GEORGIA INSTITUTE OF TECHNOLOGY
Office of Contract Administration

SPONSORED PROJECT TERMINATION

no action

Date: March 11, 1976

Project Title: X-Ray Study of Enzyme-Small Molecular Interactions

Project No: G-33-636

Project Director: Dr. James C. Powers

Sponsor: Research Corporation; New York, NY

Effective Termination Date: 3/9/76 (Final Report Submitted)

Clearance of Accounting Charges: N/A - all funds expended.

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: Chemistry (School/Laboratory)

Copies to:

Project Director
Division Chiefs(EES)
School/Laboratory Director
Dean/Director-EES
Accounting Office
Procurement Office
Security Coordinator (OCA) ✓
Reports Coordinator (OCA)
Library, Technical Reports Section
Office of Computing Services
Director, Physical Plant
EES Information Office
Project File (OCA)
Project Code (GTRI)
Other_____

RA-4(3/76)

REPORT OF RESEARCH CORPORATION GRANT

C-33-636

(Submit original and one legible copy)

ease check one)
Interim Report
Terminal Report

STITUTION AND ADDRESS
Georgia Institute of Technology
Atlanta, Georgia 30332

RINCIPAL INVESTIGATOR James C. Powers PHONE (404) 894-4038

CADEMIC RANK AND DEPARTMENT Associate Professor of Chemistry

HORT TITLE OF RESEARCH SUPPORTED BY GRANT

X-Ray studies of enzyme small-molecule interactions

STARTING DATE 6-29-71

UMMARY OR PRINCIPAL FINDINGS AND THEIR SIGNIFICANCE (State succinctly in language understandable to one not necesrily expert in this field. Include extent to which original goals have been realized and any changes to original plan made or ntemplated.)

ymotrypsin and elastase are members of a family of protein cleaving enzymes called serine oteases. These enzymes are among the most widely studied in terms of their structure and chanism of action. We intially set out to study via x-ray crystallography the binding of all molecules such as inhibitors or substrates to these enzymes. Subsequently, various mbers of the serine protease family were implicated in several diseases. In particular, lmonary emphysema is currently thought to result from the uninhibited proteolysis of lung ssue by elastase and related neutral proteases derived from leukocytes and macrophages. us, we shifted the major thrust of our research to a study of the extended substrate nding site of serine proteases. The knowledge gained was then applied in the design of ry reactive and specific active site inhibitors.

astase. A few years ago, it appeared to us that peptide chloromethyl ketones were likely ndidates for use as elastase inhibitors. We synthesized a series of five tripeptide and ur tetrapeptide chloromethyl ketones and studies their rates of inactivation of porcine ncreatic elastase. Most of the compounds were effective inhibitors and were specific nce the related serine proteases chymotrypsin and trypsin were not significantly inbited by the peptide chloromethyl ketones under identical reaction conditions.

Our next goal was a study of the inhibition of human leukocyte elastase, the enzyme tch is probably the destructive agent in emphysema. This was accomplished using enzyme iciously supplied by Aaron Janoff (Stoney Brook, N.Y). The results indicated that leukocyte astase reacts less rapidly overall with this group of peptide chloromethyl ketones than s porcine pancreatic elastase. The most effective inhibitor for both the human leukocyte l porcine pancreatic elastase in this series of compounds is Ac-Ala-Ala-Pro-AlaCH_2Cl.

Peptide Carbazates. In a search for inhibitors of elastase which were not alkylating nts such as chloromethyl ketones, we have investigated peptide carbazates. Carbazates h the appropriate substituent are analogs of amino acids in which the α-methine group has n replaced by a nitrogen atom. We synthesized Ac-Ala-Bzc-ONp(Bzc=NHN($CH_2C_6H_5$)CO-), Np= itrophenyl) and found that it acylated chymotrypsin to yield a stable derivative. Elastase not react, but did react with the methyl carbazate Ac-Ala-Ala-Mec-ONp(Mec=NHN(CH_3)CO-). se results are in accord with the substrate specificity of these two enzymes. Ac-Ala-Ala--ONp is turning into a very interesting reagent since it can be used to carry out an active e titration of porcine elastase and human leukocyte elastase, since the release of itrophenol is stochiometric.

