The Entry of CoA into Enzymes: A Case Study with Pyruvate Formate-Lyase

Marko Hanževački,[1] Karmen Čondić-Jurkić,[2] Ana-Sunčana Smith,[1,3] David M. Smith[1]

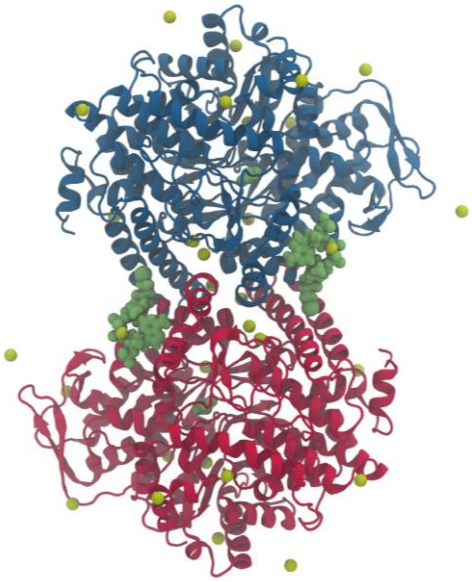
[1]*Division of Physical Chemistry, Ruđer Bošković Institute, Bijenička 54, Zagreb, Croatia*

[2]*Research School of Chemistry, Australian National University, Canberra, ACT, Australia*

[3]*Institute for Theoretical Physics, FAU Erlangen, Staudtstraße 7, Erlangen, Germany*

Coenzyme A (CoA) is an important coenzyme required, for example, in fatty acid synthesis and the citric acid cycle in aerobic organisms, where it is crucial for the oxidation of pyruvate. CoA also participates in pyruvate metabolism in anaerobic organisms, whereby pyruvate and CoA are transformed to formate and acetyl-CoA (AcCoA) in the presence of Pyruvate formate-lyase (PFL).[1]

PFL is a glycyl radical enzyme requiring activation by a member of the radical SAM enzyme superfamily.[2] Such radical enzymes are receiving increased interest because of their possible applications in biotechnology.[3] The mechanism of PFL is thought to be initiated by the formation of a radical at Gly734, which is subsequently shuttled to Cys 418 via Cys 419. The addition of radical Cys418-S· to pyruvate leads to C-C bond dissociation, resulting with formation of formyl radical and acetyl-Cys418. The latter species acts as a temporary acetyl carrier and a reactant in the subsequent half-reaction with the second substrate CoA to produce acetyl-CoA. Formation of AcCoA, the final product, closes the catalytic cycle of PFL.[4]



The investigated aspect of this mechanism concerns the process that allows CoA to enter the active site, which is a prerequisite for the second half-reaction. The problem with this step is that the binding site of CoA is located at the protein surface, while the active site is buried in the protein interior.[5] In search for possible solutions to this problem the models representing the PFL system before and after the first half-reaction with pyruvate were subjected to long unrestrained molecular dynamics (MD) simulations, to examine the possible effect that acetylation of the enzyme has on the necessary conformational changes. The PFL systems were also subjected to the free energy calculations used to estimate potential of mean force (PMF) for the process of CoA approaching the active site before and after the pyruvate cleavage.

[1] M. Müller, M. Mentel, J. J. van Hellemond, K. Henze, C. Woehle, S. B. Gould, R-Y. Yu, M. van der Giezen, A. G. M. Tielens, W. F. Martin, *Microbiol. Mol. Biol. Rev.*, **2012**, *76* (2), 444-495.
[2] J. M. Buis, J. B. Broderick, *Archives of Biochemistry and Biophysics*, **2005**, *433*, 288-296.
[3] L. Zelcbuch, S. N. Lindner, Y. Zegman, I. V. Slutskin, N. Antonovsky, S. Gleizer, R. Milo, A. Bar-Evan, *Biochemistry*, **2016**, *55* (17), 2423-2426.
[4] J.-D. Guo, F. Himo, *J. Phys. Chem. B*, **2004**, *108* (39), 15347-15354.
[5] A. Becker, W. Kabsch, *J. Biol. Chem.*, **2002**, *277* (42), 40036-40042.