The Influence of Chemical Change on Protein Dynamics: A Case Study with Pyruvate Formate-lyase

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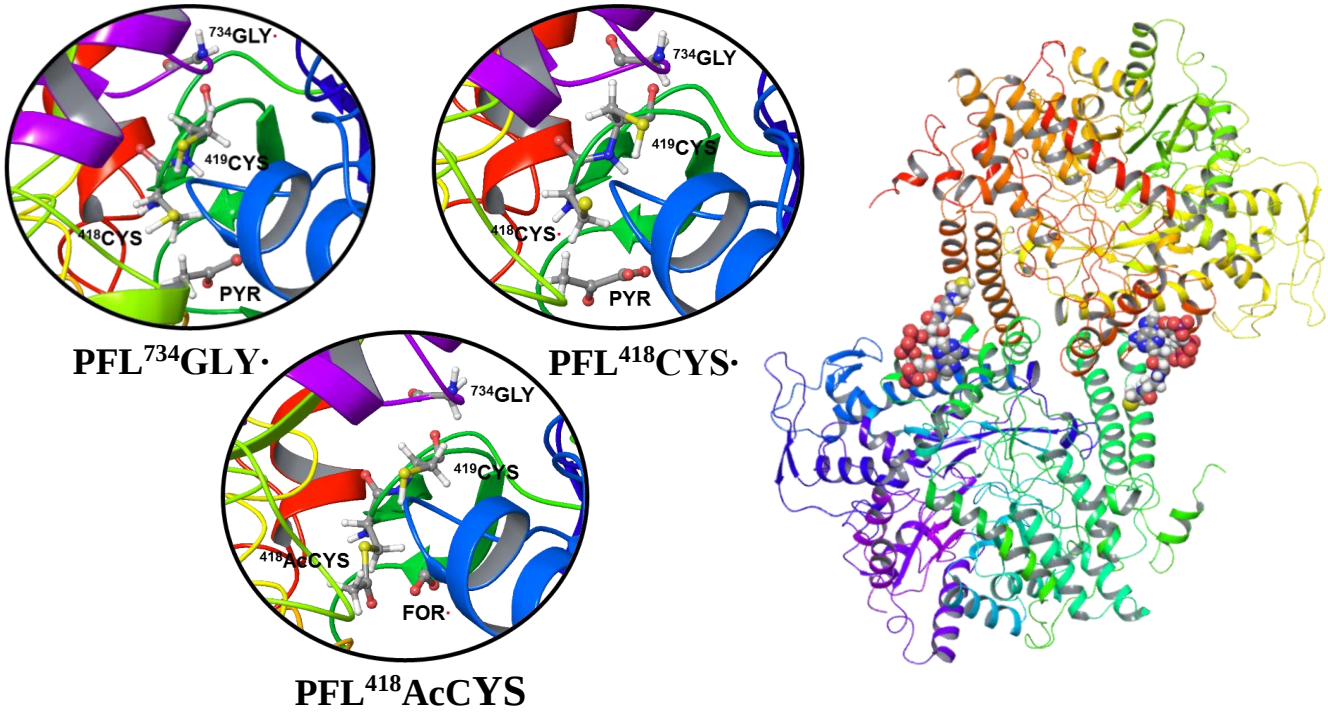

Pyruvate formate-lyase (PFL) catalyzes the break down of pyruvate into formate and the acetyl group upon the addition of a thiyl radical located at Cys418.[1] The radical is initially stored at Gly734 is shuttled to Cys418 via Cys419. The addition of radical Cys418-S· to pyruvate leads to C-C bond dissociation, 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 Ac-CoA, the final product, closes the catalytic cycle of PFL.[2]

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.[3] In search for possible solutions to this problem, the PFL system was subjected to long unrestrained molecular dynamics simulations.

The models representing the PFL system before and after the first half-reaction with pyruvate were used to examine the possible effect that acetylation of the enzyme has on the necessary conformational changes. The PFL protein comes in a homodimeric form and two sets of models were derived from the available crystal structure; one set of models was built using a single subunit (mPFL^{734}GLY·, mPFL^{418}CYS·, mPFL^{418}AcCYS), while the other contains the full dimer (dPFL^{734}GLY·, dPFL^{418}CYS·, dPFL^{418}AcCYS).

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