Kinetic Studies Pyruvate Formate Lyase and Activation of Pyruvate Formate Lyase Mutants

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Pyruvate formate-lyase (PFL) is a key enzyme of anaerobic glycosis in facultative bacteria, such as *Escherichia coli*, and in some lower eukaryotes. It catalyzes the fully reversible conversion of pyruvate and CoA into formate and acetyl-CoA (1). The enzyme shows two-step pingpong kinetics with an acetylated enzyme intermediate.

$$CH_3COCOO^- + CoA\text{-}SH \rightleftharpoons CoA\text{-}S\text{-}COCH_3 + HCO_2^-$$

Purified PFL from *Escherichia coli* is a homodimer with a molecular mass of 170 kDa and contains no metals or other cofactors (2). The PFL reaction is accomplished by a free radical on the α-carbon of Gly734 (3); PFL was the first example of a radical enzyme with a polypeptide backbone glycyl radical involved in enzymatic catalysis. The glycyl radical is transferred to Cys419 and then to Cys418 by hydrogen abstraction (4-6). The Cys418 thiyl radical is believed to attack the carbonyl C2 of pyruvate to give formate, and the radical itself becomes acetylated. After that, CoA, bound to another position of the active site, takes the acetyl group from the Cys418 to generate acetyl CoA and the active PFL radical again (6).

Here we report recent results from our laboratory in which the kinetics of PFL with ^{2}H and ^{13}C labeled pyruvate are investigated. We have also examined the activity of two PFL mutants, Y735F-PFL and R753K-PFL, compared with activity of wild type PFL. No activity is observed for R753K-PFL, while Y753F-PFL has about 1/30 activity of wild type PFL. Mechanistic implications will be discussed.

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