Engineering of a Polymeric Bacterial Protein as a Scaffold for the Multiple Display of Peptides

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ABSTRACT **Protein assemblies with a high degree of repetitiveness and organization are known to induce strong immune responses. For that reason they have been postulated for the design of subunit vaccines by means of protein engineering. The enzyme lumazine synthase from *Brucella* spp. (BLS) is highly immunogenic, presumably owing to its homodecameric arrangement and remarkable thermodynamic stability. Structural analysis has shown that it is possible to insert foreign peptides at the ten amino terminus of BLS without disrupting its general folding. These peptides would be displayed to the immune system in a highly symmetric three-dimensional array. In the present work, BLS has been used as a protein carrier of foreign peptides. We have established a modular system to produce chimeric proteins decorated with ten copies of a desired peptide as long as 27 residues and have shown that their folding and stability is similar to that of the wild-type protein. The knowledge about the mechanisms of dissociation and unfolding of BLS allowed the engineering of polyvalent chimeras displaying different predefined peptides on the same molecular scaffold. Moreover, the reassembly of mixtures of chimeras at different steps of the unfolding process was used to control the stoichiometry and spatial arrangement for the simultaneous display of different peptides on BLS. This strategy would be useful for vaccine development and other biomedical applications. Proteins 2004;00:000–000.**

Key words: immunogenicity; stability; chimeric protein; decamer; polyvalent chimeras; multiple display of peptides

INTRODUCTION

There is a close correlation between the degree of repetitiveness and organization of an antigen and the efficiency of induction of the B cell immune response[1,2] Many viruses exhibit a quasi-crystalline, highly organized surface that displays a regular array of epitopes, which efficiently crosslink epitope-specific membrane immunoglobulins on B cells.[3] A very successful example of the use of virus-like particles (VLPs) as vaccines is the surface (S) antigen of hepatitis B virus. Expression of the S domain in yeast results in the production of 22 nm VLPs with around 100 polypeptides per particle. This antigen elicits high levels of antibodies to HbsAg and is now widely used as a vaccine against Hepatitis B.[4] However, the vast majority of proteins derived from pathogens are not able to form VLPs and unmodified VLPs may only rarely be used as vaccines.

To overcome these limitations, foreign epitopes have been grafted onto VLPs by chemical linkage and especially by genetic engineering.[5,6] A critical disadvantage of chimeric VLPs is the limited size of the epitopes that can be recombinantly inserted. The inserted peptide should disturb neither the three-dimensional folding nor the quaternary arrangement of the protein particle.

In contrast with viral capsid and envelope proteins, bacterial proteins rarely have polymeric quaternary assembly. For this reason, few bacterial antigens have been proposed as carriers for subunit vaccine development. One of the exceptions is the non-toxic B subunit of the cholera toxin. This very stable pentameric protein has been used as a carrier molecule for chemically- or genetically-conjugated autoantigens for the induction of oral tolerance.[7,8] The other bacterial polymeric protein proposed for vaccine development is a protein scaffold derived form the pyruvate dehydrogenase multienzyme complex from *Bacillus stearothermophilus.*[9]

In the present work, the enzyme lumazine synthase from *Brucella spp.* (BLS) has been used as a protein carrier of foreign peptides. BLS is a highly immunogenic protein,[10–11] and behaves as a potent immunogen when injected as a protein or as a DNA vaccine.[12–13] BLS folds as

Abbreviations: BLS, *Brucella spp.* lumazine synthase; VLPs, virus-like particles; CD, circular dichroism; ELISA, enzyme-linked immunosorbent assay; BLS-OMP31, chimera formed by BLS and ten copies of the peptide OMP31; BLS-KETc1, chimera formed by BLS and ten copies of the peptide KETc1; BLS-OMP31-KETc1, polyvalent chimera formed by BLS and five copies each of the peptides OMP31 and KETc1.

