Journal Club No.819　　　　　　　　　　　　　　Jan. 22, 2019　尹　忠銖

酵素と反応基質の複合体を観察する方法の開発

多くの酵素と基質の反応は短時間で進行され酵素と基質が結合している様子を観察すのは大変難しい。酵素タンパク質と基質の共結晶が数多く報告されているが共結晶に失敗するケースも多い。特にPKSやNRPSなどの複数のドメインで構成されている酵素は反応出発基質から各ドメインによる反応を通して最終産物が生産されることから特定のドメインと基質の結合様子を共結晶で見るのは非常に難しい。今回紹介する論文では非天然アミノ酸をタンパク質に取り入れ、タンパク質と基質を固定する方法を開発したので報告する。

紹介論文

Trapping biosynthetic acyl-enzyme intermediates with encoded 2,3-diaminopropionic acid

Nicolas Huguenin-Dezot, et al & T. Martin Schmeing* & Jason W. Chin*
Medical Research Council Laboratory of Molecular Biology, Cambridge, UK.
Department of Biochemistry, MacGill University.

Nature. 2019 Jan;565(7737):112-117.

Abstract

Many enzymes catalyse reactions that proceed through covalent acyl-enzyme (ester or thioester) intermediates. These enzymes include serine hydrolases (encoded by one per cent of human genes, and including serine proteases and thioesterases), cysteine proteases (including caspases), and many components of the ubiquitination machinery. Their important acyl-enzyme intermediates are unstable, commonly having half-lives of minutes to hours. In some cases, acyl-enzyme complexes can be stabilized using substrate analogues or active-site mutations but, although these approaches can provide valuable insight, they often result in complexes that are substantially non-native. Here we develop a strategy for incorporating 2,3-diaminopropionic acid (DAP) into recombinant proteins, via expansion of the genetic code. We show that replacing catalytic cysteine or serine residues of enzymes with DAP permits their first-step reaction with native substrates, allowing the efficient capture of acyl-enzyme complexes that are linked through a stable amide bond. For one of these enzymes, the thioesterase domain of valinomycin synthetase, we elucidate the biosynthetic pathway by which it progressively oligomerizes tetradepsipeptidyl substrates to a dodecadepsipeptidyl intermediate, which it then cyclizes to produce valinomycin. By trapping the first and last acyl-thioesterase intermediates in the catalytic cycle as DAP conjugates, we provide structural insight into how conformational changes in thioesterase domains of such nonribosomal peptide synthetases control the oligomerization and cyclization of linear substrates. The encoding of DAP will facilitate the characterization of diverse acyl-enzyme complexes, and may be extended to capturing the native substrates of transiently acylated proteins of unknown function.