One-carbon metabolism enzyme Serine Hydroxy Methyl Transferase and salt tolerance in the cyanobacteria Aphanothece halophytica

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SHMT is a pyridoxal-5′-phosphate (PLP)-dependent enzyme belonging to the fold type I superfamily which plays a central role in the one-carbon unit metabolism, as it catalyzes the reversible conversion of L-serine and tetrahydropteroylglutamate (H_4PteGlu) to glycine and 5,10-methylenetetrahydropteroylglutamate (5,10-CH_2-H_4PteGlu), an essential substrate of thymidylate synthase enzyme for de novo biosynthesis of thymidylate [1]. It also catalyzes the H_4PteGlu-independent cleavage of many 3-hydroxyamino acids and the decarboxylation of aminomalonate. In plants, SHMT cooperates with the GDC (Glycine Decarboxylase Complex) to mediate photorespiratory glycine–serine interconversion [2] and has been suggested to be involved in salt tolerance [3]. Also, in cyanobacteria, SHMT has been reported to confer salt tolerance [4]. Moreover, the SHMT gene was suggested to be essential for cell survival because the complete segregation of SHMT gene could not be generated [5].
SHMT from cyanobacteria *Aphanothece halophytica* has been recently characterized [4]. Expression of *Ap*SHMT in *E. coli* lead to increase levels of osmoprotectant glycinebetaine under salinity condition and conferred salt tolerance [4]. Also expression of *Ap*SHMT gene in freshwater cyanobacterium, *Synechococcus elongatus* PCC7942 lead to transformed cells with higher tolerance to salinity than control cells [6]. Glycine level was higher in the transformed cells. Moreover, increased activities of enzymes of the phosphorylated pathway for serine biosynthesis were observed. Surprisingly, *Ap*SHMT seemed not to be inhibited by substrate THF [4], as has been reported for SHMT from other sources, such as human, rabbit, *Plasmodium vivax* [7,8].
In order to go insight in *Ap*SHMT catalytic mechanism and to elucidate if the lack of THF inhibition is important for salt stress tolerance of *A. halophytica* we decided to characterize the catalytic properties and the THF inhibition of *Ap*SHMT at different salt concentrations and pHs. To highlight the characteristics coupled to the adaptation to high NaCl concentration and high pH, the properties of the halophytic and alkalophilic enzyme have been contrasted with those of the homologous counterpart from *E. coli*, which has been structurally and functionally characterized in depth and with which it shares 57% amino acid sequence identity [4]. Moreover, in order to evaluate and compare the "in vivo" effect of the expression of *e*SHMT and *Ap*SHMT under salt conditions, we also studied the growth profile of *E.coli* cells overexpressing *Ap*SHMT in LB media with added NaCl.
Results showed that at pH7.2 and 0 M NaCl, *e*SHMT presented a higher catalytic efficiency for the THF-dependent reaction than *Ap*SHMT. However, at high pH (pH 9.5) *Ap*SHMT is more efficient than *e*SHMT. Moreover, we demonstrated that also *Ap*SHMT is inhibited by THF in the range of pHs assayed, though this inhibition tends to decrease at high pHs (pH=8.5, 9.5). NaCl dependency of the THF dependent activities were, as expected, different between the two enzymes. The catalytic efficiency of *Ap*SHMT and *e*SHMT for L-Ser decreased 30- and 227 fold, respectively when NaCl increased from 0 to 0.750 M. Similarly, the catalytic efficiency of *Ap*SHMT and *e*SHMT for THF decreased 18.6- and 32.2 fold, respectively when NaCl increased from 0 to 0.750 M. Regarding the THF-independent reactions, *Ap*SHMT resulted to present a catalytic efficiency 6.3, 5, 3.8 and 2.4 fold higher for L-allo-threonine, L-threonine, L-threophenylserine and L- erythrophenylserine than *e*SHMT. Finally, we observed that the cell growth rate of *E.coli* cells overexpressing SHMTs from different sources, *Ap*SHMT and *Psychromonas ingrahamii* SHMT (*pi*SHMT) is higher respect to the one obtained with harboring empy vector *E.coli* cells (control) under all studied conditions: 0M NaCl, 0.3M NaCl, 0.5M NaCl and 0.7M NaCl, suggesting the relevance of the SHMT for salt tolerance, although the molecular mechanism underlying this phenomenon is not clear.

