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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Work on this contract consisted of further studies on design, synthesis and catalytic activities of our synthetic "chymohelizyme" (CHZ) molecules. CHZs consist of a bundle of four amphipathic α-helices joined covalently at their carboxy-terminus and bearing on their amino ends the amino acids that constitute the active site of chymotrypsin (ChTr). The first of these molecules, CHZ-1, demonstrated ChTr-like catalysis of hydrolysis of ChTr substrates. Design studies, using molecular graphics computer software, were directed toward improving the stability of the active site. Synthesis studies were directed toward improving the synthetic methods used. Extensive studies were carried out on improving solid phase peptide synthesis resins and protecting groups for this demanding synthesis. Studies of catalysis characterized the parameters of hydrolysis of nitrophenyl esters by CHZ-1 and several analogs. Rate of hydrolysis of acylamino acid esters catalyzed by chymohelizymes depends on the nature of the acyl group, the nature of the amino acid, and the nature of the ester group.

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FINAL TECHNICAL REPORT
ONR GRANT N00014-90-J-1591
"SYNTHETIC HELIZYME ENZYMES"

PI: John M. Stewart
ORGANIZATION University of Colorado Health Sciences Center
Denver, Colorado, 80262

AWARD PERIOD 1 March 1990 - 29 February 1993
DATE OF REPORT 1 February 1994

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## STATEMENT OF THE PROBLEM

We previously reported the design and synthesis of a peptide having catalytic properties related to those of chymotrypsin (ChTr). We called the synthetic peptide "Chymohelizyme-1" (CHZ-1). Chymohelizymes (CHZs) consist of a bundle of four amphipathic α-helices joined covalently at their carboxy-terminus and bearing on their amino ends the amino acids that constitute the active site of ChTr.

The goals of this project were to study helizymes further and to design new examples.

## MOST IMPORTANT RESULTS

During this contract period we conducted further studies on catalysis of ester hydrolysis by CHZ-1, carried out design studies intended to improve the configuration of the active site of CHZs and developed improved methods for synthesis of CHZ-1 and related molecules.

Studies of catalysis characterized the parameters of hydrolysis of nitrophenyl esters by CHZ-1 and several analogs. Rate of hydrolysis of acylamino acid esters catalyzed by chymohelizymes depends on the nature of the acyl group, the nature of the amino acid, and the nature of the ester group. Higher rates of hydrolysis were observed when both the amino acid and the acylating group are hydrophobic or aromatic. Thus, tyrosine derivatives are better substrates than alanine derivatives, and the aromatic-protected benzyloxycarbonyl tyrosine esters are better substrates than the aliphatic *t*-butyloxycarbonyl-protected tyrosine. The activated p-nitrophenyl esters of these amino acids were effective substrates, but the previously reported hydrolysis of stable ethyl esters and amides could not be confirmed.

Studies of the conformation of CHZ-1 showed that the molecule was not fully helical in solution. Acting on the hypothesis that this lack of full helix conformation was due to "fraying" of the ends of the chains (with concomitant disruption of the conformation of the active site), new analogs were designed, using the SYBYL molecular graphics program, to increase the stability of the region around the active site by adding more hydrophobic amino acid residues and protecting groups on the ends of the chains. At the time of the end of the project, no conclusive results had been obtained on the efficacy of this approach.

Studies on the chemistry of synthesis of CHZs were prompted by problems in obtaining commercial synthesis resins having the required properties of swelling in the synthesis solvents, and by problems associated with use of the new 3-nitro-2-pyridinesulfenyl (Npys) blocking group that we used to give the fourth level of selectively removable blocking groups essential for synthesis of the branched chain structure of CHZs. These studies led to great improvements; the results were published in two papers (reprints enclosed).

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LIST OF PUBLICATIONS

1. Design and synthesis of a peptide having chymotrypsin-like esterase activity. K.W. Hahn, W.A. Klis and J.M. Stewart. Science 248: 1544-1547, 1990. (work done in previous grant period)

2. Design and synthesis of 'Chymohelizyme-1,' a peptide having chymotrypsin-like catalytic activity. J.M. Stewart, K.W. Hahn and W.A. Klis, in "Peptides 1990," E. Giralt and D. Andreu, Eds. ESCOM, Leiden, The Netherlands, 1991, pp. 574-576.

3. Design and synthesis of a peptide having chymotrypsin-like catalytic activity. J.M. Stewart, K.W. Hahn, W.A. Klis, J.R. Cann and M. Corey, in "Frontiers in Bioprocessing II," P. Todd, S.K. Sikdar and M. Bier, Eds. American Chemical Soc., Washington, D.C., 1992, pp. 63-69.

4. Studies on chymohelizyme-1, a designed synthetic enzyme. J.M. Stewart, J.R. Cann, K.W. Hahn and W.A. Klis, in "Peptides," J.A. Smith and J.E. Rivier, Eds. ESCOM, Leiden, 1992, pp. 335-336.

5. Solid phase synthesis of a peptide designed to hae enzymic activity. J.M. Stewart, J.R. Cann, M.J. Corey, K.W. Hahn and W.A. Klis, in "Solid Phase Synthesis," R. Epton, Ed., Intercept, Ltd., Andover, UK,1992, pp. 23-27.

6. Effects of resin swelling and substitution on solid phase synthesis. K.C. Pugh, E.J. York and J.M. Stewart. Int. J. Peptide Protein Res. 40: 208-213, 1992.

7. Synthesis and stability of 3-nitro-2-pyridinesulfenyl chloride (Npys Cl). K.C. Pugh, L. Gera, and J.M. Stewart. Int. J. Peptide Protein Res. 42: 159-164, 1993.

SCIENTIFIC PERSONNEL ON PROJECT

Supported by funds of this grant:
Michael J. Corey
Karl W. Hahn
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Wieslaw A. Klis

Persons who worked on the project, but were not supported by funds of this grant:
John R. Cann
Lajos Gera
Katherine Pugh
John M. Stewart

No degrees were awarded.

No inventions during this grant period.