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A cyclic lipopeptide surfactin is a species-selective Hsp90 inhibitor that suppresses cyanobacterial growth

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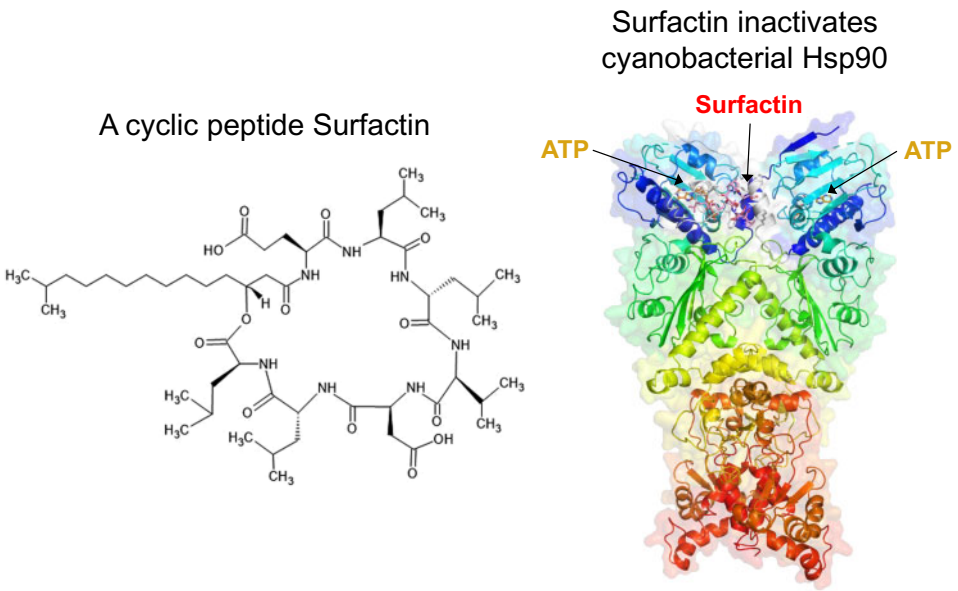
Heat shock protein 90 (Hsp90) is essential for eukaryotic cells, whereas bacterial homologs play a role under stresses and in pathogenesis. Identifying species-specific Hsp90 inhibitors is challenging because Hsp90 is evolutionarily conserved. We found that a cyclic lipopeptide surfactin inhibits the ATPase activity of Hsp90 from the cyanobacterium *Synechococcus elongatus* (*S.elongatus*) PCC 7942 but does not inhibit *Escherichia coli* (*E.coli*), yeast and human Hsp90s. Molecular docking simulations indicated that surfactin could bind to the N-terminal dimerization interface of the cyanobacterial Hsp90 in the ATP- and ADP-bound states, which provided molecular insights into the species-selective inhibition. The data suggest that surfactin inhibits a rate-limiting conformational change of *S.elongatus* Hsp90 in the ATP hydrolysis. Surfactin also inhibited the interaction of the cyanobacterial Hsp90 with a model substrate, and suppressed *S.elongatus* growth under heat stress, but not that of *E.coli*. Surfactin did not show significant cellular toxicity towards mammalian cells. These results indicate that surfactin inhibits the cellular function of Hsp90 specifically in the cyanobacterium. The present study shows that a cyclic peptide has a great specificity to interact with a specific homolog of a highly conserved protein family.

Keywords: cyanobacterium; cyclic peptide; Hsp90; molecular chaperone; surfactin.

Abbreviations: CBB, Coomassie Brilliant Blue; *E.coliEscherichia coli*; EDTA, ethylenediaminetetraacetic acid; Hsp90, heat shock protein 90; MDH, malate dehydrogenase; SDS–PAGE, sodium dodecyl sulphate–polyacrylamide gel electrophoresis; *S.elongatus,Synechococcus elongatus*; *S.pombe,Schizosaccharomyces pombe*.

The heat shock protein 90 (Hsp90) is an evolutionarily conserved molecular chaperone. In eukaryotes, it is located in the cytosol and organelles, including the nucleus, mitochondrion, chloroplast and endoplasmic reticulum (*1*). Cytosolic Hsp90 and Grp94 (the Hsp90

Graphical AbstractGraphical Abstract



homolog in the endoplasmic reticulum) are essential for cell survival (*2*, *3*). Hsp90 interacts with a wide range of protein substrates involved in various cellular processes, including DNA repair, development and the immune response (*4*). Hsp90 is also associated with various diseases including cancer and protein-misfolding diseases. Thus, Hsp90 is a drug target for the treatment of cancer and other diseases (*4*, *5*). The most notable drugs such as geldanamycin, radicicol and purine derivatives inhibit the ATPase activity of Hsp90 as competitive inhibitors of ATP binding to Hsp90 (*6*, *7*). Grp94 and mitochondrial TRAP1 also play important roles in tumorigenesis (*3*, *8*).

The prokaryotic homolog of Hsp90 (HtpG) is not essential under normal conditions but important for stress tolerances. We have shown that Hsp90 plays an important role under high/low temperature and oxidative stresses in cyanobacteria (*9–11*). The importance of Hsp90 under high/low temperature and oxidative stresses in nonphotosynthetic bacteria has also been reported (*12–14*). Furthermore, prokaryotic Hsp90 is involved in pathogenesis (*14*).

The high sequence and structural homology among Hsp90 homologs mean that developing paralog/ortholog-specific inhibitors is a challenging task. There are a few paralog-specific Hsp90 inhibitors known. These are purine-scaffold compounds (*15*) and gambogic acid (*16*). Some purine-scaffold compounds show Grp94 selectivity over cytosolic Hsp90s (α and β) and TRAP1. Gambogic acid binds Hsp90β selectively over Hsp90α and TRAP-1 to inhibit its chaperone function.

