

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, Gln151A, His179A and His112A. His112A and Ser135A* serve as hydrogen bridge partners of the deoxyribose 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.

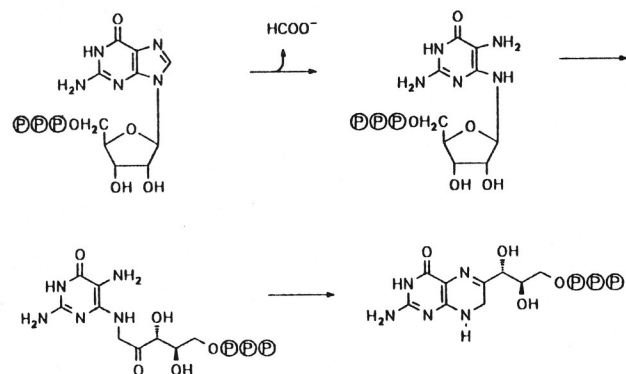


Fig. 1 : Reaction mechanism proposed by Wolf and Brown [2].

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%. Replacement of Arg139 to Ala reduced the activity to 52%.

The data are in agreement with a mechanism involving the disulfide bridge as a nucleophile. Cystine 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).

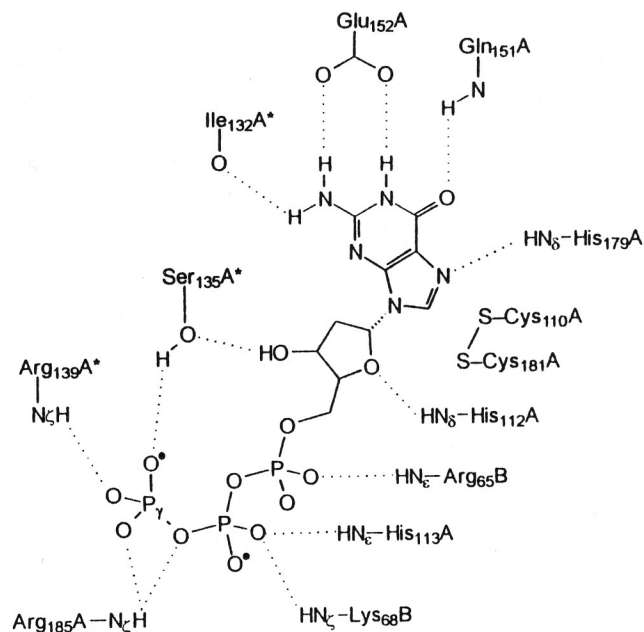


Fig. 2 : Schematic representation of the interactions of active site residues with dGTP. Residues labelled A, A* and B pertain to three different subunits. All residues involved in dGTP-binding are highly conserved, except for Lys68 which is replaced by Ser or Thr in some sequences. Hydrogen bridges from Lys136A* to the oxygen atoms marked with • were omitted for clarity.

In both cases the subsequent hydrolytic cleavage by water would lead to the elimination of formate. Hydrogen-bonding to the γ-phosphate group of the substrate could activate Ser135 for the abstraction of the proton from C2', thus conducting to an Amadori rearrangement of the carbohydrate side chain (Fig. 2). The subsequent Schiff's base formation could occur spontaneously.

The potential functional role of the disulfide motif requires further elucidation.

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