FLUOROUS PEPTIDES GET READY TO HEAL

ACS MEETING NEWS: Fluorinated analogs of natural products start to take shape as potential therapeutic drugs

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WHAT DO YOU GET when you cross a frog with a nonstick frying pan? The answer: antibiotics with fluorinated amino acids.

Since 2001, several research groups have been making progress in incorporating fluorinated amino acids into proteins to enhance the proteins’ stability and potentially their efficacy as antibiotics and agents to treat diseases such as diabetes and cancer. Two presentations made during last month’s American Chemical Society national meeting in Boston highlighted promising examples.

By creating fluorinated versions of natural antibiotics found in a frog’s skin, the University of Michigan’s Neil Marsh aims to develop new classes of antibiotics that may combat bacterial resistance more effectively. Marsh outlined his group’s progress in this arena during a talk before the Division of Fluorine Chemistry.

No known natural amino acids contain fluorine, and few fluorinated natural products are known. But synthesis of common amino acids containing fluorinated substituents has led researchers to discover the benefits of incorporating fluorinated versions of amino acids such as leucine and valine into proteins. The resulting fluorinated proteins are significantly more stable toward thermal and chemical denaturation than the nonfluorinated versions, presumably because of the extremely hydrophobic nature of fluorocarbons. Fluorine also has substantial impact on protein-protein and ligand-receptor interactions.

For Marsh, making fluorinated proteins “started off as a purely blue-sky research project to answer the question: Can we marry together the interesting and useful properties of man-made materials with the exquisite specificity associated with biological molecules?”

At first, the Michigan team focused on small α-helical proteins, a protein structure commonly found in nature. The hydrophobic interiors of the helices are packed with hydrophobic leucine residues. By replacing a few leucine residues with hexafluoroleucine, the chemists created scintific cross-fertilization occurred,” Marsh said. “We quickly realized that the properties we had designed into our fluorinated proteins might be useful for the design of more potent AMPs.”

AMPs are small proteins with sequences of 15–30 amino acids that are produced by all animals, from insects to frogs to humans, Marsh explained. They serve as the first line of defense against microbial invaders that enter through the skin and mucous membranes. AMPs exert their antibacterial effect by forming pores in the bacterial membrane, thereby killing the organism.

Scientists have been interested in exploiting the medicinal properties of AMPs since they were first discovered in the 1980s, Marsh noted. But peptide-based therapeutics usually require injection, which makes them susceptible to degradation by bacterial protease enzymes.

In Boston, Marsh presented details of a study on the synthesis and properties of a fluorinated analog of MSL-78, a potent AMP known as pexiganan. Pexiganan is a synthetic analog of magainin-2, an α-helical AMP isolated from Xenopus laevis frogs. The team replaced two leucine and two isoleucine residues in pexiganan with hexafluoroleucine residues, which resulted in a new peptide that the researchers named fluorogain-1.

“The fluorinated AMP is much more stable to degradation by proteases,” Marsh noted. When pexiganan was treated with the proteases trypsin and chymotrypsin, it degraded within 30 minutes. But under the same conditions, fluorogain-1 was still intact after 10 hours.

And fluorogain-1 “is at least as good at killing common pathogenic bacteria as its nonfluorinated counterpart,” Marsh said. Against Klebsiella pneumoniae and Staphylococcus aureus, two tough bacteria, fluorogain-1 is significantly more potent than pexiganan. The fluorinated AMP was significantly less effective against only one species tested, Streptococcus pyogenes.

Asked to comment on the significance of Marsh’s research, David O’Hagan of the University of St. Andrews, in Edinburgh, Scotland, noted that the work on the frog peptides “has significantly increased their potential as useful antibiotics.” O’Hagan spoke in Boston about his research to eluci-
date the mechanism of action of fluorinase, the only known native enzyme that catalyzes C-F bond formation between an organic substrate and fluoride ion.

**THE TYPE** of AMPs that Marsh’s group is exploring have positively charged surfaces and are thought to form coiled coil dimer structures when they interact with the negatively charged bacterial membrane. This interaction permits the AMPs to compromise the integrity of the membrane, Marsh said. In the absence of membranes, the AMPs are unstructured and susceptible to enzyme-mediated proteolysis. The stability of the fluorinated AMPs most likely arises because they form stronger interactions with the cell membrane than the nonfluorinated versions and remain inaccessible to proteases, he noted. Marsh pointed out that the exact details of the interactions are unknown at this point and that his group is continuing to conduct experiments to learn more.

“These insights are exciting and point the way to using the fluorous effect to locate and stabilize complex biomolecules in membranes,” O’Hagan said.

Marsh’s group is not the only one to meet success with fluorinated peptides. Krishna Kumar and his group at Tufts University, in Medford, Mass., also have been broadly exploring the insertion of fluorinated amino acids into proteins to imbue them with Teflon-like properties. Kumar’s work includes the design of fluorinated drug delivery systems; potential cancer drugs; and antibiotics, including soon-to-be-published work on magainin-2 and the related AMP buforin-2 that parallels Marsh’s work.

During a Division of Medicinal Chemistry session in Boston, He Meng of Kumar’s group presented a poster highlighting progress on fluorinating a different type of protein, the gut hormone glucagon-like peptide-1 (GLP-1). This peptide has potential as a drug to treat diabetes and, like other peptides, suffers rapid degradation by proteases, leaving it with a half-life in blood of only a few minutes. A nonfluorinated GLP-1 mimic named Byetta (exenatide), which is derived from the saliva of the gila monster, has increased stability to proteases and already is being marketed by Amylin Pharmaceuticals and Eli Lilly & Co. to treat type 2 diabetes.

Meng described how the Tufts researchers replaced one or two amino acid residues in GLP-1 with hexafluoroleucine. Like fluorogargin-1, fluorinated GLP-1 proved to have enhanced enzymatic stability over the native version while preserving its biological activity. It also proved to be an effective binder of the GLP-1 receptor and is just as effective as the exenatide analogs.

The findings on fluorinated AMPs and GLP-1 by the Michigan and Tufts groups “have complemented each other well. We have chosen different systems to explore how the fluorous effect might be manifested in proteins,” Marsh told C&EN.

Kumar agrees. “Both these studies show that fluorination is a powerful method for increasing the potency and stability of known peptide and protein therapeutics, especially the ones that suffer rapid proteolysis in vivo,” he said.

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