of azemethine ylides [14]. In a subsequent publication, amino-derivatized nanotubes were covalently linked to a peptide sequence derived from the foot-and-mouth disease virus (FMDV), generating monoconjugated peptide-CNT [4]. Now this functionalization method has extended to enable the linkage of two FMDV peptide sequences to amino-derivatized carbon nanotubes (bis-conjugated peptide-CNT) [13]. Standard characterization techniques such as HPLC, NMR, and microscopy were used to confirm the formation of the peptide-nanotube covalent bonds.

To establish that nanotube-linked peptides cover the same conformational space as free peptides, antigen-antibody interactions were measured in vitro using surface plasmon resonance measurements. It was found that the antibody (anti-FMDV peptide mAb with anti-mouse Fcγ antibody) did interact with free peptides and peptide-conjugated nanotubes but not with pristine carbon nanotubes. Moreover, a qualitative analysis showed no difference in response between free peptide and peptide-conjugated nanotubes, thus establishing that nanotube-linked peptides cover the same conformational space as free peptides.

Immune responses to FMDV peptide were measured in vivo using BALC/c mice. It is well known that the FMDV peptide needs to be coupled to either a carrier protein or a T-helper epitope to render it immunogenic. Now it has been established that the peptide coupling to carbon nanotubes produces the same result.

The anti-peptide antibody responses (with ovalbumin bystander help) were measured using ELISA and were most significant for bis-conjugated peptide CNT. More importantly, the responses were directed to just the peptides and not the molecular link between peptides and carbon nanotubes. As a result, no anti-carbon nanotube antibodies could be detected. This could suggest that carbon nanotubes do not trigger an immune response.

Understanding the interaction of nonbiological materials (such as carbon nanotubes) with biological systems (such as peptides) is essential for the realization of biological applications with novel nanomaterials such as carbon nanotubes.

The research described by Bianco et al. in this issue [13] advances the potential application of carbon nanotubes as drug delivery systems. One can imagine that in the (distant) future, instead of receiving a vaccine shot by syringe, a patient may lick a lollypop coated with functionalized carbon nanotubes acting as vaccine delivery systems.

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New Structural Insights into the Inhibition of Serine Proteases by Cyclic Peptides from Bacteria

The inhibition of enzymes employing a nucleophilic serine residue by natural products has been studied for many years. More recently, high-resolution structural analyses have begun to augment kinetic analyses. In this issue of *Chemistry & Biology*, Schulz and colleagues describe the crystal structure of scyptolin A, a cyclic peptide produced by cyanobacteria, complexed with elastase. Together with structures for a related inhibitor bound to trypsin, the work may assist in the design of reversible serine protease inhibitors.

Classic enzymology studies combined with pioneering structural biology have led to the serine proteases being among the best characterized of all enzyme families.

These studies identified the catalytic triad of active site residues and other important features central to catalysis such as the oxyanion hole and the P1 side chain binding pockets. Although aspects of the mechanism are still under debate, it is beyond doubt that catalysis proceeds via an acyl-enzyme complex formed with the nucleophilic serine (for recent review see [1–3]).

In addition to proteases, many other hydrolytic enzymes employ a nucleophilic serine residue to form an acyl-enzyme complex during catalysis. Perhaps the best known of these are the transpeptidases involved in bacterial cell wall biosynthesis, which are the targets of the penicillin antibiotics and the β-lactamases that mediate bacterial resistance to penicillins (for review see [4, 5]).

Due to emerging pharmaceutical applications ranging from cardiovascular disease to cancer, interest in the selective inhibition of human proteases continues to grow. The need for selective inhibitors is highlighted by the genomic assignment of hundreds of human proteases, most with little in the way of functional assignment. One approach to functional assignment is via the use of inhibitors selective for individual enzymes. In the case of serine proteases, decades of work have resulted in a plethora of inhibitors [6]. However, many of these do not fulfill the requirements of simple modification and suitability for in vivo application. There is thus a continuing interest in the development of generic templates that can be readily modified for use in pharmaceutical and functional analyses.

Although the methods of combinatorial synthesis/biosynthesis combined with high-throughput approaches may in time lead to novel and unexpected templates, at present the "ingenuity" of microorganisms and plants to produce natural products continues to provide the most interesting lead structures. A paradigm for such work comes from the development of the penicillin antibiotics, where the activity of a naturally produced nucleus or template was improved by the use of unnatural side chains. In more recent work starting from a naturally occurring steroid based inhibitor of thrombin, a serine protease involved in the blood clotting cascade, researchers were able to develop low molecular weight bicyclic "trans-lactam" templates useful for the inhibition of a range of serine proteases [7].

A current issue with respect to the chronic pharmaceutical use of protease inhibitors is the nature of the interaction with the target. There is a view that the long-term use of irreversible covalently binding inhibitors may cause toxicity problems e.g., due to the long-term accumulation of nonselective acylation. There is thus an interest in the development of generic templates that operate via noncovalent inhibition.

Protein- and peptide-based inhibitors of serine enzymes are well known [8, 9]. In humans, protease activity is regulated by a variety of mechanisms, including zymogen formation and the presence of inhibitor proteins including the serpins (serine protease inhibitors), that inhibit their targets via a remarkable mechanism involving a major conformational change [9]. Cyclic peptides are common natural products of bacteria and have proved a fruitful source of pharmaceutical leads.

