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currently in Phase I/II trials. The German-based company have developed a small molecule that inhibits uPA, together with other serine proteases. Wilex are continuing their research with the WX-678 series of potent and selective small molecule uPA inhibitors, currently at the preclinical stage of development.

Although not actively involved in drug discovery, Almholt and colleagues are looking to the future. 'What we'd like to do is find a specific uPA inhibitor and test in same model,' says Almholt. 'This might be a small molecule, or more likely we'll look to antibodies specific to the mouse uPA.'

References

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Wide-angle X-ray scattering for screening functional ligands

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'Seeing' functional protein–ligand interactions is now possible through the novel application of an established biophysical technique. Researchers at the Argonne National Laboratory (IL, USA) have just demonstrated that ligand binding that induces any type of conformational change in the secondary, tertiary or quaternary structure of the protein can be detected using wide-angle X-ray scattering (WAXS).

Multiple length scale detection

Spotting when small molecules bind functionally to proteins to screen for potentially useful drug candidates has proved difficult. Nuclear magnetic resonance spectroscopy, small-angle X-ray scattering and X-ray crystallography can observe some of the conformational changes that occur, but each sees its own limited view. 'In addition, some of techniques, notably X-ray crystallography, require good crystals, significant amounts of protein and are laborious, making it difficult to do in a moderate-throughput fashion,' says lead author Robert Fischetti. WAXS detects structural change across multiple length scales – 'in other words, it can detect changes in structure at a scale from changes in position of individual amino acid residues in the active site all the way to hinge rotations of entire protein domains,' he explains. This is unique among the biophysical approaches to observation of conformational changes.

Tom Gadek (Chief Scientific Officer, SARcode, Oakley, California, USA) comments that he is 'very impressed by the sensitivity of the technique, particularly in the case of adipocyte lipid-binding protein'. In this protein, he observes, the binding of a lipid molecule can be easily detected even though it has no effect on the backbone alpha carbon fold of the protein and the reported WAXS difference signal arises from a reorientation of just three surface residue sidechains. Glenn Hammonds (Principal Scientist, Information Biology Consulting, Berkeley, California, USA) agrees that 'the ability of WAXS to rapidly detect ligand binding seems well demonstrated.' He adds that 'it could be useful in the absence of a specific and sensitive assay system, or if there is a need to distinguish among a limited number of ligands on the basis of their effect on conformation.'

Clearing a bottleneck in drug discovery

However, he cautions that the potential of the method to clear a bottleneck in pharmaceutical drug discovery depends on three factors. Firstly, whether most proteins do undergo some degree of conformational change on binding to a ligand, as the authors believe, secondly, whether detection of low affinity (5 mm Kd) hits is a desirable characteristic of an initial screen and, finally, on the economics of protein production. 'These requirements present some difficulties, since it is not at all clear what proportion of ligand binding events will produce a clear conformational signal; most traditional screening assays are geared toward detecting somewhat stronger interactions,' he says.

Fischetti and colleagues are convinced that the technique has proved itself well enough to identify small molecule ligands that alter the function of a protein that is central to a specific disease process. 'We are presently looking for collaborators to work with on an ongoing angiogenesis project at Argonne,' he reports. The group also looks forward to increasing the speed of the technique and scaling it up. 'This study was successful because of third-generation synchrotron sources. To really go to high throughput, one would need a dedicated facility, but given the speed and breadth of the technique, a single facility could serve a large number of projects,' concludes Fischetti.

Novel approach to combating superbugs

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With superbugs now ubiquitous in many health care settings, treating them becomes increasingly difficult as therapeutic options dwindle. Devising new strategies to overcome resistance has never been more crucial. Taking a novel approach to combating superbugs, biochemist Paul Hergenrother and colleagues at the University of Illinois, Urbana, IL, have devised a method to overcome resistance by targeting the DNA that renders them antibiotic-resistant.

