Molecular basis of dihydrouridine synthase

Dihydrouridine (D) is a highly conserved modified base found in tRNAs from all domains of life. Dihydrouridine synthase (Dus) catalyzes D formation of tRNA through reduction of uracil base. We reported the crystal structures of Thermus thermophilus Dus (TthDus), which is responsible for D formation at positions 20 and 20a, in complex with tRNA, and with a short fragment of tRNA (D-loop). Dus interacts extensively with the D-arm and recognizes the elbow region composed of the kissing loop interaction between T- and D-loops in tRNA, pulling the substrate base (U20) into the catalytic center for reduction. Although distortion of the D-loop structure was observed upon Dus binding to tRNA, the canonical D-loop/T-loop interaction was maintained. These results were consistent with the observation that Dus preferentially recognizes modified rather than unmodified tRNAs, indicating that Dus introduces D20 by monitoring the complete L-shaped structure of tRNAs. In the active site, U20 was stacked on the isoalloxazine ring of FMN, and C5 of the U20 uracil ring was covalently crosslinked to the thiol group of Cys93, implying a catalytic mechanism of D20 formation. In addition, involvement of a cofactor molecule in uracil ring recognition was proposed. Based on a series of mutation analyses, we propose a molecular basis of tRNA recognition and D formation catalyzed by Dus.

Figure.1 Crystal structure of *Tth*Dus-tRNA complex