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# Expression and characterization of recombinant single-chain salmon class I MHC fused with $\beta_2$-microglobulin with biological activity

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Biosensor

**Abstract** Heterodimeric class I major histocompatibility complex (MHC) molecules consist of a putative 45-kDa heavy chain and a 12-kDa $\beta_2$-microglobulin ($\beta_2$m) light chain. The knowledge about MHC genes in Atlantic salmon accumulated during the last decade has allowed us to generate soluble and stable MHC class I molecules with biological activity. We report here the use of a bacterial expression system to produce the recombinant single-chain MHC molecules based on a specific allele Sasa-UBA*0301. This particular allele was selected because previous work has shown its association with the resistance to infectious salmon anaemia virus. The single-chain salmon MHC class I molecule has been designed and generated, in which the carboxyl terminus of $\beta_2$m is joined together with a flexible 15 or 20 amino acid peptide linker to the amino terminus of the heavy chain (Sasa$\beta_2$mUBA*0301). Monoclonal antibodies were successfully produced against both the MHC class I heavy chain and $\beta_2$m, and showed binding to the recombinant molecule. The recombinant complex Sasa$\beta_2$mUBA*0301 was expressed and isolated; the production was scaled up by adjusting to its optimal conditions. Subsequently, the recombinant proteins were purified by affinity chromatography using mAb against $\beta_2$m and $\alpha_3$. Eluates were analyzed by Western blot and refolded by the removal of denaturant. The correct folding was confirmed by measuring its binding capacity against mAb produced

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to recognize the native form of MHC molecules by biosensor analysis. This production of sufficient amounts of class I MHC proteins may represent a useful tool to study the peptide-binding specificity of MHC class I molecules, in order to design a peptide vaccine against viral pathogens.


## Introduction

Infectious salmon anaemia (ISA) is an important aquatic disease causing severe economic losses in farmed Atlantic salmon (*Salmo salar* L.). The disease was first reported in Norway in 1984, and since then, several outbreaks have occurred in Scotland, Canada, the United States, and Chile. ISA is a List I disease in the European Union, which obliges member states to undertake an eradication and control programme as specified under the fish disease control directive (93/53/EC). The ISA virus (ISAV) was identified as the causative agent of this disease, and has been classified under a new genus *Isavirus* within the family of *Orthomyxoviridae* [1–4]. Due to its commercial impact, ISAV is the first fish *orthomyxoviridae* virus to be extensively characterized at the molecular level. ISAV has been shown to be a segmented, negative-stranded RNA virus and to share several characteristics with influenza viruses in morphological, physiochemical and biochemical aspects [5,6]. Up to now, little information has been published about the vaccine development against ISA. Two commercial vaccines based on inactivated whole ISA virus particles are currently available in Canada and the USA [7,8]. Recently, DNA vaccination using plasmids expressing either the viral hemagglutinin-esterase (HE) or nucleoprotein gene has been investigated, and moderate protection against ISA was achieved after immunization with the plasmid containing the HE gene [9]. Therefore, new effective and specific vaccine development is highly demanded.

Major histocompatibility complex (MHC) class I molecules play an important role to alert the immune system against virally infected cells. In general, MHC class I molecules are heterodimers consisting of one $\alpha$ chain and one light chain ($\beta_2$-microglobulin) that are non-covalently associated with each other. The $\alpha$-subunit has three structural domains, the $\alpha1$ and $\alpha2$ domains fold to form the peptide-binding groove. The third structural $\alpha3$ domain associates with the $\beta_2$m, forming the membrane-proximal region of the molecule. The light chain is a polypeptide not encoded in the MHC, and plays a role in MHC class I intracellular transport, peptide binding, and conformational stability. MHC class I molecules are highly polymorphic and different alleles have unique peptide-binding specificities. The proteins encoded by each allele will bind only a unique set of peptides. The majority of peptides bound to MHC class I have a size restriction of 8–11 amino acids; the overall length of all ligands and epitopes is 7–13. They are bound via interactions between the side chains and backbones of both counterparts. Peptides are held in the binding grooves that are formed by $\alpha1$ and $\alpha2$ domains of MHC by a conserved network of hydrogen bonds. Anchor residues are characterized to fit into pockets formed by polymorphic MHC side chains. Short peptides (8–11a.a.) bind to this groove by two or three anchor residues that interact with the corresponding binding pockets in the groove. For example, a human class I MHC molecule HLA-A*0201 has been found to have Leu at position 2 (P2) and Val at P9 [10,11]. Most MHC class I molecules use two anchors. One of them is always located at the carboxy (C) terminus of the antigenic peptide, and is in most cases either hydrophobic or basic amino acids [10].

