



# Dissection of the bridging pattern of bovicin HJ50, a lantibiotic containing a characteristic disulfide bridge

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Received 24 February 2010 ; received in revised form 27 April 2010; accepted 8 May 2010

## KEYWORDS

Lantibiotics;  
Bovicin HJ50;  
Semi-*in vitro*  
biosynthesis;  
Structural dissection;  
Disulfide bridge

## Summary

Bovicin HJ50, a lantibiotic produced by *Streptococcus bovis* HJ50, is featured by the presence of a disulfide bridge. This study described a simplified *in vitro* synthetic strategy for producing bovicin HJ50 totally based on *Escherichia coli* expression system. In this strategy termed as Semi-*in vitro* biosynthesis (SIVB), prepeptide BovA and modification enzyme BovM were co-expressed to generate posttranslationally modified BovA. Then a specific protease BovT150 was employed to remove the leader peptide *in vitro* and produce biologically active bovicin HJ50. Via SIVB, a series of ring-broken bovicin mutants C13A, C21A, C29A and T10A/C32A were prepared by introducing site-directed mutations into *bovA* gene. Further, we analyzed the bridging patterns of these mutants through chemical modification and successfully clarified the bridging pattern of bovicin HJ50. The results showed that two thioether bridges exist between Thr8 and Cys13, and Thr10 and Cys32, respectively, and that the disulfide bond bridging Cys21 and 29 is very relevant for the antimicrobial activity of bovicin HJ50. This is the first study that reports the bridging pattern of bovicin HJ50.

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**Abbreviations:** MALDI-TOF MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; HPLC, high performance liquid chromatography; DTT, 1,4-dithiothreitol; NEM, N-ethylmaleimide; MeCN, methyl cyanide; Dha, dehydroalanine; Dhb, dehydrobutyrine; Lan, lantionine; MeLan, 3-methyllantionine; TFA, trifluoroacetic acid; MW, molecular weight.

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## Introduction

Lantibiotics are peptide-derived antimicrobial agents that are ribosomally synthesized and posttranslationally modified to their active forms (Chatterjee et al. 2005). They are featured by the presence of unusual amino acids, such as dehydroalanine (Dha), dehydrobutyrine (Dhb), lanthionine (Lan) and 3-methylanthionine (MeLan). Lantibiotics are initially synthesized as two-segment prepeptides (LanA), consisting of an N-terminal leader peptide and a C-terminal propeptide. Then the propeptide moiety undergoes extensive posttranslational modification, usually including dehydration of serine and threonine residues followed by intramolecular cyclization of the resulting dehydrated residues through reaction with cysteine thiols to form thioether rings. The last step to mature lantibiotics is the removal of leader peptide from modified prepeptide by a specific protease.

Based on the topology of their structures, lantibiotics are classified into type A and B by Jung (1991). Type A is further divided into two subtypes, which are elongated type-AI lantibiotics and type-AII polypeptides consisting of a linear N-terminus and a globular C-terminal region. Besides, the lantibiotics of the two subgroups differ in biosynthetic mechanism and gene cluster organization. For type-AI lantibiotics such as nisin and Pep5, the modification enzymes and protease are LanB/C and LanP, respectively (van der Meer et al. 1993; Meyer et al. 1995; Karakas Sen et al. 1999); for type-AII members such as lactacin 481, LanM is responsible for posttranslational modification, and LanT for transport and leader peptide cleavage (Xie et al. 2004; Furgerson Ihnken et al. 2008).

Bovicin HJ50 is a type-AII lantibiotic containing two 3-methylanthionines and a disulfide bridge (Xiao et al. 2004), while the bridging pattern is still unknown. Lantibiotics containing a disulfide bridge are uncommon in nature. In addition to bovicin HJ50, there are only three such cases among over 50 lantibiotics, including sublancin 168 (Paik et al. 1998), plw $\alpha$  (Holo et al. 2001) and thermophilin 1277 (Kabuki et al. 2009). Sublancin 168, the only disulfide bridge-containing lantibiotic with known structure, shows great differences from other type-AII members. Thus, it is intriguing to investigate the structure of bovicin HJ50 and the function of its disulfide bridge.

