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A structural model for chorismate synthase from *Mycobacterium tuberculosis* in complex with coenzyme and substrate

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Abstract

The enzymes of the shikimate pathway constitute an excellent target for the design of new antibacterial agents; chorismate synthase (CS) catalyzes the last step of this pathway. The prediction of *Mycobacterium tuberculosis* (MTB) CS three-dimensional structure and the geometric docking of the coenzyme FMN and the substrate EPSP were performed using the crystal structure of CS from *Streptococcus pneumoniae* as template. Energy minimization of the whole complex showed, as expected, that most of the template interactions are preserved in the MTB structure, except for HIS11, ARG139 and GLN255. However, novel interactions involving ARG111, GLY113 and SER317 were also observed.

Keywords: Shikimate pathway; Chorismate synthase; Molecular modeling; Energy minimization; Docking; *Mycobacterium tuberculosis*

1. Introduction

The shikimate pathway is the common way for the production of various products including folic acid, vitamin K, ubiquinone and the three aromatic amino acids, tryptophan, phenylalanine and tyrosine. In bacteria, fungi, plants and apicomplexan parasites, chorismate, the final product of the shikimate pathway, is the branch point in the biosynthesis for all these products that are essential for these species. The absence of the shikimate pathway in all other species makes it an attractive target for the development of new antibacterial agents [1,2].

Chorismate synthase (CS), the seventh and final step of the shikimate pathway, catalyzes the conversion of 5-enolpyruvylshikimate 3-phosphate (EPSP) to chorismate in the presence of a reduced flavin mononucleotide (FMN) as a coenzyme [3]. The reaction mechanism of the shikimate pathway has been studied extensively and revealed that the reaction of CS is unique in nature. The reaction involves a 1, 4 elimination of phosphate and the loss of a proton of the C-6 hydrogen. This consists in the formation of the second out of three necessary double bonds to build an aromatic ring (Fig. 1). The enzyme activity requires a reduced FMN molecule which is not consumed during the reaction [4].

The function of the reduced FMN in catalysis was extensively studied. The most accepted mechanism suggests a direct role of reduced FMN in the elimination reaction. FMN transfers the electron transiently to phosphate and the substrate donates an electron to regenerate the FMN. This reaction does not involve an overall change in the redox state [3,5].

Recently, with the first high-resolution X-ray structure of CS from *Streptococcus pneumoniae* (SPN) with the substrate and the coenzyme in the oxidized form [6], the structure of CS from *Saccharomyces cerevisiae* [7], and the structure of CS from *Helicobacter pylori* with the coenzyme in the reduced form [8], studies on the binding mode of substrate and coenzyme in the active site has been started.

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How reduced FMN is obtained divides the CS into two classes, monofunctional and bifunctional. Bifunctional CS has an intrinsic ability to reduce flavin (specifically FMN) using NADPH. In monofunctional CS this catalytic activity is not present. The bifunctional enzyme is present in fungi and the monofunctional form in plants and bacteria [3].

The active site of SPN CS is very hydrophilic and extremely basic, with six arginine and two histidine residues. The two histidines in the active site, HIS10 and HIS110, are present in both classes of CS and across all known species, being HIS10 part of a characteristic CS signature sequence [6]. HIS110 is involved in FMN binding while HIS10 protonates the

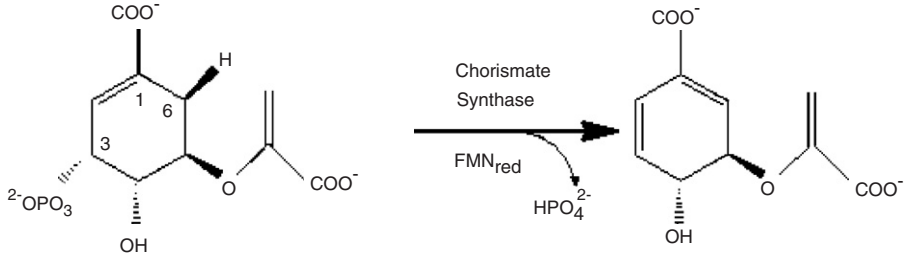

Fig. 1. Reaction catalyzed by CS. The elimination of the 3-phosphate and the loss of a proton in C-6 introduce a second double bond in the ring.