The MHC gene in a teleostean fish was first discovered in the common carp in 1990 [12]; salmonid MHC sequences were reported 3 years later [13,14]. Subsequently, Atlantic salmon has been identified to have a well-defined MHC system with MHC class I and class II genes located on different linkage groups, and each region contains one major and dominant-expressed locus. At least 12 different alleles were identified, and evidence has been found for exon shuffling to produce new MHC I alleles in Atlantic salmon [15].

Extensive research work has been carried out to study the structure of the peptide-binding specificity in human and mouse, but there are no crystallographic models of fish MHC molecules, neither are there data on the peptide-binding specificity. Therefore, production of sufficient amounts of stable class I MHC proteins represents a valuable tool to study the peptide-binding specificity of MHC class I molecules, and to design a peptide vaccine against viral pathogens. Here we report the first use of a bacterial expression system to produce recombinant single-chain MHC molecules based on a specific allele Sasa-UBA*0301. A flexible 15 or 20 amino acid peptide linker was applied to the carboxyl terminus of $\beta_2$m and to the amino terminus of the heavy chain. Monoclonal antibodies were successfully produced against MHC class I and $\beta_2$m and showed binding to the recombinant molecule. Recombinant Sasa$\beta_2$mUBA*0301 was purified by affinity chromatography using mAb against $\beta_2$m and $\alpha_3$. The correct folding was confirmed by measuring its binding capacity against mAb produced to recognize the native form of MHC molecules by biosensor analysis. Thus, this system appears to produce sufficient amounts of class I MHC proteins, and may represent a valuable tool to study the peptide-binding specificity of MHC class I molecules in salmon.

## Materials and methods

### Cells, plasmid, and production of monoclonal antibodies

*Escherichia coli* BL21 and pET-22b(+) were purchased from Novagen Co. All chemicals used were of analytical reagent grade. Sasa-UBA*0301 $\alpha3$ (clone 7) and $\beta_2$m (clone 43) specific monoclonal antibodies were kindly provided by Dr. Karsten Skjødt, University of Southern Denmark.

## Construction of single-chain MHC/$\beta_2$m complex

The regions encoding the extracellular domains of MHC heavy chain and $\beta_2$m were amplified from cloned cDNA by polymerase chain reaction (PCR). The heavy and light chains were amplified from plasmid containing Sasa-UBA*0301 (animal number 401, female, stored in our laboratory) and pCR2.1 (kindly provided by Unni Grimholt, Norway). The PCR products were cloned into pGEM-T easy vector (Promega) and sequenced. Using the sequenced plasmid DNA as template, the heavy and light chains were joined by a linker of 15 amino acid residues ($GGGGS_3$) by splicing overlap extension PCR (SOE-PCR) with oligonucleotide pairs pHCf and pHCr 5′-GC GCG GTG CATATG AAA GAA TCT CCC CCC AAG GTG-3′ and 5′-GCC GCC ACC CGA GCC GCC TCC GCC CAT ATC TGC TTC CCA GGT-3′; primer pairs pLCf and pLCr 5′-GGC GGC TCG GGT GGC GGC GGC TCT GGC GGA GGT GGA TCT GCC GCT ACG CAT TCA CTG-3′ and 5′-CGC GAT GAG CTCGAT GTT GGG GTC ATT CCA GTT GG-3′ (Table 1). These PCR products were purified using a Qiagen PCR purification kit and mixed together. The two hybrid primers pHCr and pLCf were overlapped, followed by re-amplification of the assembled products with external flanking oligonucleotides pHCf and pLCr. The final products were purified, digested with NdeI and XhoI, and cloned into the T7 expression vector pET-22b(+) (strain Rosetta™(DE3)-pLysS, Novagen Inc., WI) digested with the same restriction enzymes, giving the plasmid pJZsc$\alpha\beta$. The T7 expression vector pET-22b(+) contains a 6 × His tag at the C-terminus for purification purposes. The plasmids were sequenced through the entire encoding sequence to verify the absence of undesired mutations introduced by PCR.