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TABLE I. Sequence of the Inserted Peptides and Stability of Chimeric Proteins†

Protein	Peptide sequence	Peptide Length	Peptide pl*	Protein MW (Da)*	†ΔG_{H20}[a] (Kjoule/mole)	T_m (°C)[b]
BLS	SNQSCP	6	5.2	174,429[c]	305 ± 30	86.3 ± 0.1
BLS-KETc12	GNLLLSCLGS	10	5.5	178,495	335 ± 9	87.7 ± 0.1
BLS-MEAM	NIIRTKKQGS	10	11.2	180,177	n.d.	88.8 ± 0.4
BLS-RSVM	HWSISKPQGS	10	8.5	179,996	343 ± 18	88.3 ± 0.1
BLS-TRP2	VYDFFVWLGS	10	5.1	181,055	n.d.	87.5 ± 0.1
BLS-MEAL	INQDPDKILTYGS	13	5.2	183,370	258 ± 14	88.6 ± 0.1
BLS-KETc1	APMSTPSATSVRGS	14	9.8	182,219	340 ± 10	88.1 ± 0.1
BLS-OMP31	NAGYAGGKFKHPFSSFDKEDNEQVSGS	27	6.0	197,775	342 ± 15	86.5 ± 0.1

†For each chimera the sequence of the inserted peptide, its length and its theoretical isoelectric point (pl) is shown as well as the predicted molecular weight of the whole protein. The peptide from the wild-type protein (BLS) replaced in the chimeras is also shown.
[a]The free energy of unfolding in water (ΔG_{H20}) was obtained for each protein from guanidinium equilibrium denaturation curves.
[b]The apparent melting points (Tm) were obtained from thermal denaturation curves.
[c]The MW of the monomer is 17,442.9.
n.d.: not determined.
*Theoretical values were obtained with ProtParam, Swiss Institute of Bioinformatics.[31]

a highly stable dimer of pentamers, displaying an intertwined structure where each monomer has approximately 45% of its exposed surface area buried on monomer–monomer and pentamer–pentamer contacts.[14–15] In that sense, BLS resembles the highly stable pentameric structure of the immunogenic B subunit of the cholera toxin.[7–8] Noteworthy, BLS is resistant to urea denaturation and is more stable to thermal denaturation than the cholera toxin (Tm of 84 and 75°C, respectively). Crystallographic and spectroscopic solution studies allowed us to determine the mechanisms of dissociation and unfolding of the decameric structure, serving as a platform for future protein engineering purposes.[15]

One striking feature shown by the three-dimensional crystal structure of BLS is the disordered nature of its N-terminus. The first ten residues show no electron density, indicating a high degree of conformational freedom.[14] The crystal structure of the homologous enzyme from *S. cerevisiae*, shows the same degree of flexibility at the N-terminus, which is not essential for the overall stability and the enzymatic activity of the protein.[16] Given this fact, we reasoned that the native N-terminus could be exchanged for peptides derived from other proteins, without affecting the folding and stability of the decamer. To probe this strategy, we have set up a modular system to produce chimeric proteins and show that the folding and stability of these chimeric constructs is similar to that of the wild-type protein. We show also that this strategy allows the engineering of polyvalent chimeras able to display different predefined peptides on the same molecular scaffold. The simultaneous display of different peptides can be modulated taking advantage of the particular assembly of BLS. We show that both the stoichiometry and the spatial order can be modified by reassembling of mixtures of chimeras at different steps of the unfolding process. This strategy has potential for vaccine development and other biomedical applications.

MATERIALS AND METHODS

Construction of a Cassette Plasmid for the Engineering of Chimeras

A pET11a plasmid containing the open reading frame of *Brucella* spp. lumazine synthase[17] was digested with the restriction enzymes BamH I and Xba I and the insert was subcloned in the vector pALTER-Ex1 (Promega). Using the Altered Sites II (Promega) site directed mutagenesis kit, two new restriction sites were introduced: an Nsi I site in the two first codons of the 5′ extreme and an Afl II site at the two codons comprising residues 8 and 9 of the wild-type amino acid sequence. The resulting sequence contains a His residue instead of Ala at position 1, and a Leu residue instead of Asn at position 8 of the coding sequence of the wildtype protein. After checking the mutations by sequencing, the construction was subcloned again in the pET11a vector. The mutated pET11a plasmid was digested with the restriction enzymes Nsi I and Afl II, removing the coding sequence of the first eight residues. The wild-type sequence was exchanged for seven different sequences pertaining to the inserted peptide (see Table I). T1
To insert these sequences, two oligonucleotides were synthesized (Integrated DNA Technologies, Inc.) for each peptide in a way that, after annealing, they contain the coding sequence plus the corresponding overhangs for ligation at the Nsi I (5′) and Afl II (3′) ends. The seven generated double-stranded oligonucleotides were ligated with the previously digested mutated pET11a by overnight incubation at 16°C with T4 DNA ligase (Promega). The ligation mix was used to transform competent *E.coli* DH5α cells. The insertion was verified by colony-PCR using as primers one of the peptide-specific oligonucleotides and the T7 terminator primer. The constructions were finally checked by automated sequencing.