In this issue of *Chemistry & Biology*, Schulz and coworkers [10] describe the crystal structure of porcine pancreatic elastase complexed with scyptolin A [11], a

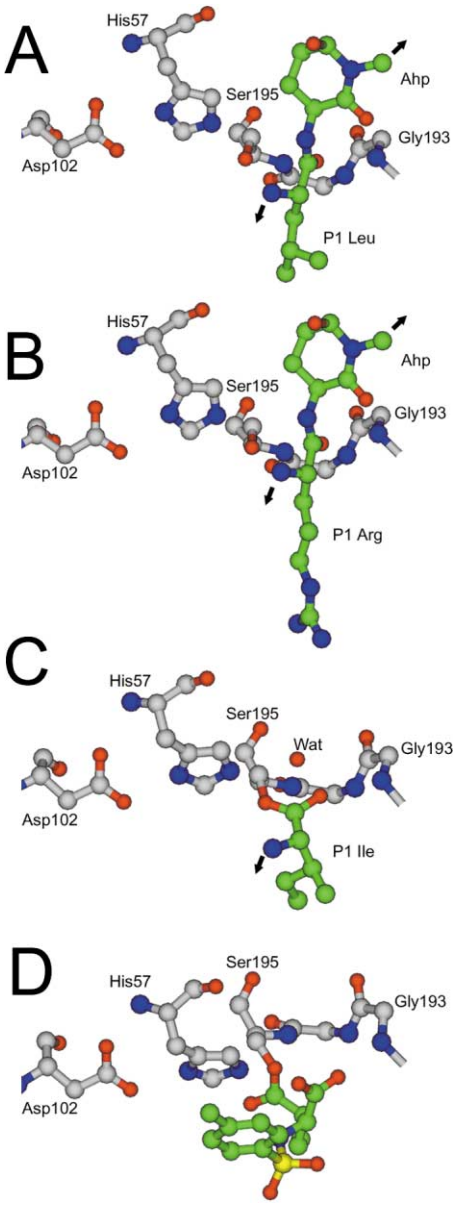

Figure 1. Acyl-Enzyme Complexes of Serine Proteases

Views from crystal structures of (A) P1 Leu and Ahp residues of scyptolin A at the active site of elastase (PDB ID code 1OKX), (B) P1 Arg and Ahp residues of A90720A at the active site of trypsin (PDB ID code 1TPS) (note the similarity with scyptolin A), (C) P1 Ile of the acyl-enzyme complex of human β-casomorphin-7 peptide substrate at the active site of porcine pancreatic elastase (PPE) (PDB ID code 1HAX), and (D) a stable acyl-enzyme complex formed by a monocyclic β-lactam inhibitor and PPE (PDB ID code 1BTU). Note the presence of the hydrolytic water in (C) but not (A), (B), or (D) and the rotation of the carbonyl of the ester out of the oxyanion hole in (D). The Ser-His-Asp catalytic triad and oxyanion hole formed by the main chain nitrogens of Ser195 and Gly193 are shown. The classical serine protease numbering scheme is used. Protein carbon atoms are colored gray, while ligand carbon atoms are green. Oxygen, nitrogen, and sulfur atoms are red, blue, and yellow, respectively. Additional ligand residues of (A), (B), and (C) are omitted for clarity; the arrows indicate the direction of omitted ligand binding.

member of the cyanopeptolin family of depsipeptides produced by *Scyptonema hofmanni*, a species of cyanobacterium [12] (Figure 1). Elastases characteristically catalyze the degradation of elastin found in connective tissue. Porcine pancreatic elastase is closely related to human elastase, which is a current pharmaceutical target for diseases including emphysema, arthritis, and cystic fibrosis. Together with a previously reported and closely related structure of a cyanopeptolin (A90720A) [13] complexed with trypsin from the Clardy group and studies on substrate complexes [14, 15], the work suggests how a peptide can bind at the active site yet manage to avoid hydrolysis.

Both scyptolin A and A90720A possess a 19-membered ring containing one lactone (involving the side chain hydroxyl of a threonine residue), five lactam links, and an unusual Ahp residue. The latter comprises a glutamate semi-aldehyde bound as hemi-aminal to the amide nitrogen of the succeeding amino acid. The selectivity of the scyptolins and A90720A for elastase and trypsin, respectively, is probably primarily governed by the residue in position 4, which corresponds to the P1 (i.e., on the N-terminal side of the scissile bond) position of a substrate, i.e., leucine in the scyptolins cf. arginine in A90720A. The conformations of protease-bound scyptolin A and A90720A are very similar, and both structures reveal binding at the S1 to S4 subsites as observed for substrates. Despite the fact the "backbone" carbonyl group of the P1 equivalent residue projects toward the oxyanion hole, no evidence for hydrolysis of the macrocyclic ring is observed. Significantly, the Ahp residue occupies space adjacent to His57, and comparison with acyl-enzyme complexes obtained with simple peptide substrate/inhibitors indicates that it may disrupt hydrolysis by excluding the hydrolytic water. Such exclusion has been proposed to occur with small molecule inhibitors including β- and γ-lactams [16, 17]. Clearly, there is more to the mechanism of action of the cyanopeptolin inhibitors than displacement of the hydrolytic water, but the observation that the same complex scaffold can inhibit more than one protease should stimulate studies aimed at defining the essential inhibitory components and refining them into an accessible and readily modifiable template that binds reversibly.

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