## Protein expression in *E. coli* and analysis

Bacteria containing expression plasmid were grown in LB containing 100 μg/ml ampicillin and 34 μg/ml chloramphenicol; protein expression was induced for 3—4 h by adding 1 mM isopropyl-β-D-thiogalactopyranoside at the mid-log phase with shaking. The recombinant proteins were overexpressed as inclusion bodies. Bacterial pellets were resuspended in 20 mM Tris—HCl, pH 8.0, 15 mM NaCl and disrupted by sonication on ice. After centrifugation (16,000*g*, 30 min), the cell pellets were washed three times with resuspension buffer adding 1 mM DTT, 0.5 mM EDTA, 0.5% (V/V) Triton X-100, and mixed with ice-cold lysis buffer A containing 8 M urea, 10 mM Tris—HCl, 100 mM $NaH_2PO_4$, pH 8.0, for 1 h.

**Table 1** Primers designed for cloning the full length $\beta_2$m-linker-$\alpha$ gene

| | |
|---|---|
| Primer 1 | 5′-gc gcg gtg cat atg aaa gaa tct ccc ccc aag gtg-3′ |
| Primer 2 | 5′-gcc gcc acc cga gcc gcc tcc gcc_cat atc tgc ttc cca ggt-3′ |
| Primer 3 | 5′-gcc gcc acc cga gcc gcc tcc gcc cga acc gcc acc tcc_cat atc tgc ttc cca ggt-3′ |
| Primer 4 | 5′-ggc ggc tcg ggt ggc ggc ggc tct ggc gga ggt gga tct_gcc gct acg cat tca ctg-3′ |
| Primer 5 | 5′-cgc gat gag ctc gag gtt ggg gtc att cca gtt gg-3′ |

Western blotting was performed using the Westernbreeze Chemiluminescent Immunodetection System (Invitrogen) according to the manufacturer's instructions. Briefly, proteins were separated on a 10% SDS—PAGE and electrotransferred onto nitrocellulose membranes. After blocking with bovine serum albumin (BSA), the membranes were subjected to immunodetection with Sasa-UBA*0301 $\alpha$3 (clone 7) and $\beta_2$m (clone 45) specific monoclonal antibodies for 1 h at room temperature. The secondary HRP-conjugated GAM antibody (1:1000, Invitrogen) was added and incubation was carried out for 45 min. The membranes were finally developed with horseradish peroxidase staining solutions.

## Purification and *in vitro* refolding of recombinant complex

Purification of recombinant proteins under denaturing conditions was performed according to Qiagen's protocols. The urea-solubilized supernatant was mixed with the nickel-charged matrix for 1 h. The mixture was loaded onto the Ni-NTA nickel column (Qiagen) and centrifuged at 300*g* at 4 °C for 2 min. The column was extensively washed with 3 × 0.6 ml of lysis buffer A containing 20 mM imidazole (WB-1), 3 × 0.6 ml of washing buffer WB-2 (20 mM Tris—HCl, pH 8.0, 100 mM KCl, 5 mM $MgCl_2$, 10 mM 2-mercaptoethanol, 20% glycerol, 0.1% NP40, 30 mM imidazole), 3 × 0.6 ml of washing buffer WB-3 (WB-2 with 50 mM imidazole), followed by 3 × 0.6 ml of WB-4 (WB-3 but with 100 mM imidazole). The recombinant protein was eluted from the column with WB-3 containing 250 mM imidazole. The eluted fractions were finally checked for purity using a 12% polyacrylamide gel. Clarification by centrifugation at 10,000*g* for 10 min was applied to get rid of cell debris, and the supernatant containing the solubilized protein was transferred into a clean tube. Refolding and complex formation were initiated by extensive dialysis against a Redox system buffer containing 50 mM Tris—HCl (pH 8.0), 3 mM reduced glutathione, 0.3 mM oxidized glutathione, 400 mM arginine, 0.1 mM EDTA, 0.1 mM PMSF. The refolding mixture was left undisturbed at 4 °C for 2 days. The refolding mixture was concentrated with PEG (MW: 12,000). The final concentration of MHC was determined by the Bradford method.