Surfactin is a cyclic lipopeptide antibiotic/biosurfactant produced by various strains of the genus *Bacillus* (*17*), and is an anionic heptapeptide linked with a fatty acid chain (Fig. 1). Surfactin has been reported to have/induce antiviral, antibacterial, antitumor and antiinflammatory activity (*18*). In the present study, we found that surfactin inhibits a cyanobacterial Hsp90 in an ortholog-specific manner.

Materials and Methods

Materials and bacterial cell culture

Malate dehydrogenase (MDH) from porcine heart was obtained from Sigma-Aldrich (St. Louis, MO, USA). Adenylate kinase and hexokinase from yeast were obtained from Oriental Yeast Co., Ltd (Tokyo, Japan). *Synechococcus elongatus* (*S.elongatus*) PCC 7942 was cultured photoautotrophically in BG-11 inorganic liquid medium as described previously (*19*). Cultures were grown at 30 or 42°C under a light intensity of 30 $\mu E\,m^{-2}\,s^{-1}$. *Escherichia coli* (*E.coli*) K-12 W3110 cells were cultured at 30 or 42°C in Luria-Bertani broth. Arthrofactin, which was produced by *Pseudomonas* sp. MIS38, was purified as described previously (*20*).

Purification of various molecular chaperones from the cyanobacterium S.elongatus PCC 7942, the fission yeast Schizosaccharomyces pombe (S.pombe) and Homo sapiens

Construction of strains that overexpress His-tagged Hsp90 protein, His-tagged GroEL1 or His-tagged DnaK2 from *S.elongatus* PCC 7942 and their purification protocols were described previously (*19*, *21*, *22*). A strain that overexpresses nontagged Hsp90 from *S.pombe* was a gift of Dr. Masafumi Yohda, Tokyo University of Agriculture and Technology, Japan. Purification of the Hsp90 was performed as described previously (*23*). Recombinant human Hsp90α was purified as described previously (*24*) with modifications described below. A frozen *E.coli* cell pellet was solubilized on ice in phosphate-buffered saline supplemented with 1% Triton X-100 and 1:100 (v/v) of a bacterial protease inhibitor cocktail (Sigma-Aldrich) using a BioRuptor sonicator (200 W 30 s ON/30 s OFF, 10 times). Purification of GST-Hsp90α and cleavage with PreScission Protease (GE Healthcare Life Sciences, Tokyo, Japan) to remove the GST moiety from the fusion protein were performed as described previously (*25*). ResourceQ and then MonoQ column chromatography (GE Healthcare Life Sciences) using a linear gradient of 0–1,000 mM NaCl in 50 mM Tris–HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid (EDTA) and 1 mM dithiothreitol were used to further purify Hsp90α. The purified Hsp90α was desalted with a PD10 column (GE Healthcare Life Sciences) with a buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol and 10% glycerol, and concentrated with a centrifugal filter (Ultra-4 30K cut-off, Amicon, Merck, Tokyo, Japan). The recombinant Hsp90α was >98% pure and ran as a single band on a sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) gel stained with Coomassie Brilliant Blue (CBB).

ATPase assay for Hsp90, GroEL1 and DnaK2

The ATPase activity of Hsp90 was measured at 37°C with an ATP regeneration system, as described previously (*22*). All measurements were evaluated in the presence of the Hsp90 specific inhibitor radicicol to confirm that the activity measured was because of Hsp90 and not from any contaminating ATPase activity. The ATPase activity measurements of GroEL1 and DnaK2 were described previously (*21*, *26*).

Hexokinase and adenylate kinase assay

The hexokinase and adenylate kinase activities were measured according to the manufacturer's instructions (Oriental Yeast). The hexokinase assay mixture (total volume, 1 ml) contained triethanolamine–HCl–NaOH (pH 7.5), glucose, ATP, $MgCl_2$, $NADP^+$, glucose 6-phosphate dehydrogenase and 0.5 pmol hexokinase. The adenylate kinase assay mixture contained triethanolamine–HCl–NaOH (pH 7.5), AMP, ATP, $MgCl_2$, KCl, phosphoenolpyruvate, NADH, lactate dehydrogenase, pyruvate kinase and 1 pmol/ml myokinase.

Molecular modelling simulations

Homology models of ligand-free (open), ADP-bound (semiclosed) and ATP-bound (closed) states of Hsp90 were created using SWISS-MODEL (*27*) or PHYRE2 (*28*) based on structures of ligand-free *E.coli* Hsp90 (PDB ID: 2IOQ) (*29*), ADP-bound *E.coli* Hsp90 (PDB ID: 2IOP) (*29*) and ATP-bound *Saccharomyces cerevisiae* Hsp90 (PDB ID: 2CG9) (*30*), respectively. A model of surfactin was also created using open-source PyMOL (version 1.7, Schrödinger, LLC) and Prodrg (*31*) via energy minimization. Molecular docking was performed using Autodock Vina (*32*). N-terminal domain of each Hsp90 model was covered by a grid box as a ligand-search space (number of points in *x*, *y*, *z*-dimensions = 126, 126, 126 with a spacing of 0.375 Å). The initial position of surfactin was set within the grid box for the docking simulations. The other parameters (*e.g.* the maximum number of binding modes and exhaustiveness of search) were used as defaults. The binding modes of surfactin were ranked in the order of calculated binding affinity. The highest ranked, energetically favourable results were selected as the considerable docking model structures. Figures of surfactin-bound Hsp90 models were depicted using PyMOL.

Primary amino acid sequence alignments

Amino acid sequence alignments were performed using Clutal omega (*33*). The figure of the alignment was created using ESPript 3 (*34*).

