Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c7ob01272a

Exploring the catalytic mechanism of dihydropteroate synthase: elucidating the differences between the substrate and inhibitor†

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Dihydropteroate synthase (DHPS) catalyzes the condensation of 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) with *p*-aminobenzoic acid (*p*ABA) and is a well validated target for anti-malarial and anti-bacterial drugs. However, in recent years its utility as a therapeutic target has diminished considerably due to multiple mutations. As such, considerable structural biology and medicinal chemistry effort has been expended to understand and overcome this issue. To date no detailed computational analysis of the protein mechanism has been made despite the detailed crystal structures and multiple mechanistic proposals being made. In this study the mechanistic proposals for DHPS have been systematically investigated using a hybrid QM/MM method. We aimed to compare the energetics associated with S_N1 and S_N2 processes, whether the S_N1 process involves a carbocation or neutral DHP intermediate, uncover the identity of the general base in the catalytic mechanism, and understand the differences in substrate *vs.* inhibitor reactivity. Our results suggest a reaction that follows an S_N1 process with the rate determining step being C–O bond breaking to give a carbocation intermediate. Comparative studies on the inhibitor STZ confirm the experimental observations that it is also a DHPS substrate.

Received 24th May 2017,
Accepted 12th June 2017

DOI: 10.1039/c7ob01272a

rsc.li/obc

Introduction

Dihydropteroate synthase is a key enzyme in the folate pathway of prokaryotes and primitive eukaryotes. It catalyzes the condensation of *p*-aminobenzoic acid (*p*ABA) with 6-hydroxymethyl-7,8-dihydropterin pyrophosphate (DHPPP) to form 7,8-dihydropteroate and pyrophosphate (Scheme 1). Humans lack the enzyme meaning it represents an ideal target for the treatment of infections including those associated with bacteria and parasites. Since the 1930s members of the sulfonamide class of DHPS inhibitors have been used as *p*ABA competitive inhibitors for bacterial infections (*i.e.* sulfanilamide),[1–3] and as 2nd line anti-malarial treatment in combination with the inhibitors of another folate enzyme dihydrofolate reductase (DHFR) (*i.e.* sulfadoxine/pyrimethamine).[4] Sulfonamides act as competitive inhibitors of DHPS and dead-end substrate analogs.[5,6] However, in the past 2 decades their effectiveness has been reduced greatly as a result of mutations in the enzyme.[4,7,8]

Given its status as a validated drug target there has been a renewed scientific effort to better understand the protein from a structural perspective, the role of mutations on substrate and inhibitor binding and reactivity, and the implementation of new strategies to allow the development of next generation inhibitors.[9–12]

DHPS has a classic TIM barrel α/β structure in which the eight α-helices and β-strands form alternating, parallel repeating units.[6,7,9,13,14] The DHPPP binding site is located towards the center of the enzyme (Fig. 1). The pterin scaffold of DHPPP makes two strong interactions with Asp101 and Lys220 while its pyrophosphate forms 4 interactions with the active site Mg^{2+} ion, Arg254 and His256. The octahedral Mg^{2+} interacts with a further 3 water molecules and the carbonyl of Asn27. *p*ABA binds towards the entrance of the active site pocket which is defined by two flexible loops. The substrate forms π-stacking interactions with Phe33 and Phe189, and comes into contact with the hydrophobic portion of the sidechains of Pro69 and Lys220 and a hydrogen bond (H-bond) with Ser221.[7] Earlier research on DHPS suggested that the reaction

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† Electronic supplementary information (ESI) available: Active distances, charges and additional energies. See DOI: 10.1039/c7ob01272a