## Binding assay using the IAsys biosensor

Successful refolding was determined by the biosensor assay using conformational Sasa-UBA*0301 $\beta_2$m (clone 45) and $\alpha$3 (clone 7) specific antibodies. The complex to be tested was coated onto the IAsys carboxymethyl-dextran (CMD) cuvette, and the binding analysis was based on the IAsys resonant mirror biosensor (Affinity Sensors, Cambridge, UK). Recombinant MHC class I/$\beta_2$m (75 μg) was first coupled to the surface of the cuvette with a 200 nm thick layer of CMD after activating the CMD hygrogel, non-coupled activated CMD sites were blocked with ethanolamine, and the binding assays were carried out. All binding experiments were performed in distilled water at room temperature. The contents of the reaction cuvette were stirred

continuously with the aid of a propeller. The cuvette was regenerated by repeated washes with formic acid and PBS-T. The data readout from the biosensor were measured in units of arc seconds.

## Results

### Construction of the recombinant scMHC/$\beta_2$m complex

We constructed a bacterial system expressing soluble MHC class I heavy chain, Sasa-UBA*0301, designated pJZ$\alpha$his, with extra GGGGS sequences at its N-termini and a histidine tag at its C-termini (Figs. 1, 2a). We also constructed salmon $\beta_2$-microglobulin that presents naturally at different chromosomes on the salmon genome, designated pJZ$\beta$, with extra GGGGS sequences at its C-termini (Figs. 1, 2a). Furthermore, we constructed a recombinant construct, designated pJZsc$\alpha\beta$, which linked two molecules together by overlap extension PCR reaction (Figs. 1, 2b, c). Sequencing results indicated that the flexible 15-amino acid or 20-a.a. linker was introduced between the C-termini of $\beta_2$m gene and the N-termini of the heavy chain gene, and the C-termini of the heavy chain coupled with a His tag motif (LEHHHHHH) encoded by the pET-22b(+) plasmid. This shows that we have successfully made a recombinant molecule consisting of a truncated heavy chain which lacks the transmembrane domain, and $\beta_2$-microglobulin covalently linked to the N-termini of this truncated heavy chain by two flexible bridges. These resulting single-chain molecules have the advantage of efficiently binding low concentrations of peptides with the expected specificities in the further peptide-binding studies. Sequencing analysis of the full length constructs confirmed that there were no unwanted sequence-altering mutations and deletions introduced by PCR and cloning.

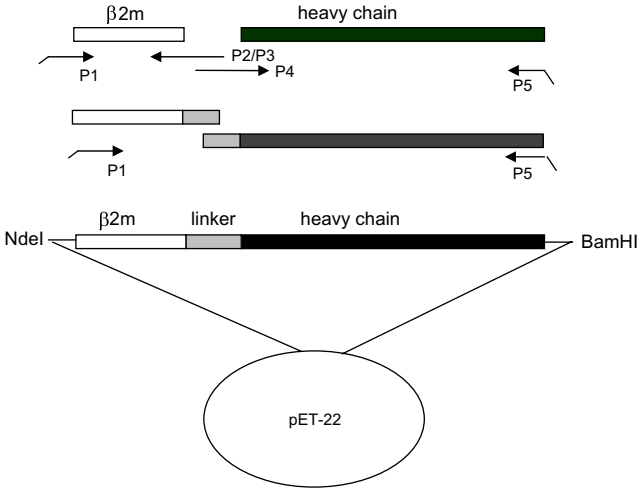


**Figure 1** Map and diagram of the single-chain Sasa$\beta_2$mUBA*0301 construct. Splicing by overlap extension was used to connect the carboxyl terminus of $\beta_2$m to the amino terminus of the Sasa-UBA*0301 heavy chain via a flexible 15 or 20 amino acid peptide linker. Primers 1, 2, 4, and 5 were applied to insert $(GGGGS)_3$ linker; primers 1, 3–5 were used to insert $(GGGGS)_4$ linker. Restriction sites are also shown for the insertion of the MHC complex into the expression vector pET-22b(+).

