2P37 Mechanism for Biosynthesis of Antibiotics in Isopenicillin N Synthase Studied by Active-site and QM/MM methods

○Marcus Lundberg and Keiji Morokuma 京都大学 福井謙一記念研究センター
E-mail: lundberg@fukui.kyoto-u.ac.jp

Isopenicillin N synthase (IPNS) catalyzes a key step in the biosynthesis of the important β-lactam antibiotics penicillin and cephalosporin. Industrial production of penicillins requires post-synthetic modifications of the IPNS product. Genetically engineered IPNS that directly make novel penicillin compounds could therefore be an efficient route to design new antibiotics.

To improve the understanding of the natural catalytic reaction in IPNS, the enzyme's activity has been analyzed using an active-site (DFT) model together with an ONIOM QM/MM (B3LYP/Amber) model that includes the full protein. Full optimizations of QM/MM transition states have been performed for seven different reaction steps. This is possible with the use of a novel coupled Hessian algorithm. Based on these calculations, a detailed reaction mechanism can be proposed that is in agreement with experimental observations.

IPNS is an oxygen-activated non-heme iron enzyme and belongs to an enzymatic family where iron is coordinated by two histidines and one carboxylate ligand. In addition to their structural similarity, enzymes in this family also show important similarities when it comes to their reaction mechanisms. The general scheme is: binding of O_2 to iron to form a ferric-superoxo (Fe(III)-OO^-) species, two-electron oxidation of the substrate (or a cofactor), generation of a ferryl-oxo (Fe(IV)=O) species by heterolytic O-O bond cleavage and finally two-electron oxidation of the substrate by the ferryl-oxo intermediate. Iron is in a high-spin configuration and the reaction mainly proceeds on the quintet surface.

IPNS uses O_2 to transform the substrate ACV into the penicillin precursor isopenicillin N (see Fig. 1). Compared to other reactions in the 2-histidine-1-carboxylate family, this reaction is unique in that all four electrons required to reduce dioxygen comes from the substrate. Despite the unique substrate reaction, the present calculations instead highlight the similarities that exist between isopenicillin N synthase and other enzymes of the same family. The proposed mechanism follows the general scheme outlined above. However, compared to previous proposals for IPNS, an alternative mechanism is suggested for the O-O bond cleavage step. This new proposal includes electron transfer from iron concerted with protonation of the distal oxygen by an iron-bound water ligand (see Fig. 1), rather than hydrogen abstraction from the substrate itself. The transition states have direct analogues in the related enzymes pterin-dependent amino acid hydroxylases and α-keto-acid-dependent dioxygenases.

The results show that modeling is an important tool to identify similarities between different enzymes that can be difficult to detect by experiments only.

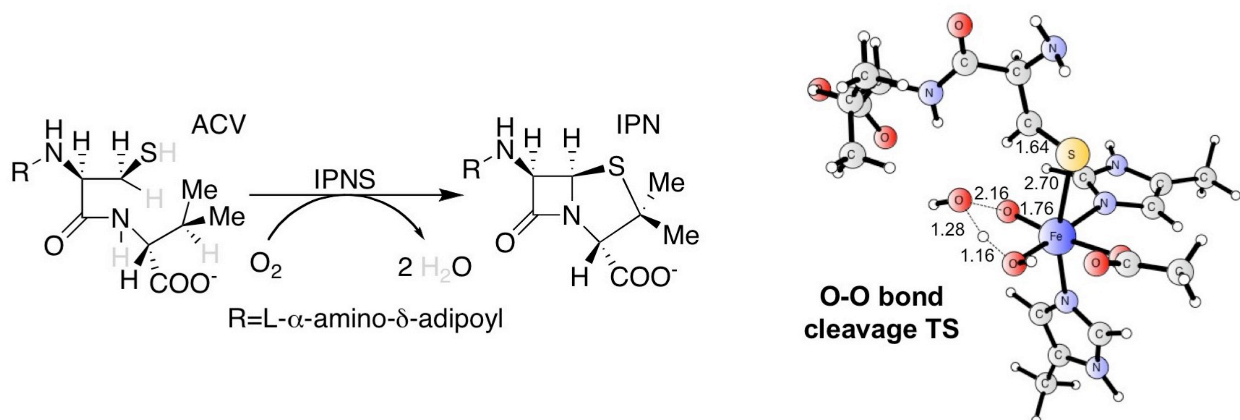

Figure 1. Substrate reaction in isopenicillin N synthase (left) shown together with a transition state for O-O bond cleavage (right). Labels show selected bond distances in Å.