Crystal Structural Studies of Glutathioylspermidine Synthetase from *Helicobacter pylori*

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Glutathionylspermidine synthetase (GspS) catalyzes ATP-dependent conjugation of GSH and spermidine to form glutathionylspermidine (GSP) and it belongs to the ATP-grasp superfamily. Since the major function of GSH-spermidine conjugates are defense against oxidative stress, GspS is considered as drug targets. GspS from *H. pylori* (HpGspS) has been overexpressed in *E.coli* and purified as a monomer with a molecular weight of 45 KDa. The crystals of apo-HpGspS, HpGspS-ADP-Pi complex, and HpGspS-AMPPNP complexes were grown in a similar condition using PEG3350 as a precipitate. All these crystals belong to C2 space group and contain two molecules per asymmetry unit. The crystal structure of the HpGspS-ADP-Pi complex has been determined to 2.25 Å resolution by multwavelenght anomalous dispersion (MAD) using selenomethionine derivative. The overall structure of HpGspS shows a mixed α/β fold with an equilateral triangle shape, including an antiparallel β sheet, a parallel β sheet, and a lid domain. The ATP binding site of HpGspS structure is located at the central antiparallel β-sheet and is surrounded by five loops. The adenine ring and ribose of AMPPNP are buried in a hydrophobic pocket in HpGspS. We find some conserved residues (Arg98, Asp100, Glu114, Asn116, Lys276, Lys308 and Arg372) participating in ATP binding.

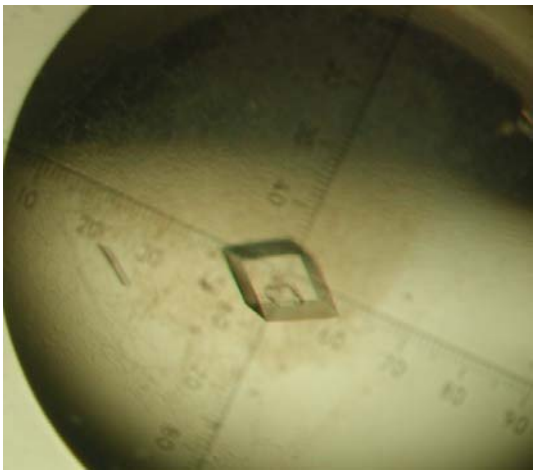

Fig. 1: The HpGspS crystals grew in 100mM bis-tris propane pH8, 18% PEG3350 within one weak at 12ºC using hanging-drop vapor diffusion method, and the size of crystal were about 0.30 x 0.15 x 0.05 mm^3

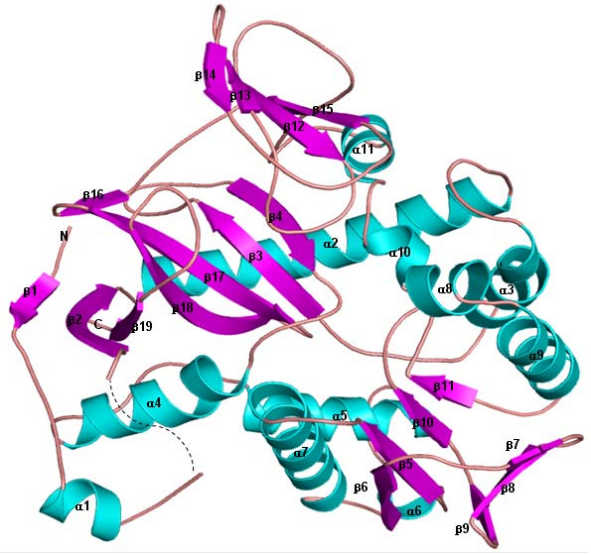

Fig. 2: Overall structure of HpGspS: A ribbon diagram of the structure of HpGspS. The α-helices are colored in blue and numbered in α1~α11. The β-strands are colored in pink and numbered in β1~β19. The figure was drawn using PyMOL

Table1: X-ray diffraction data statistics of the HpGspS

Table: X-ray diffraction data statistics of the HpGspS

Crystal	Apo-HpGspS	HpGspS-ADP-Pi	HpGspS-AMPPNP	SeHpGspS		
beamline	NSRRC_13C1	NSRRC_13C1	NSRRC_13C1	Spring8_12B2		
Space group	C2	C2	C2	C2		
Unit cell parameters (Å)	a = 203.88 b = 51.20 c = 93.31 β = 98.41	a = 203.50 b = 51.49 c = 93.23 β = 98.98	a = 203.89 b = 51.20 c = 93.31 β = 98.42	a = 203.12 b = 51.65 c = 93.11 β = 98.98	a = 203.12 b = 51.65 c = 93.11 β = 98.98	a = 203.10 b = 51.64 c = 93.10 β = 98.97
Wavelength (Å)	0.9762 Å	0.9762 Å	0.9762 Å	0.964511 Å (high remote)	0.979754 Å (edge)	0.979438 Å (peak)
Resolution (Å)	30.00-2.40 (2.49-2.40)	30.00-2.0 (2.07-2.25)	30.00-2.0 (2.07-2.0)	30.00-2.3 (3.0-2.3)	30.00-2.3 (3.0-2.3)	30.00-2.3 (3.0-2.3)
Total # of Reflections	140697	174584	257378	140443	141259	140505
# of Unique Reflections	37626	44211	63434	40613	40813	40427
Redundancy	3.8 (2.8)	3.9 (3.2)	4.1 (3.6)	3.5 (3.1)	3.5 (3.1)	3.4 (3.1)
Completeness (%)	98.7 (95.2)	96.5 (85.8)	98.7 (91.7)	94.6 (97.9)	94.9 (98.1)	94.7 (98.2)
I/σ (I)	24.8 (2.9)	27.7 (4.7)	36.1 (3.4)	25.2 (3.0)	25.5 (2.9)	25.5 (3.1)
R_{sym} (%) [a]	4.8 (30.6)	6.4 (35.7)	3.5 (26.7)	7.5 (33.4)	7.7 (34.9)	7.5 (33.8)
Refinement statistics						
Resolution range (Å)	30.00-2.40 (2.49-2.40)	30.00-2.0 (2.07-2.25)	30.00-2.0 (2.07-2.0)			
R-factor (%)[b]	20.8	19.8	20.2			
R_{free} (%)[c]	26.8	26.1	24.1			
No. of protein residues	751	753	757			
No. of water	463	582	608			
RMSD bond length (Å°)	0.003	0.007	0.005			
RMSD, angles	0.717	1.093	0.984			
Ramachandran plot (%) Most favored/allowed regions	88.1/100	88.1/100	89.9/100			
Average B factor (Å°)						
Total	50.67	28.94	33.76			
Substrate	no	31.30	34.90			

Reference

[1] C. H. Pai, B. Y. Chiang, T. P. Ko, C. C. Chou, C. M. Chong, F. J. Yen, S. Chen, J. K. Coward, A. H. Wang, and C. H. Lin, EMBO J. **25**, 5970 (2006).