Structure of the trypsin–cyclotheonamide A complex: an all-natural approach

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Natural products play a crucial role in chemistry, biology, and the rapidly growing area where these two disciplines meet. They are nature's answer to controlling biological processes with small molecules, and as such they are valued as biochemical probes by biologists and as novel drug templates by pharmaceutical scientists. Contemporary topics such as chemical diversity and structure-based drug design have remarkable parallels in the wealth of the natural products library and the evolutionary imperatives for optimizing potency and specificity. While natural products research is arguably the oldest branch of experimental science, recent developments have transformed the field. The trypsin–cyclotheonamide A story illustrates these changes [1].

In the last two decades, chemists have begun exploring the ocean as a source of novel natural products, and the sponges of the genus *Theonella* have been a prolific source of biologically active secondary metabolites [2]. Recently, a Japanese group isolated two cyclic peptides, cyclotheonamides A and B, that were potent inhibitors of serine proteases — a family of enzymes involved in numerous biological processes [3]. The cyclotheonamides are cyclic peptides with several unusual features: a vinylogous tyrosine in which the Cα is separated from the CO by a double bond, an arginine with an extra CO inserted between the Cα and the amide carbonyl, and a D-phenylalanine — the mirror image of the more common L-phenylalanine. These variations are precisely why natural products are so appealing; nature makes molecules with astounding chemical diversity. After a compound is isolated, chemists turn their attention to characterizing it, and in the cyclotheonamide case the final characterization was done through total chemical synthesis [4]. Total synthesis clarified a part of the three-dimensional structure that had not been assigned and another part that had been assigned incorrectly. The synthesis also established the technology to make analogs.

The complete characterization and synthesis of the cyclotheonamides mark the traditional end of natural products chemistry, but today we can push further and ask: Why are the cyclotheonamides such potent serine protease inhibitors? We will answer this question in structural terms because chemistry and biology are predicated on molecular structure. In recent years, X-ray crystallography has been developed into a powerful method for defining large structures at the atomic level in a short period of time. The results from a single crystal X-ray diffraction analysis of cyclotheonamide A bound to bovine trypsin are shown in Figure 1. This X-ray structure reveals the molecular basis of cyclotheonamide's potent inhibitory ability. Most of the inhibitor fills the catalytic site of trypsin with a charged guanidinium group making the deepest penetration (Fig. 1a). The extra carbonyl is crucial for cyclotheonamide's high affinity and is probably responsible for its slow binding kinetics, since it forms a covalent bond with the active site serine (Ser195) (Fig. 1b) [5]. The hemiketal oxygen is located in the oxyanion hole (Fig. 1c) where it accepts hydrogen bonds from main chain NHs. The phenyl ring of the D-phenylalanine is located in an aromatic binding pocket where it can interact with several other aromatic rings (Fig. 1d). Phenylalanine of the usual chirality, L-phenylalanine, would not have its phenyl ring disposed to make these contacts. The guanidinium group (Fig. 1e) is deeply anchored in a specificity pocket through a series of hydrogen bonds to side chain residues.

Unfortunately, the structure does not answer every question, and cyclotheonamide still retains some of its secrets. For example, the structure doesn't clarify the function of the vinylogous tyrosine; the residue lies outside the binding pocket exposed to solvent. An explanation might have to await the identification of the real target of cyclotheonamide, for surely a Pacific sponge doesn't make this compound to inhibit bovine trypsin. Overall, the structure provides an extraordinarily satisfying explanation of how cyclotheonamide works; this explanation can now be refined by making analogs and studying structures of their complexes with serine proteases. Understanding the structural basis of the binding of natural products to their protein receptors is a goal that now seems to be within our grasp.