Immunoprecipitation assays

A mixture containing MDH (0.5 nmol) and/or the cyanobacterial Hsp90 (1.0 nmol monomer) was incubated at 45°C for 25 min in 1 ml of 50 mM HEPES-KOH (pH 8.0) with or without a cyclic lipopeptide whose concentration is indicated in Fig. 4 and centrifuged at 10,000 *g* for 30 min at 4°C. The resulting supernatant fraction was used for immunoprecipitation assays with Protein G Sepharose 4 Fast Flow beads (GE Healthcare Life Sciences) with coupled *S.elongatus* PCC 7942 Hsp90 polyclonal antibodies as described previously (*26*).

Mammalian cell culture, imaging and counting of cells

COS7 and NIH3T3 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum in humidified air

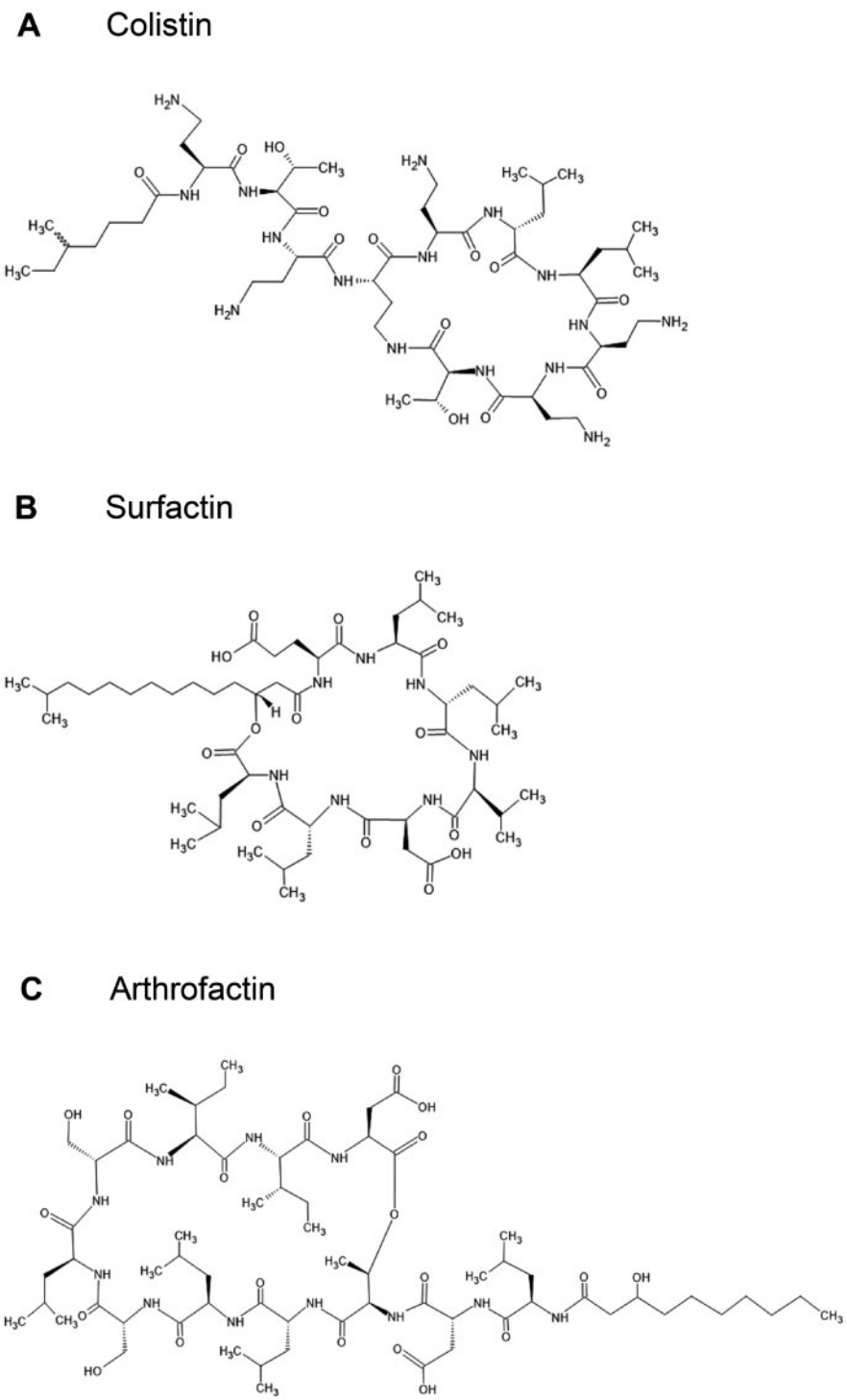


Fig. 1. Chemical structures of colistin (A), surfactin (B) and arthrofactin (C). The amino acid sequences of the cyclic peptide moiety of colistin, surfactin and arthrofactin (in clockwise direction) are Dab-Thr-Dab-Dab-Dab-D-Leu-Leu-Dab-Dab, D-Asp-Leu-D-Leu-D-Glu-D-Leu-Leu-D-Val and D-Asp-D-Thr-D-Leu-D-Leu-D-Ser-Leu-D-Ser-Ile-Ile-Asp-D-Leu, respectively. Dab: diaminobutyric acid. Note that colistin has no acidic amino acids, whereas there are two acidic amino acids in surfactin and arthrofactin.

containing 5% CO_2. Cells were treated with the indicated concentrations of drugs for 30 h. Phase contrast cell images were obtained and cell numbers were counted as described previously (*35*).

Results

We showed previously that cationic, cyclic lipopeptide antibiotics including colistin sulphate salt and polymyxin B bind to Hsp90 (HtpG) from the cyanobacterium *S.elongatus* to inhibit its chaperone activity (*22*). These lipopeptides reduce the ability of the Hsp90 to suppress aggregation of heat-denatured proteins. In the present study, we evaluated whether other cyclic lipopeptides, surfactin and arthrofactin, affect the functions of Hsp90. In contrast to colistin, surfactin and arthrofactin carry negative charges (Fig. 1).