Origin of the Inserted Peptides

The sequences of two peptides of eight and 12 amino acids namely KETc1 and KETc12 respectively, originally

described as highly protective against murine and porcine cysticercosis, were employed to produce chimeric constructions.[18,19] RSVM and MEAM represent 8mer peptides that mimic conformational B-cell epitopes on the F protein of respiratory syncytial virus (RSV) and Measles Virus (MV), respectively.[20–21] MEAL comprises a linear epitope of 11 residues from the F protein of Measles Virus (MV).[22] OMP31 peptide comprises a sequence included in an exposed loop of the Outer Membrane Protein OMP31 of *Brucella melitensis.*[23]

Protein Expression and Purification

Chimeric proteins were recombinantly expressed and purified as was previously described for the wild-type protein.[17] The peptides used in this work were synthesized at W. M. Keck Facility, Yale University, USA.

Stability Studies of the Chimeras

The stability studies were conducted using a Jasco J-810 spectropolarimeter, with a spectrum speed of 100 nm/min and a bandwidth of 1 nm. A 5-mm optical path cuvette (Hellma) was used. The proteins were dialyzed against 50 mM phosphate buffer (pH 7.0) and diluted to a protein concentration of 0.2 μM. For chemical denaturation studies, *Brucella* LS and the generated chimeras were incubated with increasing concentrations of guanidinium during 3 h at 25°C, and were centrifuged before measurement. The denaturation was followed by measuring the molar elipticity at 220 nm in function of the increase in guanidinium concentration. Data points were fitted to an equation describing the two-state unfolding transition from a folded pentamer to five unfolded monomers.[15]

Thermal denaturation studies were conducted using the Peltier system (Jasco). The sample in the cuvette was heated from 25 to 95°C at a speed of 4°C/min. The molar ellipticity was measured at 220 nm every 0.5°C. Apparent T_m values were obtained as the transition midpoints after fitting the data points to an equation describing the curve.[15]

Protein Immunization

A rabbit was immunized with 200 μg of LS-OMP31 protein emulsified in complete Freund's adjuvant at day 0 and in incomplete Freund's adjuvant at days 22, 45, and 155 by intramuscular and subcutaneous routes alternatively. The rabbit was bled on days 31, 52, and 180 from the ear's central artery. Six-weeks-old BALB/c mice were immunized with 40 μg of BLS-OMP31-KETc1 mixed chimera emulsified in Stimune adjuvant (Cedi Diagnostics B. V.) at days 0, 22, 45, and 60 by intraperitoneal route. Retro-orbital bleeding of the mice was done on day 70 of the immunization protocol.

ELISA assays

Standard indirect ELISA procedures were followed. rOMP31 Protein was used as antigen (0.1 ug/well) and reactivity of the sera was assayed by incubation with peroxidase-conjugated polyclonal antibodies to rabbit IgG (Dako). For the ELISA against BLS protein and OMP31 and KETc1 peptides, 0.4 ug/well of antigen were used.

