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Studies on the Biosynthesis of Bialaphos (SF-1293) 8 Purification and Characterization of 2-Phosphinomethylmalic acid Synthase from *Streptomyces hygroscopicus* SF-1293*

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2-Phosphinomethylmalic acid (PMM) synthase catalyzes the condensation of phosphinopyruvic acid (PPA), an analog of oxalacetic acid, and acetyl-CoA to form PMM. The enzyme was purified approximately 700-fold from a cell-free extract of *Streptomyces hygroscopicus* SF-1293, a bialaphos producing organism, to an electrophoretically homogeneous state. The purified PMM synthase has a subunit molecular weight of 48,000 by SDS-polyacrylamide gel electrophoresis and a native molecular weight of 90,000 ~ 98,000 by gel filtration. PMM synthase was relatively unstable, showed maximum activity at pH 8.0 and 30°C, and was inhibited strongly by *p*-chloromercuribenzoate, iodoacetamide and EDTA. Enzyme activity suppressed by EDTA was completely restored by adding Co^{++} or Mn^{++} and partially restored by addition of Ca^{++}, Fe^{++} or Mg^{++}.

The specific substrates of this enzyme are PPA or oxalacetic acid in addition to acetyl-CoA. The enzyme does not catalyze the liberation of CoA from acetyl-CoA in the presence of α-keto acids, such as pyruvate, α-ketoglutarate, deamino-α-keto-demethylphosphinothricin or phosphonopyruvate. The condensation reaction did not take place when propionyl-CoA or butyryl-CoA was used as a substrate in place of acetyl-CoA. The Km values of the enzyme were 0.05 mM for acetyl-CoA, 0.39 mM for PPA and 0.13 mM for oxalacetate. PMM synthase is very similar to (*R*)-citrate synthase of *Clostridium* in the inhibition pattern by sulfhydryl compounds, its metal ion requirement and stereospecificity; unlike (*R*)-citrate synthase PMM synthase was not inhibited by oxygen.

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