### Expression and purification of the recombinant scMHC/$\beta_2$m complex

As shown in Fig. 3, SDS–PAGE analysis revealed that inclusion bodies had formed when the *E. coli* system was applied for the expression of salmon MHC class I molecules. Under 1% IPTG induction, the recombinant scMHC/$\beta_2$m complex was expressed as inclusion bodies. In order to purify the non-native scMHC/$\beta_2$m molecules, the inclusion bodies were isolated using a modified detergent extraction protocol. Crude inclusion bodies were treated with urea solubilization under reducing conditions, followed by further purification steps. The yield of recombinant protein reached 10 mg/l of bacterial culture. The resulting recombinant proteins were further purified on a Ni-NTA-agarose column.

It has been found that most MHC class I molecules are not expressed efficiently on the cell surface in the absence of $\beta_2$m because of its conformational instability [16,17]. Though no research work has been done about the conformational stability of fish MHC class I and fish $\beta_2$m, we did not want to run the risk and assumed that they would behave the same way as those in human. Our expression approach has helped us to achieve the yield of 5 mg/l of bacterial culture, while other research studies show that the approach of expressing the heavy chain and $\beta_2$m separately would not be possible. Our idea of linking the heavy chain and $\beta_2$m together has simplified the analysis of measurements of different ratios between the two molecules of heavy chain and $\beta_2$m for the refolding test. In addition, we have tried rapid removal and dilution of the solubilizing substances, which resulted in a large amount of aggregation and only a small amount of functional scMHC/$\beta_2$m complex (data not shown). Gradual removal of the solubilizing substances by using a continuous dialysis system was quite essential to increase the efficiency of the recovery of biological activity.

### *In vitro* refolding and purification

*In vitro* refolding was achieved by extensive dialysis against 10 mM Tris–HCl, pH 7.5, and the refolding buffer. In order to purify the refolded scMHC/$\beta_2$m, affinity purification was performed. The soluble refolded fractions were applied to a mAb (clone 43) coupled-Sepharose column and almost no flow-through soluble protein was observed, indicating that these molecules had affinities for the monoclonal antibody. No aggregation of recombinant scMHC/$\beta_2$m molecules was observed under these elution conditions (Fig. 4A). Both SDS–PAGE and the subsequent Western blot analysis of the recombinant protein revealed bands with 53 kDa (Fig. 4B). The final concentration of the recombinant complex was 200 $\mu$g/ml by using the Bradford method.

### Biosensor assay

Biosensor assay was performed in order to confirm that the recombinant MHC class I/$\beta_2$m complex was conformational

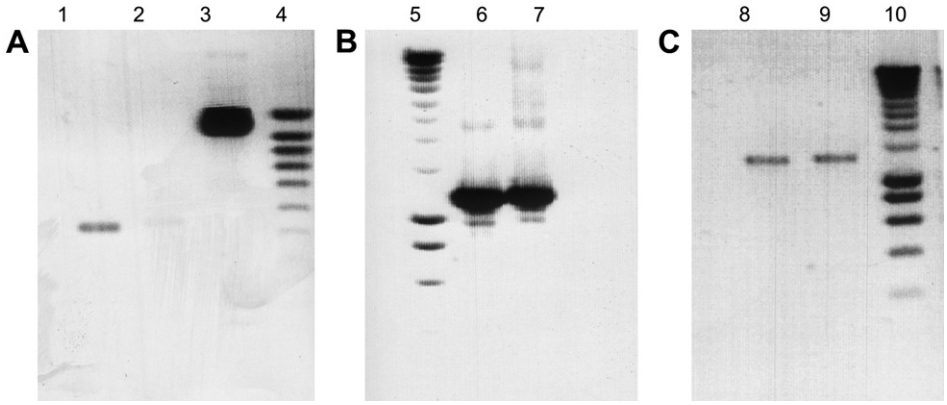


**Figure 2** Identification of cloning of the single-chain Sasa$\beta_2$mUBA*0301 with the correct size. The putative signal peptide of the heavy chain was predicted by signal P program and excluded in the construct. The cDNA fragments of the heavy chain encode the putative full length mature protein with deletion of the transmembrane and cytoplasmic domain. (A) Cloning of light chain ($\beta_2$m) and heavy chain (UBA*0301). Lane 1, $\beta_2$m with partial sequences designed for linker $(GGGGS)_3$; 2, $\beta_2$m with partial sequences designed for linker $(GGGGS)_4$; 3, heavy chain; 4, Smartladder SF. (B) Full length cDNA of Sasa$\beta_2$mUBA*0301. Lane 5, Smartladder; 6, full length cDNA with linker $(GGGGS)_3$; 7, full length cDNA with linker $(GGGGS)_4$. (C) Full length cDNA of Sasa$\beta_2$mUBA*0301 after purification. Lane 8, full length cDNA with linker $(GGGGS)_3$; 9, full length cDNA with linker $(GGGGS)_4$; 10, Smartladder.

