Analysis and Phylogeny of Small Heat Shock Proteins from Marine Viruses and Their Cyanobacteria Host

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Abstract

Small heat shock proteins (sHSPs) are oligomeric stress proteins characterized by an α-crystallin domain (ACD) surrounded by a N-terminal arm and C-terminal extension. Publications on sHSPs have reported that they exist in prokaryotes and eukaryotes but, to our knowledge, not in viruses. Here we show that sHSPs are present in some cyanophages that infect the marine unicellular cyanobacteria, Synechococcus and Prochlorococcus. These phage sHSPs contain a conserved ACD flanked by a relatively conserved N-terminal arm and a short C-terminal extension with or without the conserved C-terminal anchoring module (CAM) L-X-I/V, suggested to be implicated in the oligomerization. In addition, cyanophage sHSPs have the signature pattern, P-P-[YF]-N-[ILV]-[IV]-x(9)-[EQ], in the predicted β2 and β3 strands of the ACD. Phylogenetically, cyanophage sHSPs form a monophyletic clade closer to bacterial class A sHSPs than to cyanobacterial sHSPs. Furthermore, three sHSPs from their cellular host, Synechococcus, are phylogenetically close to plants sHSPs. Implications of evolutionary relationships between the sHSPs of cyanophages, bacterial class A, cyanobacteria, and plants are discussed.

Introduction

The small heat shock proteins (sHSPs) are a family of stress proteins, found in archaea, bacteria, fungi, plants and animals [1-4]. sHSPs monomers (12-42 kDa) are characterized by a conserved domain of approximately 90 amino acids called α-crystallin domain (ACD), consisting of eight beta strands which form a β-sandwich fold (Pfam PF00011: Hsp20/alpha-crystallin). This domain is flanked by an N-terminal arm and C-terminal extension variable in both length and sequence between orthologues and may reflect functional specificity and/or preferential chaperone activity [5,6]. sHSPs generally exist as oligomers that are usually polydisperse and change size and organization on exposure to stress and when interacting with substrate [6]. In vitro sHSPs have been shown to prevent the irreversible aggregation of non-native proteins during heat shock. Mutations in sHSPs are associated with a variety of severe diseases, including myopathies, dystrophies, and cataracts [7,8]. Phylogenetic analyses indicated that sHSPs were already present in the last common ancestor of prokaryotes and eukaryotes [9,10].

Phages are very important in marine systems. They are the most abundant forms of life in the Earth’s oceans with concentrations exceeding 10 million per milliliter of seawater [11]. They influence marine biogeochemical cycles by controlling host abundance and community composition as well as recycling photosynthetically fixed organic carbon as dissolved organic material via viral lysis [12]. Cyanophages infect the marine unicellular cyanobacteria, Synechococcus and its sister group Prochlorococcus which dominate the picophytoplankton in the oceans [13,14]. To date, the vast majority of phages that are known to infect cyanobacteria are myoviruses [15,16], which are related to phage T4 [17,18]. It has been reported that the sequenced genomes of Synechococcus and Prochlorococcus phages contain genes with an hsp20/alpha-crystallin domain (PF00011) [18-20].

Materials and Methods

Sequence databases, alignment and phylogeny

We searched the presence of sHSPs in the complete sequenced genomes of viruses from the biological databases (GenBank, protein database, and genomes database) using BLASTp, tBLASTn and HMM profile. We have also searched sHSPs in complete sequenced genomes of their host cyanobacteria, Synechococcus and Prochlorococcus. We
aligned sequences of small heat shock proteins (sHSPs) from several species with ClustalW. Secondary structures indicated in the alignment are assigned according to the determined crystal structure of wheat HSP16.9 [21]. GeneBank accession numbers of sequences of cyanophages and cyanobacteria used in this alignment are listed in the Tables 1 and 2, respectively. Phylogenetic tree was constructed using PhyML [22] and BioNJ [23]. Only the ACD and C-terminal extension were used for the phylogenetic analysis. For PhyML, WAG Substitution model and the statistical confidence of the nodes was calculated by aLRT test.

