JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY

COMMUNICATION

pubs.acs.org/JACS

Molecular Mechanism for Eliminylation, a Newly Discovered Post-Translational Modification

Zhihong Ke,[†] Gregory K. Smith,[†] Yingkai Zhang,[‡] and Hua Guo*,[†]

[†]Department of Chemistry and Chemical Biology, University of New Mexico, Albuquerque, New Mexico 87131, United States
[‡]Department of Chemistry, New York University, New York, New York 10003, United States

Supporting Information

ABSTRACT: The newly discovered bacterial phosphothreonine lyases perform a post-translational modification of host cell signaling proteins through a novel catalytic mechanism that irreversibly removes the phosphate group from a phosphorylated threonine via β-elimination. This "eliminylation" reaction is shown by ab initio QM/MM studies to proceed via an E1cB-like pathway, in which the carbanion intermediate is stabilized by an enzyme oxyanion hole provided by Lys104 and Tyr158 of SpvC.

Post-translational modifications (PTMs), such as methylation and phosphorylation, covalently modify amino acid residues in peptides,[1] altering the structural properties of the peptides and their interactions with other molecules. PTMs play an important role in cell signaling and gene expression, and their malfunction has been implicated in many diseases. This communication is concerned with a newly discovered PTM process called eliminylation[2] that leads to irreversible removal of the phosphate group from a phosphorylated threonine residue. Interestingly, the strategy used by phosphothreonine lyases, which catalyze eliminylation, is quite novel and differs from the well-known phosphoryl transfer mechanism used by protein phosphatases. Instead of cleaving the $P-O_\gamma$ bond, these enzymes catalyze breaking of the $C_\beta-O_\gamma$ bond and formation of a $C_\alpha=C_\beta$ double bond via β-elimination.[3,4] The resulting π system presents an easy target for Michael addition, thus eliminating the possibility of future phosphorylation of the threonine residue.

While eliminylation has yet to be identified in eukaryotic cells, its usage by bacteria has been well-documented.[2] Many bacteria have been found to evade host immune responses,[5,6] often by injecting into the host cell protein effectors that disrupt host signal transduction pathways.[7,8] One class of such bacterial effectors consists of phosphothreonine lyases, which have recently been found in *Salmonella*,[4] *Shigella*,[3,9] and *Pseudomonas syringae*.[10] The other example of eliminylation is found in bacterial biosynthesis of lantibiotics, which are macrocyclic peptides with thioether cross-links. Lanthionine synthetase catalyzes the removal of the OH group from a serine/threonine residue by initial phosphorylation with a kinase domain followed by β-elimination of the phosphate group with a lyase domain, in the same fashion as phosphothreonine lyases. The addition of a terminal Cys residue to the π bond completes the macrocyclization.[11] Although no crystal structure is known, the lyase domain of the enzyme shares some active-site residues with phosphothreonine lyases.[12]

The crystal structure of the phosphothreonine lyase SpvC from *Salmonella typhimurium* has recently been solved,[4,13] and its

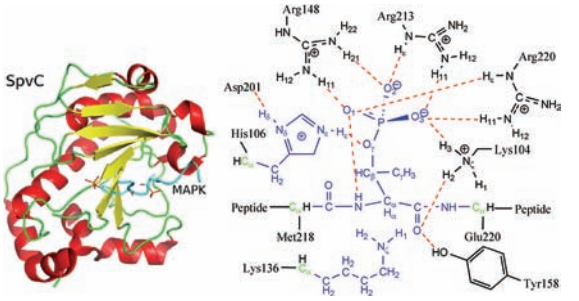


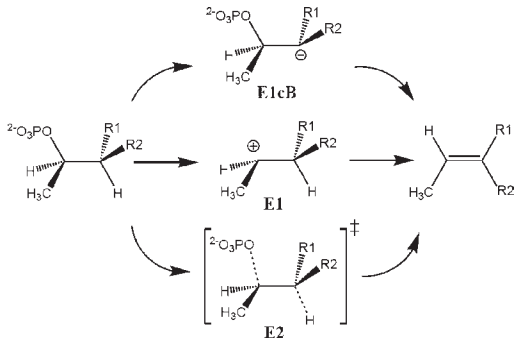
Figure 1. (left) Overall structure of SpvC. (right) Arrangement of the active site. The QM region includes the atoms colored in blue and the boundary atoms colored in green. Hydrogen bonds are colored red.