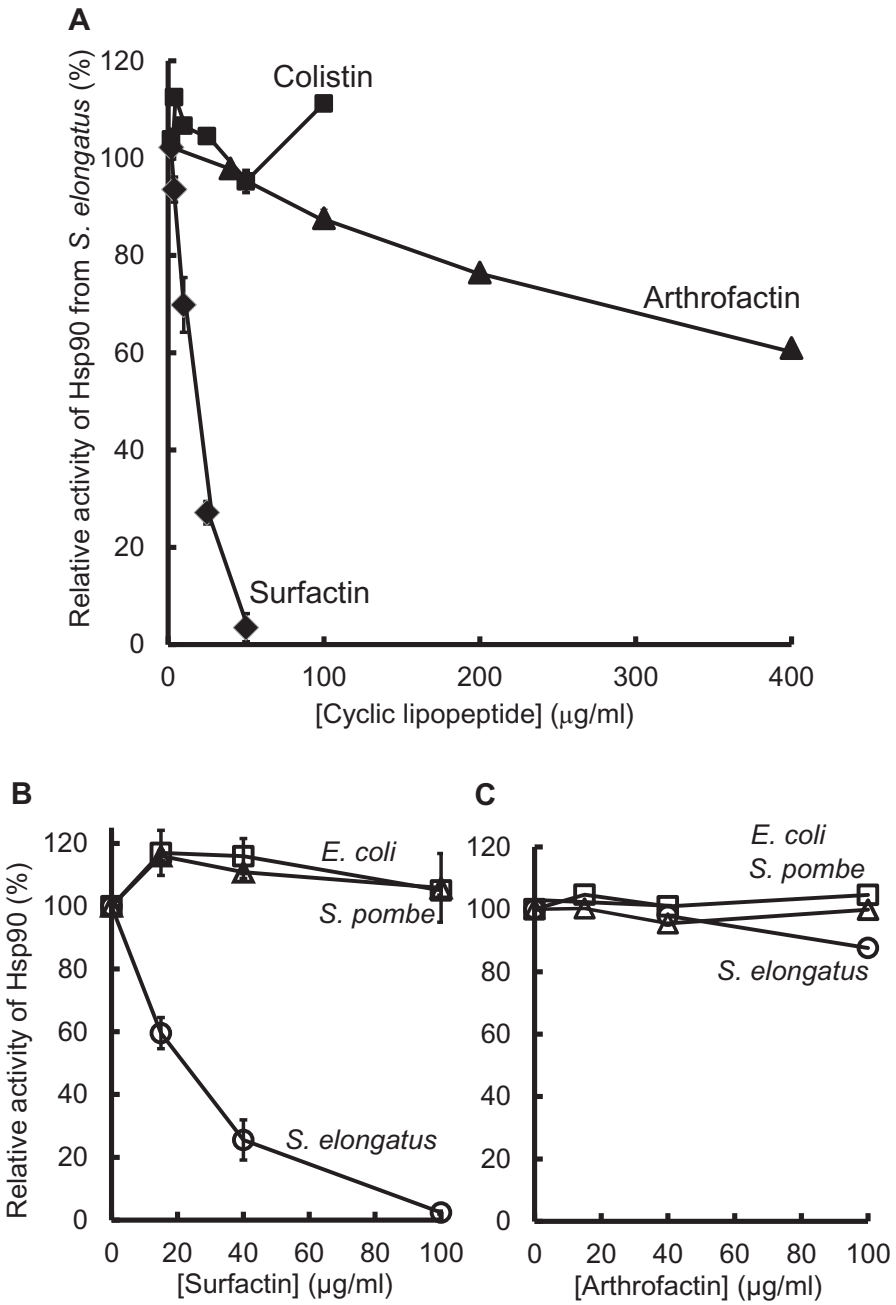


Fig. 2. Surfactin, which was the strongest inhibitor of the cyclic lipopeptides tested, showed specificity towards Hsp90 from cyanobacteria.
(**A**) Concentration-dependent inhibition of the ATPase activity of Hsp90 from *S.elongatus* by colistin, surfactin and arthrofactin.
(**B**) Concentration-dependent inhibition of the ATPase activity of Hsp90 from *E.coli*, *S.pombe* and *S.elongatus* by surfactin.
(**C**) Concentration-dependent inhibition of the ATPase activity of Hsp90 from *E.coli*, *S.pombe* and *S.elongatus* by arthrofactin. Data from three replicates are presented as means ± SEM.

Results from our previous study showed that colistin sulphate salt does not significantly affect the ATPase activity of Hsp90 from *S.elongatus* PCC 7942 even though it binds to the N-terminal domain of Hsp90 where the ATP-binding site is located (*22*). We tested whether other cyclic lipopeptides affect the ATPase activity of the cyanobacterial Hsp90 (Fig. 2A). In contrast to the colistin sulphate salt, both anionic cyclic lipopeptides inhibited the activity, although surfactin showed much stronger inhibitory activity than arthrofactin. The half maximal inhibitory concentration for surfactin was approximately 20 $\mu g\,ml^{-1}$ (19 μM), whereas that for arthrofactin was higher than 400 $\mu g\,ml^{-1}$ (>296 μM).

The activity of surfactin towards other major molecular chaperones and enzymes that use ATP as a substrate/cofactor was examined to test if the inhibition by surfactin is specific to Hsp90. Surfactin at 50 $\mu g\,ml^{-1}$ inhibited the ATPase activity of DnaK slightly but had no effect on the activity of GroEL (Supplementary Fig. S1). Surfactin at 50 $\mu g\,ml^{-1}$ did not inhibit the activities of adenylate kinase and hexokinase (Supplementary Fig. S1). These results indicate that the activity of surfactin is rather specific to Hsp90.

