Structural Basis for Substrate Channelling of a Fatty Acid â-oxidation Multienzyme Complex

Momoyo Ishikawa, Daisuke Tsuchiya, Takuji Oyama, Yasuo Tsunaka, Kosuke Morikawa, *Biomolecular Engineering Research Institute. Osaka, Japan*. E-mail: ishikawa@beri.or.jp

Many enzymes are organized into multienzyme complex to catalyze sequential reactions termed the channelling mechanism. The purpose of our structural study is to elucidate this mechanism at the atomic level, focusing the fatty acid â-oxidation multienzyme complex from *Pseudomonas fragi*. We have determined two distinct crystal structures of the bacterial multienzyme complex that catalyzes the last three sequential reactions in the fatty acid â-oxidation cycle. The $á_2â_2$ heterotetrameric structure shows the uneven ring architecture, where all the catalytic centers of 2-enoyl-CoA hydratase (ECH), L-3-hydroxyacyl-CoA dehydrogenase (HACD) and 3-ketoacyl-CoA thiolase (KACT) face a large inner solvent region. The substrate, anchored through the 3'-phosphate ADP moiety, allows the fatty acid tail to pivot from the ECH to HACD active sites, and finally to the KACT active site. Coupling with striking domain rearrangements, the incorporation of the tail into the KACT cavity and the relocation of 3'-phosphate ADP bring the reactive C2-C3 bond to the correct position for cleavage. The á-helical linker specific for the multienzyme contributes to the pivoting center formation and the substrate transfer through its deformation. This channelling mechanism could be applied to other â-oxidation multienzymes, as revealed from the homology model of the human mitochondrial trifunctional enzyme complex.

Keywords: beta-oxidation, multienzyme complex, three-dimensional protein structure