s1.m4.p3 **Ultra-High Resolution Structure of a ß-alanyl-CoA Ammonia Lyase (ACL).** Andreas Heine[a], Thorsten Selmer[b], Gerhard Klebe[a] and Klaus Reuter[a], [a]*Department of Pharmaceutical Chemistry, Philipps-University Marburg, Germany,* [b]*Department of Biology, Philipps-University Marburg, Germany. E-mail: heinea@mailer.uni-marburg.de*

Keywords: Ammonia lyase; MAD phasing; Ultra-high resolution

The ACL enzyme from *Clostridium propionicum* catalyzes the deamination of β-alanyl-CoA to form acrylyl-CoA, a reaction involved in the degradation of β-alanine in this organism. Since this reaction is fully reversible, it is also of interest for the biosynthesis of ß-alanine and represents an example of the formation of an amino-acyl-CoA derivative [1]. In order to gain an insight into the mechanism of the reaction catalyzed by the enzyme structural studies were instigated. The crystal structure of the ACL protein was determined by multiple-wavelength anomalous dispersion collecting three data sets close to the zinc edge. The protein consists of 144 residues and contains one zinc atom per molecule, which was introduced during protein crystallization. Fortunately, crystals diffracted to ultra-high resolution and a high, medium and low resolution data set was recorded resulting in a very complete data set at 0.97Å resolution. The position of the zinc atom was unambiguously determined and used for initial phasing with SHELXE [2]. Automated model building was performed with ARP/wARP [3] and resulted in 139 out of 144 residues. The structure is being refined with SHELXL [4] with current refinement statistics of R=15.8% and R_{free}=17.5%. The protein consists of a five-stranded anti-parallel β-sheet with a long α-helix lying across and shows a high structural similarity to 4-hydroxybenzoyl CoA thioesterase (PDB code 1bvq) [5].

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s1.m4.p4 **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*

Keywords: X-ray structure; Pyruvate oxidase; FAD linked Enzyme

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, FADbinding 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.