[1]Anderson and Stover, 2009
[2] Bauwe et al., 2010
[3] Zhou et al., 2012
[4]Waditee-Sirisattha et al. 2012
[5] Hagemann et al., 2005
[6] Waditee-Sirisattha et al. 2017
[7] Pinthong et al. 2014
[8] Amornwatcharapong et al. 2017

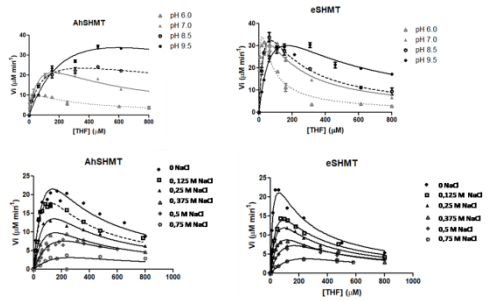

Fig.1 Effects of pH and NaCl on *Ap*- and *e*-SHMT Bi-substrate steady-state kinetics

ApSHMT						
NaCl (M)	K_M		Kcat (min^{-1})	$kcat/K_M$		KiTHF (µM)
	L-Ser (mM)	THF (µM)		L-Ser	THF	
0	0.53 ± 0.08	56 ± 13	740 ± 8	1213	13.20	300 ± 70
0.125	1.10 ±0.11	74 ± 19	775 ± 12	704	10.50	191 ± 50
0.250	1.54 ± 0.24	113 ± 22	669 ± 12	434	5.92	185 ± 50
0.375	2.14 ± 0.27	137 ± 25	519 ± 10	242	3.78	171 ± 48
0.500	2.41 ± 0.34	224 ± 60	443 ± 15	184	1.98	218 ± 30
0.750	6.02 ± 1.06	327 ± 70	240 ± 17	40	0.71	160 ± 50
eSHMT						
NaCl (M)	K_M		Kcat (min^{-1})	$kcat/K_M$		KiTHF (µM)
	L-Ser (mM)	THF (µM)		L-Ser	THF	
0	0.39 ± 0.06	33 ± 9	1064 ± 150	2728	32.2	96 ±26
0.125	2.03 ± 0.21	63 ± 14	747 ± 92	368	11.8	131 ± 29
0.250	4.36 ± 0.38	85± 8	763 ± 43	175	9.0	102 ± 9
0.375	10.30 ± 1.50	71 ± 22	431 ± 75	41	6.1	180 ±55
0.500	9.86 ± 3.66	164 ± 30	480 ± 102	48	3.0	163 ± 50
0.750	20.03 ± 4.59	241 ± 50	244 ± 65	12	1.0	250 ± 40

Fig. 2 Bi-substrate stedy-state kinetic parameters of the *Ap*- and *e*-SHMT at various NaCl concentrations

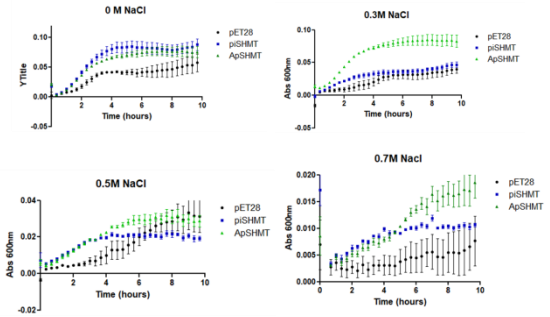

Fig. 3 Growth profile of *Escherichia coli* cells (BL21) expressing *Ap*- and pi-SHMT