and folded properly. The instrument can detect changes in refractive index occurring within the evanescent field when one bio-molecule, the ligand, in the solution binds to its partner. Refolded MHC class I/$\beta_2$m were immobilized onto the sensor surface, allowing for the capture of different monoclonal antibodies on this surface. Three conformational antibodies were tested, clone 7 (anti-$\alpha$3), clone 45 (anti-$\beta_2$m), and clone 15 (anti-$\beta_2$m). The interactions between the recombinant molecules and three conformational antibodies could be followed in real-time with the

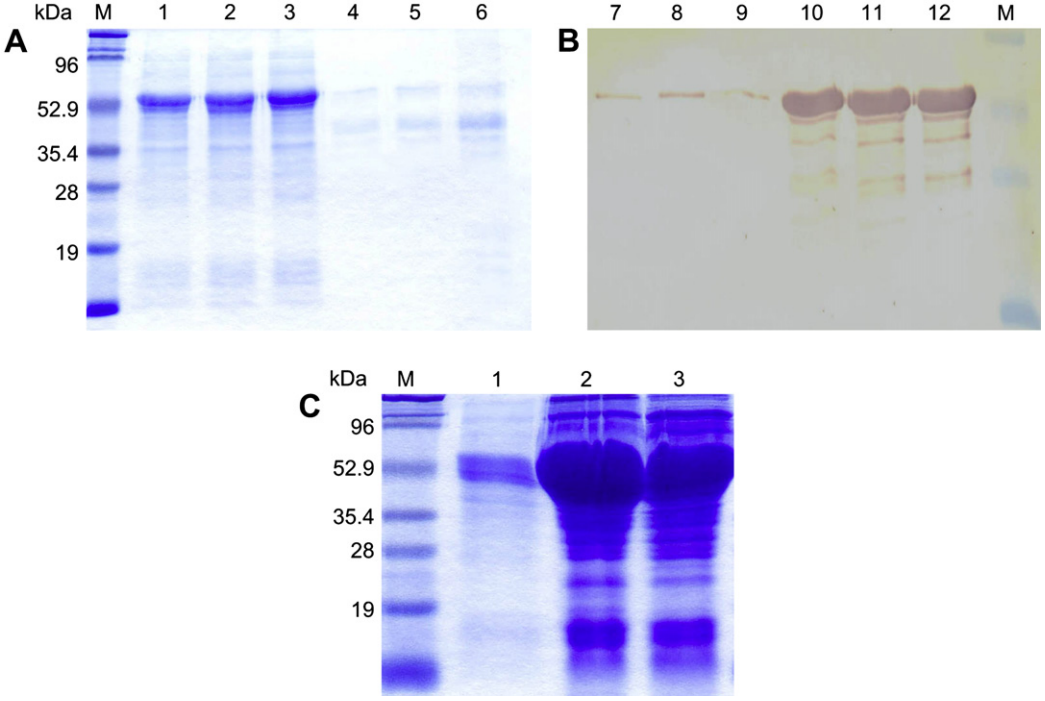


**Figure 3** Expression and production of the recombinant Sasa$\beta_2$mUBA*0301 MHC complex. The plasmid pET22-Sasa$\beta_2$m/UBA0301 expressing the $\beta_2$m and heavy chain was transformed into *E. coli* strain BL20 and Rosetta. (A) SDS—PAGE analysis of recombinant protein Sasa$\beta_2$m/UBA*0301 in *E. coli* stained with Coomassie blue R-250. Lane M, MW marker; 1—3, soluble fraction of lysate from bacteria strain BL20 with $(GGGGS)_3$, $(GGGGS)_4$, and strain Rosetta with $(GGGGS)_4$, respectively; 4—6, insoluble fraction of lysate from strain BL20 and Rosetta. (B) Western blot analysis using mouse IgG mAb against $\beta_2$m. Lanes 7—12 are the mirror images of lanes 1—6. (C) Large scale production of recombinant Sasa$\beta_2$m/UBA*0301. Lane M, MW marker; 1, small scale production; 2, large scale production with linker $(GGGGS)_3$; 3, large scale production with linker $(GGGGS)_4$.

