Viral Proteases and Antiviral Protease Inhibitor Therapy

PROTEASES IN BIOLOGY AND DISEASE

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Editors

Viral Proteases and Antiviral Protease Inhibitor Therapy

Proteases in Biology and Disease

Springer

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Preface

This, the eighth volume in the *Proteases in Biology and Disease* series, focuses on the role of proteases in virus function and their potential as anti-viral targets.

The respiratory illness 'severe acute respiratory syndrome' (SARS) was first reported in Asia in November 2002, and rapidly spread to several other countries across the world. The SARS coronavirus (SARS-CoV) causes SARS and a key step in the replication of the virus is the cleavage of the viral polyproteins by the SARS-CoV main protease. In the first chapter of this volume, Wei-Zhu Zhong and colleagues describe the functional importance of this protease in the viral life cycle, which is an attractive target for developing drugs against SARS, and outline a rational, structure-based approach to inhibitor design.

More than 30 million people worldwide suffer from infection by human immunodeficiency virus type 1 (HIV-1) and today there are several types of anti-HIV drugs that target the three enzymes, reverse transcriptase, protease and integrase. The aspartic protease encoded by HIV-1 is an important target for antiviral therapy for AIDS. In Chapter 2 Jozsef Tözsér and colleagues describe the basic properties of HIV-1 protease, its importance in structure-guided drug design for AIDS, and discuss recent developments in antiviral therapy based on targeting the HIV-1 protease and drug resistant mutants.

It is currently estimated that 2.2% of the world's population is infected with Hepatitis C virus (HCV) which can be transmitted mainly through intravenous drug use and contaminated blood products. Currently therapeutic options for HCV are limited and in Chapter 3 Philip Tedbury and Mark Harris describe the HCV protease which is one of the principle novel targets for new anti-HCV agents.

The focus of Chapter 4 by Marion Kaspari and Elke Bogner is human cytomegalovirus (HCMV), one of eight human herpesviruses that can cause life threatening diseases in newborns and immunocompromised patients. Like many viruses, HCMV has evolved strategies to redistribute host proteins and to take over host functions to promote viral replication. The host cell's ubiquitin-proteasome system (UPS) mediates degradation of misfolded proteins but is also required for specific processing events important for apoptosis, the cell cycle, protein sorting, etc. Since

these processes interfere with viral replication, proteasome inhibitors are now in focus as new targets for antiviral therapy.

Human T-cell lymphotropic virus type 1 (HTLV-1), like its better known relative HIV-1, is a human single-stranded RNA retrovirus that infects 20–30 million people worldwide. In Chapter 5 Jeffrey-Tri Nguyen and Yoshiaki Kiso describe the HTLV-1 aspartic protease and its potential as an anti-viral target.

The family of picornaviruses includes a number of important human and animal pathogens such as poliovirus, hepatitis A virus, coxsackievirus, human rhinovirus and foot-and-mouth disease virus. Although the last 25 years have seen an enormous increase in our knowledge and understanding of the molecular biology and pathogenicity of these viruses, at present no anti-viral substances have been approved for clinical use against picornaviral infections. In Chapter 6 Tim Skern and colleagues begin by explaining the situations in which an anti-viral against a particular picornavirus would be advantageous and identify the possible proteolytic activities against which anti-viral substances can be directed.

From this volume we are sure that the reader will see the potential for proteases to be the targets for effective anti-virals and hope that this volume in the *Proteases in Biology and Disease* series will provoke further research in this important area and be a valuable source of information on viral proteases. Finally, we would like to thank all the authors for their scholarly contributions.

January 2009

Uwe Lendeckel
Nigel M. Hooper

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Chapter 1
Study of Inhibitors Against SARS Coronavirus by Computational Approaches

Kuo-Chen Chou, Dong-Qing Wei, Qi-Shi Du, Suzanne Sirois, Hong-Bin Shen, and Wei-Zhu Zhong

Abstract Called by many as the biology's version of Swiss army knives, proteases cut long sequences of amino acids into fragments and regulate most physiological processes. They are vitally important in life cycle and have become a main target for drug design. This Chapter is focused on a special protease that plays a key role in replicating SARS (Severe Acute Respiratory Syndrome) coronavirus, the culprit of SARS disease. The progresses reported here are mainly from various computational approaches, such as structural bioinformatics, pharmacophore modelling,