**Molecular modeling and docking**

3D models of *Synechococcus* phage sHSP S-MbCM6 (HspSP-MbCM6) and *Synechococcus* sp. PCC 7335.1 sHSP (HspS-PCC7335.1) were constructed using I-TASSER which combines the methods of threading, *ab initio* modeling and structural refinement [24]. Structures of Hsp16.0 from *Schizosaccharomyces pombe* (PDB: 3w1z), Hsp16.9 from *Triticum aestivum* (PDB: 1gme) and αB-crystallin from human (2ygd) were used as templates for HspSP-MbCM6. 3w1z, 1gme and Hsp16.5 from *Methanocaldococcus jannaschii* (PDB: 4eld) served as template for HspS-PCC7335.1. Search of structure similarity of obtained 3D models was conducted by PDBeFold [25] against PDB database. The electrostatic potential surface of sHSP 3D models was realized with PyMOL software (http://pymol.org/). Pairwise 3D models alignment was performed using Matras software [26]. Docking of the C-terminal extension of cyanophage (HspSP-MbCM6) and cyanobacteria (HspS-PCC7335.1) into hydrophobic pockets of β4/β8 strands region revealed by electrostatic potential surface analysis, was conducted by structure alignment to tetramer of wheat Hsp16.9 (PDB: 1gme).

**Results and Discussion**

Publications on sHSPs have reported that they are present in archaea, bacteria, fungi, plants and animals but not in viruses. Here, we searched for sHSPs in the complete sequenced genomes of viruses from the biological databases (GenBank, protein database, and genomes database) using BLASTp, tBLASTn and HMM profile. These searches showed that sHSPs are present only in marine viruses (cyanophages) that infect the unicellular cyanobacteria, *Synechococcus* and *Prochlorococcus* (Table 1). We found that the genomes of

**Table 1. Cyanophages’ nomenclature.**

| Cyanophages                       | Accession number | Nomenclature        |
|-----------------------------------|------------------|---------------------|
| *Synechococcus* phage S-RSM4      | YP_003097310.1   | HspSP-RSM4*         |
| *Synechococcus* phage S-PM2       | YP_195165.1      | HspSP-PM2           |
| *Synechococcus* phage S-SM1       | YP_004323062.1   | HspSP-SM1           |
| *Synechococcus* phage S-SSM5      | YP_004324766.1   | HspSP-SSM5          |
| *Synechococcus* phage Syn19        | YP_004323990.1   | HspSP-Syn19         |
| *Synechococcus* phage S-SM2       | YP_004322303.1   | HspSP-SM2           |
| *Synechococcus* phage S-CBM2      | AFK66310.1       | HspSP-CBM2          |
| *Synechococcus* phage S-MbCM6     | YP_007001883.1   | HspSP-MbCM6         |
| *Synechococcus* phage syn9        | YP_717838.1      | HspSP-Syn9          |
| *Synechococcus* phage metaG-MbCM1 | YP_007001660.1   | HspSP-MbCM1         |
| *Synechococcus* phage S-RIM8 AHR1 | YP_007518247.1   | HspSP-RIM8          |
| *Synechococcus* phage S-ShM2      | YP_004322832.1   | HspSP-ShM2          |
| *Synechococcus* phage S-SSM7      | YP_004324229.1   | HspSP-SSM7          |
| *Synechococcus* phage S-CRM01     | YP_004508578.1   | HspSP-CRM01         |
| *Synechococcus* phage S-CAM8      | AET72746.1       | HspSP-CAM8          |
| *Synechococcus* phage S-RIM2 R1_1999 | YP_007675621.1 | HspSP-RIM2          |
| *Synechococcus* phage S-SKS1      | YP_007674470.1   | HspSP-SKS1          |
| *Synechococcus* phage S-CAM1      | YP_007673074.1   | HspSP-CAM1          |
| *Synechococcus* phage S-SSM4      | YP_007677312.1   | HspSP-SSM4          |
| *Prochlorococcus* phage Syn1      | YP_004324522.1   | HspPP-Syn1**       |
| *Prochlorococcus* phage P-SSM4    | YP_214702.1      | HspPP-SSM4          |
| *Prochlorococcus* phage P-RSM4    | YP_00432305.1    | HspPP-RSM4          |
| *Prochlorococcus* phage Syn33     | YP_004323772.1   | HspPP-Syn33         |
| *Prochlorococcus* phage P-SSM2    | YP_214406.1      | HspPP-SSM2          |
| *Prochlorococcus* phage P-SSM7    | YP_004325000.1   | HspPP-SSM7          |
| *Prochlorococcus* phage P-HM2     | YP_004323516.1   | HspPP-HM2           |
| *Prochlorococcus* phage P-HM1     | YP_004322573.1   | HspPP-HM1           |