We then examined if the inhibition is also observed for Hsp90s from various species. To our surprise, surfactin did not inhibit the ATPase activity of Hsp90s from *E.coli* and the fission yeast *S.pombe*, but at 20 and 40 $\mu g\,ml^{-1}$ increased their ATPase activity by ~20% (Fig. 2B). In the case of human Hsp90α, k_{cat} in the absence and presence of 100 $\mu g\,ml^{-1}$ surfactin were 0.099 and 0.136 min^{-1}, respectively. Thus, surfactin did not inhibit the ATPase activity of human Hsp90α but caused an increase by 40% at 100 $\mu g\,ml^{-1}$. Arthrofactin up to 100 $\mu g\,ml^{-1}$ did not show any significant effect on these Hsp90s (Fig. 2C).

Molecular docking simulations were performed to identify surfactin-binding sites of Hsp90s from *S.elongatus*, *E.coli*, *S.pombe* and *H.sapiens*. The open states of all the Hsp90s showed no obvious difference in the surfactin-binding sites (Supplementary Figs S7 and S8), suggesting that the open states are not targeted by surfactin in the inhibition of *S.elongatus* Hsp90.

By contrast, the surfactin-binding sites of semi-closed and closed states of *S.elongatus* Hsp90 were found to be distinct from those of the other Hsp90s (Fig. 3A–D). In ATP-bound *S.elongatus* Hsp90, surfactin bound to the N-terminal dimerization interface (Fig. 3A). The amino acid residues comprising this surfactin-binding site (*e.g.* Asn15, Lys21 and Tyr108) were unique to *S.elongatus* Hsp90 among the Hsp90s (Fig. 3E). By contrast, surfactin in the other Hsp90s bound around the interface between the N-terminal and middle domains (Supplementary Figs S3 and S4).

The ADP-bound state of *S.elongatus* Hsp90 also showed the difference in the surfactin-binding site, as compared with the other Hsp90s (Fig. 3C and D, Supplementary Figs S5 and S6). Surfactin in ADP-bound *S.elongatus* Hsp90 was located within a groove formed between helix A (residues 9–25) and a region consisting of helix B (residues 87–95), a loop (residues 96–97) and helix C (residues 98–107). The loop and helix C form the lid (*36*). The lid region is close to the ATP-binding site. It is demonstrated in the isolated N-terminal domain of yeast Hsp90 that helix A (see Fig. 3E) interacts with helix C of the lid (*36*). Surfactin in *S.elongatus* Hsp90 is mainly in contact with helices A and C. By contrast, surfactin in the other Hsp90s was located much closer to helix B rather than helices A and C, resulting in surfactin-binding sites 10 Å-away from the site on *S.elongatus* Hsp90 (see Supplementary Fig. S6). ADP-bound *S.elongatus* Hsp90 utilized not only hydrophobic, but also polar amino acid residues for binding of surfactin, whereas the other Hsp90s mostly utilized nonpolar amino acid residues. Thus, the difference in surfactin-binding sites was most probably caused by the difference in the amino acid residues of the N-terminal domains. In total, the ATP- and ADP-bound states rather than the open state of *S.elongatus* Hsp90 could be targeted by surfactin in the ATPase inhibition.

We showed previously that colistin sulphate salt inhibits the interaction of Hsp90 with a denatured substrate, causing a decrease in the anti-aggregation activity of Hsp90 (*22*). Thus, we examined the effect of the cyclic lipopeptides on the interaction between the cyanobacterial Hsp90 and a model substrate (*i.e.* heat-denatured MDH) by immunoprecipitation assays. As shown in Fig. 4, surfactin whose concentration is higher than 20 $\mu g\,ml^{-1}$ had a significant effect on the interaction of the heat-denatured MDH with the cyanobacterial Hsp90. Colistin sulphate salt completely diminished the interaction at 10 $\mu g\,ml^{-1}$, which is consistent with our previous study (*22*). In contrast to surfactin and colistin sulphate salt, arthrofactin (up to 200 $\mu g\,ml^{-1}$) did not have any effect on the interaction.

We have shown that Hsp90 plays an essential role under heat stress because mutations in the *htpG* gene encoding Hsp90 cause high-temperature sensitivity in *S.elongatus* (*9*). This high-temperature sensitivity of the *htpG* mutant can be used to detect the dysfunction of the cyanobacterial Hsp90 *in vivo*. We showed previously that colistin sulphate salt leads to high-temperature sensitivity similar to that observed with the *htpG* mutant (*22*). If surfactin inhibits the cyanobacterial Hsp90 function in a cell, then it is expected that the cell will lose heat tolerance like that observed for the *htpG* mutant. This is what we observed as shown in Fig. 5B. No significant effects by surfactin on the growth of cells at 30°C were observed (Fig. 5A), but surfactin at 15 $\mu g\,ml^{-1}$ inhibited the growth at 42°C markedly. We also measured the survival rate of *S.elongatus* in the presence or absence of surfactin. In this experiment, *S.elongatus* cells in liquid culture were preheat-treated at a mild temperature (42°C) for 2 h in the absence or presence of various concentrations of surfactin, and then the culture temperature was increased to a lethal temperature of 50°C. After heat treatment, the temperature of the culture was lowered to 30°C and cells were grown on an agar plate for 10 days to allow colony growth. The mild preheat-treatment induces expression of Hsp90 (*9*). In contrast to eukaryotic cells, the accumulation level of Hsp90 in *S.elongatus* cells under a normal temperature is very low (*11*). Thus, cells were heat-treated at 42°C to increase the level of Hsp90 to examine the effect of surfactin on its chaperone function in cells. Surfactin up to 5 $\mu g\,ml^{-1}$ had no effect, but surfactin at 25 and 125 $\mu g\,ml^{-1}$ caused a significant decrease in the survival rate at a lethal temperature (Supplementary Fig. S2). The results shown in Fig. 5B and Supplementary Fig. S2 indicate that surfactin is permeable to membranes and inhibits the cellular function of Hsp90. In contrast to *S.elongatus*, the growth of *E.coli* at 37°C and 42°C was not affected by surfactin even at a much higher concentration (100 $\mu g\,ml^{-1}$) (Fig. 5C and D). This indicates that the compound does not affect the activity of Hsp90 and other proteins in *E.coli* at concentrations up to 100 $\mu g\,ml^{-1}$.

