PROTEIN BIOCHEMISTRY

Showing 'support' for chemical protein synthesis

Researchers demonstrate that in addition to protein ligation, folding and functional assays can be performed on proteins while they are still attached to a water-compatible solid support.

With recent advances in recombinant expression technology, researchers can make just about any protein of interest. But it is not always a simple pursuit, especially for complicated endeavors such as incorporating spectroscopic probes or making membrane proteins. Stephen Kent of the University of Chicago is one of the champions of the somewhat overlooked but quite useful alternative techniques of chemical protein synthesis.

Solid phase peptide synthesis (SPPS) is used to chemically synthesize peptides of less than 50 amino acid residues. To assemble larger proteins, native chemical ligation (NCL) is an established method to stitch together synthetic peptide pieces. It exploits the highly specific reaction between an N-terminal cysteine and an α-thioester, and transpires in that most gentle solvent of all, water. Recently, by using a water-compatible solid support upon which to assemble a protein, Kent and his M.D.-Ph.D. student Erik Johnson showed that it is possible to carry out ligation, folding and functional assays, all while the protein is still attached to the support.

Kent, Johnson and colleagues discovered ideal properties for performing support-based NCL in a poly(ethyleneoxide)-based commercially available resin, the water-compatible superpermeable organic combinatorial chemistry (SPOCC) resin. Using SPOCC and NCL, they synthesized the small protein EETI-II, a trypsin inhibitor found in squash seeds. Because SPOCC is compatible with water, EETI-II could be folded on the resin. Even more interestingly, EETI-II retained its trypsin-inhibiting activity while it was still attached to the support. Because SPOCC is highly permeable, large molecules such as proteins can diffuse in and out without much trouble, facilitating functional assays.

Kent acknowledges that the most obvious practical application for this method would be to perform combinatorial protein synthesis, such as assembling one-bead–one-compound libraries for functional studies. But he is more engaged in exploring the intriguing properties of various resins for improving the efficiency of support-based NCL to demonstrate its often straightforward application in comparison to recombinant expression. "We are much more interested in [applying NCL] to make larger proteins, especially membrane proteins, for which we can keep the polypeptide chain well-solvated and available for reaction until we want to put it in a lipid bilayer," says Kent. "We are more interested in being able to recover proteins in functional form rather than assay them directly on the resin."

Allison Doerr

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Johnson, E.C.B. *et al*. Total chemical synthesis, folding, and assay of a small protein on a water-compatible solid support. *Angew. Chem. Int. Ed.* **45**, 3283–3287 (2006).

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CHEMICAL BIOLOGY

GETTING A DNA TO DO AN RNA'S JOB

The successful *in vitro* evolution of a DNA enzyme based on a ribozyme could offer insights into early evolutionary events and provide guidelines for designing hardier catalytic oligonucleotides.

A variety of catalytic RNA molecules, or ribozymes, have been identified in nature and engineered in the laboratory; DNA-based versions of these ribozymes, however, consistently lack catalytic activity. Some people are surprised by this, says Gerald Joyce of The Scripps Research Institute, in part because it is easy to overlook the importance of the biochemical differences that distinguish ribonucleotides from deoxyribonucleotides. "But by the time they're functional molecules," he says, "by the time it's an RNA or DNA that folds into a particular shape and carries out an enzymatic function, all of the little differences between RNA and DNA make all the difference in the world."

This bears directly on one leading theory of the earliest stages of life, which posits a 'pre-RNA world' in which an RNA precursor mediated the earliest biological functions. At some point in time, pre-RNA gave way to RNA, but somehow these functional properties also transferred over to the more sophisticated macromolecule. This led Joyce to ask, "if you start with one kind of informational macromolecule that has a function, let's say RNA in this case, can you evolve it over to another informational macromolecule that retains the function or reacquires the function?"

To answer this, Joyce's group used R3C—an engineered RNA ligase ribozyme—as the starting point, synthesized its (inactive) DNA counterpart and then subjected it to multiple rounds of test-tube evolution, each time selecting for DNAs with the ability to ligate two RNA substrate molecules. After ten rounds, they isolated clone 10-18, a deoxyribozyme with approximately one-half the catalytic rate of the template ribozyme.

Clone 10-18 seems to retain the same general catalytic mechanism, although the ligation bond it forms is chemically different than that formed by R3C. Nonetheless, says Joyce, "it's the first time that anybody's ever taken function 'over the falls', from one macromolecule to another through an evolutionary transition." As such, this work marks a milestone in understanding the earliest stages of prebiotic evolution. This work has practical benefits as well, according to Joyce: "If you have an RNA enzyme that's interesting, but you wish it was more rock-hard like DNA... you could take the RNA and evolve it into being a DNA enzyme. So one way to harden an RNA enzyme is by 'DNA-izing' it."

Michael Eisenstein

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Paul, N. *et al.* Conversion of a ribozyme to a deoxyribozyme through *in vitro* evolution. *Chem. Biol.* **13**, 329–338 (2006).