* Hsp for Small heat shock protein; SP for Synechococcus phage and S-RSM4 for strain
** Hsp for Small heat shock protein; PP for Prochlorococcus phage and Syn1 for strain

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Table 2. Cyanobacteria's nomenclature and number of genes.

| Cyanobacteria | Gene number | Accession number | Nomenclature |
|---------------|-------------|------------------|--------------|
| Synechococcus sp. WH 5701 | 3 | ZP_01083513.1; ZP_01084874.1; ZP_01086483.1 | HspS-WH5701.1; HspS-WH5701.2; HspS-WH5701.3 |
| Synechococcus sp. PCC 7335 | 3 | ZP_05035247.1; ZP_05037140.1; ZP_05039628.1 | HspS-PCC7335.1; HspS-PCC7335.2; HspS-PCC7335.3 |
| Synechococcus sp. CB0101 | 2 | ZP_07972696.1; ZP_07973042.1 | HspS-CB0101.1; HspS-CB0101.2 |
| Synechococcus sp. CB0205 | 2 | ZP_07971592.1; ZP_07969614.1 | HspS-CB0205.1; HspS-CB0205.2 |
| Synechococcus sp. JA-3-3Ab | 2 | YP_474873.1; YP_475298.1 | HspS-JA-3-3Ab.1; HspS-JA-3-3Ab.2 |
| Synechococcus sp. JA-2-3B'a.2(2-13) | 2 | YP_477816.1; YP_476514.1 | HspS-JA-2-3B'a.1; HspS-JA-2-3B'a.2 |
| Synechococcus sp. PCC 6312 | 1 | YP_007081156.1 | HspS-PCC6312 |
| Synechococcus elongatus PCC 6301 | 1 | YP_172414.1 | HspS-PCC6301 |
| Synechococcus sp. PCC 7502 | 1 | YP_007106253.1 | HspS-PCC7502 |
| Synechococcus sp. PCC 7002 | 1 | YP_001733915.1 | HspS-PCC7002 |
| Synechococcus sp. WH 7805 | 1 | ZP_01125036.1 | HspS-WH7805 |
| Synechococcus sp. RCC307 | 1 | YP_001228840.1 | HspS-RCC307 |
| Synechococcus sp. WH 7803 | 1 | YP_001226126.1 | HspS-WH7803 |
| Synechococcus sp. PCC 7336 | 1 | ALWC01000004.1 | HspS-PCC7336 |
| Synechococcus sp. RS9917 | 1 | ZP_01079326.1 | HspS-RS9917 |

* Hsp for Small heat shock protein; S for Synechococcus sp.; WH5701 for strain and .1 for Hsp number

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many, but not all, of these cyanophages contain a single-copy shSHPs gene. Small cyanophage genomes such as *Synechococcus* phage P60 (47872 bp) and *Synechococcus* phage Syn5 (46214 bp) do not contain any shSHP genes. It is interesting to note that *Prochlorococcus* phage P-SSM2 and P-SSM4 lack core T4-like chaperonin genes (rnlA, 31, and 57A), although, both phages contain shSHPs [19]. shSHPs could play the same function as core T4-like chaperonin genes intervening in scaffolding during maturation of the capsid [27].