We also tested if surfactin affects cultured mammalian cells. Surfactin has been reported to be cytotoxic to certain cell types (*37*) and shows inhibitory effects on human cancer cells (*38*). We examined the effect of various concentrations of surfactin on COS7 and NIH3T3 cell lines and found that surfactin up to 25 $\mu g\,ml^{-1}$ did not significantly show cytotoxicity, did

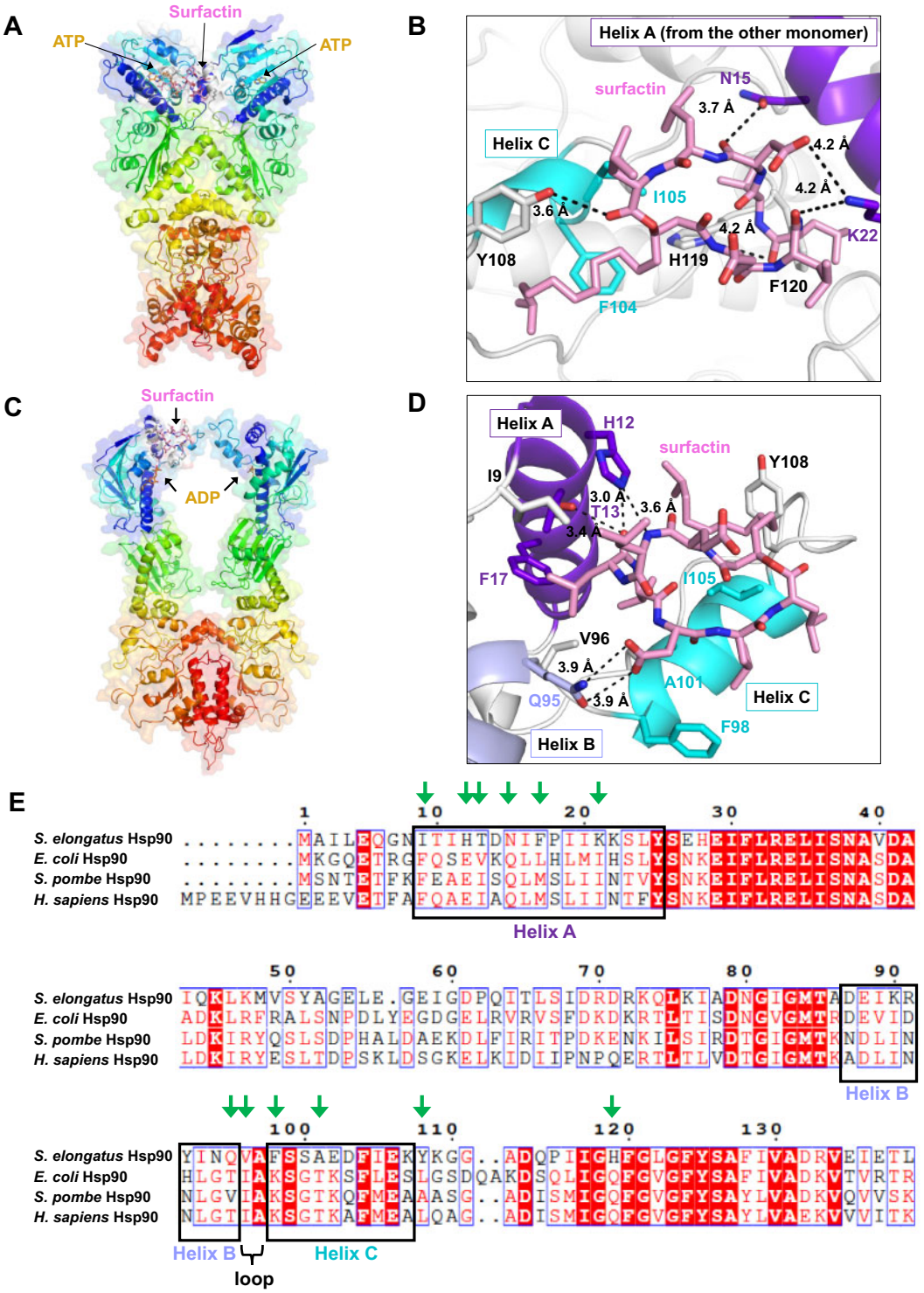


Fig. 3. Molecular docking simulations (A–D) and amino acid sequence alignments (E). The docking model structure of surfactin- and ATP-bound *S.elongatus* Hsp90 (A) and the surfactin-binding site (B). The docking model structure of surfactin- and ADP-bound *S.elongatus* Hsp90 (C) and the surfactin-binding site (D). Overall structures are shown in cartoon-and-surface models. The rainbow colouring indicates the direction of the polypeptide chain from the N-terminal (blue) to the C-terminal region (red), except for the surfactin-binding site (white and purple). ATP or ADP and surfactin are shown in orange and pink stick models, respectively. Helices A, B and C were coloured in purple, light blue and cyan, respectively. The amino acid sequence alignments of N-terminal regions of Hsp90s from *S.elongatus*, *E.coli*, *S.pombe* and *H.sapiens* (E). In the alignments, strictly conserved amino acid residues are indicated by white letters in a red shaded box. Regions containing well-conserved amino acids are indicated by red letters in a blue framed box. Green arrows indicate the amino acid residues that are uniquely identified in *S.elongatus* Hsp90 rather than the other Hsp90s. Residues comprising key α-helices (designated as helices A, B and C) at the surfactin-binding sites are in black boxes.