Site-directed mutagenesis is an effective approach for structural analysis of lantibiotics (Krull et al. 2000). Traditionally, variants of lantibiotics, such as nisin, mutacin II and mersacidin, were generated via *in vivo* peptide engineering

which relies on effective engineered expression systems (Kuipers et al. 1996; Chen et al. 1998; Szekat et al. 2003). Such strategy provided a wealth of knowledge on structure–activity relationships, but also exposed some intrinsic problems, e.g. cytotoxic effect and the loss of regulatory ability for certain products, as well as laborious work for constructing expression systems. Comparing with *in vivo* engineering, *in vitro* engineering of lantibiotics has several conceptual advantages. Van der Donk et al. first conducted research on *in vitro* synthesis of lantibiotic lactacin 481. They reconstituted the *in vitro* activity of synthetase LctM (Xie et al. 2004) and protease LctT and successfully produced authentic lactacin 481 via step-by-step *in vitro* biosynthesis (Furgerson Ihnken et al. 2008). During the same time, Nagao et al. (2005) reported that co-expression of *nukA* and *nukM* in *Escherichia coli* could introduce unusual amino acids into the prepeptide of nukacin ISK-1. To realize convenient preparation of active lantibiotic and its variants, we developed a simplified synthetic strategy named Semi-*in vitro* biosynthesis (SIVB). Using SIVB, we produced bioactive bovicin HJ50 and a series of bovicin mutants, and successfully clarified the structure of bovicin HJ50 through chemical modification analysis.

## Materials and methods

### Bacterial strains, plasmids and growth conditions

*E. coli* strains DH5 $\alpha$  for DNA cloning and sequencing, and BL21 (DE3) for expressing heterologous proteins, were both cultured in Luria–Bertani (LB) medium. *Streptococcus bovis* HJ50 was grown anaerobically at 37°C in M17 medium supplemented with 0.5% glucose (GM17). The indicator strain *Micrococcus flavus* NCIB 8166 was cultured in S1 medium at 30°C. Vector pET-28a was used for heterologous expression in *E. coli* BL21 (DE3). If necessary, media were added with 50  $\mu$ g/ml kanamycin for pET-28a derivatives.

### Molecular biology protocols

General molecular biology techniques were carried out according to the standard protocols (Sambrook et al. 1989). PCR was performed with TransStart FastPfu DNA Polymerase (Transgene, China). PCR products and DNA fragments were purified by Cycle-Pure Kit and Gel Extraction Kit, respectively (Omega, US). Plasmids from *E. coli* were isolated

by Plasmid Mini Kit (Omega, US). Tricine-SDS-PAGE was performed according to the protocol described by Schagger (2006).

### Overexpression and purification of unmodified prepeptide BovA (His<sub>6</sub>-BovA)

To generate the unmodified prepeptide with an N-terminal His tag, *bovA* gene was amplified by PCR and cloned into pET-28a, resulting in pET-bovA. The *E. coli* strain BL21 (DE3) containing pET-bovA was cultured at 37 °C till OD<sub>600</sub> reached 0.6. Then the cells were induced with 1 mM IPTG and grown at 37 °C for additional 4 h. The purification of His<sub>6</sub>-BovA from inclusion body was performed according to the procedure reported by Xie et al. (2004).

### Overexpression and purification of the protease domain of BovT

A 450-bp fragment corresponding to the N-terminal protease domain of BovT (BovT150) was amplified and cloned into pET-28a, resulting in the expression vector pET-bovT150. The BL21 (DE3) cells carrying pET-bovT150 were incubated in 1 l of LB medium at 37 °C till OD<sub>600</sub> reached 0.6 and then induced with 0.5 mM IPTG at 20 °C for 12 h. After that, the cells were harvested, resuspended in 30 ml of start buffer (50 mM Na<sub>2</sub>HPO<sub>4</sub>, 500 mM NaCl, pH 7.4) plus 20 mM imidazole, and then lysed by sonification. The supernatant was transferred to another centrifuge tube and mixed with 1 ml of Co<sup>2+</sup>-NTA chelating resin by rotation at 4 °C for 1 h. The resin mixture was then moved into a column, which was drained and washed with 20 ml of wash buffer (start buffer plus 40 mM imidazole). Finally, the BovT150 was eluted with 4 ml of elution buffer (start buffer plus 120 mM imidazole). Glycerol was added to a final concentration of 15% (v/v) before storage at -80 °C.

### Construction of the *bovAM* co-expression vector and site-directed mutagenesis

A 2.8-kb DNA fragment containing two successive genes *bovA*-*M* was amplified from the genome of *S. bovis* HJ50 and cloned into pET-28a, resulting in the co-expression vector pET-bovAM. Site-directed mutagenesis was performed according to the protocols reported by Chiu et al. (2004), generating a series of pET-bovAM derivatives.

