies developed from the original project aimed at evaluating the lactose repressor protein-DNA operator interaction through the use of drug probes. Unfortunately the original goal was not achieved due to technical problems. The studies which have been made are in the related area of site specific ligand-DNA interactions.

Studies on block DNA oligomers have been made to examine conformational and thermodynamic interactions between contiguous DNA regions. The DNA oligomers have a block of A·T base pairs (5 to 15 base pairs long) covalently joined to a block of 15 to 20 G·C base pairs. These DNAs provide model systems for determining the influence of one region of DNA on the properties of adjacent regions. The possibility of an action at a distance effect may be important to understanding the regulation of transcription of bacterial genes. There are at least two examples where a protein binding at one DNA site maybe influencing the binding of a second protein at a DNA site 30 to 100 base pairs away (1,2). The experimental unwinding transition of these block DNA oligomers have been obtained and examined theoretically. These DNAs have 15G.C/15 A.T
20 G·C/15 A·T and 20 G·C/10 A·T pairs. The analysis indicated that the unwinding temperature of the A·T pairs are substantially increased by the presence of the G·C region. The increase in stability is larger than previous studies indicated. A reprint of this work has been sent previously. Further work is under way to examine the conformation of block DNAs with different lengths of A·T and G·C blocks. Laser Raman spectroscopy will be employed for this study. The synthesis of large amounts of a 5. A·T/15. G·C block DNA is in progress.

The binding of distamycin and netropsin to DNA has been studied by Laser Raman spectroscopy. These two oligopeptide antibiotics have been shown to be specific for A·T rich regions of duplex DNA. They are of interest as simple models for site specific protein-DNA interactions. Raman vibrational spectra of the drugs were obtained on the free state and when bound to DNA. By assigning bands of the drugs' Raman spectra to specific vibrations of these molecules, it was possible to probe which portions of the drugs are effected upon binding. The results show that certain portions of the drugs are altered upon binding DNA whereas other portions are not. A detailed description of this study is described in the enclosed preprint.
STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.)

Mr. James C. Martin: Mr. Martin has worked on the interactions of distamycin and netropsin with DNA using laser Raman spectroscopy. The major portion of his Ph. D. thesis research is involved with this study. Mr. Martin plans on assuming a postdoctoral research position upon completion of his degree.

Mr. Dennis Howell: Mr. Howell is working on the studies of the block DNA polymers using laser Raman Spectroscopy.

Dr. Rino Salvo: Dr. Salvo is a research associate who is working on the synthesis of 5 A·T/15 G·C block DNA. He is now supported by the National Institutes of Health grant described below.

PAPERS AND SCIENTIFIC TALKS (Give titles and references to papers or talks resulting from the work. Attach two copies of any reprints available, if not previously forwarded.)

1. Evidence For Long Range Interaction in DNA; Analysis of Melting Curves of Block DNA Polymers d(C15A15)·d(T15G15), d(C20A15)·d(T15G20) and d(C20A10)·d(T10G20), Biopolymers. 15, 1461, 1976.


OTHER SUPPORT (List amounts and sources—including institutional—of other contributions received or expected for this work.)

National Science Foundation—$80,000 for studies on long range interactions along DNA. March 1976-Feb. 1980.


EXPENDITURE OF RESEARCH CORPORATION GRANT FUNDS (The terminal report should be approved by an authorized officer of the institution.)

a. Equipment, supplies (Itemize major expenditures)

   i) 9 watt Laser, Coherent Radiation Inc. (partial cost) $5,389.00
   temperature probe and voltage bridge 317.35
   digital multimeter 289.00
   Precision temperature resistors 108.00 totals 6103.35

   ii) Material & Supplies $1,500.00

   totals $1500.00

b. Stipends (Academic status, rates, periods of appointment)

Research Associate—Rino Salvo 1/1/77-7/31/77 2,657.50
(partial support)

Graduate Students: C. Fordyce 6/1/75-6/30/76 2,499.98
A. Oliver 6/1/75-8/31/76 200.00
C. Heilker 3/1/76-3/31/76 120.00

C. Other expenditures (Itemize and give purpose) D. Howell 8/1/76-8/31/76 200.00 totals $6177.48

Retirement benefits for R. Salvo — $227.42
Travel to Princeton, N.J. for use of computer graphics for drug-DNA studies — 100.00 327.42 7/13/78

Signature of principal investigator

Signature of authorized officer of institution (required for terminal report only)

Name and position of authorized officer of institution