Production of Polyvalent Chimeras

The chimeras BLS-OMP31 and BLS-KETc1 at 300 μM concentration were denatured by adding guanidinium hydrochloride to a final concentration of 6 M [Step 2, see Fig. 4(A)]. Alternatively, both chimeras were dissociated by adding guanidinium hydrochloride to a final concentration of 2 M [Step 1, see Fig. 4(A)]. After incubation for 3 h at room temperature, both proteins were mixed and dialyzed extensively against phosphate saline buffer (PBS) containing 1 mM EDTA and 1 mM PMSF. As controls, both proteins were treated in the same form separately. After centrifugation, the supernatants of dialysis were purified by gel filtration in a Superdex 200 column. The proteins eluting at the expected volume for the decamers were collected and concentrated using Centriprep (Millipore) ultrafiltration/filtration. The samples were analyzed by isoelectrofocusing in a PhastSystem (Amersham Pharmacia) using PhastGel IEF5-8 gels. Native gels were run in the same apparatus, using Phastgel homogeneous 12.5% acrylamide. The samples were also analyzed by MonoQ (Amersham Pharmacia) anion exchange chromatography using as a buffer 50 mM Tris (pH 8.5) and a linear gradient until 1 M NaCl.

RESULTS AND DISCUSSION

Construction, Expression and Stability of Chimeric Proteins

Over recent years, a clear relationship between protein quaternary (and higher order) organization and immunogenicity has emerged.[1,2,24–27] Molecular order could affect immunogenicity in at least two related ways. One is the capacity to efficiently crosslink antigen-specific surface immunoglobulins on B cells (BCR). A repetitive and spatially ordered presence of the same epitope would produce a strong signal transduction mediated by BCRs, as has been described using haptens as model antigens.[28] The other effect is that a homopolymeric quaternary structural order implies an increase on the overall stability of the polymer. The higher stability increases the half-live and thus the probability of effectively stimulating antigen-specific B-lymphocytes. In this regard, the high immunogenicity and stability of the decameric BLS resembles the cases of the *cholera* toxin B-subunit and the heat-labile toxin of *E.coli,* two pentameric and highly stable bacterial proteins.[29–30] Noteworthy, BLS is almost as stable to thermal denaturation assays as the homolog LS from *B.subtilis,*[15] which form a 1 MDa icosahedric particle, similar to VLPs. The decameric order of BLS would be an advantage over VLPs for the display of foreign peptides because it would be able to accommodate longer peptides, given its smaller structure and its thermodynamic stability.[15] Besides, BLS can be easily crystallized in several conditions,[14,31] and this property would allow for structural studies to be conducted on the chimeric proteins. These studies will be of value to correlate the immunogenicity of the inserted peptides with their conformation in the

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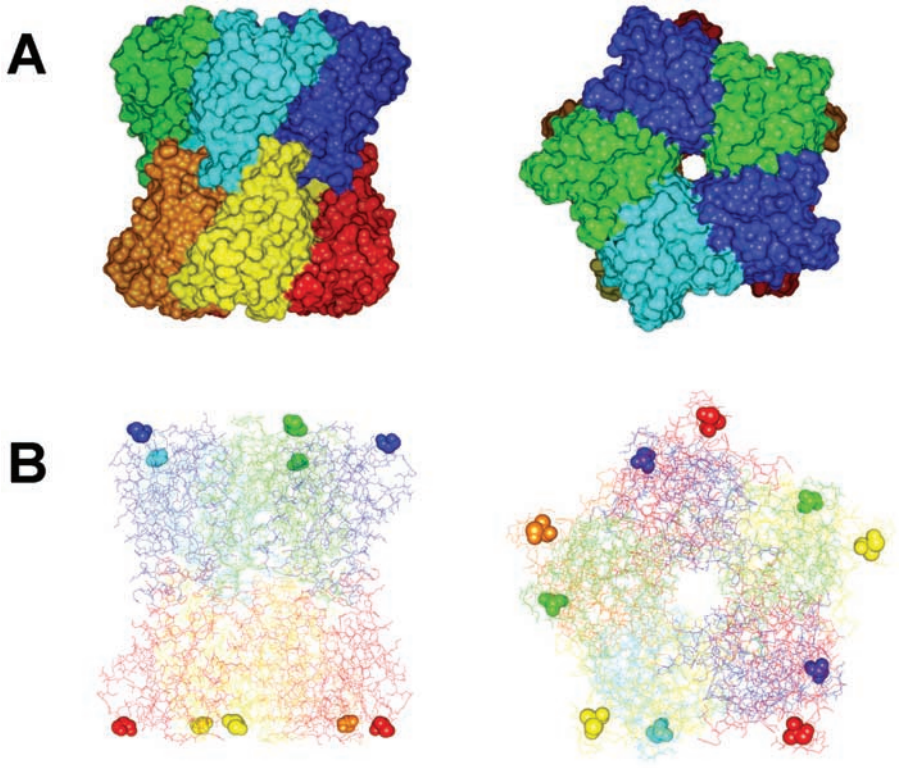