### Expression and purification of modified His<sub>6</sub>-BovA and its variants

The BL21 (DE3) cells containing pET-bovAM was incubated at 37 °C till OD<sub>600</sub> reached 0.6. IPTG was added to a final concentration of 0.5 mM, after which the culture was grown at 18 °C for 20 h. Then the cells were harvested and resuspended in start buffer plus 30 mM imidazole. After sonification lysis and centrifugation, the supernatant was placed into a centrifuge tube and mixed with Ni<sup>2+</sup>-NTA chelating resin. The resin mixture was moved into a column, drained and washed with wash buffer (start buffer with 80 mM imidazole added). Finally, the peptide was eluted with elution buffer (start buffer plus 500 mM imidazole).

### Removal of leader peptide *in vitro* and purification of bovicin analogues

Purified His<sub>6</sub>-BovA and BovT150 were co-incubated at 25 °C for 8 h in the presence of 50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 7.4, 50 mM Na<sub>2</sub>SO<sub>4</sub>, 1 mM DTT (Ferguson Ihnken et al. 2008). The final concentration of substrate and protease are 0.5 and 0.3 mg/ml, respectively. Then bovicin HJ50 was purified by reverse-phase HPLC with a C18 analysis column (Dalian Elite, China), using a gradient of 30–50% B over 25 min (B = 100% MeCN/0.1% TFA). Antimicrobial activity was tested against indicator strain by agar well diffusion assay as described by Cintas et al. (1995).

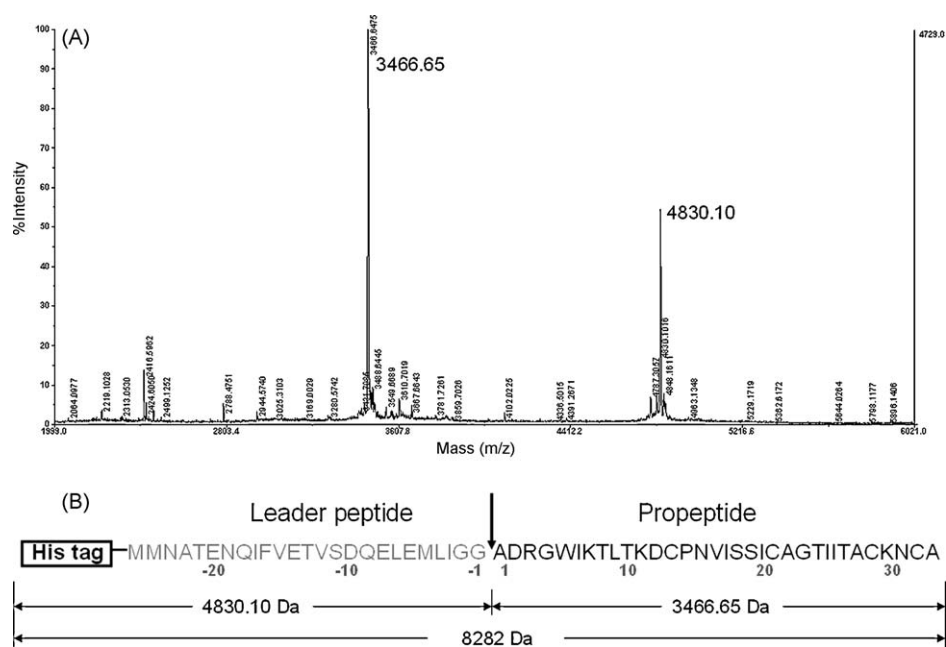
### Quantitation of cysteine residues through chemical modification

N-ethylmaleimide (NEM, MW 125.1, SCRC, China), a thiol-selective modification agent, was added into peptide solution (pH 6.5–7.0) to a final concentration of 10 mM. The reaction system was placed on ice for 30 min and monitored by MOLDI-TOF MS.