Protein sequence analysis of cyanophage shSHPs showed that they contain a conserved ACD (~ 92 amino acids) flanked by a relatively conserved N-terminal arm and a short C-terminal extension. The length of the arm and the extension is variable. Conserved C-terminal anchoring motif (CAM) L-X-I/L/V, implicated in the inter-dimer interactions is present in 12 of 19 Synechococcus phages (Figure 1). The Prochlorococcus phages do not contain a classical CAM but A-X-P, L-X-G and L-X-A motives are present in the C-terminal extension of Prochlorococcus phages Syn33, P-SSM2 and P-SSM7, respectively. It was reported that shSHP Tsp36 also contains a non-classical CAM, I-X-P [28]. The end of N-terminal arm contains a double conserved proline and another conserved proline is present at the beginning of the C-terminal extension (Figure 1). Furthermore, an A-G doublet characteristic of bacterial class A shSHPs is also present in cyanophage shSHPs [29,30]. This doublet is sandwiched by hydrophobic residues, aliphatic residue L and aromatic F/Y/W. Aromatic residues in this position are found only in bacterial class A and animals shSHPs [29]. Cyanophages also have a conserved arginine, important for dimerization and associated with human diseases in the predicted β7 strand (Figure 1). *Synechococcus* phage S-PM2, S-CAM1 and Prochlorococcus phage Syn1 contain a hydrophilic amino acid asparagine in the place of arginine, and *Synechococcus* phage S-CRM01 contains a lysine. The ACD contains a variable region corresponding to the L57 loop (residues 109-121) (Figure 1). Arg in beta7 strand could form salt bridge with Asp or Glu in the L57 loop (residues 109-121) of the neighbor monomer, probably with Asp or Glu in position 117 (Figure 1). Using I-TASSER, we have constructed a 3D model of the sHSP from *Synechococcus* phage S-MbCM6 (HspSP-MbCM6). Figure 2A shows that 3D model is similar to the structure of wheat Hsp16.9 [21]. 3D structure alignment between HspSP-MbCM6 and wheat Hsp16.9 (Figure 2B) showed that the best conserved region is the ACD domain. 3D alignment by PDBRefold of the 3D model against PDB database revealed a high similarity (RMSD of 1.40 Å and 20% of identity) with 1gme.

We have also searched for shSHPs in the genomes of their host cyanobacteria, *Synechococcus* and *Prochlorococcus*, in order to know if shSHPs in cyanophages are the result of lateral gene transfer (LGT) from cyanobacteria to phage. LGT from cyanobacteria to cyanophages is well documented for photosynthesis genes [31]. Fifteen sequenced genomes of *Synechococcus* contain 1, 2 or 3 shSHP genes (Table 2) while seven others *Synechococcus* genomes do not. Surprisingly 13 genomes of *Prochlorococcus* do not contain any shSHPs gene. It is possible that genomes of *Prochlorococcus* and some *Synechococcus* have not acquired shSHPs gene by LGT or have lost it. Alignment of cyanobacterial shSHPs (Figure 3) revealed the presence of the P-G doublet characteristic of plants and bacterial class B. It is important to note a novel organization of CAM, three hydrophobic amino acids residues IV-X/X-L/V instead of classical two hydrophobic amino acids residues separated by a non-hydrophobic residue. Moreover, *Synechococcus_PCC7502* contains the classical CAM (V-X-L) and *Synechococcus_PCC7336* without CAM (Figure 3). The electrostatic potential surface of 3D models of *Synechococcus* phage S-MbCM6 (HspSP-MbCM6) and the cyanobacteria *Synechococcus sp. PCC 7335.1* shSHP (HspS-PCC7335.1) revealed the presence of three hydrophobic pockets formed by 64/68 strands. Docking of the C-terminal extension into the β4/β8 strands grooves revealed that
Figure 1. Sequence alignment of cyanophage sHSPs. Amino acids comprising predicted β-strands in *Synechococcus* phage S-ShM2 are in yellow background. The ACD comprises β2-β9. The CAM L-X-I/L/V and non-classical CAM in the C-terminal extension is in cyan and green background, respectively. Alignment was generated using ClustalW. Secondary structures indicated above are assigned according to the crystal structure of wheat HSP16.9 (1gme) [21]. GeneBank accession numbers of sequences used in this alignment are listed in the Table 1.

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According to the work of Fu et al. [30] based on the relationship between phylogeny and oligomeric polydispersity, we could suppose that cyanophage sHSPs exist in oligomeric polydispersity as in their bacterial class A ancestor sHSP. It is important to note that three sHSPs from their cellular host, *Synechococcus*, form a monophyletic clade that is phylogenetically close to plants (Figure 5). Cyanobacteria are among the most ancient organisms on Earth, and fossils of these photosynthetic bacteria indicate a striking resemblance between current species and ones extant over 2 billion years ago [32]. Thus, the ACD of sHSP gene family must be at least 2 billion years old. We could suppose that plants acquired sHSPs gene from cyanobacterial endosymbionts that gave rise to the chloroplast.

