Proteases: The Tip of the Iceberg

Proteases of Infectious Agents
Edited by Ben M. Dunn
San Diego, CA: Academic Press (1999). 282 pp. $69.95

Handbook of Proteolytic Enzymes
Edited by Alan J. Barrett, Neil D. Rawlings, and J. Fred Woessner
San Diego, CA: Academic Press (1998). 1666 pp. With CD-ROM $250.00

"It is the power of man to make parasitic maladies disappear from the face of the globe."

—Louis Pasteur

We live our lives unavoidably bathed in an ocean of viruses, bacteria, and parasites. The physical barriers of skin and mucosal membranes and the intricacy of the immune system allows us to live in peaceful equanimity with microbes...for the most part. The delicate nature of the equilibrium between host and microbe has been evident from the earliest accounts of civilization. Indeed, the battle between humans and infectious disease has only in this past century been one of tempered success for humans. Attenuation of death from disease has been due to an increased understanding of infectious agents and their modes of transmission as well as the identification of successful treatments. Introduction of physical processes like pasteurization, plastered walls, and condoms significantly reduced the incidence of tuberculosis, Chagas disease, and sexually transmitted diseases, respectively. The identification and development of antibiotics in the early half of the twentieth century led many to proclaim that the book was closing on infectious disease. Despite these advances, infectious disease has not been eradicated, in fact, 25% of all deaths are still attributed to infectious disease (The World Health Report, 1999). Worse still is the resistance of pathogens to drugs that once held them in check. The reemergence of tuberculosis as a major health problem is due to the increasing resistance of the mycobacterium to antibiotics. Malaria is becoming prevalent once again due to its ability to evade current antimalarial drugs and the list of drug-resistant infectious agents grows longer every day. The rapid replication times of microbes make the occurrence of drug resistance seem inevitable. Indeed, it has been estimated that every single point mutation of the HIV genome occurs between 10^4 and 10^5 number of times each day in an HIV-infected individual (J. M. Coffin Science *267*, 483–489, 1995). Therefore, the rapid evolution of HIV results in it surmounting obstacles in an expansive manner. But like the turtle and the hare, faster is not always better.

While the battle against microbes may never be won, keeping them at bay requires disruption of essential processes in the pathogen life cycle. One class of proteins that has recently become an attractive target for therapeutic intervention is that of proteolytic enzymes. Cleavage of the peptide bond is a reaction that is vital in almost every aspect of life for both host and pathogen. The peptide bond has a half-life of seven and a half years and requires selective catalysis to hydrolyze it. The proteolytic enzymes, or proteases, that catalyze peptide-bond hydrolysis represent one of the largest known functional classes of proteins. Through the catalysis of an essentially irreversible reaction they regulate commitment to such critical tasks as digestion, secretion, migration, assembly, proliferation, and cell death. Furthermore, proteolytic mechanisms are well studied and elements of a protease's catalytic mechanism can be exploited in the drug design process.

It appears to be against the backdrop of identifying new pathogenic targets for drug design that the editor compiled the book *Proteases of Infectious Agents*. The contents of this book can be broken down into four categories: six chapters on proteases involved in viral infection (HIV protease, hepatitis C protease, herpesvirus proteases, picornavirus proteases, host viral processing proteases, plant virus proteases), two chapters on protozoan infection (malarial proteases, Chagas disease proteases), one chapter on fungal infection (*Candida* proteases), and one chapter on bacterial infection (bacterial signal peptidases). The book was not written as an integrated approach to proteases involved in pathogenesis, but rather as separate stand-alone chapters contributed by researchers in the field. That being said, a theme that unifies each chapter is not only the presentation of the basic science of proteases involved in particular infectious diseases but also the possible therapeutic consequences of protease inhibition. While the focus on drug design is not evident from the title, *Proteases of Infectious Agents*, it becomes very obvious after reading the last sentence of the preface: "The task before us is clear: Find the critical protease and develop a potent and selective inhibitor." (p. xvii). The emphasis of therapeutic intervention through protease inhibition works better for some of the chapters than for others.

The theme of drug design is unmistakable in the chapter on HIV protease, the first and longest chapter in the book. In many ways, devoting one-fifth of the book to HIV protease is justifiable; the success of drugs designed and targeted against this enzyme has fueled interest for proteases as drug targets. Like many viruses, successful replication of HIV requires the proteolytic processing of multiple sites in the polyprotein encoded by the viral genome. Deletion of the virally encoded HIV protease gene results in noninfectious virus particles, demonstrating the essential nature of the proteolytic activity and validating it as a target for drug design. The historical and structural presentation of the drug design efforts against HIV protease is beautifully presented and well referenced in the book. Ultimately, one of the biggest issues permeating the long-term usefulness of any antiviral therapy is drug resistance. The high mutability of HIV means that drug design efforts against viral enzymes inevitably have to aim at moving targets. However, there may be limits to the mutational accessibility of the protease for maintaining activity and substrate specificity while escaping drug inhibition. While the authors present what is known about resistance, even offering some potential strategies to combat it, they truthfully state that "the full implications of resistance for disease outcome have not yet been realized" (p. 36).