## Results

### *In vitro* proteolytic activity of BovT towards unmodified prepeptide (His<sub>6</sub>-BovA)

BovT is a putative protease responsible for the cleavage of leader peptide. To examine its *in vitro* activity and substrate specificity, the proteolytic activity assays were performed with His<sub>6</sub>-BovA as the substrate. Due to the presence of membrane spanning domain, the heterologous expression of full-length BovT in *E. coli* was unsuccessful (data not shown). So the protease domain correspond-



**Figure 1.** *In vitro* proteolytic activity of BovT towards unmodified prepeptide (His<sub>6</sub>-BovA). (A) Molecular weights of the two products were measured by MALDI-TOF MS after co-incubation of His<sub>6</sub>-BovA and protease BovT150. 3466.65 and 4830.10 Da correspond to the propeptide and leader peptide, respectively. (B) His<sub>6</sub>-BovA, with a calculated molecular weight of 8282 Da, consists of a leader peptide (4830.10 Da) and a propeptide (3466.65 Da). The cleavage site is indicated by the arrow.

ing to the N-terminal 150 amino acids of BovT (BovT150) were expressed. BovT150 with a C-terminal His tag was purified by Co<sup>2+</sup>-NTA affinity chromatography.

His<sub>6</sub>-BovA, with a calculated MW of 8282 Da (by Protein Calculator v3.3) was incubated with BovT150 for 4 h. Fig. 1A presents the MALDI-TOF MS of the two fragments generated in the reaction. The peak at 3466.65 Da corresponds to the C-terminal propeptide containing 33 amino acid residues. The N-terminal product (44 aa) containing the leader peptide plus an N-terminal His tag (GSSHHHHHSSGLVPRGSH-) was observed at 4830.10 Da. The result indicated that His<sub>6</sub>-BovA was effectively cleaved by BovT150 *in vitro* and the cleavage site was between Gly-2/Gly-1 and Ala1 as expected (Fig. 1B). This suggested that cleavage of the leader peptide by BovT150 was not affected by the presence of His tag and absence of ring structures.

### Semi-*in vitro* biosynthesis (SIVB) of biologically active bovicin HJ50

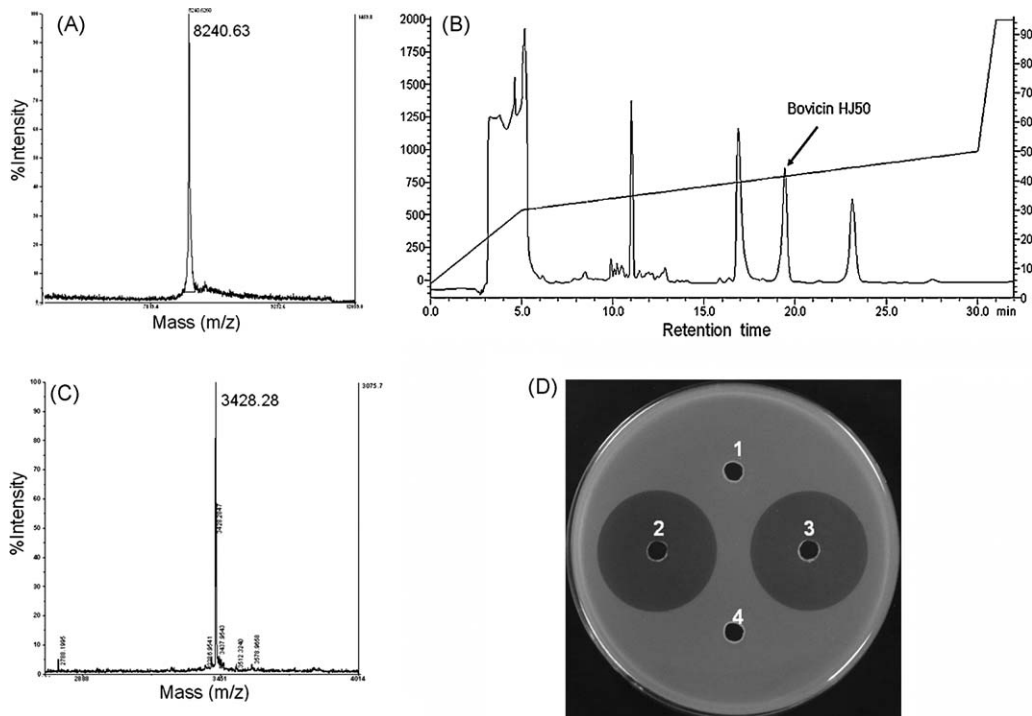
To obtain modified His<sub>6</sub>-BovA (His<sub>6</sub>-mBovA), *bovA* and *bovM* were co-expressed in *E. coli*. His<sub>6</sub>-mBovA was purified via Ni<sup>2+</sup>-NTA affinity chromatography. MALDI-TOF MS of His<sub>6</sub>-mBovA indicated about 40-Da decrease in its MW (8240.63 Da, Fig. 2A) compar-

ing with that of unmodified His<sub>6</sub>-BovA, suggesting that posttranslational modification took place at this stage. Then the leader peptide was removed from His<sub>6</sub>-mBovA by BovT150 *in vitro*, resulting in mature bovicin HJ50 that was further purified by reverse-phase HPLC (Fig. 2B).

