

Reaction Mechanism for Formation of the Tear-Inducing Chemical Compound from Onion

In onions, a chemical compound that makes us weep is formed by the action of an enzyme called lachrymatory factor synthase (LFS). LFS can catalyze this reaction from a highly labile substrate in a strictly stereocontrolled manner. The detailed reaction mechanism of LFS was investigated using the Photon Factory. Through crystallographic observations, we found that the distorted conformation of a substrate analog inside LFS facilitated the catalysis. An integrated study in combination with biochemical and computational approaches revealed the detailed reaction mechanism, which proceeds through an intramolecular proton shuttling mediated by the key amino acid in the pocket.

Most people weep tears when chopping up bulb onions (*Allium cepa*) for cooking. The volatile and irritating compound (lachrymatory factor or *syn*-propanethial S-oxide, PTSO) is formed by a specific enzyme called lachrymatory factor synthase (LFS) (Fig. 1) [1]. LFS was discovered in 2002, and the study gathered significant attention again in 2013 when it was awarded the Ig Nobel Prize in Chemistry. Damage to onion cells causes vacuolar enzymes to come into contact with cysteine sulfoxides that are abundant in cytosol, and *trans*-S-1-propene L-cysteine sulfoxide (*trans*-1-PRENCISO) is cleaved by an enzyme called alliinase to generate (*E*)-1-propene-sulfenic acid (1-PSA). 1-PSA is a highly labile compound with a half-life of several milliseconds in ambient conditions, and its spontaneous decomposition and polymerization produces various volatile end products that contribute to alliaceous smells and flavors. Although LFS is a small protein (169 amino acids in total) without any cofactors or apparent modifications, it can catalyze the specific reaction from the labile compound to the *syn*- (not *anti*-) PTSO. To solve this mystery, we clarified the three-dimensional

structure of LFS using the macromolecular crystallography beamlines of the Photon Factory. By combining biochemical and computational techniques in collaboration with bioinformaticians and researchers of House Foods Group Inc., we established the detailed catalytic mechanism of LFS [2].

The crystal structures of LFS were determined at a resolution of 1.7–2.1 Å in four different states (+glycerol, +1,2-propanediol, +crotyl alcohol and with no solute molecule in the active site). LFS has a helix-grip fold (Fig. 2, left), which is also adopted by receptors for a plant hormone, abscisic acid. Inside the LFS molecule complexed with the stable substrate analog, crotyl alcohol, a flat and U-shaped electron density map of (*E*)-2-buten-1-ol appeared, indicating that the distorted conformation is stabilized in the pocket (Fig. 2, upper-right). Therefore, the (*E*)-isomer, not the (*Z*)-isomer, is the catalytically competent conformation of the substrate, 1-PSA. The hydroxy group of (*E*)-2-buten-1-ol is fixed by a network of hydrogen bonds with E88, Y102, and Y114. A set of mutagenesis analysis on the component residues of the pocket indicated that the con-

