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Peptide Nanoparticle Catalysis

Michael J. Heller, Michelle Cheung and Tsukasa Takahashi
University of California San Diego, Departments of Nanoengineering and Bioengineering, Atkinson Hall, Rm 2312, La Jolla, CA, USA 92093-0448

ABSTRACT

Improved catalysis for more efficient chemical processing, biomass conversions and water oxidation-hydrogen production will be important for achieving new clean technologies. Our research work involves a novel approach to produce catalytic mechanistic properties in synthetic peptide nanoparticle structures that mimic the active sites of enzymes. A series of six peptides have now been designed and synthesized which contain a cysteine sulfhydryal group (nucleophile) and histidine imidazole group (general base) in close proximity. Upon acylation of the cysteine sulfhydryal group, some of the peptides with closely proximated imidazole groups, exhibit accelerated deacylation in the presence of a trapping reagent. Peptides with phenylalanine groups between the cysteine and histidine have deacylation rates more than 10 times higher than in the peptides without a histidine. This accelerated deacylation indicates that the histidine imidazole group is functioning much like it does in the catalytic sites of the natural cysteine and serine proteases. These peptides will ultimately be attached to derivatized nanoparticles which are designed to bind appropriate substrates (esters and amides) through hydrophobic and/or electrostatic interactions. The final goal being to use the peptide nanoparticle structures to accelerate both the acylation and deacylation rates and produce true substrate turnover.

Keywords: catalysis, synzymes, peptides, biomimetic, nanocatalysis

INTRODUCTION

Of all the magnificent macromolecules in living organisms, enzymes represent those which are the most complex in terms of mechanistic properties. Enzymes are able to catalyze the transformation of all other biomolecules, providing the dynamics and very essence of life[1-3]. In these new days of nanotechnology, enzymes can aptly be considered natural bio-nanomachines which do chemistry, and they do it very well. These bio-nanomachines catalyze reactions with high specificity and enormous rate accelerations, some having turnover numbers of millions of substrate molecules per second. Over the past three decades considerable efforts have been made to create synthetic versions of enzymes with are sometimes called *synzymes.* Most have failed, and the few so-called successes are at best only marginal exhibiting properties that can barely be described as catalytic [4-5]. Many of the synzyme models are based on peptide, macromolecule and more recently nanostructures that are designed to closely resemble the active site of an enzyme. While these synthetic structures look similar to the enzyme active site they do not have the unique mechanical or dynamic catalytic properties to transform a substrate molecule into the desired product molecule in a repeated process i.e., turnover.

By way of an example, the enzyme Papain is a cysteine protease from papaya. Papain‘s active site contains a catalytic triad is composed of Cys25, His 159, and Asp 158 which is used for breaking peptide bonds [6-7]. The catalysis reaction of substrate is initiated by a nucleophilic attack from the cysteine’s thiol group. Acetyl exchange transfers the acyl group from thiol to imidazole before the acetyl group leaves the complex [8].

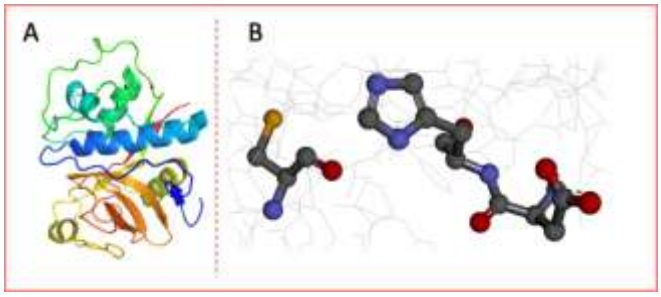

Figure 1 – (A) shows the basic 3D structure of the cysteine protease called Papain. (B) shows the catalytic triad is composed of Cys 25-His 159-Asp 158.

Our goal has been to design and synthesize novel peptide synzyme sequences which begin to mimic the acylation and deacylation mechanism of the cysteine proteases. Six synthetic peptides (synzyme) composed of nine amino acids containing a combination of Serine (hydroxyl), Cysteine (thiol), Histidine (Imidazole) were designed. Synzyme-substrate reactions were performed and the corresponding acylation/deacylation rate constants were calculated. Intramolecular interactions of the Cys-His and Cys-Ser peptides were studied using molecular modeling and NMR.

RESULTS

A series of peptides have been designed and synthesized which contain a cysteine sulfhydryal group (nucleophile) and histidine imidazole group (general base) in close proximity (see Table below). Peptides 1 and 3 do not contain a histidine, and serve as controls for the study.

Synzyme 1	Gly-Gly-Ala-Ala-Cys-Ala-Ser-Ala-Asp
Synzyme 2	Gly-Gly-Ala-Ala-Cys-Ala-His-Ala-Asp
Synzyme 3	Arg-Gly-Ala-Ala-Cys-Ala-Ser-Ala-Asp
Synzyme 4	Arg-Gly-Ala-Ala-Cys-Ala-His-Ala-Asp
Synzyme 5	Arg-Gly-Ala-Phe-Cys-Phe-His-Ala-Asp
Synzyme 6	Lys-Gly-Ala-Phe-Cys-Phe-His-Ala-Asp

Figure 2 and 3 below show the CPK space filling structures of peptide 2 and 6.