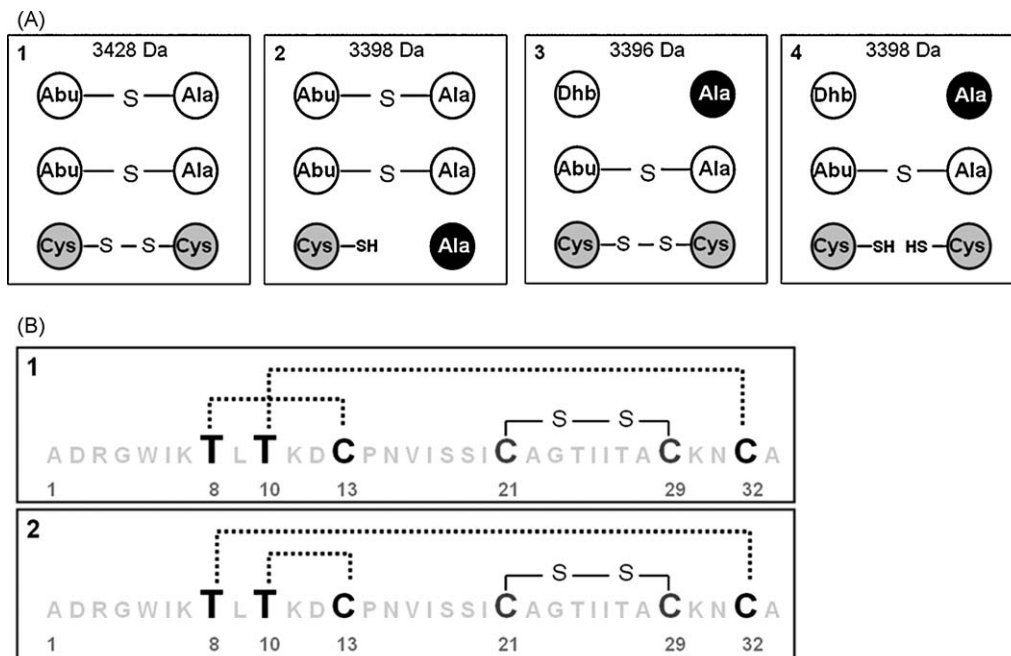
The molecular weight (3428.28 Da, Fig. 2C) and antimicrobial activity (Fig. 2D) of the resulting lantibiotic are the same as that of the bovicin HJ50 extracted from the producing strain (Xiao et al. 2004). Comparing with the propeptide (3466.65 Da, Fig. 1A), the 38-Da loss of mature bovicin HJ50 corresponds well to the formation of two methyl-lanthionines and a disulfide bridge. So it is evident that the bovicin HJ50 produced via SIVB is identical to that secreted by *S. bovis* HJ50. In addition, the yield of bovicin HJ50 via SIVB is 300 μg per liter culture, while it is only 80 μg according to the purification method described by Xiao et al. (2004) from the producing strain.

### Dissection of the structure of bovicin HJ50 via site-directed mutagenesis and chemical modification

Our previous study revealed that bovicin HJ50 contains two thioether rings and a disulfide bridge (Fig. 3A-1). Of the four Cys residues (Cys13, 21, 29, 32), two are involved in the formation of



**Figure 2.** Semi-*in vitro* biosynthesis of bovicin HJ50. (A) MALDI-TOF MS of modified His<sub>6</sub>-BovA generated via co-expression of *bovA* and *bovM* in *E. coli*. (B) Bovicin HJ50 was finally purified from the *in vitro* proteolytic system through reverse-phase HPLC. The absorbance was measured at 220 nm. The peak corresponding to bovicin HJ50 is indicated. (C) MALDI-TOF MS of bovicin HJ50 produced via SIVB. (D) Antimicrobial activity assays. Inhibition zones of 1 μg modified His<sub>6</sub>-BovA (1), bovicin HJ50 produced by SIVB (2), bovicin HJ50 extracted from *S. bovis* HJ50 (3) and the disulfide bridge-broken mutant C21A (4) tested against *Micrococcus flavus* NCIB8166.