novel α/β fold (shown in Figure 1) differs from all other known folds. The peptide substrate contains the signature pThr-X-pTyr motif of mitogen-activated protein kinases (MAPKs), which is recognized by SpvC. Specifically, pTyr is bound with Lys160, Lys134, and Arg80, while pThr inserts its phosphate group into a pocket formed by Arg220, Arg213, Arg148, and Lys104. The binding of the substrate induces a conformational change in the loop containing Arg220, which seals the positive pocket surrounding the pThr phosphate. The active-site arrangement of SpvC is also depicted in Figure 1. All phosphothreonine lyases share two critical residues that have been implicated in catalysis by mutagenesis experiments, kinetic data, and their proximity to the substrate in the X-ray structure.[4] The putative base for the β-elimination is Lys136 in SpvC, which abstracts the α-proton from pThr in the substrate. A conserved His106 is thought to protonate the phosphate leaving group. Despite this general description, however, the detailed mechanism by which the β-elimination is catalyzed is still unresolved. Information concerning the microscopic reaction pathway, such as the structure and charge distribution of the transition state(s), might be helpful for designing agents to regulate such PTM events.

As shown in Scheme 1, there are three possible mechanistic scenarios for a β-elimination reaction. The E1cB mechanism envisions a carbanion intermediate created by the abstraction of the α-proton. The E1 mechanism, on the other hand, assumes a carbocation intermediate generated by the unimolecular loss of inorganic phosphate. Finally, there is the concerted, or E2, mechanism, wherein the two processes occur simultaneously. To identify the correct molecular mechanism, we investigated the reaction using

Received: May 12, 2011
Published: June 28, 2011

Scheme 1. Three Possible Reaction Mechanisms for β-Elimination



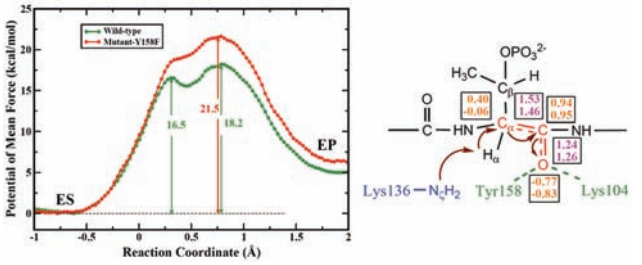


Figure 2. (left) Calculated PMFs for wild-type SpvC (green) and the Y158F mutant (red). (right) Scheme for charge stabilization of the carbanion intermediate. The atomic charges are given in orange and the bond lengths in purple. The first and second numbers are for ES and INT, respectively.

the quantum mechanical/molecular mechanical (QM/MM) approach, a proven method for studying enzyme catalysis.[14−16] While the simulation protocols are described in detail in the Supporting Information (SI), we note here that the QM system, which is colored blue in the right panel of Figure 1, included the phosphothreonine and the side chains of both Lys136 and His106, totaling 52 atoms. On the basis of pH-titration curves of wild-type and mutant SpvC,[4] Lys136 and His106 were assigned to be deprotonated and protonated, serving as the general base and general acid, respectively. The effects of several important secondary residues, such as Asp201, Tyr158, and Lys104, were approximated via the MM force field. The QM region was treated with density functional theory (DFT) at the B3LYP/6-31G* level using the pseudobond formalism.[17,18] The nonempirical treatment of the QM region is important for a reliable description of the chemistry.

To address the mechanistic questions related to the catalyzed reaction, we first calculated the QM/MM minimal-energy surface in both the α-proton abstraction coordinate and the $C_\beta-O_\gamma$ bond-breaking coordinate. The resulting two-dimensional surface, which is shown in Figure S1 in the SI, strongly suggests that the proton abstraction occurs prior to the $C_\beta-O_\gamma$ bond cleavage. From the reaction coordinate, which was chosen to be $d_{C_\beta-O_\gamma} - d_{H_\alpha-N_\zeta}$, the potential of mean force (PMF) was then calculated for wild-type SpvC using the same QM/MM Hamiltonian. The free-energy profile (displayed in green in Figure 2) indicates a stepwise E1cB-like reaction mechanism with a shallow minimum flanked by two transition states. As shown in Figure 3, the first transition state (TS-I) is primarily for the abstraction of the α-proton by Lys136, while the second and rate-limiting transition state (TS-II) is for

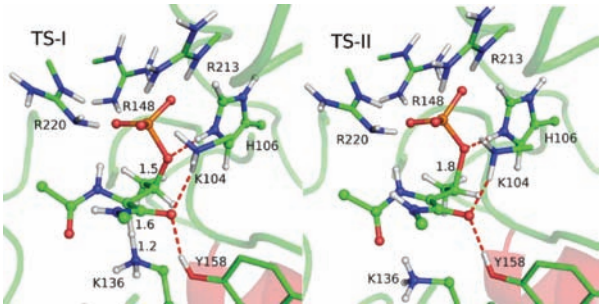


Figure 3. Structures of TS-I and TS-II for wild-type SpvC. The hydrogen bonds of interest are represented by red dashed lines, and some key distances are given.