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molecular docking, and peptide-cleavage site prediction, among others. It is highlighted that the compounds $C_{28}H_{34}O_4N_7Cl$, $C_{21}H_{36}O_5N_6$ and $C_{21}H_{36}O_5N_6$, as well as KZ7088, a derivative of AG7088, might be the promising candidates for further investigation, and that the octapeptides ATLQAIAS and ATLQAENV, as well as AVLQSGFR, might be converted to effective inhibitors against the SARS protease. Meanwhile, how to modify these octapeptides based on the "distorted key" theory to make them become potent inhibitors is explicitly elucidated. Also, a brief introduction is given for how to use computer-generated graphs to rapidly diagnose SARS coronavirus. Finally, a step-by-step protocol guide is given on how to use ProtIdent, a web-server developed recently, to identify the proteases and their types based on their sequence information alone. ProtIdent is a very user-friendly bioinformatics tool that can provide desired information for both basic research and drug discovery in a timely manner. With the avalanche of protein sequences generated in the post-genomic age, it is particularly useful. ProtIdent is freely accessible to the public via the web-site at http://www.csbio.sjtu.edu.cn/bioinf/Protease/.

Keywords SARS • coronavirus proteinase • KZ7088 • AG7088 • binding pocket • octapeptide inhibitors • distorted key theory • ProtIdent web server

Abbreviations CoV: Coronavirus; M^{pro}: Main proteinase; SARS: Severe acute respiratory syndrome

1.1 Introduction

Proteases, also termed as proteinases, or peptidases, are enzymes that are essential for all stages of life cycle, such as conception, birth, growth, ageing, and death of all organisms. Functioning as biology's version of Swiss army knives, they cleave long sequences of amino acids into fragments, a process that is indispensable for the synthesis of all proteins, controlling protein composition, size, shape, turnover and ultimate destruction. Therefore, it can help us better understand the biology of life and the treatment of diseases to study proteases, their receptors and inhibitors.

A respiratory illness called "severe acute respiratory syndrome" (SARS) was first reported in Asia in November 2002. The illness spread to more than two dozen countries in North America, South America, Europe, and Asia within only a few months. Patients suffering from SARS usually begin having a high fever (>38°C or 100.4°F) with symptoms such as headache, malaise, chilly, rigor, diarrhoea, and body aches, followed by developing a dry (non-productive) cough and having trouble breathing that might be accompanied by or progress to hypoxia, a condition in which there is insufficient oxygen reaching body tissues. Most patients developed pneumonia with a fatality rate around 15%.

It is known that the culprit that causes SARS is the SARS coronavirus (sometimes shortened to SARS-CoV) (Peiris et al., 2003). It is also known that a key step for the replication of the culprit is cleaving the SARS-coronavirus polyproteins

by a special protease, the so-called SARS coronavirus main protease (SARS CoV Mpro). The functional importance of the protease in the viral life cycle has made it an attractive target for developing drugs against SARS. To conduct the rational (or structure-based) drug design, a key step is to understand the binding interaction of SARS CoV Mpro with its ligands.

Progress in synthesis of novel test compounds for antiviral chemotherapy of SARS has been summarized by Kesel (Kesel, 2005), and that in drug discovery against SARS-CoV reported in a recent review (Wu et al., 2006). This chapter will focus on the progress mainly from the approaches of computer-aided drug discovery.

1.2 Binding Interactions

Based on the atomic coordinates of SARS-CoV Mpro (Anand et al., 2003), two binding models were developed (Chou et al., 2003). One is for the binding interaction of SARS-CoV Mpro with a compound called KZ7088 (Fig. 1.1a), and the other is for that with the octapeptide AVLQSGFR.

