Peptidic mimetics of hydrolases

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Abstract

Rational design of artificial enzymes that can catalyze reactions with efficiency comparable to native enzymes and possess similar properties is an extremely challenging task. Knowledge of known reaction mechanisms and examples of native enzymes can serve as a valuable foundation for such projects, where it is possible to recreate the active site of an enzyme or introduce a non-protein cofactor. This is feasible because the general catalytic mechanisms of enzymes within a given class are quite similar to one another. There are few examples in the literature of successfully recreating a catalytically active hydrolase mimic on a small peptide scaffold. Creating a cysteine hydrolase mimic, in particular, is a challenging task due to the highly reactive nature of the cysteine residue, which plays a key role in the function of these enzymes.

In this work, enzyme mimics based on naturally occurring hydrolases, including metallohydrolases and cysteine hydrolases, were designed, synthesized, and studied. The peptides and miniproteins used in the design did not exceed 50 amino acid residues and were obtained using the SPPS method, purified, and examined for their structural properties (CD studies) and catalytic activity (spectrofluorometric studies) towards ester organic substrates. The work was organized based on the complexity and size of the peptide enzyme mimics. Initially, the placement of key residues (cysteine and histidine) on foldameric, helical peptides containing 2-amino-1-cyclopentanecarboxylic acid residues was investigated. Subsequently, by increasing the protein scaffold, a short foldameric zinc finger analog was developed, which was able to fold having only 3 histidine residues able to coordinate zinc ion. The zinc-binding site potentially served as a metalloenzyme analog. A similar approach was taken in the case of work on the MvaT protein domain.[1] In this small, 43-amino acid miniprotein with a well-defined tertiary structure, hydrophobic core residues responsible for folding were mutated. They were replaced with four residues capable of coordinating a zinc ion, thereby recovering the original tertiary structure upon metal ion binding. Analogs with three amino acid ligands were synthesized, but they did not show catalytic activity.

The MvaT miniprotein domain was also used to create a cysteine hydrolase mimic. Out of 11 examples of incorporating an active site with the appropriate geometry, one was selected and optimized multiple times. Catalysis optimization was carried out by stabilizing intermediates by changing residues around the active site, altering the global dipole moment by neutralizing the charged residues of the miniprotein, and increasing substrate affinity by adding hydrophobic residues in the second coordination sphere. This resulted in a more than 1,000-fold acceleration of the hydrolysis reaction compared to the non-catalyzed reaction, with an enzymatic hydrolysis profile.

Using in silico design methods, 21 mini-proteins containing varying numbers of 2-amino-1-cyclopentanecarboxylic acid residues were also synthesized. Structural analysis using CD and DSC methods allowed for the selection of three examples that exhibited partial folding. Based on these, several cysteine hydrolase analogs were `proposed, primarily incorporating cysteine and histidine residues. These mimics demonstrated faster catalysis compared to the initial scaffolds.