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**Archaeal tetrathionate hydrolase goes viral: secretion of a sulfur  
metabolism enzyme in the form of virus-like particles**

**Running title:** Tetrathionate hydrolase as a virus-like particle

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32 **Abstract**

33 In the course of screening for virus-host systems in extreme thermal environments, we have  
34 isolated a strain of the hyperthermophilic archaeon *Acidianus hospitalis* producing unusual  
35 filamentous particles with zipper-like appearance. The particles were shown to represent a  
36 secreted form of a genuine cellular enzyme, tetrathionate hydrolase, involved in sulfur  
37 metabolism.

38

39 **Text**

40 Thermal aquatic areas with temperatures above 80°C are common habitats of  
41 archaea and their astoundingly diverse viruses (6). Due to frequent difficulties associated  
42 with isolation and cultivation of pure archaeal strains, studies of the viral diversity often rely  
43 on successful establishment of enrichment cultures from environmental samples (7). While  
44 exploring the viral community associated with a culture enriched in crenarchaeon *Acidianus*  
45 *hospitalis*, we have observed a variety of unique virus-like particles (9). One particle type  
46 was shown to represent infectious virions of a new virus, *Acidianus* filamentous virus 1,  
47 AFV1 (2). The nature of other particles, however, remained unknown. The most unusual  
48 among them were filamentous particles with a surface demonstrating a zigzag-like periodic  
49 pattern, zipper-like particles, ZLPs (9).

50 To study the ZLPs, we have isolated a producer strain, *Acidianus hospitalis* YS8, from  
51 the enrichment culture (Supplemental Material). The ZLPs were collected and purified from  
52 cell-free culture supernatant of *A. hospitalis* YS8 by sequential filtration through filters with  
53 pore sizes of 0.80, 0.45 and 0.20  $\mu\text{m}$ , followed by concentration of ZLPs on ultrafilters with  
54 the exclusion size of 100 kDa (Supplemental Material). The purified ZLPs appeared as  
55 cylindrical particles uniform in their width,  $15\pm 1$  nm, and variable in length, 100–200 nm (Fig.  
56 1A). The periodic zigzag-like pattern on the particle surface (Fig. 1A) most likely resulted  
57 from regular assemblage of identical structural units.

58 By electron microscopic analysis of the growing cells of *A. hospitalis* YS8, we could  
59 observe extrusion from cells of ZLPs (Fig. 1B). The amount of ZLPs, estimated as described  
60 in Supplemental Material, increased after treatment of the cells with mitomycin C (final  
61 concentration of 5%, v/v) and UV-light (7 min in the layer of 3 mm), and by freezing cells in  
62 liquid nitrogen and their rapid thawing (not shown). The results suggested that the ZLP  
63 production may be under general stress response regulatory network in *A. hospitalis*.  
64 Consistently, when the cells were allowed to adapt to the growth conditions, the presence of  
65 the ZLPs in the culture supernatant decreased to non-detectable levels (Supplemental  
66 Material).

67 The possibility to induce the production of ZLPs with mitomycin and UV irradiation  
68 was in line with our initial assumption that ZLPs represent genuine viruses (9). However,

69 upon examination of the molecular constituents of the purified ZLPs, no nucleic acids—  
70 neither DNA nor RNA—could be isolated from purified particles by phenol extraction (10). An  
71 SDS-PAGE analysis revealed two major protein bands with apparent molecular masses of  
72  $55\pm 5$  and  $110\pm 10$  kDa as well as three minor bands of proteins larger than 200 kDa (Fig. 2A).  
73 N-terminal sequencing of proteins from both the 55 and 110 kDa bands revealed an identical  
74 sequence (PIVYTY). Thus, the larger 110 kDa protein was apparently a dimer of the 55 kDa  
75 protein, while the larger proteins likely represent multimers of the 110 kDa dimer.

76 The N-terminal sequence enabled identification of the ZLP-coding gene on the  
77 genome of *A. hospitalis* W1 (11). The gene was annotated as coding for tetrathionate  
78 hydrolase (TetH; GenBank accession number: YP\_004458846) (11). Notably, an orthologue  
79 of the ZLP-forming protein from *Acidianus ambivalens* (99% identical), a very close relative  
80 of *A. hospitalis*, has been recently characterized biochemically and confirmed to possess the  
81 predicted activity (8). TetH is one of the key players in sulfur metabolism, oxidizing  
82 tetrathionate into thiosulfate and sulfate (8). The N-terminal sequence of the ZLP-forming  
83 protein matched to the residues Pro<sub>22</sub>-Tyr<sub>27</sub> of the annotated *tetH* gene product, indicating  
84 that the protein is a subject to N-terminal processing. This is consistent with previous reports  
85 on presence of the N-terminal signal sequence in bacterial and archaeal TetH proteins (4, 8).