$C_\beta-O_\gamma$ bond cleavage. The shallow minimum in the PMF represents a carbanion intermediate (INT). The overall barrier is 18.2 kcal/mol, which is in reasonably good agreement with the experimental value of 16.0 kcal/mol estimated from the value of 10.60 s^{-1} for k_{cat}.[4]

It is interesting to note that the QM/MM results differ from those obtained in our earlier DFT study using a truncated active-site model, which suggested a concerted mechanism.[19] This discrepancy is likely due to the small size of the truncated active-site model used in our earlier work. An analysis of charges along the reaction path shows that as the α-proton is extracted, C_α develops a negative charge. However, this charge can be stabilized via a resonance structure featuring an enolate form of the adjacent backbone carbonyl, as illustrated in Figure 2. This hypothesis is supported by the fact that the charge of the carbonyl O changes from -0.77 ± 0.04 at the enzyme−substrate complex (ES) to -0.83 ± 0.06 at INT. The development of a negative charge on the carbonyl O is accompanied by a shortening of the $C_\alpha-C$ bond from 1.53 ± 0.07 Å (ES) to 1.46 ± 0.07 Å (INT) and an elongation of the carbonyl bond from 1.24 ± 0.05 Å (ES) to 1.26 ± 0.05 Å (INT). The spread of the negative charge is facilitated by an oxyanion hole provided by the enzyme. Indeed, the hydrogen bonds of the carbonyl to Lys104 and Tyr158 become stronger as the negative charge develops at the enolate O, as indicated by the shortening of the hydrogen-bond lengths from 1.95 ± 0.19 and 1.81 ± 0.16 Å (ES) to 1.83 ± 0.12 and 1.79 ± 0.15 Å (INT), respectively. These two residues were not included in our earlier model.

To further confirm our hypothesis, we performed PMF calculations for the Y158F mutant, in which the hydrogen bond between Tyr158 and the carbonyl O is abolished. However, the hydrogen bond involving Lys104 is still maintained in all stationary states except the enzyme−product complex (EP), where the interaction with the phosphate group is the sole strong interaction. The removal of the hydrogen bond to residue 158 in the Y158F mutant increases the overall barrier by 3.3 kcal/mol, but the shape of the barrier remains roughly the same, as shown in Figure 2. This increase in the barrier height is consistent with mutagenesis data, which showed that the Y158F mutant retains only 5% of the catalytic activity of the wild type.[4] To further understand the role played by secondary residues and the enzyme itself, we computed the nonbonded contributions of all MM residues to the barriers. As discussed in the SI, these results are also consistent with the mutagenesis data, providing additional support for the proposed reaction mechanism.

While the strategy used by the phosphothreonine lyase SpvC is unique and novel, it shares some features with those used by several well-studied enzymes. The enolase superfamily, for

example, is known to abstract the α-protons of carboxylic acids, although the subsequent events may differ significantly for different members of the superfamily.[20,21] Much discussion has been devoted to the acidity of the α-proton and the mechanism of the catalysis.[22−24] Convincing evidence based on kinetic isotope effects (KIEs) indicated that the reaction is stepwise and involves a carbanion intermediate.[25] This conclusion was confirmed by QM/MM calculations,[26−28] which suggested that the negative charge developed on C_α is spread to an enolate oxygen stabilized by metal ions (e.g., Mg^{2+}). Phosphothreonine lyases differ in several aspects. First, the α-proton is from the backbone of the peptide substrate instead of the carboxylic acid. Second, no metal ion is involved. Instead, the oxyanion hole is stabilized by hydrogen-bonding interactions. Finally, the *β*-elimination removes a phosphate group rather than H_2O as in the case of enolases. In the last aspect, SpvC is perhaps more closely related to methylglyoxal synthase (MGS), which catalyzes the elimination of the phosphate group of dihydroxyacetone phosphate (DHAP) initiated by proton abstraction.[29]

In conclusion, our ab initio QM/MM calculations have revealed that eliminylation catalyzed by SpvC utilizes an E1cB-like mechanism in which the reaction is initiated by α-proton abstraction by Lys136. A metastable enolate intermediate has been identified in which the negative charge developed on C_α is spread via a conjugated resonance to the backbone carbonyl oxygen. The carbanionic intermediate is stabilized by an enzyme oxyanion hole consisting of Tyr158 and Lys104. The second and rate-liming elimination step in this enzymatic reaction is catalyzed by His106, which protonates the leaving group. In addition, several cationic residues, namely Arg220, Arg213, Arg148, and Lys104, provide further electrostatic stabilization of the phosphate leaving group. Their catalytic roles are consistent with structural and mutagenesis data.

■ ASSOCIATED CONTENT

Supporting Information. Details of the simulation protocol, the minimal-energy surface, and analysis of QM−MM interactions. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author
hguo@unm.edu

■ ACKNOWLEDGMENT

This work was supported by the NIH (R01-GM079223 to Y.Z., R03-AI071992 to H.G.). The calculations were performed at the National Center for Supercomputing Applications (NCSA) and the New Mexico Center for Computational Applications. We thank Alvan Hengge for several useful discussions.

