Given that the tail comprises only ~40% of the total surface area of the toxins and the fact this feature is highly conserved across different cytotoxin families, the work by Perrins et al. raises important questions about why marine organisms go to the trouble to produce complex macrocycles. Their observation that the functionality of the tail segment was significantly enhanced with the addition of an aromatic group (a *para*-methoxyphenyl acetal protecting group), which is in actuality an artifact of the chemical synthesis, indicates that the macrocycle is not entirely superfluous, but can be modified or substituted with alternative hydrophobic moieties to help stabilize the actin-drug complex. Cursory docking of the most bioactive analog onto the structural coordinates of actin in its reidispongiolide A-bound conformation lends support to this hypothesis (Figure 2). The aromatic group could, in part, reconstitute important hydrophobic interactions with the shallow hydrophobic patch on actin that is contacted by analogous regions of many diverse barbed end-binding macrolides. Notably, this portion of the ring was previously been described as a likely component of the pharmacophore of these compounds (Allingham et al., 2006; Melville et al., 2007). Elaboration of the tail segment with other hydrophobic moieties in place of this portion of the ring could serve a dual role of stabilizing the interaction of the tail with actin, likely by reducing the entropy of binding, and providing a scaffold for appending cell-specific targeting ligands or optical probes.

In addition to the direct therapeutic applications, the work of investigators like Perrins et al. could also stimulate novel areas of interest in the development of actin-binding ligands as diagnostic or prognostic tools based on alterations of the cytoskeletal properties of tumor cells. On a more general note, such work may have important implications on the design of drugs that are intended to disrupt protein-protein interactions based on the filament-disrupting mechanism of these compounds.

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How to Identify a Pharmacophore

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The inhibition of chitinases by argifin and progressively dissected analogs had been studied by a combination of kinetic and crystallographic methods (Andersen et al., 2008). This work also leads to a general understanding of structure-activity relationships for inhibitors with one distinct pharmacophor.

In this issue of *Chemistry & Biology*, van Aalten and colleagues describe the identification of dimethylguanylurea as a pharmacophor for family 18 chitinases (Andersen et al., 2008). Dimethylguanylurea forms the terminus of the major side chain of argifin, a modified cyclopentapeptide which is a potent chitinase inhibitor. In hindsight, that finding may have been expected, since the molecule dimethylguanylurea is to the molecule argifin what the symbol + is to the symbol ♀. The beauty of this work lies in the complete set of methods applied. Five successively shortened linear fragments of argifin were synthesized. Their activity was tested against family 18 chitinases from three different species, fungus, human, and mouse. Crystals of one of these (chitinase B1 from the fungus *Aspergillus fumigatus*) were soaked with those fragments. The X-ray structures of the complexes were solved and compared to the known complex with argifin (Houston et al., 2002). All

of them were related to their enzymatic activity. This study is a textbook-like case to decipher a structure-activity relationship, and it sets a benchmark for the molecular identification of conceptual pharmacophors (Ehrlich, 1909). It may be regarded as the fruit of several years of experience with the enzyme and with the techniques applied.

Taken by itself, dimethylguanylurea is not much of a drug. Its affinity was in the millimolar range. Of three chitinases tested, its affinity for the fungal chitinase was highest, but even there, the measured IC_{50} value only was 0.5 mM. This is a 20,000-fold decrease in affinity as compared to argifin. However, that argument may be reversed: the natural product argifin has incorporated tremendously effective modifications of the pharmacophor dimethylguanylurea which led to an increase in affinity by a factor 20,000. Which steps attribute to the high affinity of argifin? How rigid are the bound structures? How flexible is the pharmacophor when bound to the active site? The answers obviously are of general interest.

The largest change in affinity (by a factor of 200) was observed when the intact argifin, the cyclopentapeptide, was compared with a liner tetrapeptide, missing one aspartic acid cut off opposite to the pharmacophore side chain, but otherwise identical. That large affinity change was by no means reflected in the crystal structure, where both inhibitors roughly occupy the same sites and were involved in basically the same molecular interactions. The authors rightfully argue that the different affinities likely result from entropic penalties which will accompany the binding of the flexible tetrapeptide to give the same backbone structure (within 0.6 Å) as argifin. Or, putting it as the not unexpected simplified take-home message: a cyclic peptide has a much higher affinity toward a target protein as compared to the linear peptide, when the conformations fit. Flexibility comes at a price.

When that tetrapeptide was truncated by an N-terminal alanine residue not involved in significant interactions with the enzyme active site, the resulting affinity of the tripeptide was only marginally lower. This finding was in accordance with minor changes detected in the crystal structure of the complex. But when a C-terminal aspartic acid involved in the network of hydrogen bonds also was removed, considerable changes (up to 3.4 Å) in the backbone of the bound peptide were observed. These changes were accompanied with major changes in hydrogen bonds for the peptide, but not for the pharmacophor. The affinity for the dipeptide was decreased, but only by a factor 2.4 as compared to the tripeptide. The different orientation of the dipeptide backbone obviously had led to an energetically more favorable array of molecular interactions. Again, there is a simple and not unexpected take-home message. Modification of a small molecule may lead to a different orientation of that molecule within the same protein site. That different orientation may leave parts of that molecule (in this case the pharmacophor with its four hydrogen bonds and one stacking interaction with one chitinase tryptophan) unaffected. Further truncation of the dipeptide toward the acetylated single amino acid monopeptide and finally to dimethylguanylurea resulted in affinities lower by factors of 6.7 and 6.2, respectively. This was to be expected and was in full accordance with the decreasing number of molecular interactions detected in the crystal structures.

With one exception, the amino acids interacting specifically with dimethylguanylurea are conserved in evolution. The ranking of argifin fragments is in agreement with chitin-argifin interactions identified in a previous mutagenesis study (Rao et al., 2005a). The same active site residues of chitinases employed in the detection of argifin interactions had been identified previously for other family 18 chitinase inhibitors (Terwisscha van Scheltinga et al., 1994; Rao et al., 2005b). Therefore, the results are in accordance with the literature. With this background, the contribution of van Aalten and his group (Andersen et al., 2008) may be regarded as one rare example of an original paper with conclusive results. Of course, dimethylguanylurea with its low affinity is not a drug. It is a small promising pharmacophor which may be used as a building block for future syntheses of enzyme inhibitors.

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