**Conclusions**

This study revealed the presence of sHSPs in viruses and highlighted their structural characteristics and phylogenetic relationships with those of prokaryotes and eukaryotes. We expect that the study of sHSPs in a simple system such as viruses and cyanobacteria will help answer many questions not yet resolved such as the mechanism of their interaction with the substrate. Moreover, they could help to know the origin and evolution of this ancient, at least 2 billion years old, gene family.
Figure 3. Sequence alignments of cyanophages, prokaryotes and eukaryotes. Amino acids comprising β-strands are in gray background. The ACD comprises β2-β9. The CAM L-X-I/L/V of cyanophages and non-classical CAM I/V-X-I/L/V-X-I/L/V of cyanobacteria in the C-terminal extension is in cyan background. Alignment was generated using ClustalW. Secondary structures indicated above are assigned according to the crystal structure of wheat HSP16.9 [21]. GeneBank accession numbers of sequences of cyanophages and cyanobacteria used in this alignment are listed in the Tables 1 and 2, respectively. IBPA_ECOLI (Escherichia coli) small heat shock protein IbP, NP_290325), IBPB_ECOLI (Escherichia coli, NP_290324), IBPA_SALET (Salmonella enterica, NP_458130), IBPB_SALET (Salmonella enterica, WP_000605929), IBPA_ENTCL (Enterobacter cloacae, YP_004949877), IBPB_ENTCL (Enterobacter cloacae, YP_004949878), Hsp20_BACAN (Bacillus anthracis, NP_844651), Hsp20_CLOAB (Clostridium acetobutylicum, NP_350294), Hsp20_STRT (Streptococcus thermophiles, YP_796431), HSP16_SCHPO (Schizosaccharomyces pombe, NP_596091), HSP20_SCHCM (Schizopyllum commune, XP_003031590), HSP16.5_METJA (Methanocaldo菌ococcus jannaschii, NP_247258), HSP20_METM6 (Methanococcus maripaludis, YP_001548257), HSP17.4_ARATH (Arabidopsis thaliana, NP_190209), HSP17.6_II_ARATH (Arabidopsis thaliana, NP_196763), HSP23.6_ARATH (Arabidopsis thaliana, NP_194250), HSP21_ARATH (Arabidopsis thaliana, NP_194497), HS16B_WHEAT (Triticum aestivum, Q41560), HSP17.2I_FUNHY (Funaria hygrometrica, AAD09178), HSP16.4II_FUNHY (Funaria hygrometrica, AAD09184), CRYAB_HUMAN (Homo sapiens, NP_001876), HSPB3_HUMAN (Homo sapiens, NP_006299), HSP_16.48_CAEEL (Caenorhabditis elegans, NP_505355), HSP23_DROME (Drosophila melanogaster, NP_523999), hspb7_DANRE (Danio rerio, NP_001006040).

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**Figure 4.** Electrostatic potential surface representation of CAM docking of cyanophages and cyanobacteria into hydrophobic β4 and β8 pockets. A. The CAM I-X-I connects dimers in oligomers of wheat Hsp16.9 by interacting with a hydrophobic pockets formed by β4 and β8 (PDB: 1gme_AJ). B. Cyanophage dimers interaction. C. Cyanobacterial dimers interaction. D. Cyanophage-cyanobacteria dimer interaction. The surfaces are coloured by electrostatic potential with negative charge shown in red and, positive charge in blue. For clarity one monomer of each dimer is represented and one monomer is in ribbon form. PyMOL software (http://pymol.org/).

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Figure 5. Phylogenetic relations of sHSPs from cyanophages, prokaryotes and Eukaryotes obtained by maximum likelihood. Only the ACD and C-terminal extension were used for the phylogenetic analysis. WAG Substitution model and the statistical confidence of the nodes were calculated by aLRT test. Branches with aLRT values lower than 50% were collapsed.

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Author Contributions

Conceived and designed the experiments: HM RMT. Performed the experiments: HM. Analyzed the data: HM RMT. Contributed reagents/materials/analysis tools: HM. Wrote the manuscript: HM. Manuscript revision: RMT.