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Studies of SpnF-Catalyzed [4+2]-Cycloaddition in the Biosynthesis of Spinosyn A

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Abstract

The Diels–Alder reaction is a [4+2]-cycloaddition reaction in which a cyclohexene ring is formed between a 1,3-diene and an electron deficient alkene via a single pericyclic transition state. This reaction has been proposed as a key transformation in the biosynthesis of many cyclohexene-containing secondary metabolites. Although several purified enzymes have been implicated in biotransformations that are consistent with a Diels–Alder reaction, these enzymes typically demonstrate more than one catalytic activity, leaving their specific influence on the cycloaddition step uncertain.

In our studies of the biosynthesis of spinosyn A, a tetracyclic polyketide-derived insecticide from *Saccharopolyspora spinosa*, we identified a cyclase, SpnF, that catalyzes a transannular [4+2]-cycloaddition to form the cyclohexene ring in the final product. SpnF is unique, because it is the first enzyme that was characterized to specifically catalyze a [4+2]-cycloaddition without introducing any other changes to its

substrate. The same cycloaddition also takes place nonenzymatically, but at a much reduced rate. If the reaction catalyzed by SpnF is a concerted process with a single pericyclic transition state, then SpnF would be the first example of a naturally occurring Diels-Alderase. In order to investigate this possibility, *alpha*-secondary deuterium kinetic isotope effects were measured at all points of rehybridization in the diene during both the nonenzymatic and SpnF-catalyzed [4+2]-cycloaddition reactions. This was accomplished using regiospecifically deuterated substrates and electrospray ionization, time-of-flight mass spectrometry to follow changes in deuterium enrichment of the substrate as the reaction progressed. This presentation will describe the measurement of these KIEs and offer a mechanistic discussion of their implications for understanding the SpnF catalyzed [4+2]-cycloaddition.

Selected Publications

1. Ruszczycky, M. W.; Zhong, A.; Liu, H.-w. "Following the Electrons: Peculiarities in the Catalytic Cycles of Radical SAM Enzymes." *Nat. Prod. Rep.* **2018**, *53*, in press.
2. Ko, Y.; Lin, G.-M.; Ruszczycky, M. W.; Liu, H.-w. "Mechanistic Implications of the Deamination of TDP-4-amino-4-deoxy-fucose Catalyzed by the Radical SAM Enzyme DesII." *Biochemistry* **2018**, *57*, in press.
3. Chen, J.-K.; Lin, W.-L.; Chen. Z.; Liu, H.-w. "PARP-1-Dependent Recruitment of Cold Induced RNA-Binding Protein Promotes Genome Stability." *Proc. Natl. Aca. Sci. USA.* **2018**, *115*, in press.
4. Yang, Z.; Yang, S.; Yu, P.; Li, Y.; Doubleday, C.; Park, J.; Patel, A.; Jeon, B.-s.; Russell, W. K.; Liu, H.-w.; Russell, D. H.; Houk, K. N. "Influence of Water and Enzyme SpnF on the Dynamics and Energetics of the Ambimodal [6+4]/[4+2] Cycloaddition." *Proc. Natl. Aca. Sci. USA.* **2018**, *115*, in press.
5. Besandre, R.; Liu, H.-w. "Biochemical Basis of Vosevi, a New Treatment for Hepatitis C." *Biochemistry* **2018**, *57*, 479-480.
6. Lin, G.-M.; Romo, A. J.; Liem, P. H.; Chen, Z.; Liu, H.-w. "Identification and Interrogation of the Herbicidin Biosynthetic Gene Cluster: First Insight into the Biosynthesis of a Rare Undecose Nucleoside Antibiotic." *J. Am. Chem. Soc.* **2017**, *139*, 16450-16453.
7. Kim, H. J.; Liu, Y.-n.; McCarty, R. M.; Liu, H.-w. "Biochemical Characterization and Stereochemical Analysis of Reactions Catalyzed by GenK, a Cobalamin-Dependent Radical SAM Methyltransferase Involved in Gentamicin Biosynthesis." *J. Am. Chem. Soc.* **2017**, *139*, 16084-16087.
8. Ruszczycky, M. W.; Liu, H.-w. "The Surprising History of an Antioxidant." *Nature* **2017**, *551*, 37-38.

Honors and Awards

Camille & Henry Dreyfus Grant for Distinguished New Faculty in Chemistry (1984)
American Cancer Society Junior Faculty Research Award (1985)
Eli Lilly Life Science Young Investigator Award (1985)
NIH Research Career Development Award (1990-5)
Horace S. Isbell Award, American Chemical Society, Division of Carbohydrate Chemistry (1993)
National Institute of General Medical Sciences MERIT Award (1999)
Distinguished McKnight University Professor, University of Minnesota (1999)
Honorary Professor of Chemistry, University of Hong Kong (2001-2007)
American Association for the Advancement of Science Fellow (2005)
American Academy of Microbiology Fellow (2006)
Japan Society for the Promotion of Science Fellow (2006)
Nakanishi Prize, American Chemical Society, Division of Organic Chemistry (2007)
Repligen Award in Chemistry of Biological Processes, American Chemical Society, Division of Biological Chemistry (2008)
Elected Academician of Academia Sinica (2008)
Tunghai University Distinguished Alumni Award (2008), Honorary Professor (2011)
Honorary Professor, National Tsing Hua University, Taiwan (2011)
A. I. Scott Medal for Excellence in Biological Chemistry Research (2011)
American Chemical Society Fellow (2014)
Arthur C. Cope Late Career Scholars Award, American Chemical Society (2014)