Significance. Synthetic elastase inhibitors have proven to be useful reagents for the dy of the biologic function of elastolytic enzymes. In the past two years we have supplied ples of the chloromethyl ketones Ac-Ala-Ala-Pro-AlaCH_2Cl and Ac-Ala-Ala-Ala-AlaCH_2Cl to r 35 other principal investigators in this and other countries for in vivo and in vitro dies with human leukocyte enzymes. At present, half a dozen papers have appeared in which se compounds have been utilized. Peptide carbazates, being of more recent origin, have s far only been requested by 13 other principal investigators. It is my belief that these pounds or related ones will eventually be used in the treatment of emphysema and similar eases.

STUDENT PARTICIPATION (Give names of students working on the project, their role in the research, their achievements and their career plans.) Barry Chelm, B. Lee Baker, Janice Brown, Philip Andrews and Mark Lively were undergraduates who did research working in my group at various times during the past few years. The results of the first three are published in a paper in JACS (ref. below). Janice Brown is currently finishing her PhD at U. of Illinois in biochemistry, Barry Chelm is at the U. of Colorado in biochemistry, B. Lee Baker is in Medical School, Philip Andrews is in graduate school at Purdue and Mark Lively is a graduate student at Georgia Tech. Peter Tuhy and Ron Whitley received their PhD's last year from Georgia Tech and are currently doing post-doctorial research at Brookhaven National Labs and the Mayo Clinic. Peter's results have been published (refs. below). Dave Carroll didn't complete his PhD and is currently employed at Colgate. However, his research resulted in several publications. Dave Rasnick is currently a graduate student at Georgia Tech. All of the students made substantial contributions to the research as the publications (past and future) indicate. With the exception of Mr. Baker, all are pursuing careers in biochemistry.

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.)

(see attached sheet)

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

National Institute of Health 1971 - present (ca 18,000-20,000/year)

National Instutute of Health 1975 - present (ca 40,000/year)

Georgia Tech Biomedical Research Funds - $6,000 for equipment

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)

Ultraviolet chromatography monitor	$1,584.50
Table	97.10
Electrophoresis Equipment	595.00
Electrophoresis Power Supply	506.60
Materials & Supplies	981.72

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

Undergraduate Student Assistants
Barry Chelm (843.20), Philip Andrews (332.00), Mark Lively (96.00)
Graduate Research Assistants
Ron Whitley (325.00), Dave Rasnick (638.88)

c. Other expenditures (Itemize and give purpose)

Signature of principal investigator — Date: March 1, 1976

Signature of authorized officer of institution (required for terminal report only) — Date: March 4, 1976

Evan Crosby, Associate Director of Financial Affairs
Name and position of authorized officer of institution

PUBLICATIONS :

"Active-Site Specific Inhibitors of Elastase," James C. Powers and Peter M. Tuhy, Biochemistry 12, 4767 (1973).

"Inhibition of Chymotrypsin A$_\alpha$ with N-Acyl and N-Peptidyl-2-phenylethylamines. Subsite Binding Free Energies," James C. Powers, B. Lee Baker, Janice Brown and Barry K. Chelm, J. Amer. Chem. Soc., 96, 238 (1974).

"Inhibition of Human Leukocyte Elastase by Peptide Chloromethyl Ketones", P. M. Tuhy and J. C. Powers, FEBS LETTERS, 50, 359 (1975).

"Synthetic Active Site-Directed Inhibitors of Elastolytic Proteases," J. C. Powers, D. L. Carroll and P. M. Tuhy, Ann. N.Y. Acad. Sci., 256, 420 (1975).

"Reaction of Acyl Carbazates with Proteolytic Enzymes", J. C. Powers and D. L. Carroll, Biochem. Biophys. Res. Comm., 67, 639 (1975).

G-33-636

Final Report

Research Corporation Grant

X-ray Studies of Enzyme-Small Molecule Interactions

by

James C. Powers
School of Chemistry
Georgia Institute of Technology
Atlanta, Georgia 30332

December 1975

Introduction. Chymotrypsin and elastase are members of a family of protein cleaving enzymes called serine proteases. These enzymes are among the most widely studied in terms of their structure and mechanism of action. Recently various members of the serine protease family have been implicated in several diseases. In particular, pulmonary emphysema is currently thought to result from the uninhibited proteolysis of lung tissue by elastase and related neutral proteases derived from leukocytes and macrophages. The major thrust of our research has been a study of the extended substrate binding site of serine proteases. The knowledge gained is then applied in the design of very reactive and specific active site inhibitors. Synthetic elastase inhibitors in particular would be expected to be useful reagents both for the treatment of emphysema and related diseases and for the study of the biological function of elastolytic enzymes.