86 The near-identity of the ZLP protein to the TetH from *A. ambivalens* suggested that  
87 the ZLPs are not viruses, but rather represent homomultimers of TetH. To investigate  
88 whether such filamentous particles might represent a physiologically-relevant form of TetH,  
89 we tested the biochemical activity of the purified ZLPs. The TetH activity was measured in a  
90 continuous assay by monitoring the increase in absorbance ( $\lambda=290$  nm), resulting from  
91 accumulation of long chain sulfur intermediates as described previously (3). The assay  
92 mixture contained 1 M ammonium sulfate (pH 3.0), 1 mM sodium tetrathionate and 20  $\mu$ l of  
93 ZLP preparation (Supplemental Material), in 0.1 ml volume. The presence of purified ZLPs in  
94 the assay mixture resulted in hydrolysis of the tetrathionate, as documented by continuous  
95 increase in the absorbance at 290 nm over 4 hours of incubation at 70 °C (Fig. 2B). The  
96 experiment was conducted in duplicate and resulted in an increase of the  $A_{290}$  by 0.35 units.  
97 No changes in the  $A_{290}$  were observed in the control experiment in the absence of  
98 tetrathionate (Fig. 2B), excluding a possibility that the increase in absorbance was due to  
99 solubilization of the ZLP-constituent protein. Similarly, when ZLPs were omitted, no changes  
100 in the absorption at 290 nm were detected (not shown). Thus, we confirm that the secreted  
101 ZLPs possess the tetrathionate hydrolase activity. Notably, secretion of TetH from *A.*  
102 *ambivalens* and *Acidithiobacillus ferrooxidans* have been reported previously (1, 8).  
103 However, this is the first time that TetH is shown to form virus-like particles.

104 To gain insights into the remarkable ability of TetH from *A. hospitalis* to assemble into  
105 filamentous structures, we built a structural model of the ZLP-forming protein using I-

106 TASSER (12). Similarly to the TetH from *A. ambivalens* (8), *A. hospitalis* TetH was found to  
107 adopt an eight-bladed  $\beta$ -propeller topology (Fig. 3A). Analysis of the electrostatic surface  
108 charge distribution revealed an overall opposite charge on the two faces of the disc-shaped  
109 molecule (Fig. 3B), suggesting that electrostatic interaction-mediated head-to-tail stacking of  
110 the TetH building blocks might play a role in ZLP formation. It should be noted, however, that  
111 stacking of the TetH monomers, with an estimated diameter of  $\sim 4.5$  nm (Fig. 3A), would be  
112 insufficient to produce ZLPs with the diameter of 15 nm (Fig. 1A). Consequently, the “stacks”  
113 used for ZLP formation are likely to consist of TetH multimers. Interestingly, electron  
114 microscopy data suggests that the assembly of ZLPs occurs prior or concomitantly with the  
115 extrusion of the filaments from *A. hospitalis* cells (Fig. 1B).

116 In the present study, we have demonstrated that filamentous particles previously  
117 assumed to correspond to archaeal viruses (9), in fact, represent a secreted form of a  
118 cellular enzyme, TetH, involved in sulfur metabolism. Consequently, caution should be taken  
119 when exploring and interpreting the diversity of virus-like particles in different environments.  
120 Our results also pave a way for further structural and biochemical studies, which should  
121 reveal a detailed mechanism of assembly and secretion as well as physiological role of the  
122 remarkable ZLP structures.

