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FIELD	GROUP	SUB-GROUP	Ligand + receptor interactions, molecular modelling, drug design, X-ray crystallography.

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

The short-term objective of this project is to establish a data base which defines the binding mode of various classes of inhibitors of the serine proteases, especially elastase. The long-term goal is to determine general rules of binding specificity and to apply these rules to the design of novel or improved (more specific, more potent) inhibitors. By means of high-resolution X ray crystallography, the structure of native porcine pancreatic elastase (PPE, Acta Cryst. B, in press) has been refined to 1.65Å resolution. It forms the basis for comparison with 12 high-resolution complexes of PPE. The homologous structure of human leucocyte elastase (EMBO Journal, 1987) has been determined. These two enzyme structures form a highly interesting pair for subsequent modelling studies. The variety of binding modes prompts one to be cautious in using molecular modelling in the absence of additional (experimental) information.

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FINAL PROJECT REPORT

OFFICE OF NAVAL RESEARCH

N00014-85-K-0662

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INTRODUCTION

For four years this grant was the principle source of funds for support of equipment, maintenance, and salaries of this research group. As a result of this crucial support, the research efforts described below reached fruition and have generated seminal contributions to related fields. The grant was terminated as of July, 1988 with an extension through September, 1988. During this time, three doctoral students were, at one time or another, supported: Leonard Presta (now a post-doc with George Rose, Hershey Medical Center), Gail Carlson (now a post-doc with John Katzenellebogen at the University of Illinois and student in the Veterinary Medicine program at the University of Illinois), and Lori Takahashi (employed by a biotech firm in the Bay Area of San Francisco). A fourth student, Robin Crook, received the M.S. degree.

PROJECT GOALS

1. Determine the structures of small molecule complexes with the serine protease, porcine pancreatic elastase.
2. Using molecular modelling, study the binding modes of small molecule inhibitors so as to define important binding interactions and predict ways of improving such interactions.

ACCOMPLISHMENTS

1. During the past four years, initial investigations in this series were completed and published (54-67) forming the data base for subsequent studies.
2. A strong collaborative component in this research was made possible by annual visits (6 weeks-2 months) with Dr. Wolfram Bode, MPIB, Martinsried, West Germany. Out of this collaboration came the structure of human leucocyte elastase (HLE), the causative agent in human pulmonary emphysema (58). With only a 41% sequence homology between and porcine pancreatic elastase (PPE), the degree of homology in the extended receptor region (528 common backbone (NCCO) atoms may be

superimposed with a R.M.S. fit of 0.48Å) is clearly established. This homology is all the more striking when active site (57, 102, 189-195, 213-216, 226, 228) backbone atoms are superimposed, 55 atoms agree to a R.M.S. fit of 0.26Å, the greatest divergence occurring at amino acids 192 and 226.

3. Two classes of elastase inhibitors were studied, heterocyclics and derivatized peptides. A total of 7 papers reporting these results have been published (54, 56, 57, 61, 62, 64, 67). Two more papers are being submitted.

4. Due to our success in capturing a "Michaelis complex" of PPE with a hexapeptide (64), follow up studies of two pentapeptides (Leu-Leu-Arg*X-Tyr; X=Pro and Sar=sarcosine) complexed to trypsin are now being investigated.

Thus, a total of 16 high-resolution crystallographic studies were either completed or initiated (or both) during these 4 years of ONR support. This therefore comprises the definitive data base of small-molecule binding to elastase.

INTERPRETATION AND EVALUATION

Two clear patterns emerged from these studies:

1. Derivatized peptides of the length of 3 or 4 amino acids, without definitive, covalent or electrostatic attachment to the enzyme, preferred to bind backwards in the active site. Longer (penta-, hexa-) peptides were found bound in the "forward" orientation (as compared with trypsin + BPTI).

2. Heterocyclic complexes like isocoumarins (54) and benzoxazinones (61) bind in totally unpredictable fashion, each markedly different from the other, beyond the fact that each is covalently attached to Ser 195. This puts molecular modelling of such complexes on a very shaky basis and calls for more structure analysis in order to determine the rules of binding.

A review paper is being jointly authored by Bode, Powers, and myself; it will summarize chemical and structural data and compare the crystallographic results of HLE and PPE.

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