/THE FIRST WORD

Solvent Mapping and Molecular Recognition

February 4, the day before the 1995 Miami *Bio/Technology* Winter Symposium on Protein Engineering and Structural Biology began, was a landmark day for meteorology, as dire theoretical predictions of winter storms and arctic fronts were experimentally vindicated. Although a few featured speakers were lost to the inclement weather, most let neither sleet nor snow nor grant renewals keep them from their appointed rounds.

Among the stalwarts was Dagmar Ringe of Brandeis University (Waltham, MA), who described an ingenious experimental method that can be used to design potential drug leads by examining the pattern of bound solvent in the active sites and specificity pockets of proteins, among other things.

Called "solvent mapping," the method involves using organic solvents as probes for functional group binding sites. It can, in principle, be used to map the binding surface of any crystalline macromolecule, including those of nonenzymatic regulatory molecules interacting with receptors. It brings together observations about water, organic solvents, and protein structure, "double-sited" solutions to the target inhibitor problem, and computational methods in one neat package.

The problems associated with designing a target inhibitor to, or exploring the receptor interactions of, a protein are numerous. An experimental approach such as mutagenesis can be used to map these interactions, but this is a difficult and time-consuming process. Other current approaches are computational, and include MCSS, the multiple copy simultaneous search method, developed by Martin Karplus of Harvard University (Cambridge, MA). In the MCSS approach, computer graphics are used to construct molecular "probes" that are then used to explore the interaction surface of the protein in question. This approach will correctly identify the general region of the active site or receptor binding. Unfortunately, it cannot capture all the details of a specific match—and specificity is everything.

An example of true specificity is the hirudin-thrombin complex. Hirudin inhibits the active site of thrombin; however, a second part of hirudin interacts with a second region of the protein, which gives it its specificity. Is it possible to locate these second sites by design, rather than by accident?

Solvent mapping, which extends the MCSS method to an experimental situation, makes this much more possible. Instead of using computer-generated probes, it uses experimental ones—organic solvents such as methanols and ketones—to search for possible target sites. After the protein crystal is dipped in solvent, solvent clusters are seen in the active site. Solvent molecules are also often found clustered on other, secondary, sites on the molecule. By "connecting the dots" between the solvent clusters at the two sites, it becomes possible to design an inhibitor that is specific to that enzyme.

In a series of experiments with crystalline elastase, Ringe et al. took crystals of elastase, placed them in different organic solvents, and mapped the surface interactions—which turn out to be surface interactions of the solvent with the molecule and with the water attached to its surface. Elastase in solvent was compared to elastase in the absence of solvent to make sure that the enzyme was intact, and a set of potential binding sites was revealed by the mappings. The first inhibitor made incorporating these sites was totally insoluble. A subsequent, more hydrophilic, version fit very nicely.

Solvent mapping is an improvement over computational approaches because it is able to take water into account—something that is too difficult to do computationally. Another advantage is speed—it is possible to imagine running these experiments overnight and collecting new target data in the morning.

Solvent-protein interactions have been studied in detail because of their importance in enzyme purification and other protein separation processes. As this writing was completed, *Protein-Solvent Interactions* (1995, Marcel Dekker, New York), edited by Roger B. Gregory from Kent State University (Kent State, OH), came across my desk. It would make interesting ancillary reading for those thinking about applying Ringe's method to their own work.

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