Structural comparison between the open and the closed forms of citrate synthase from *Thermus thermophilus* HB8

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Citrate synthase (E.C. 4.1.3.7), an enzyme involved in the first step of tricarboxylic acid cycle, catalyzes a bi-bi reaction by which acetyl-coenzyme A condenses with oxaloacetate to yield citrate and coenzyme A (CoA).

oxaloacetate + acetyl-coenzyme A + H_2O citrate + coenzyme A

Crystal structures of the enzymes isolated from eukaryotic cells, archaebacteria and gram-positive bacteria have been determined. The citrate synthase from a higher animal exists as a dimeric form and each subunit consists of a 45kDa polypeptide chain, which is folded into 20 α-helices.

In this study we determined the crystal structures of the *Thermus thermophilus* HB8 citrate synthase (TtCS) in the open and the closed forms at resolution of 1.7Å and 2.3Å, respectively. In the absence of substrates or products, TtCS crystallizes into an orthorhombic crystal ($P4_32_12$, a=b=84.55 Å, c=153.52 Å, α=β=γ=90°). When the enzyme is crystallized with citrate and CoA, an orthogonal crystal ($P2_12_12_1$, a=80.01, b=110. 6 Å, c=184.4 Å, α=β=γ=90°) is obtained. In the orthorhombic crystal, one monomer of TtCS is contained in the asymmetric unit. In the orthogonal crystal, four monomers are contained in the asymmetric unit.

In the closed form, on the other hand, each subunit of TtCS contains citrate and CoA in the active site. Citrate is tightly bound to basic residues located in the cleft between the large and the small domains. In the open form, one bicarbonate ion and one glycerol molecule are found in the active site. Comparison of the open and the closed forms indicates that binding of citrate induces a large-scale conformational change, which is approximately described by 30° rotation of the small domain with respect to the large domain. When citrate comes into the active site, basic residues in the active site move so as to form hydrogen bonds with citrate, causing a large-scale rotation of the small domain with reference to the large domain. Three residues (H219, G220, G221) between two domains are found to act as a hinge.