**Figure 3.** Bridging pattern analysis of bovicin HJ50. (A) Bridging status of wild-type bovicin HJ50 and its mutants. Bridging status and molecular weight of wild-type bovicin HJ50 (1), disulfide bond-broken mutant (2), expected (3) and actual (4) bridging status of MeLan-broken mutant are presented. Grey-colored circles represent the disulfide bond-forming cysteines. Black-colored circles indicate the mutated residues. There is no particular order for the bridges. (B) Two possible bridging patterns of bovicin HJ50. Dotted lines link the possible bridging partners forming 3-methylanthionine. Disulfide bridges are indicated as '—S—S—'.

**Table 1.** Molecular weight of bovicin mutants before and after modification by NEM.<sup>a</sup>

Mutant	Molecular weight (Da)		
	Unreacted	NEM-modified	Increase <sup>b</sup>
C13A	3398.61	3648.67	250.1 (2 × NEM)
C21A	3398.60	3523.62	125.0 (1 × NEM)
C29A	3398.61	3523.61	125.0 (1 × NEM)
T10A/C32A	3386.63	3636.67	250.0 (2 × NEM)

<sup>a</sup> NEM (N-ethylmaleimide, MW 125.1) is a thiol-selective modification agent.

<sup>b</sup> The increase of molecular weight corresponds to the number of NEM molecules, indirectly indicating the number of Cys residues existing in the peptide.

3-methylanthionines with Thr8 and Thr10, respectively, and the other two form a disulfide bond (Xiao et al. 2004), but the exact bridging pattern is unknown. The first step for dissecting the structure of bovicin HJ50 was to identify the two disulfide bond-forming cysteines. We built three single mutants (C13A, C21A and C29A) to break either a disulfide bridge or a 3-methylanthionine in each mutant for chemical analysis. Theoretically, disulfide bond-broken mutants should possess an MW of 3398 Da (Fig. 3A-2), and MeLan-broken mutants 3396 Da (Fig. 3A-3). However, the MALDI-TOF MS showed 3398.6 Da for all the three mutants (Table 1), indicating two extra daltons for the expected MeLan-broken mutant. This phenomenon suggested that, in the MeLan-broken mutant, the two disulfide bond-forming cysteines did not form a bridge but remained unbonded (Fig. 3A-4).

Despite no difference between their molecular weights, the disulfide bridge-broken mutant can be distinguished from the MeLan-broken mutant according to the number of free Cys thiols. So, the mutants above were chemically modified by N-ethylmaleimide (NEM), a thiol-selective alkylating agent that could result in a 125-Da increase in MW by reacting with each Cys thiol. Mass spectra showed that MW of both C21A and C29A increased by 125 Da after chemical modification (Table 1), suggesting that one Cys existing in each mutant (Fig. 3A-2); while the MW of C13A increased by 250 Da, suggesting that C13A contains two Cys residues (Fig. 3A-4). Thus, we concluded that Cys21 and 29 form the disulfide bridge, while Cys13 and 32 are involved in the formation of 3-methylanthionines.

So far two bridging patterns (Thr8-Cys13/Thr10-Cys32 or Thr8-Cys32/Thr10-Cys13) are possible as shown in Fig. 3B. To identify the correct bridging pattern between the two candidates, a double mutant T10A/C32A was produced via SIVB. The

measured MW of this mutant (3386.63 Da, Table 1) corresponds to a product with disulfide bridge-forming residues (Cys21 and 29) unbonded. The bridging pattern could also be determined by quantitation of Cys residues existing in this peptide. If Fig. 3A-1 shows the correct pattern, one MeLan (Thr10-Cys32) is broken, and there should be only two Cys residues (Cys21 and 29) existing in the peptide. If Fig. 3B-2 is correct, both two thioether rings are broken and there should be an additional cysteine (Cys13) besides Cys21 and 29. After NEM modification, MW of this mutant increased by 250 Da, indicating there are two NEM-reactive Cys residues in total. So the correct bridging pattern is as Fig. 3B-1 shows. The structure of bovicin HJ50 is thus deduced and presented in Fig. 4.