The immune compromising effects of HIV infection have given rise to an increase in opportunistic pathogens such as the *Candida* and human herpesviruses,

both discussed in the book. Various *Candida* species express and secrete a family of aspartyl proteases that have been implicated as virulence factors. Characterization and structural determination of the protease encoded by several herpesviruses reveal a unique catalytic mechanism and a novel protein fold not previously seen in mammalian proteases. Other examples of non-host-like proteases are discussed in the book, such as the hepatitis C protease's unusual requirement for a peptide cofactor. These may be examples of the viruses being too smart for their own good. The novelty of the protease folds and mechanisms allows for the design of drugs that distinguish viral proteases from host proteases and may, in the end, be the Achilles' heel of the virus.

But the host's proteases are not always innocent. An additional aspect of viral infection is the role of host proteases in determining the infectivity and tropism of certain viruses. Evidence for the assistance of a host protease, tryptase clara, in the cleavage and activation of the fusion glycoprotein of Sendai virus or the hemmagglutinin of the influenza virus is presented. Tryptase clara is only expressed in the epithelial cells of the respiratory tract and therefore might explain the localization of the virus to these cells.

Chapters on malaria and Chagas disease represent infectious disease caused by protozoa. These protozoa have complex life cycles involving multiple stages and transmission through an insect vector. Proteases are postulated to play various roles in the parasite life cycle, from invasion to differentiation to digestion. The omnipresence of proteases in the parasitic life cycle makes them attractive targets for drug design. However, the role of individual proteases has been implied from inhibitor studies and has not been conclusively established in these systems. Unlike most viral proteases, the downstream substrates of many parasitic proteases are not known. Identification of the pathways they operate in may lead to additional avenues for therapeutic intervention.

Resistance to antibiotics represents a major public health issue. A novel bacterial target presented in the book, *Proteases of Infectious Agents*, is the type I signal peptidase. This protease is pivotal to the exportation of proteins from the cytoplasm since removal of the signal peptide is required for proper release of the secreted protein as well as for proper localization of membrane proteins with cleaved signal peptides. The enzyme is essential for bacterial viability. The drug design process may be hampered by cross-reactivity with the host microsomal signal peptidases that appear to be distant relatives of the bacterial peptidase. Nonetheless, this target holds promise for a new class of broad spectrum antibiotics, particularly with the availability of key crystal structures and mechanistic information of various members of the family of signal peptidases forthcoming.

While several classes of infectious agents and the proteases they use to facilitate infection are discussed in *Proteases of Infectious Agents*, the editor does not claim it to be encyclopedic, and it is not. A comprehensive assembly of proteases, even narrowed down to pathogenic proteases, into a single handbook is a daunting task due to the sheer numbers of proteases. However, this heroic task of collecting and compiling information on over 500 proteases has been taken on by Barrett, Rawlings, and Woessner who edited the mammoth *Handbook of Proteolytic Enzymes*. It provides a much needed classification system to help organize the relationship amoung each of the members. The short chapters on the individual proteases are uniform and well organized starting with classification and databank identifiers of the protease followed by sections on its history, activity and substrate specificity, structure, preparation, biology, distinguishing features, related proteases, and references. The accompanying CD-ROM not only provides the text and figures of the handbook in a completely searchable format but there are additional sequence alignments, three-dimensional structures, and cross-references to links on the world wide web. The *Handbook of Proteolytic Enzymes* is an important piece of literature and absolutely indispensable for those working in the field of proteolysis.

For those interested in a comprehensive description of proteases, the *Handbook of Proteolytic Enzymes* is an excellent resource. For a more in-depth view of the involvement of proteases in specific infectious diseases and drug design strategies to combat them, *Proteases of Infectious Agents* offers current and detailed analysis. The editors of the *Handbook of Proteolytic Enzymes* point out that an estimated 2% of genes in a genome encode proteases. Although not all of these proteases will be attractive targets for drug design, *Proteases of Infectious Agents* shows us that we could be looking at the tip of the iceberg. Given the emergence and reemergence of infectious agents, these books offer a timely look at a class of enzymes that may yield weapons in our arsenal to contain disease.

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