Is the 5,10-methylenetetrahydrofolate cofactor synthesized through a non-enzymatic or enzymatic mechanism?

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INTRODUCTION

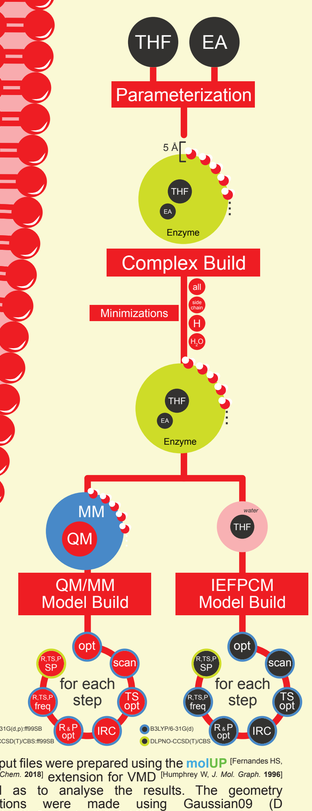
The 5,10-methylenetetrahydrofolate (**5,10-mTHF**) plays a key role in several cellular functions, in particular, the synthesis of amino acids and nucleic acids.[Froese DS, *Nature comm.* **2018**]. Among all these reactions, the 5,10-mTHF is crucial as a supply of carbon units required for the synthesis of thymidine monophosphate (**dTMN**) by the thymidylate synthase.

The 5,10-mTHF is synthesized through the methylation and cyclization of tetrahydrofolate (**THF**), whose most significant supply is catalyzed by the serine hydroxymethyltransferase (**SHMT**). However, in the literature, there are two hypotheses for the conversion of THF into 5,10-mTHF.[Schirch V, *Curr. Opin. Chem. Biol.* **2005**] Some evidence supports that the full mechanism occurs in an enzymatic way where the SHMT assists the entire pathway. On the other hand, some authors suggested that the THF can leave the SHMT after the hydroxymethyl group transfer. In this work, the full catalytic mechanism of SHMT was addressed by computational means and these two hypotheses were also tested.

METHODS

The crystallographic structure of SHMT, deposited on Protein Data Bank with the code 1DFO [Scarsdale JN, *J. Mol. Biol.* **2000**], was used to build the ONIOM QM/MM model to study the catalytic mechanism. One of the three available homodimers was selected to proceed with the calculations. The enzyme was co-crystallised with the external aldimine (**EA**), the product of the reaction (glycine) bonded to PLP, and an analogue of the THF cofactor. The THF analogue was modulated to fit the natural THF configuration. After the two first steps of the mechanism, the 5-hydroxymethyl-THF intermediate was used to perform the calculations in water solvent using the IEFPCM formalism (ε = 78.3553).

The study of the catalytic mechanism followed the scheme:



The input files were prepared using the **molUP** [Fernandes HS, *J. Comput. Chem.* **2018**] extension for VMD [Humphrey W, *J. Mol. Graph.* **1996**] as well as to analyse the results. The geometry optimisations were made using Gaussian09 (D version)[Frisch MJ, *Gaussian, Inc.: Wallingford* **2009**] and the single-point energy calculations (SP) were calculated using ORCA (4.0.1.2 version)[Frank N, *Wiley Interdiscip. Rev.: Comput. Mol. Sci.* **2018**].

CONCLUSION

The SHMT catalyzes the conversion of L-serine and THF into glycine and 5,10-mTHF, with the help of the pyridoxal-5'-phosphate (**PLP**) cofactor. The catalytic mechanism occurs in three main stages: the α-elimination of the -CH2OH from the L-serine, the cyclisation of the 5-hydroxymethyl-THF intermediate into 5,10-methylene-THF, and the protonation of the quinonoid intermediate (**QI**). One of the biggest discussion topics about this mechanism lies on the possibility of the 5,10-mTHF being produced through a purely enzymatic mechanism or whether the last steps could occur in the cytoplasm through a non-enzymatic process.

The results [Fernandes HS, *ACS Catal.* **2018**] show that both hypotheses are feasible, although the mechanism takes two different pathways with a different number of steps. The non-enzymatic conversion of 5-hydroxymethyl-THF into 5,10-mTHF requires only three steps, but it is kinetic and thermodynamically less favorable by 0.8 and 24.3 kcal/mol, respectively.

Although the reaction is not impossible in solution, it is very improbable that the 5-hydroxymethyl-THF intermediate might be released to the cytoplasm to overcome a set of reactions that are less favorable when compared to the ones that would occur in the SHMT. Moreover, the final step of the 5,10-mTHF synthesis provides a proton that is needed for the protonation of the QI.

Synthesis of **RNA** and **DNA**

Thymidylate synthase

dTMP ← dUMP

5,10-mTHF

DiHydroFolate (DHF)

DHF Reductase

NADPH → $NADP^+$

TetraHydroFolate (THF)

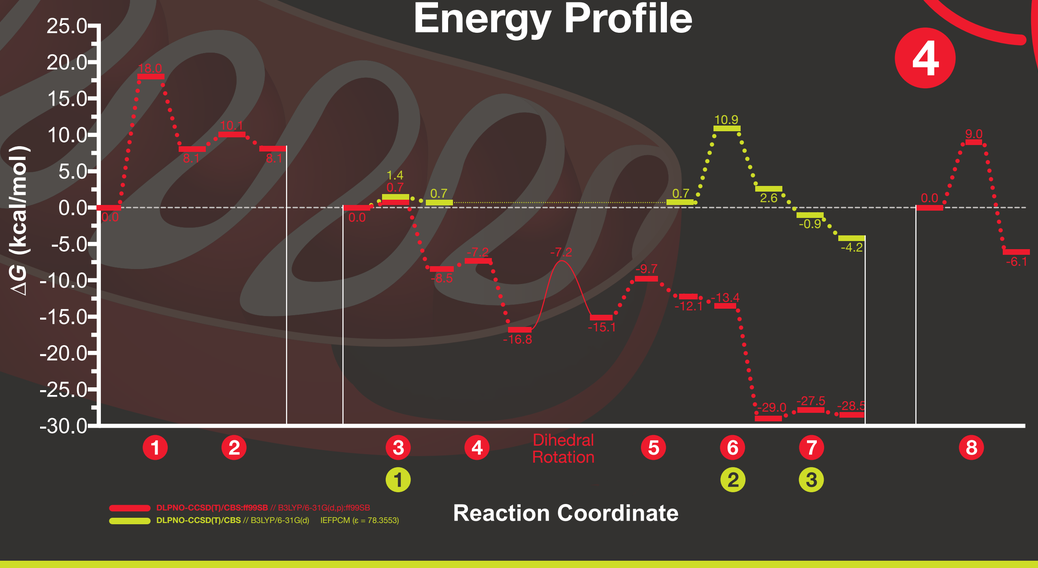
L-Serine

$H^+ + H_2O$

5-hydroxymethyl-THF

Glycine + Water

Energy Profile



Enzymatic

Serine HydroxyMethylTransferase

Non-Enzymatic

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