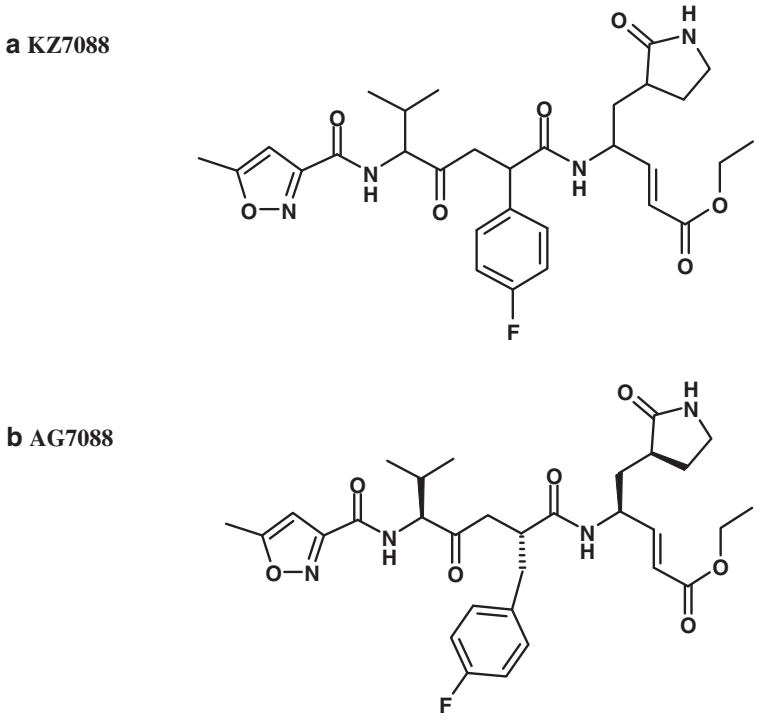

Fig. 1.1 Chemical structure of KZ7088: (**a**) a derivative of AG7088; (**b**) by removing –CH2 from its fluorophenylalanine side chain (Reproduced from Chou et al., 2003. With permission)

KZ7088 (Chou et al., 2003) is a derivative of AG7088 (Fig. 1.1b). The latter was developed by Pfizer Inc. and is currently in clinical trials for the treatment of rhinovirus, a pathogen that can cause the common cold. As shown in Fig. 1.1, AG7088 has a p-fluorophenylalanine side chain (p-fluorobenzyl), which is too long (or bulky) to fit into the binding pocket of SARS-CoV Mpro. By removing –CH2 from the side chain, the compound KZ7088 thus obtained could well fit into the binding pocket, as shown in Fig. 1.2. The constituents of the binding pocketare defined by those residues that have at least one heavy atom (i.e., other than hydrogen) with a distance ≤5 Å from a heavy atom of KZ7088. A similar bindingpocket was defined for ATP in the Cdk5-Nck5a*-ATP complex (Chou et al., 1999) and other studies (see, e.g., Zhou and Troy, 2003, 2005a, b; Chou, 2004b; Wei et al.,2006a; Wang et al., 2007a, b; Li et al., 2007a, b; Gu et al., 2009; Gong et al., 2009). The binding pocket of SARS-CoV Mpro for KZ7088 involved 23 residues, and the ligand was tethered to the enzyme by six hydrogen bonds, as detailed in (Chou et al., 2003). A series of follow-up discussions about the binding mechanism can be found in (Wu et al., 2006; Zhang and Yap, 2004; Samee, 2005; Clercq, 2006).

The binding interaction of SARS-CoV Mpro with the octapeptide AVLQSGFR is illustrated in Fig. 1.3, from which we can see that the octapeptide is tethered to Arg-40, His-41, Phe-185, Asp-187, and Gln-189 of SARS-CoV Mpro by six hydrogen bonds. The binding interaction mode had the important implications in stimulating rationally designing drugs for SARS therapy due to the following considerations: (1) The protease-susceptible sites in proteins usually extend to an octapeptide, as generally formulated by $P_4P_3P_2P_1P_{1'}P_{2'}P_{3'}P_{4'}$ with the scissile bond located between the subsites P_1 and $P_{1'}$, as generally expressed by P1↓P1′ (Schechter and Berger, 1967; Miller et al., 1989; Chou, 1993b). (2) The SARS coronavirus enzyme and several viral proteinases exhibit Gln↓ (Ser, Ala, Gly) specificity (Anand et al., 2003). (3) According to the "lock-and-key" mechanism in enzymology, the octapeptide cleavable by the SARS protease must have a good fit for binding to the active site. However, such a peptide, after a modification of its scissile bond with some simple routine procedure, will completely lose its cleavability but it can still bind to the active site. Actually, the molecule thus modified can be compared to a "distorted key" (Chou, 1996), which can be inserted into a lock but can neither open the lock nor be pulled out from it, spontaneously becoming an ideal competitive inhibitor against the SARS proteinase.

Stimulated by the above binding interaction modes, a series of follow-up studies were conducted, as described below.

1.3 Narrow down the Compounds-Searching Scope by Pharmacophore Approach

As mentioned above, both KZ7088 and the octapeptide AVLQSGFR are tethered to SARS-CoV Mpro by six hydrogen bonds. It is instructive to point out that analyzing the hydrogen bonding interactions of a receptor with its ligand often provided