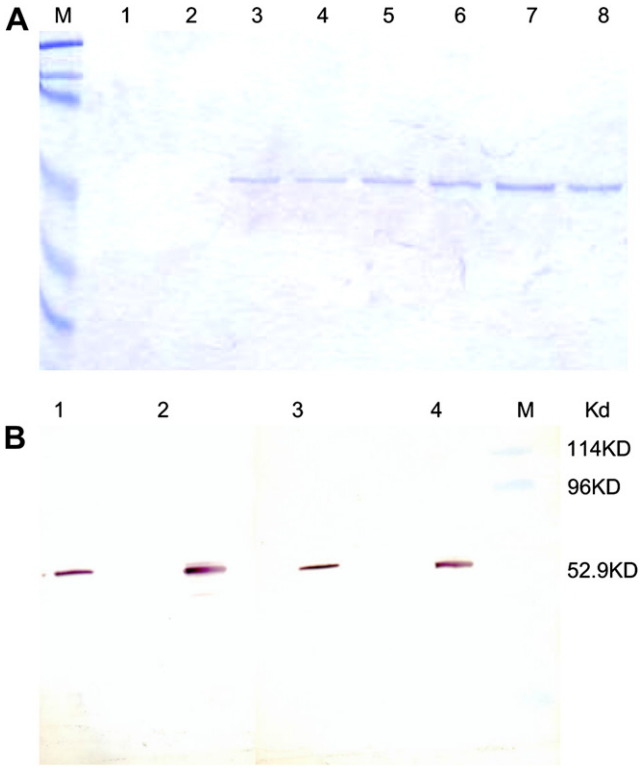


**Figure 4** Purification of recombinant class I molecule Sasa$\beta_2$-mUBA*0301 by affinity chromatography using mAb against $\beta_2$m (clone 45) and $\alpha_3$ (clone 7). Eluates were analyzed by Western blotting and quantified by the Bradford assay. (A) SDS—PAGE. Lane M, molecular mass markers (from top to bottom, 200, 114, 96, 52.9, 36.8, and 30 kDa; 1, flow-through; 2—4, washing fractions; 5—8, eluted fractions. (B) Western blot. Lanes: 100 ng of purified Sasa$\beta_2$m/UBA*0301.

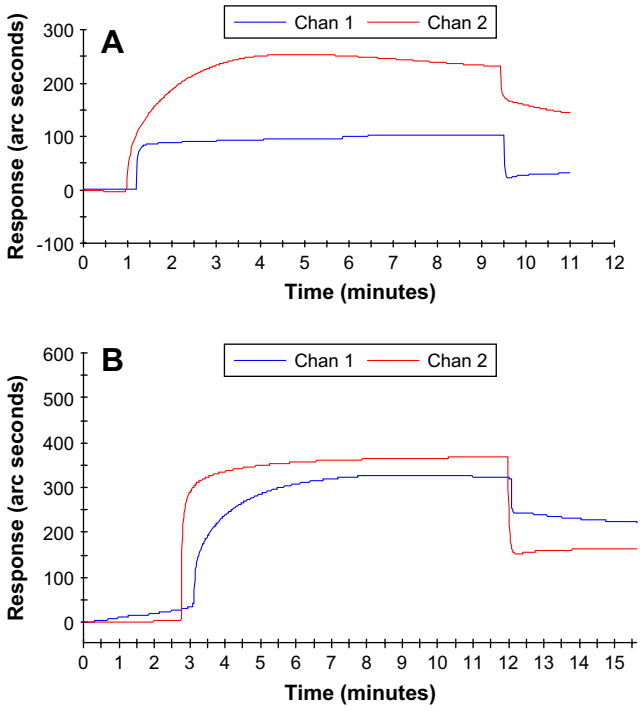


**Figure 5** Biosensor measurement of recombinant class I molecule Sasa$\beta_2$m/UBA*0301 binding to different mAbs, Sasa$\beta_2$m/UBA*0301 was immobilized to a CMD cuvette. (A) Channel 1, negative control; channel 2, binding of Sasa$\beta_2$m/UBA*0301 to mAb 45 (against $\beta_2$m) in arc seconds. (B) Channel 1, binding to mAb 15 (against $\beta_2$m) in arc seconds; channel 2, binding to mAb 7 (against $\alpha$3).

instrument producing a plot of the response, and measured in arc seconds against time. As shown in Fig. 5, a range of responses from 150 to 230 arc seconds was achieved by the recombinant MHC class I/$\beta_2$m solution.