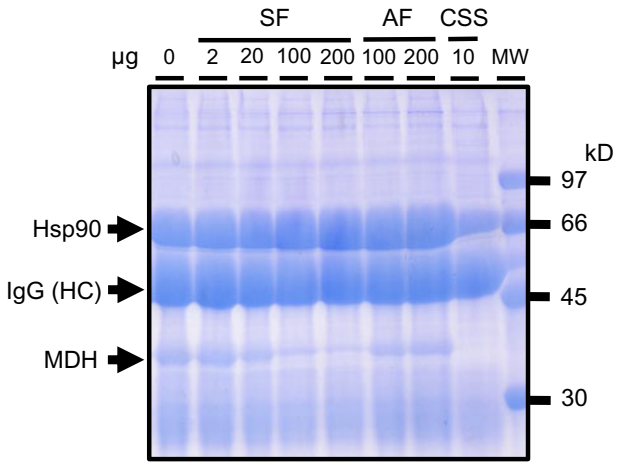


Fig. 4. Surfactin inhibited the physical interaction of Hsp90 from *S.elongatus* with heat-denatured MDH. For immunoprecipitation assays, a mixture containing MDH and Hsp90 was incubated in the absence or presence of varying concentrations of surfactin, arthrofactin or colistin sulphate salt at 45°C for 25 min. Proteins that coprecipitated with the Protein G Sepharose beads with coupled Hsp90 antibodies were separated on SDS–PAGE (12%) gels and stained with CBB. Arrows indicate Hsp90, MDH and the heavy chain of IgG [IgG (HC)] separated in the SDS–PAGE gel. SF, AF, CSS and MW are abbreviations for surfactin, arthrofactin, colistin sulphate salt and protein molecular weight markers, respectively.

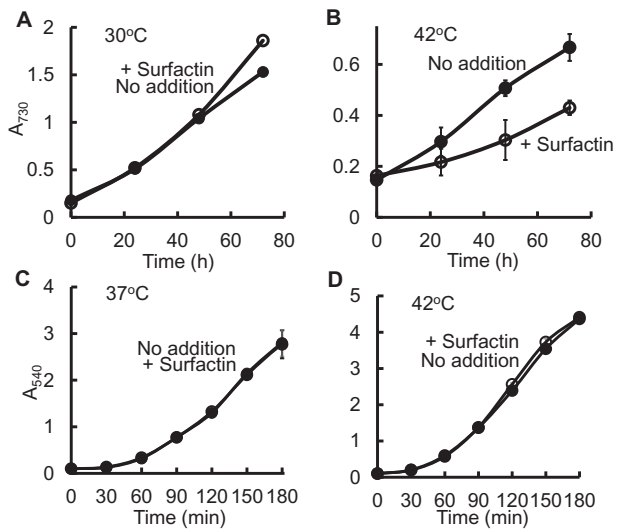


Fig. 5. Surfactin led to a high-temperature sensitive phenotype in *S.elongatus*, whereas surfactin did not affect the growth of *E.coli*. Growth of *S.elongatus* (**A** and **B**) and *E.coli* (**C** and **D**) at a normal growth temperature and under a mild high temperature in the presence (open symbols) or absence (closed symbols) of 15 $\mu g\,ml^{-1}$ (for *S.elongatus*) or 100 $\mu g\,ml^{-1}$ (for *E.coli*) surfactin. The apparent absorbance at 730 and 540 nm was used to measure the relative number of cells of *S.elongatus* and *E.coli*, respectively. All points represent data from three replicates that are presented as means ± SEM. Some error bars are masked by plot symbols.

not suppress cell proliferation and survival and did not change cell morphology (Fig. 6A). At 50 $\mu g\,ml^{-1}$, surfactin slightly suppressed cell proliferation and induced cell morphology changes in COS7 and NIT3T3 cells. In contrast, Hsp90 inhibition by much lower concentrations of geldanamycin induced significant cell death, cytotoxicity and cell morphology changes (Fig. 6B). These results agree well with our present results that surfactin does not inhibit the ATPase activity of human Hsp90, although geldanamycin does (*6*).

Discussion

Our present study showed that a cyclic lipopeptide surfactin inhibits Hsp90 in a species-selective way. Surfactin inhibited Hsp90 from the cyanobacterium *S.elongatus* PCC 7942 but not Hsp90s from *E.coli*, yeast and human (Fig. 2 and data described in the text). In contrast, arthrofactin did not inhibit any of these Hsp90s significantly up to 100 $\mu g\,ml^{-1}$ (Fig. 2C). Noteworthy, both surfactin and arthrofactin are effective anionic biosurfactants with critical micelle concentrations of 7.0×10^{-5} and 1.0×10^{-5} M, respectively (*20*). They commonly contain two acidic amino acid residues, Asp or Glu. The fact that only surfactin inhibited Hsp90 from *S.elongatus* suggests that neither its surfactant activity nor anionic character is a critical factor for inhibition.

Because of the high sequence and structural homology among Hsp90 homologs, it is difficult to identify and develop paralog/ortholog-specific inhibitors. As described in the Introduction, only a limited number of studies have reported paralog-selective Hsp90 inhibitors. Purine-scaffold compounds and gambogic acid inhibit the ATPase activities of Grp94 and Hsp90β, respectively, and selectively over other Hsp90 paralogs in eukaryotic cells (*15*, *16*). In contrast to these previous studies, our study indicates a cyclic lipopeptide as a promising candidate that inhibits Hsp90 (HtpG) in a species-selective or in an ortholog-specific way. Currently, there are no ortholog-specific Hsp90 inhibitors reported.