Chymotrypsin Subsite Binding Free Energies. A series of N-acyl and N-peptidyl-2-phenylethylamines was synthesized in order to measure subsite binding free energies. These substrate analogs are expected to bind to chymotrypsin's extended substrate binding site in much the same manner as a normal peptide substrate. The dissociation constants of complexes of chymotrypsin with various acyl phenyl ethylamines were measured and the free energies calculated. The free energies of binding became more negative with increasing number of interactions as predicted from a crystallographic model. The major contributors to the binding free energy were the interaction of the phenylethyl side chain of inhibitors with a hydrophobic pocket on the enzyme surface and other hydrophobic interactions. Comparison of the data in the literature provides support for the hypothesis that serine proteases place the scissionable peptide bond of a substrate on a stereoelectronic "rack" favoring formation of a tetrahedral intermediate.

Elastase. A few years ago, it appeared to us that peptide chloromethyl ketones were likely candidates for use as elastase inhibitors. The crystallographic determinations of the binding modes of peptide chloromethyl ketones to chymotrypsin and subtilisin had provided insights into the interactions of inhibitors with these serine proteases. The inhibitors were covalently bound to imidazole ring of the active site hiotidine residue and the extended peptide chain of the inhibitor formed a β-sheet structure with a three residue section of the enzyme's backbone. We synthesized a series of five tripeptide and four tetrapeptide chloromethyl ketones and studied their rates of inactivation of porcine pancreatic elastase. Most of the compounds were effective inhibitors with Ac-Ala-Ala-Pro-AlaCH_2Cl being the most reactive. The inhibitors were specific

$$AlaCH_2Cl = \begin{array}{l} NHCHCOCH_2Cl \\ | \\ CH_3 \end{array}$$

since the related serine proteases chymotrypsin and trypsin were not significantly inhibited by the peptide chloromethyl ketones under identical reaction conditions.

Our next goal was a study of the inhibition of human leukocyte elastase, the enzyme which is probably the destructive agent in emphysema. This was accomplished using enzyme graciously supplied by Aaron Janoff (Stoney Brook, N.Y.). The results indicated that leukocyte elastase reacts less rapidly overall with this group of peptide chloromethyl ketones than does porcine pancreatic elastase. The most effective inhibitor for both the human leukocyte and porcine pancreatic elastase in this series of compounds is Ac-Ala-Ala-Pro-AlaCH_2Cl. Interestingly, its isomer Ac-Ala-Pro-Ala-AlaCH_2Cl is a relatively good inhibitor for the leukocyte enzyme, but is not effective toward the pancreatic enzyme, which is strong evidence that the pancreatic

and leukocyte enzymes are distinct entities. More recently, we have discovered that Ac-Ala-Ala-Pro-Val-CH_2Cl is more effective than the alanine compound at inhibiting the human leukocyte enzyme.

Peptide Carbazates. In a search for inhibitors of elastase which were not alkylating agents such as chloromethyl ketones, we have investigated peptide carbazates. Carbazates with the appropriate substituent are analogs of amino acids in which the α-methine group has been replaced by a nitrogen atom. These would be expected to acylate a serine protease with the appropriate specificity in much the same fashion as simple synthetic peptide substrate. The acylated enzymes should be considerably more stable toward deacylation than a normal "acyl enzyme" due to the influence of the adjacent nitrogen atom. We synthesized Ac-Ala-Bzc-ONp (Bzc =-NHN($CH_2C_6H_5$)CO-, Np = p-nitrophenyl) and found that it acylated chymotrypsin to yield a stable derivative. Elastase did not react, but did react with the methyl carbazate Ac-Ala-Ala-Mec-ONp (Mec =-NHN(CH_3)CO-). Theses results are in accord with the substrate specificity of these two enzymes. Ac-Ala-Ala-Mec-ONp is turning into a very interesting reagent since it can be used to carry out an active site titration of porcine elastase and human leukocyte elastase, since the release of p-nitrophenol is stochiometric.

Significance. It is likely that synthetic elastase inhibitors will be useful reagents for the treatment of emphysema and related diseases are for the study of the biologic function of elastolytic enzymes.

In the past two years we have supplied samples of the chloromethyl ketones Ac-Ala-Ala-Pro-AlaCH_2Cl and Ac-Ala-Ala-Ala-AlaCH_2Cl to over 35 other principal investigators in this and other countries for *in vivo* and *in vitro* studies with human leukocyte enzymes.

At present half a dozen papers have appeared in which these compounds have been utilized. Peptide carbazates, being of more recent origin, have thus far only been requested by three other principal investigators. It is my belief that these compounds or related ones will eventually be used in the treatment of emphysema and similar diseases.