■ REFERENCES

(1) Wold, F. *Annu. Rev. Biochem.* **1981**, *50*, 783.
(2) Brennan, D. F.; Barford, D. *Trends Biochem. Sci.* **2009**, *34*, 108.
(3) Li, H.; Xu, H.; Zhou, Y.; Zhang, J.; Long, C.; Li, S.; Chen, S.; Zhou, J. M.; Shao, F. *Science* **2007**, *315*, 1000.
(4) Zhu, Y.; Li, H.; Long, C.; Hu, L.; Xu, H.; Liu, L.; Chen, S.; Wang, D. C.; Shao, F. *Mol. Cell* **2007**, *28*, 899.
(5) Brodsky, I. E.; Medzhitov, R. *Nat. Cell Biol.* **2009**, *11*, 521.
(6) Ribet, D.; Cossart, P. *Cell* **2010**, 694.
(7) Cornelis, G. R. *Nat. Rev. Microbiol.* **2006**, *4*, 811.
(8) Galan, J. E.; Wolf-Watz, H. *Nature* **2006**, *444*, 567.
(9) Arbibe, L.; Kim, D. W.; Batsche, E.; Pedron, T.; Mateescu, B.; Muchardt, C.; Parsot, C.; Sansonetti, P. J. *Nat. Immunol.* **2007**, *8*, 47.
(10) Zhang, J.; Shao, F.; Li, Y.; Cui, H.; Chen, L.; Li, H.; Zou, Y.; Long, C.; Lan, L.; Chai, J.; Chen, S.; Tang, X.; Zhou, J.-M. *Cell Host Microbe* **2007**, *1*, 175.
(11) Goto, Y.; Li, B.; Shi, Y.; Bibb, M. J.; van der Donk, W. A. *PLoS Biol.* **2010**, *8*, No. e1000339.
(12) Goto, Y.; Okesli, A.; van der Donk, W. A. *Biochemistry* **2011**, *50*, 891.
(13) Chen, L.; Wang, H.; Zhang, J.; Gu, L.; Huang, N.; Zhou, J.-M.; Chai, J. *Nat. Struct. Mol. Biol.* **2008**, *15*, 101.
(14) Warshel, A. *Annu. Rev. Biophys. Biomol. Struct.* **2003**, *32*, 425.
(15) Gao, J.; Ma, S.; Major, D. T.; Nam, K.; Pu, J.; Truhlar, D. *Chem. Rev.* **2006**, *106*, 3188.
(16) Senn, H. M.; Thiel, W. *Angew. Chem., Int. Ed.* **2009**, *48*, 1198.
(17) Zhang, Y.; Lee, T.; Yang, W. *J. Chem. Phys.* **1999**, *110*, 46.
(18) Zhang, Y. *J. Chem. Phys.* **2005**, *122*, No. 024114.
(19) Smith, G. K.; Ke, Z.; Hengge, A. C.; Xu, D.; Xie, D.; Guo, H. *J. Phys. Chem. B* **2009**, *113*, 15327.
(20) Babbitt, P. C.; Mrachko, G. T.; Hasson, M. S.; Huisman, G. W.; Kolter, R.; Ringe, D.; Petsko, G. A.; Kenyon, G. L.; Gerlt, J. A. *Science* **1995**, *267*, 1159.
(21) Babbitt, P. C.; Hasson, M. S.; Wedekind, J. E.; Palmer, D. R. J.; Barrett, W. C.; Reed, G. H.; Rayment, I.; Ringe, D.; Kenyon, G. L.; Gerlt, J. A. *Biochemistry* **1996**, *35*, 16489.
(22) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1992**, *114*, 5928.
(23) Guthrie, J. P.; Kluger, R. *J. Am. Chem. Soc.* **1993**, *115*, 11569.
(24) Gerlt, J. A.; Gassman, P. G. *J. Am. Chem. Soc.* **1993**, *115*, 11552.
(25) Anderson, S. R.; Anderson, V. E.; Knowles, J. R. *Biochemistry* **1994**, *33*, 10545.
(26) Alhambra, C.; Gao, J.; Corchado, J. C.; Villa, J.; Truhlar, D. G. *J. Am. Chem. Soc.* **1999**, *121*, 2253.
(27) Liu, H.; Zhang, Y.; Yang, W. *J. Am. Chem. Soc.* **2000**, *122*, 6560.
(28) Feierberg, I.; Aqvist, J. *Theor. Chem. Acc.* **2002**, *108*, 71.
(29) Zhang, X.; Harrison, D. H.; Cui, Q. *J. Am. Chem. Soc.* **2002**, *124*, 14871.