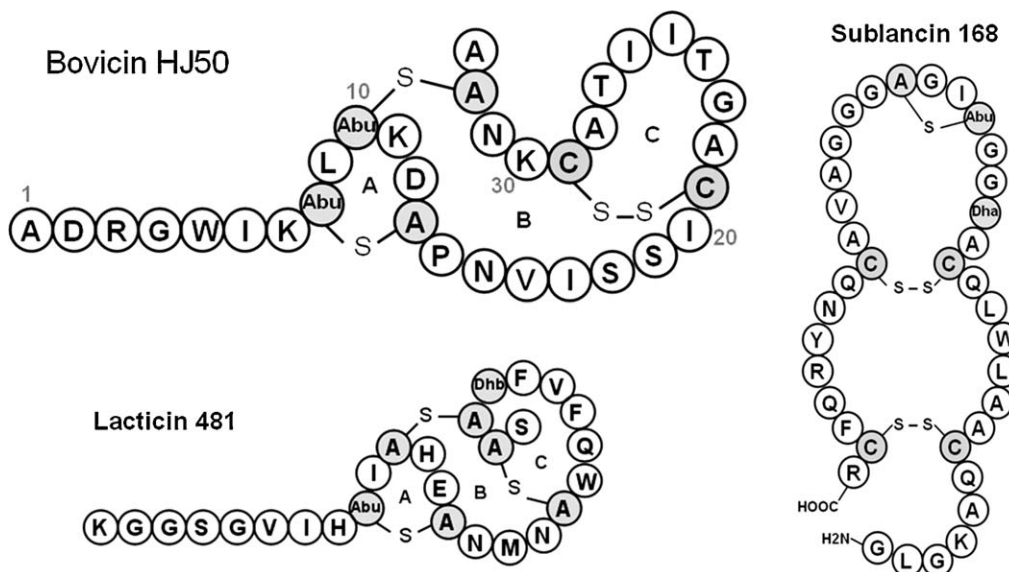
### Importance of the disulfide bridge to the activity of bovicin HJ50

The structure of bovicin HJ50 reveals that the disulfide bridge leads to the formation of ring C which is usually formed by a thioether bridge in other lantibiotics, e.g. lactacin 481. To investigate the function of this disulfide bridge, we prepared a disulfide bridge-broken mutant C21A and performed agar well diffusion assays. The result showed that the inhibition ability of C21A against *M. flavus* NCIB 8166 decreased dramatically comparing with that of the wild-type bovicin HJ50 (Fig. 2D). This suggested that the disulfide bridge is critical for the antimicrobial activity of bovicin HJ50.

### Discussion

Semi-*in vitro* biosynthesis, which combines *in vivo* modification and *in vitro* cleavage of leader peptide, is a convenient lantibiotic synthetic approach that exhibits several advantages. First, *in vitro* processing can avoid unwanted regulatory properties and toxic effects of some mutants. Moreover, comparing with step-by-step *in vitro* synthesis, SIVB has simpler procedures and higher modification efficiency. Most importantly, SIVB merely employ the widely-used *E. coli* expression system, so the laborious work for constructing specific engineered expression systems is not necessary. In principle, it can be generalized to other type-AII lantibiotics, as long as *lanA*, *lanM* and *lanT* genes exist in their biosynthetic gene clusters. This is very meaningful especially for those without established engineered expression systems.

Previously, bovicin HJ50 was classified as type-AII lantibiotic because of the similarity of primary



**Figure 4.** Bovicin HJ50 compared with lactacin 481 and sublancin 168. The deduced bridging pattern of bovicin HJ50 is presented and shown to have a linear N-terminus and a globular C-terminal region. It shows structural similarity to lactacin 481 (van den Hooven et al. 1996) but differs mainly in the disulfide bridge and several undehydrated Ser and Thr residues. Bovicin HJ50 exhibits great differences from another disulfide bridge-containing lantibiotic sublancin 168 (Paik et al. 1998). Dark-colored circles indicate the unusual amino acids. A-S-A, Abu-S-A and C-S-S-C represent lanthionine, 3-methylanthionine and disulfide bond, respectively.