123

124 **References**

- 125 1. **Beard, S., A. Paradel, J. P. Albar, and C. A. Jerez.** 2011. Growth of  
126 *Acidithiobacillus ferrooxidans* ATCC 23270 in thiosulfate under oxygen-limiting  
127 conditions generates extracellular sulfur globules by means of a secreted tetrathionate  
128 hydrolase. *Front Microbiol* **2**:79.
- 129 2. **Bettstetter, M., X. Peng, R. A. Garrett, and D. Prangishvili.** 2003. AFV1, a novel  
130 virus infecting hyperthermophilic archaea of the genus *Acidianus*. *Virology* **315**:68-  
131 79.
- 132 3. **de Jong, G. A., W. Hazeu, P. Bos, and J. G. Kuenen.** 1997. Polythionate  
133 degradation by tetrathionate hydrolase of *Thiobacillus ferrooxidans*. *Microbiology*  
134 **143**:499-504.
- 135 4. **Kanao, T., K. Kamimura, and T. Sugio.** 2007. Identification of a gene encoding a  
136 tetrathionate hydrolase in *Acidithiobacillus ferrooxidans*. *J Biotechnol* **132**:16-22.
- 137 5. **Pettersen, E. F., T. D. Goddard, C. C. Huang, G. S. Couch, D. M. Greenblatt, E.  
138 C. Meng, and T. E. Ferrin.** 2004. UCSF Chimera--a visualization system for  
139 exploratory research and analysis. *J Comput Chem* **25**:1605-1612.
- 140 6. **Pina, M., A. Bize, P. Forterre, and D. Prangishvili.** 2011. The archeoviruses. *FEMS*  
141 *Microbiol Rev* **35**:1035-1054.
- 142 7. **Prangishvili, D.** 2006. Hyperthermophilic virus-host systems. *Methods in*  
143 *Microbiology* **35**:331-347.
- 144 8. **Protze, J., F. Müller, K. Lauber, B. Naß, R. Mentele, F. Lottspeich, and A.  
145 Kletzin.** 2011. An extracellular tetrathionate hydrolase from the thermoacidophilic  
146 archaeon *Acidianus ambivalens* with an activity optimum at pH 1. *Front Microbiol*  
147 **2**:68.
- 148 9. **Rachel, R., M. Bettstetter, B. P. Hedlund, M. Haring, A. Kessler, K. O. Stetter,  
149 and D. Prangishvili.** 2002. Remarkable morphological diversity of viruses and virus-  
150 like particles in hot terrestrial environments. *Arch Virol* **147**:2419-2429.
- 151 10. **Sambrook, J., and D. W. Russel.** 2001. *Molecular cloning: a laboratory manual*, 3rd  
152 ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- 153 11. **You, X. Y., C. Liu, S. Y. Wang, C. Y. Jiang, S. A. Shah, D. Prangishvili, Q. She, S.  
154 J. Liu, and R. A. Garrett.** 2011. Genomic analysis of *Acidianus hospitalis* W1 a host  
155 for studying crenarchaeal virus and plasmid life cycles. *Extremophiles* **15**:487-497.
- 156 12. **Zhang, Y.** 2008. I-TASSER server for protein 3D structure prediction. *BMC*  
157 *Bioinformatics* **9**:40.
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163 **Figure legends**

164

165 **FIG 1.** Negative contrast electron micrographs of the ZLPs. A: Purified ZLPs. B:  
166 Extrusion of ZLPs from a cell of *A. hospitalis* (indicated by an arrow). Scale bars, 200  
167 nm.

168

169 **FIG 2.** Protein composition of the ZLPs and their enzymatic activity. A: SDS-PAGE of  
170 purified ZLP preparation; molecular masses of the major proteins are indicated. B:  
171 Tetrathionate hydrolase activity of the ZLPs; the absorbance at 290 nm of the assay  
172 mixture, from duplicate experiments (indicated with squares and circles), is plotted  
173 over time. The average values are shown by a black line. The results of the control  
174 experiment in the absence of tetrathionate are shown with diamond symbols.

175

176 **FIG 3.** Structural model of the TetH monomer from *A. hospitalis*. A. Ribbon  
177 representation of the eight-bladed  $\beta$ -propeller topology of the TetH viewed down the  
178 central axis of the disc-shaped molecule. The model is colored according to the  
179 secondary structure elements:  $\alpha$ -blue, red;  $\beta$ -strands, magenta; coils, gray. B. TetH  
180 model colored according to the electrostatic surface potential following the Coulomb's  
181 law. The color scale is from -7 (red) to +7 (blue) kcal/(mol·e). The view on the left  
182 corresponds to the orientation depicted in panel A, while the one on the right shows a  
183 flipside of the molecule. The figure was prepared in UCSF Chimera (5).







