SAXS Studies on Human Branched-Chain Alpha-Ketoacid Dehydrogenase Complex

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The mammalian mitochondrial branched-chain-alpha-ketoacid dehydrogenase (BCKD) complex catalyzes the oxidative decarboxylation of branched-chain-alpha -ketoacids derived from leucine, isoleucine and valine to give rise to branched-chain acyl-CoAs. The human BCKDC is a 4-million dalton catalytic machine. There are three catalytic components in human BCKDC: a heterotetrameric . . branched-chain -ketoacid decarboxylase (E1), a homo-24 meric dihydrolipoyl transacylase (E2), and a homodimeric dihydrolipoamide dehydrogenase (E3). E1 and E2 components are specific for the BCKDC, whereas the E3 component is common among the three -ketoacid dehydrogenase complexes. The BCKDC is organized around the cubic E2 core, to which 12 copies of E1, and unspecified copies of E3, the BCKD kinase and the BCKD phosphatase are attached through ionic interactions. The transacylase (E2) subunit of the branched-chain -ketoacid dehydrogenase (BCKD) complex carries three independently folded domains which are linked together by flexible loops. We have employed multidimensional heteronuclear NMR techniques to determine the structure of the hbLDB (a.a. 1-84) domain and hbSBD (a.a. 104-152) domain; however, the di-domain (hbDD aa. 1-168) structure has not been solved yet. The hbLBD and hbSBD play central role in substrate channeling and substrate recognition, thus we would like to understand the domain (hbLBD and hbSBD) orientation using SAXS. In addition, it will be of great interest to dissect the mode of interactions between hbDD and E1 or E3.

Using small angle X-ray scattering (SAXS), we have studied the structure of the di-domain protein (LBD+SBD) from BCKD complex in a buffer solution. The SAXS was measured at BL17B3 SWAXS endstation, with 0.5 mm dia. photon beam of 10 keV and a sample-to-detector 1250 cm. With the 2-D area detector, the Q range measured covered from 0.015 to 0.35 $Å^{-1}$, where the wavevector transfer Q (=$4\pi\sin(\theta/2)/\lambda$) was defined by θ and λ for the scattering angle and wavelength of the photons. At a concentration of 1mM, the SAXS data shown that di-domain protein (hbDD) is a rod-like molecule with the length of 110 Å and radius of 13 Å (the radius of gyration ~30 Å). To orientate individual domains (hbLBD and hbSBD) to this model indicating that the 20 residues linker between LBD and SBD is not totally extended but somehow twisted. This result consists with our NMR observation where the dynamics data shown the linker is not totally flexible but somehow restricts the motion of these two domains.

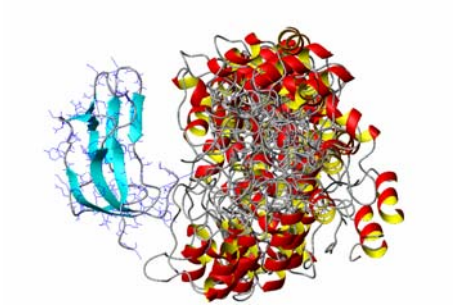

Figure 1. Models of hbSBD (helix) and hbLBD (beta sheets) orientation based on SAXS data (using Bunch to generate 50 models, and overlap hbLBD to show the orientation)

On the other hand, to study E1/DD complex, we also collect data for E1 and E1/DD complex. The SAXS data shown the E1 component of BCKD is very compact, and the shape of E1 is closer to sphere model which is consisting with the X-ray structure of E1. Further analysis is necessy to understand E1/DD complex .

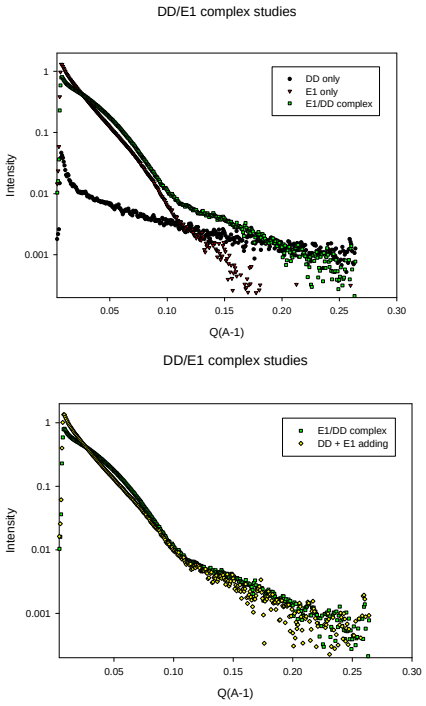

Figure 2. SAXS data comparison for hbDD, E1 and hbDD/E1 complex.