Structure and mechanism of GTP cyclohydrolase I of Escherichia coli

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GTP cyclohydrolases I and II catalyze the first committed steps in the pathways of pteridine and flavin biosynthesis [1]. A reaction pathway for GTP cyclohydrolase I involving the release of C8 of GTP as formate, followed by Amadori rearrangement and ring closure (see Fig. 1), has been proposed earlier [2]. The enzyme was first purified and characterised by Yim and Brown [3].

Recombinant GTP cyclohydrolase I of Escherichia coli was crystallised, and the structure was solved by X-ray analysis assisted by freeze etching electron microscopy [4,5]. The protein is a torus-shaped decamer with D5 symmetry and with approximate dimensions of 100 Å in diameter and 65 Å in height. The structure is characterised by a novel 20 strand β-barrel.

A pocket at the interface of three adjacent subunits A, A* and B located by X-ray analysis of a dGTP complex was identified as the active site. The topology of amino acid residues at the active site is summarised in Fig. 2. The guanine moiety is fixed in a cleft constituted by Ile132A*, Glu152A, Gln115A, His119A and His112A. His112A and Ser135A* serve as hydrogen bridge partners of the desoxyribosyl side chain. The entrance to the pocket is lined by a cluster of five basic amino acid residues, Arg65B, His113A, Lys68B, Arg185A and Arg139A*.

A highly conserved cystine motif (Cys181A and Cys110A) is in close proximity to C8 of the purine nucleotide substrate.

Site directed mutagenesis was used to analyze the possible function of amino acid residues at the active site. Replacement of each of the amino acids Arg65, Cys110, His112, His113, Ser135, Lys136, Glu152, Cys181 reduced the enzyme activity to less than 10%.

The data are in agreement with a mechanism involving the disulfide bridge as a nucleophile. Cysteine could facilitate the addition of water by a prior nucleophilic attack of the C8 atom.

Alternatively, water could act directly as a nucleophile at C8 of the imidazole ring. The opening of the ring could be assisted by protonation of the ring oxygen of the furanose moiety by His112 followed by the conversion of the glycoside to a Schiff's base (Fig. 2).