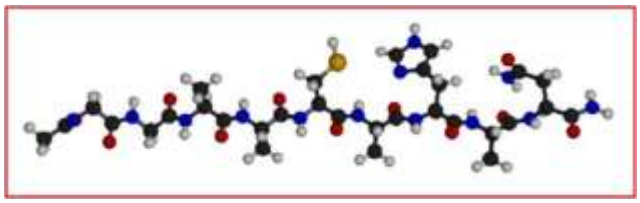

Figure 2 – Shows the CPK space filling structure of the synzyme peptide 2

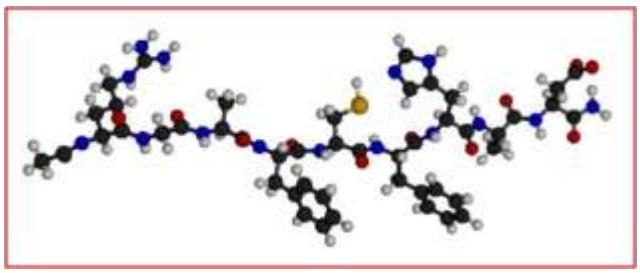

Figure 3 – Shows the CPK space filling structure of the synzyme peptide 3.

Upon acylation of the cysteine sulfhydryal group, some of the peptides with closely proximated imidazole groups, exhibit accelerated deacylation in the presence of a trapping reagent. Peptides 5 and 6 in particular, have deacylation rates more than 10 times higher than in the peptides without the histidine. This accelerated deacylation indicates that the histidine imidazole group is functioning much like it does in the catalytic sites of the natural cysteine and serine proteases. In the case of peptides 5 and 6, the presence of the phenylalanines between the cysteine and histidine apparently causes the sulfydryal and imidazole groups to be in better proximity, leading to the improved deacylation.

CONCLUSIONS

Our peptide synzyme structures demonstrated a noticeable level of deacylation catalytic ability compared to controls. Strict dimensional constraint created by the peptide synzymes begins to mimic the catalytic triad of papain and demonstrated a high deacylation rate. However, rates are not as high as true enzymes which shift the equilibrium of the acyl-exchange within the catalytic triad much more efficient [9]. Our plans include making a variety of new peptides that contain multiple histidines to aid the catalysis reaction[10]. In order to reduce the thiol back-attack, dynamic molecular motion of the acyl exchange must be controlled to achieve a greater distance once acyl-imidazole compound to formed. The ability to develop synthetic catalysts which operate under mild conditions (temperature, pressure, etc.) would have a large impact on many biotechnology and biomedical areas[4]. Even more important, synzymes could have major impact in the chemical, renewable energy, food processing, waste processing and environmental related industries. A few uses could include synthetic catalysis for production of hydrogen, ethanol production, high fructose corn syrup production and as catalyst for fuel cells.

REFERENCES

[1] Hunter T. Protein kinases and phosphatases: the yin and yang of protein phosphorylation and signaling. Cell, 80, 225-236, 1995. [2] Bachovchin WW. Review: Contributions of NMR spectroscopy to the study of hydrogen bonds in the serine protease active sites. Magnetic Resonance Chemistry, 39, S199-S213, 2001. [3] Fricker SP. Cysteine proteases as targets for metal-based drugs. Metallomics, 2, 366-377, 2010. [4] Kisailus D, Truong Q, Amemiya Y, Weaver JC, Morse DE. Self-assembled bifunctional

surface mimics an enzymatic and templating protein for the synthesis of a metal oxide semiconductor. PNAS, 103-15, 5652-5657, 2006. [5] Bommarius AS and Riebel BR. Biocatalysis - Fundamentals and Applications. 2004. [6] Vernet T, Tessier DC, Chatllier J, Plouffe C, Lee TS, Thomas DY, Storer AC, and Menard R. Structural and Functional Roles of Asparagine 175 in the Cysteine Protease Papain. J. Bio. Chem. 270, 16645-16652, 1995. [7] Dodson G and Wlodawer A. Catalytic triads and their relatives. Trends Biochem Sci., 23, 347-352, 1998. [8] Verdegem PJE, Helmle M, Lugtenburg J, and de Groot HJM. Journal of the American Chemical Society, 119, 169-174, 1997. [9] Heller MJ, Walder JA, and Klotz IM. Intramolecular catalysis of acylation and deacylation in peptides containing cysteine and histidine. Journal of the American Chemical Society, 99, 2780-2785, 1977. [10] Kirsch JF and Jencks WP. Nonlinear Structure-Reactivity Correlations. The Imidazole-Catalyzed Hydrolysis of Esters. Journal of the American Chemical Society, 86, 837-846, 1964.