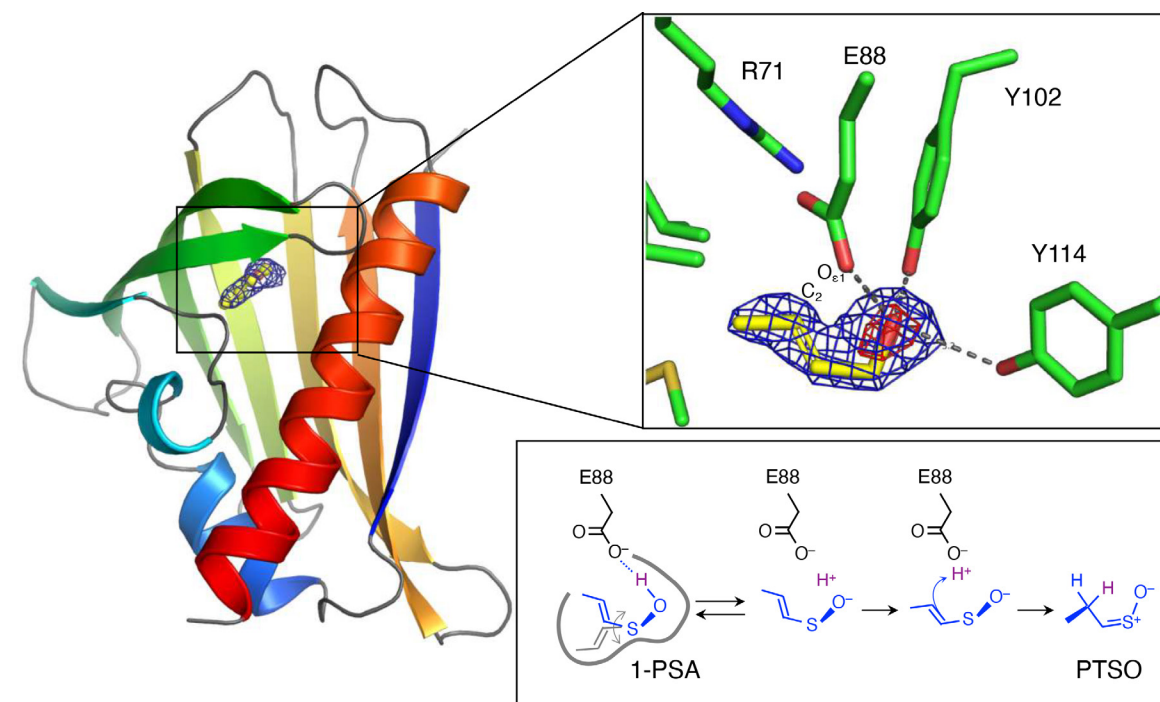


Figure 2: The crystal structure of LFS and the proposed reaction mechanism.

formation is maintained by amino acid residues on the wall, and the catalysis is ruled by a dyad of two charged residues (R71 and E88). The hydroxy group of Y114 is critical for the activity, whereas that of Y102 does not contribute more than its aromatic moiety. These results enabled us to investigate the detailed catalytic mechanism of LFS by computational methods. Molecular dynamics (MD) simulations and hybrid quantum mechanics and molecular mechanics (QM/MM) calculations starting from the crystal structures were performed. The MD simulations confirmed that the 1-PSA molecule in the pocket prefers the *syn*-like conformation, and the C₂ atom of 1-PSA is almost always located close to the O_{ε1} atom of E88. By potential energy surface searches employing QM/MM methods, the minimum-energy pathway of the catalytic reaction was explored. The proposed reaction pathway consists of two sequential proton-transfer reactions involving E88 (Fig. 2, lower-right). The pK_a of the E88 side chain is controlled by the charge interaction with R71, and this effect triggers the proton shuttling. A molecular simulation study using variants of the tyrosine residues suggested a key role of Y114 in the reaction by arranging the C₂ atom at a position adjacent to E88.

In this study, we could determine a plausible complex structure with the substrate analog that mimics the catalytically competent conformation. This was achieved by exploiting the automated robotic system of the beamlines to screen many crystals suitable for the diffraction data collection. A combination of structural, biochemical, and computational analyses revealed the intriguing chemistry that makes us cry. This work provides inspiration for future investigations, such as the synthesis, reaction design, and use of transient sulfur compounds for nutritional and health benefits for human beings.

REFERENCES

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BEAMLINES

BL-5A, AR-NE3A and AR-NW12A

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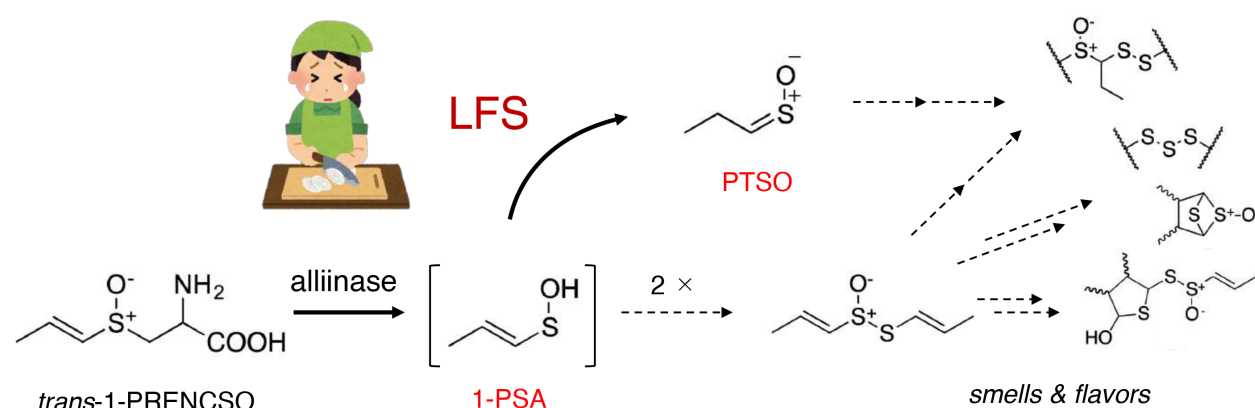


Figure 1: Production of PTSO and other compounds in crushed onion.