structure and genetic organization (Xiao et al. 2004; Liu et al. 2009). In this study, we found that bovicin HJ50 shows structural similarity to typical All members, such as lactacin 481 (van den Hooven et al. 1996) and mutacin II (Krull et al. 2000), although it differs a lot from another disulfide bridge-containing lantibiotic sublancin 168 (Paik et al. 1998) (Fig. 4). Bovicin HJ50 contains a linear N-terminus and a globular C-terminus, which are the features of All lantibiotics. The ring A of bovicin HJ50 shows high similarity to that of lactacin 481, from the number and property of amino acid residues to the position of three (Me) Lan-forming residues. However, bovicin HJ50 shows its own features in other aspects. First, a disulfide replaces a lanthionine and forms the ring C, which is the unique case in this type of lantibiotics. In addition, bovicin HJ50 contains no dehydrated amino acids (Dha or Dhb) but four unmodified Thr/Ser, which is uncommon in other members. Besides, our attempt to replace Cys21 with Ser resulted in neither an additional thioether bridge nor a Dha (data not shown). These phenomena indicated a high fidelity of the modification enzyme BovM, which is the basis for structural analysis via site-directed mutagenesis.

Disulfide bonds are generally thought to enhance the stability of proteins but rarely exist in the world of lantibiotics. The formation of disulfide bridges can be mediated by either specialized enzymes or

other electron-accepting reagents such as oxygen (Bulaj 2005). In *B. subtilis* 168, two thio-disulfide oxidoreductases BdbB and BdbC are essential for the production of active sublancin 168 (Dorenbos et al. 2002). However, there may be different formation mechanism for the disulfide bridge in bovicin HJ50. Heterologous expression of *bovA* in *E. coli* resulted in a linear polypeptide without any bridges; while co-expression of *bovA* and *bovM* generated a product with two thioether bridges and a disulfide bridge. Seemingly, it was BovM that catalyzed the formation of this disulfide bridge. Nevertheless, there were evidences showing that BovM is not directly related to the formation of the disulfide bridge. Previous study showed that heterologous expression of bovicin HJ50 biosynthetic gene cluster in *Lactococcus lactis* MG1363 resulted in a product containing two thioether bridges but no disulfide bridge, with two Cys residues unbonded (Liu et al. 2009). Besides, the present study showed that the ring A-broken (C13A) and ring B-broken (T10A/C32A) mutants both failed to form the disulfide bridge. These results suggested that there might be two necessary factors for the disulfide bridge formation in bovicin HJ50. One is the spacial proximity of the two disulfide bridge-forming cysteines, which may be achieved by the presence of two thioether rings. The other one is concerning the potential electron-accepting reagent, which may be properly provided in *E. coli*, but not in *L.*

*lactis*. Thus, we proposed, given the two above-mentioned conditions, the disulfide bridge could be formed without direct dependence on BovM.

This study indicated that the antimicrobial activity of the disulfide bridge-broken mutant C21A decreased dramatically, providing direct evidence that the disulfide bridge is of great importance to the activity of bovicin HJ50. Nevertheless, it was reported that DTT-treated bovicin HJ50, in which the disulfide bond was reduced, showed no change in its antimicrobial activity (Xiao et al. 2004). Besides, heterologously expressed bovicin HJ50 from *L. lactis* MG1363 contained no disulfide bridge but exhibited similar inhibition ability and spectrum comparing with wild-type bovicin HJ50 (Liu et al. 2009). One possible explanation was that the two proximal Cys residues were unstable and prone to be oxidized spontaneously. So the disulfide bridge might form again when these lantibiotics were exposed to a favorable environment. Interestingly, replacement of nisin's thioether ring C with a disulfide bridge led to only slight structural change but the loss of antimicrobial activity (van Kraaij et al. 2000). This phenomenon suggested that disulfide bridges cannot help maintain the activity for all lantibiotics.

In summary, Semi-*in vitro* biosynthesis is an effective approach for producing bovicin HJ50 analogues. It exhibits simpler procedure, higher productivity and more extensive applicability than conventional methods. In addition, revelation of the unique structure of bovicin HJ50 enriches the structure pool of type-All lantibiotics. It is expected that, with SIVB as a platform, more investigations can be performed on the structure-activity relationships, as well as the mechanisms of modification and regulation of lantibiotics. Still, there is potential to further increase the productivity of SIVB by optimizing the *in vitro* cleavage system.

## Acknowledgements

The work was supported by grants from National Natural Science Foundation of China (30500013), the National Program for High Technology Research and Development of China (2006AA10Z319) and the Key Project of the Chinese Academy of Sciences (KSCXZ-YW-G-016). We thank Dr. Yuanming Luo and Dr. Qian Wang with MALDI-TOF MS measurements.

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