

Crystal structure of a reaction intermediate of pyruvate oxidase from *Aerococcus viridans*, Ella Czarina Magat Juan^a, Tofazzal Hossain^a, Kaoru Suzuki^b, Masaru Tsunoda^c, Shigeyuki Imamura^d, Tamotsu Yamamoto^d, Takeshi Sekiguchi^b and Akio Takénaka^{a*}, ^a*Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, Japan*, ^b*College of Science and Engineering, Iwaki Meisei University, Japan*, ^c*School of Pharmaceutical Sciences, Showa University, Japan*, and ^d*Asahi Kasei Corporation, Japan*. E-mail: atakenak@bio.titech.ac.jp

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Pyruvate oxidase (POPG) catalyzes oxidative decarboxylation of pyruvate to form hydrogen peroxide and a high-energy compound acetylphosphate. For catalytic activity, the enzyme requires two cofactors FAD (Flavin adenine dinucleotide) and TPP (thiamin pyrophosphate), and a divalent cation such as Mn^{2+} , Mg^{2+} . To establish the enzymatic reaction mechanism catalyzed by POPG, three crystal structures of POPG in the absence and in the presence of the TPP and Mg^{2+} , and a complex of the latter with the substrate pyruvate have been determined at 1.8 angstrom, 1.6 angstrom and 1.96 angstrom resolutions, respectively. The overall structures are similar to each other. In every case, four subunits are associated to form a tetramer. Each subunit is composed of three domains, FAD-binding domain, TPP-binding domain and a core domain. A magnesium cation bound to the TPP-binding domain facilitates the coordination of the pyrophosphate moiety of TPP. The active site is found between the TPP-binding domain and the FAD-binding domain of the adjacent subunit. In the structure of POPG-TPP-Pyruvate complex an acetyl group covalently bound to the C2 atom of TPP is found, which indicates the reaction intermediate of pyruvate oxidative decarboxylation reaction. It is considered that the thiazole ring of TPP contains a reactive carbon atom (C2) and that a proton attached to this atom can be dissociated to make carbonium form of TPP so that it can attack pyruvate as a substrate. Therefore, to form oxyethyl-TPP as an intermediate, FAD must be in the reduced state. When it is oxidized, oxyethyl-TPP is converted to acetyl-TPP as found in the present study.