References

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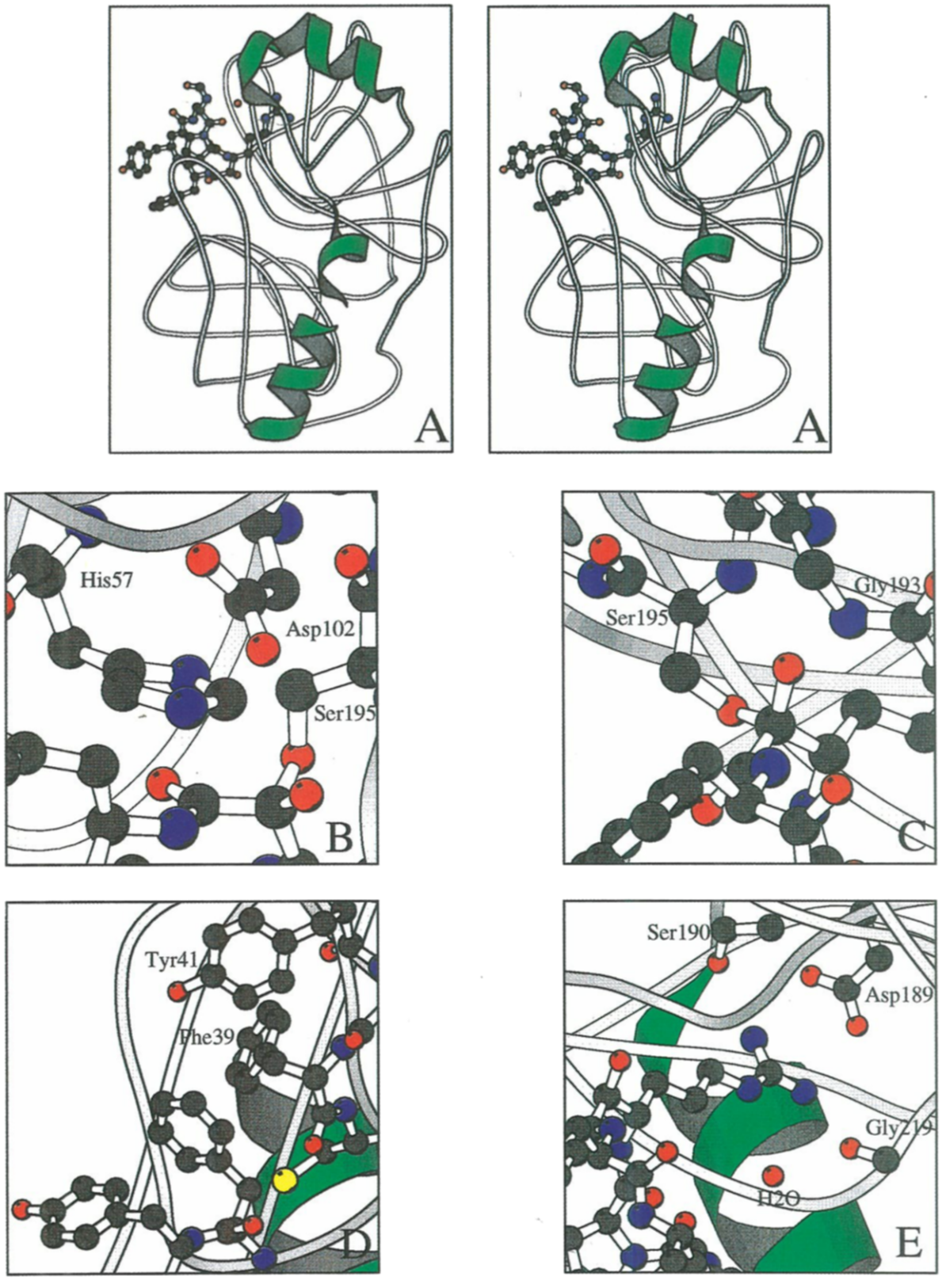

Fig. 1. (a) A stereo pair of the complete bovine β trypsin–cyclotheonamide A structure. The protein is shown as a ribbon diagram (α-helices are green); cyclotheonamide A, in ball and stick convention (atom colors: carbon, black; nitrogen, blue; oxygen, red; sulfur, yellow). **(b)** The catalytic triad of trypsin, Asp102, His57 and Ser195. Note that Ser195 makes a covalent bond with cyclotheonamide. **(c)** The hemiketal oxygen of cyclotheonamide in the oxyanion hole of trypsin. Hydrogen bonds are formed from main chain NHs to the oxygen atom. **(d)** The aromatic pocket of the complex. The bottom two aromatic rings are from cyclotheonamide, the top two from trypsin. Note the extensive edge-to-side contacts. **(e)** The deeply buried arginine side chain of cyclotheonamide. There are hydrogen bonds between the guanidinium and Ser190, Asp189 and Gly219. (For a related study on thrombin see B.E. Maryanoff, *et al.* (1993). *Proc. Natl. Acad. Sci. USA* **90**, 8048–8052.)