COLOR

Fig. 1. Spatial arrangement of the inserted peptides. The crystallographic structure of BLS is shown. Each monomer of the homodecamer is displayed in a different color. **A**: Space-fill representation in lateral and frontal views highlight the compact spool-like shape of BLS. **B**: Wireframe representations of BLS in the same orientations as in A. The N-terminal residue of each monomer is displayed in space-fill to show the ten points of insertion of the peptides in the decamer.

F1 chimeric proteins. As can be seen in Figure 1, the peptides could be inserted at ten different points located at the vertices of two planar pentagons, separated by a regular distance of around 40 Å and would be displayed to the solvent in a regular array, increasing their intrinsic immunogenicity.

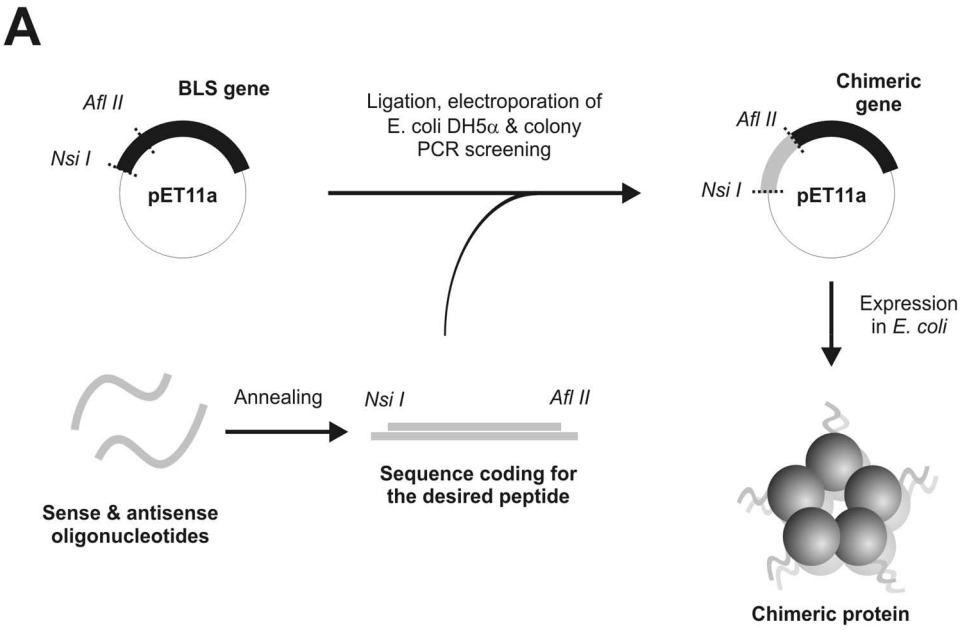
A cassette vector was designed to allow the insertion of different foreign peptide sequences at the N-terminus of
F2 the protein [Fig. 2(A)]. Seven different chimeric proteins were constructed in which the sequence, length, and charge of the inserted peptide were varied (Table I).[31]The insertion of the different peptides did not affect the level of expression of the recombinant proteins [Fig. 2(B)]. The yield of the purified proteins after refolding procedures was also not affected, suggesting that the inserted peptides do not change the folding pathway and overall stability of the decameric assembly.

The far UV circular dichroism spectra of the chimeras are practically superimposable with that of the wild-type protein [see Fig. 3(A) for the analysis of BLS-OMP31 as an
F3 example] indicating that the overall secondary structure is not changed following peptide insertions. As has been described,[15] chemical denaturation of BLS with guanidinium chloride measures the thermodynamic stability of the intermediate pentamers, leading to a cooperative and reversible change in the circular dichroism spectra minima at 222 nm. As can be seen in Figure 3(B) and Table I, the analyzed chimeras were as stable as the wild-type protein, further demonstrating the lack of influence of the inserted peptides on the overall stability of the protein.