The resistance problem

In recent years, only one new class of antibiotics with a novel mechanism of action surfaced from the pharmaceutical pipeline since

1359-6446/04/$ – see front matter PII: S1359-6446(04)03343-4

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fluoroquinolones were introduced in the 1970s. Identifying new bacterial targets poses another challenge. Available antibiotics aim at one of three targets: cell wall biosynthesis, protein synthesis, and enzymes involved in bacterial DNA replication. 'There are so few targets when trying to develop antibiotics compared with the plethora of targets in mammalian disease states,' says Mark Spaller, a chemist at Wayne State University in Detroit, MI. Compounding matters further, pharmaceutical giants are abandoning their anti-infectives programs for more lucrative pastures.

Bacterial exorcism

Bacteria acquire resistance when they take up foreign plasmids, carrying one or more antibiotic-resistance genes. However, should the bacteria lose its plasmid, antibiotic-susceptibility can be restored. 'The notion of eliminating plasmids could be a potential new target for treating drug-resistant bacteria,' explains Hergenrother. 'We wanted to mimic a very specific process known as plasmid incompatibility.'

Plasmid incompatibility is a natural mechanism for eliminating plasmids from bacteria. Two plasmids are deemed 'incompatible' with each other if they fail to co-segregate into daughter cells during cell division, resulting in elimination of one of the plasmids. For many plasmids, replication is controlled by the reversible binding of a small piece of untranslated RNA to the replication machinery, preventing translation and subsequent plasmid replication.

Their goal was to mimic binding of the small untranslated RNA with a small molecule, says Hergenrother. 'The compound causes plasmid elimination.' 'His strategy of targeting bacteria by plasmid ejection is like a bacterial 'exorcism' of sorts,' says Spaller.

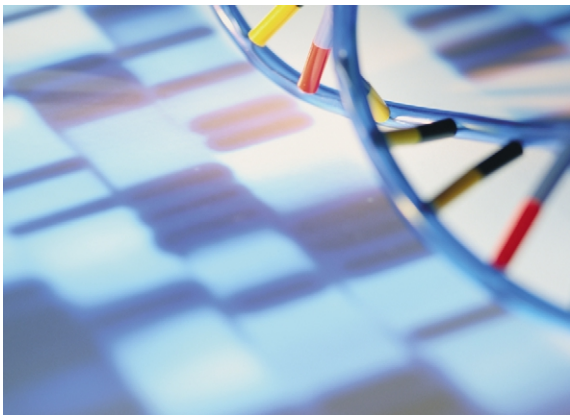
Proof of concept

Screening various aminoglycoside antibiotics, known to bind to RNA, Hergenrother and colleagues showed that one aminoglycoside in particular, apramycin, had notable affinity for the replication machinery of an already well-characterized plasmid. Not only did apramycin successfully mimic binding of the small RNA, it triggered loss of the β-lactamase encoding plasmid. Susceptibility to the β-lactam antibiotic, ampicillin, was restored, demonstrating proof of concept.

'We wanted to mimic a very specific process known as plasmid incompatibility'

'In principle, this opens the door to a novel way of treating antibiotic-resistant bacteria,' says chemical biologist, Scott Lokey, University of California, Santa Cruz. Furthermore, these findings have implications for drug therapies beyond those geared at treating bacterial infections. 'It's one thing to find compounds that bind RNA *in vitro*,' he explains, 'but it's another to find a small molecule-RNA interaction that actually works *in vivo*.'

Now, with proof of concept established, Hergenrother says their next steps involve moving toward clinically-important bacterial strains. 'Clinically-relevant plasmids often replicate via a different mechanism and that have multiple resistance genes,' he explains. 'What we need now is that well-characterized information for clinical plasmids.'



An attractive feature of some of these plasmids might very well prove useful as a weapon against their bacterial hosts, says Hergenrother. 'These plasmids have evolved an addiction system to encode a toxin and an antidote,' he says. 'If the cell divides and the daughter lacks the plasmid, it still has the toxin and dies. If we can destabilize one of these interactions, we can kill the cells from within.'

Further studies entail characterizing these clinical plasmids to identify potential new drug targets. From there, Hergenrother says they will use HTS to identify candidate small molecules. 'There really is a need for new bacterial targets that target replication machinery.'

Reference

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