In order to find how surfactin exerts its effect ortholog-specifically, we performed molecular docking simulations. The simulations with Hsp90s from *S.elongatus*, *E.coli*, *S.pombe* and *H.sapiens* indicate that the binding region of surfactin on *S.elongatus* Hsp90 was distinct from regions of the other Hsp90s. This differential binding was observed when Hsp90s was in ATP-bound or ADP-bound states. The Hsp90 ATPase cycle consists of the apo, ATP and ADP conformational states (*4*, *39*). In the absence of ATP, Hsp90 adopts mainly an open conformation. ATP binding by Hsp90 results in a closed state in which the N-terminal domain of the two monomers dimerizes. This closure is the rate-limiting step in ATP hydrolysis. Upon the ATP binding, the lid segment (residue 94–125 as described in Ref. (*40*), see Fig. 3E) closes over the ATP-binding pocket, leading to the N-terminally closed state. This dimerization is coupled to the first 24 amino acids of the N-terminus that form a swapped β-strand that stabilizes dimerization of the N-terminal domain (*39*). Thus, the lid restructuring and the β-strand swapping plays an important role in the N-terminal closure and ATP hydrolysis. Our present study indicates that surfactin binds the lid and its interacting region of *S.elongatus* Hsp90 (helix A in Fig. 3E), suggesting that this interaction inhibits the ATP-driven conformational cycle/ATP hydrolysis.

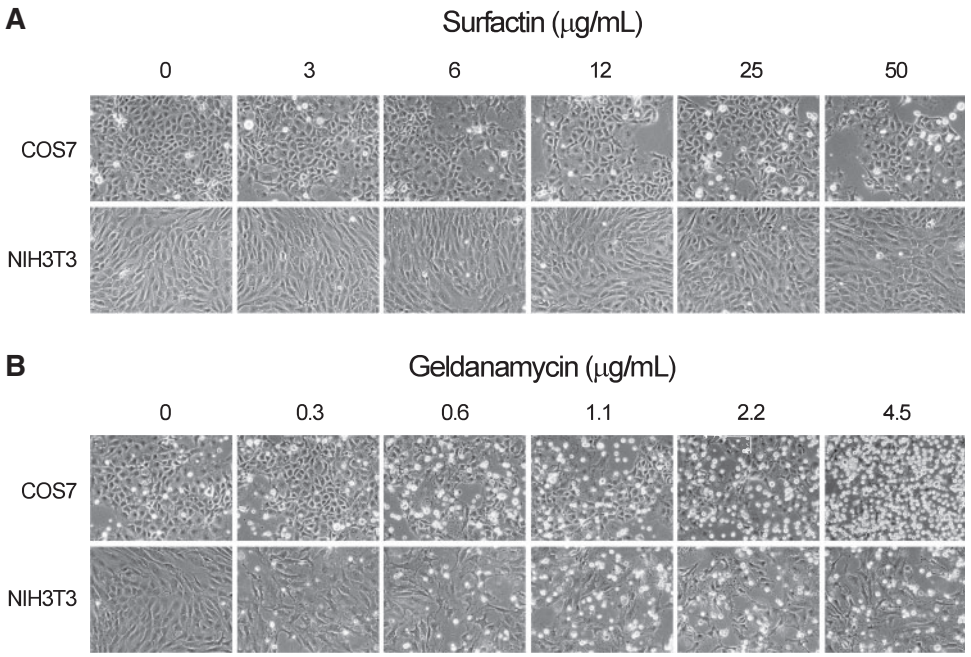


Fig. 6. Geldanamycin, but not surfactin, showed mammalian cell cytotoxicity. COS7 (upper row) and NIH3T3 (lower row) cells were treated with indicated concentrations of surfactin (**A**) or geldanamycin (**B**) for 30 h and phase contrast cell images are shown.

Surfactin inhibited growth of *S.elongatus* at a high temperature, whereas it did not have any effect on *E.coli* growth under both normal and high temperature conditions. Mutation of the *htpG* gene in *S.elongatus* causes sensitivity to high temperature (*9*), whereas mutation of the *E.coli htpG* gene does not show a significant effect on normal and high-temperature growth (*41*). Thus, surfactin treatment and *htpG* mutations cause similar phenotypes; supporting the concept that surfactin targets Hsp90 in *S.elongatus*.

Previously, it was shown that a cell-free culture broth of *B. subtilis* C1 containing surfactin ($10\,\mu g\,ml^{-1}$) completely inhibited the growth of the cyanobacterium *Microcystis aeruginosa* (*M. aeruginosa*), a bloom-forming cyanobacterium in highly eutrophic lakes (*42*); however, the mechanism of inhibition was not characterized at the molecular level. *M. aeruginosa* causes environmental and mammal/human health problems by producing toxic compounds such as microcystins (*43*). Thus, it is important to control growth of toxic cyanobacteria including *M. aeruginosa*. Interestingly, the transcript level of *htpG* encoding Hsp90 in *M. aeruginosa* NIES-298 is much higher than those of chaperone genes encoding GroEL, DnaK and ClpB at a normal growth temperature (25°C). In addition, the *htpG* gene of *M. aeruginosa* is the most highly heat-induced chaperone gene expressed among various molecular chaperones (*44*). These results indicate that Hsp90 plays an important role for this cyanobacterium under normal conditions as well as at high temperatures. The results herein should aid in elucidating a compound that controls the prevalence of toxic cyanobacteria via Hsp90 inhibition.

Cyclic peptides have been receiving increasing attention as a class of underexplored compounds that exhibit a wide spectrum of biological activities (*45*, *46*). They exhibit higher target specificity, metabolic stability/bioavailability and membrane permeability than conventional small molecule drugs and noncyclic peptides. The present study shows that a cyclic peptide surfactin has an exceptional specificity to interact with a specific homolog of an evolutionarily conserved protein family. It also shows that a cyclic peptide has great potential as a species/ortholog-specific inhibitor.

Supplementary Data

Supplementary Data are available at *JB* Online.

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Conflict of Interest

None declared.

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