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                    10         20         30         40         50         60         70
                     |          |          |          |          |          |          |
TARGET       MLRWITAGESHGRALVAVVEGMVAGVHVTSADIADQLARRRLGYGRGARMTFERDAVTVLSGIRHGSTLG
TEMPLATE     -MRYLTAGESHGPRLTAIIEGIPAGLPLTAEDINEDLRRRQGGYGRGGRMKIENDQVVFTSGVRHGKTTG
              :*::*******  *.*::**: **: :*: ** ::* **: *****.**.:*.* *.. **:***.* *

                    80         90        100        110        120        130        140
                     |          |          |          |          |          |          |
TARGET       GPIAIEIGNTEWPKWETVMAADPVDPAELADVARNAPLTRPRPGHADYAGMLKYGFDDARPVLERASARE
TEMPLATE     APITMDVINKDHQKWLDIMSAEDIEDRLKSKRK----ITHPRPGHADLVGGIKYRFDDLRNSLERSSARE
             .**:::: *.:  **  :*:*: ::    :.      :*:******* .* :** *** *  ***:****

                   150        160        170        180        190        200        210
                     |          |          |          |          |          |          |
TARGET       TAARVAAGTVARAFLRQALGVEVLSHVISIGASAPYEGPPPRAEDLPAIDASPVRAYDKAAEADMIAQIE
TEMPLATE     TTMRVAVGAVAKRLLAELDMEIANHVVVFGGKEIDVPENLTVAEIKQRAAQSEVSIVNQEREQEIKDYID
             *: ***.*:**: :* :     .   *:  * .       . **       * *   ::  * ::   *:

                   220        230        240        250        260        270        280
                     |          |          |          |          |          |          |
TARGET       AAKKDGDTLGGVVEAVALGLPVGLGSFTSGDHRLDSQLAAAVMGIQAIKGVEIGDGFQTARRRGSRAHDE
TEMPLATE     QIKRDGDTIGGVVETVVGGVPVGLGSYVQWDRKLDARLAQAVVSINAFKGVEFGLGFEAGYRKGSQVMDE
               *:****:*****:*. *:******:.. *::**::** **:.*:*:****:* **::. *:**:. **

                   290        300        310        320        330        340        350
                     |          |          |          |          |          |          |
TARGET       MYPG-PDGVVRSTNRAGGLEGGMTNGQPLRVRAAMKPISTVPRALATVDLATGDEAVAIHQRSDVCAVPA
TEMPLATE     ILWSKEDGYTRRTNNLGGFEGGMTNGQPIVVRGVMKPIPTLYKPLMSVDIETHEPYKATVERSDPTALPA
             :  .  ** .* **. **:*********: **..****.*: :.* :**: * :   *  :***  *:**

                   360        370        380        390        400
                     |          |          |          |          |
TARGET       AGVVVETMVALVLARAALEKFGGDSLAETQRNIAAYQRSVADREAPAARVSG
TEMPLATE     AGMVMEAVVATVLAQEILEKFSSDNLEELKEAVAKHRDYTKNY---------
             **:*:*::** ***:  ****..*.* * :. :* ::  . :

Identity (*): 172 is 42.79 %
Strongly similar (:): 77 is 19.15 %
Weakly similar (.): 33 is 8.21 %
Different: 120 is 29.85 %
```

Fig. 2. CLUSTAL W pairwise sequence alignment between the target (MTB CS) and template (1QXO). The amino acid residues of the CS signatures (G-[DES]-S-H-[GC]-x(2)-[LIVM]-[GTIV]-x-[LIVT]-[LIV]-[DEST]-[GH]-x-[PV], [GE]-x(2)-S-[AG]-R-x-[ST]-x(3)-[VT]-x(2)-[GA]-[STAVY]-[LIVMF], R-[SHF]-D-[PSV]-[CSAVT]-x(4)-[SGAIVM]-x-[IVGSTAPM]-[LIVM]-x-E-[STAHNCG]-[LIVMA]) are underlined.