CHAPTER 1

INTRODUCTION
The inherent ability of animals to fight and prevent the entry of infection causing pathogens is called immunity. The immune system in higher animals has two functional arms which work together, called cellular and humoral immunity. The effector molecules involved in immune system of higher animals have been well studied by X-ray crystallography and other structural techniques. Insects account for more than 90% of the total known animal species. Their tremendous success not only depends on their ability to adapt to a wide spectrum of ecological niches, but also in their innate capacity to ward off attacks from pathogenic microorganisms. It was evident in as early as 1921 that insects develop immunity to infection after a first challenge with *E. coli*, and that the insect immune response has both cellular and humoral components (Metalnikov, 1921). However, recent studies have shown that the immune system of insects have few parallels with the immune system of higher animals. Insect immunity has come into great scrutiny in recent times with a view to understand the nature and biology of the immune response, and to use the knowledge in the discovery of novel anti-microbial compounds. Cellular immunity and the process of immune recognition is not well understood in the insects. A large number of proteins and peptides involved in the humoral immune system of the insects have been isolated, identified and characterized biochemically. The immune peptides and proteins have been classified into different families on the basis of their activity and primary structure. They include the cecropins, defensins, attacins and the proline-rich peptides. Additionally, homologues of several well known protein families have been discovered to play a role in insect immunity (Faye and Hultmark, 1995). However,
the mechanism of action of all the insect immune peptides and their regulation during the immune response are far from understood.

Understanding any biological phenomenon is critically dependent on the three-dimensional structure of the macromolecule associated with these processes. X-ray crystallography has emerged as a powerful tool for solving the three-dimensional structures of biological molecules like proteins and nucleic acids. In the last few years, a large number of protein structures have been solved by this method to atomic resolution. This has mainly been possible due to the rapid advances in computing technology and the design of better diffraction methods. In parallel, advances in molecular biology have resulted in the expression of proteins in large quantities suitable for crystallization experiments. The knowledge database accruing from the crystallographic studies have been used for rational drug design and the design of molecules with desired specificity and activity for use in biotechnology.

The main focus of this thesis is the X-ray crystallographic study on an antibacterial protein from Tasar Silkworm (Antheraea mylitta). This protein belongs to the insect immune system and is overexpressed in the hemolymph of the insect larvae on E. coli infection. The A. mylitta immune protein has antibacterial activity against both gram negative and gram positive bacteria. The protein was shown to belong to the chicken lysozyme family by N-terminal sequencing. It has high homology with the lysozymes from other members of the insect order Lepidoptera. The protein was crystallized in the orthorhombic space group C221. Diffraction data were collected to a resolution of 2.4 Å. The present work describes the structure determination of the A. mylitta immune protein (AmP) by the molecular replacement
method using a low homology model. Although both chicken lysozyme and the AmP share a common structural fold, significant differences exist in AmP particularly in the variable loop regions and the N- and C-terminals. To the best of our knowledge, this is the first report of any crystallographic study on an insect immune protein.

As this thesis describes studies on an insect protein of immune origin, it is pertinent to review the current understanding of the insect immune system. Chapter 2 presents a brief review on the different facets of insects immunity. The primary technique used in the work under study is X-ray crystallography. Chapter 3 discusses the principles and application of the X-ray crystallographic techniques used in protein structure determination. The subsequent chapters, namely Chapters 4,5,6 and 7 describe the crystallographic studies on the major immune protein of A. mylitta covering preliminary characterization, crystallization and X-ray structure determination.