## Discussion

Recently outbreaks of viral diseases on cultured fish have led to high morbidity and mortality and resulted in great economic losses. Therefore, understanding the immune response process is extremely important. MHC class I molecules play crucial roles in antigen presenting processes that trigger specific T-cell responses [18]. It has been shown that an artificially manufactured MHC I complex is useful for studying the epitope specific T-cell responses and for enhancing these responses *in vitro*. We have described here a bacterial system which can be used to express salmon MHC I molecules. Large amounts of MHC I molecules were obtained, and the correct folding was achieved and confirmed by measuring its binding capacity against mAb produced to recognize the native form of MHC molecules. Thus, this system can be applied for the further study of peptide-binding specificity of MHC class I molecules in salmon.

Since MHC I involves the assembly of three non-covalently bound components, this makes the recombinant expression and folding in the periplasma cumbersome. One of the solutions is to generate a single-chain construct; this strategy has been proven successful in the case of scFv fragment. It has been shown that artificially manufactured MHC class I complexes could be applied for further study on peptide-binding specificity in human and mouse. Several methods have been reported to produce the MHC I complex. These range from making the three components separately and mixing them [19]; to making two molecules by fusing a heavy chain with $\beta_2$m [20,21]; or making a single-chain construct of the three components [22]. In this study, we have designed and generated a single-chain salmon MHC class I molecule, in which the carboxyl terminus of $\beta_2$m is joined together with a flexible 15 or 20 amino acid peptide linker to the amino terminus of the heavy chain. The truncated heavy chain lacks the transmembrane domain. These resulting single-chain molecules are useful for the further peptide-binding studies.

Our experience suggested that it is not always possible to achieve successful target molecules by splicing of extension (SOE) by PCR; we suggest an intermediate step to test the appropriate conditions when difficulties are encountered. A major disadvantage of SOE by PCR is its possibility of unwanted mutations that might be introduced over conventional shuffling techniques. Conventional splicing by the shuffling of restriction fragments is more

accessible when it is dependent on the introduction and removal of restriction sites. However, this conventional method will introduce at least six extra nucleotides encoding for the restriction sites, which might interfere with the peptide-binding specificity. We suggest the PCR regime should involve a smaller numbers of cycles, cutting it down to 15 cycles.

A number of studies have shown that the folding of MHC class I by $\beta_2$m in the absence of added MHC I binding peptide is much more efficient at room temperature than at 37 °C; $\beta_2$m was a critical factor in this low-temperature folding of MHC class I [23—26]. However, no such research work has been carried out in fish, and a model of MHC/$\beta_2$m folding should account for the species. We assume that the folding of salmon MHC class I should be more efficient at a temperature of 4 °C, significantly lower than room temperature.

The current instruments are not suitable for the determination of the interactions between very small molecules that have a molecular mass in the range of 5 kDa, because the biosensors read the changes in the sensor surface which is caused by the binding and dissociation of the target proteins. In addition, biosensors can continuously provide a real-time picture of the binding/dissociation events, and are extremely useful when the concentration of the target protein is relatively low and in small volume. The most common sensor surface is a flexible hydrogel matrix composed of carboxymethylated dextran chains of a thickness of 100—200 nm. As the sensor surface can be regenerated, multiple binding determinations can be done on the same surface. Compared with ELISAs, the flow rate and the stirred rate can be adjusted to reduce the mass transport limitations.

Although we have described one example of the application of a single-chain MHC class I complex in fish, the procedures we have developed here should be applicable to the expression and refolding of other fish MHC molecules. Allele Sasa-UBA*0301 was selected because previous work showed it to be related to the resistance to ISAV, and there is a significant association between MHC polymorphism and the disease resistance [27]. The results presented in this study will be helpful for a better understanding of salmon MHC class I antigen presentation and to design a peptide vaccine against ISAV in salmon.

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