Irreversible thermal denaturation analysis showed that the chimeras behave as very stable decamers as compared with the wild-type [Fig. 3(C) and Table I]. These results suggest that in this setting, the inserted peptides would have the stability conferred by the carrier protein, increasing their in vivo half-life by several-fold. Thus, the insertion of different peptides at the N-terminus of BLS does not affect its overall stability, recombinant expression, and refolding properties. The retained properties of the chimeric constructs highlight the usefulness of BLS as a general carrier for the display of peptides to the immune system. The development of this novel carrier system for delivering both linear and conformational epitopes using BLS opens the possibility of assessing these peptide models and to effectively use this new system, which exploits concepts of protein engineering and structural immunology.

Generation of Polyvalent Chimeras

The display of different peptides on the same protein scaffold, opens the possibility for the generation of polyvalent molecules, combining the effect of the individual peptides, or alternatively, changing the way a given peptide is presented to the immune system.[33] These molecular



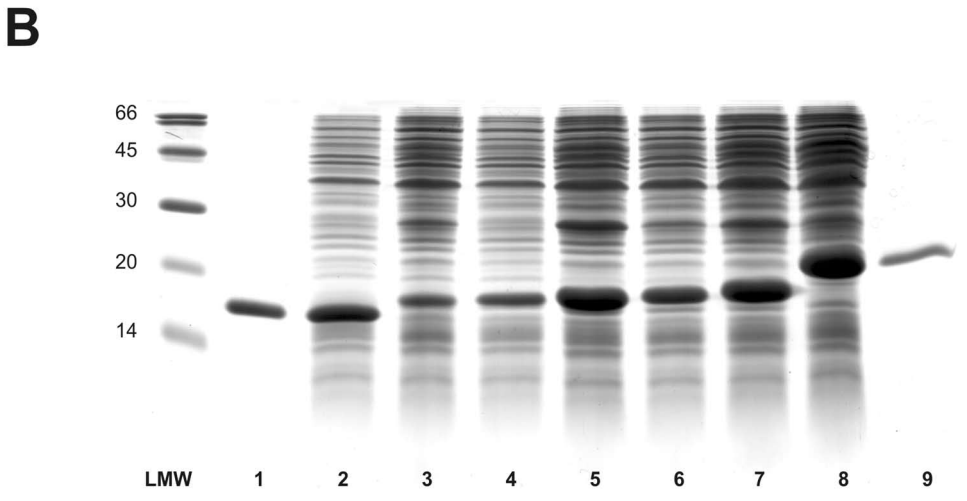


Fig. 2. **A**: Schematic outline of the strategy used to construct the chimeras. A cassette vector was generated to insert different peptides at the N-termini of the enzyme. **B**: Overexpression of chimeric proteins. Crude extracts of induced bacterial cultures expressing LS (**lane 2**) and chimeric proteins LS-KETc12 (**lane 3**), LS-MEAM (**lane 4**), LS-RSVM (**lane 5**), LS-MEAL (**lane 6**), LS-KETc1 (**lane 7**) and LS-OMP31 (**lane 8**) were run on SDS-PAGE. Purified LS (**lane 1**) and LS-OMP31 (**lane 9**) are shown. LMW: Low Molecular Weight markers (kDa).

assemblies would be composed of monomers from two different chimeras displaying a different peptide, giving rise to decamers with these two peptides displayed with different stoichiometries at their ten N-termini. These polyvalent chimeras would be useful tools for the display of different peptides on the same molecular scaffold. For example, a mixed or polyvalent chimera could contain a peptide bearing an accessory T-helper epitope and a second peptide containing a B-cell or cytotoxic T-cell epitope towards which direct a specific immune response. In this way, the immunogen would be able to carry all the necessary elements to elicit a desired protective immune response.[33]

In a previous work, we have elucidated the mechanisms of dissociation and unfolding of the decameric BLS [Fig. 4(A)]. Incubation of the enzyme with 2 M Guanidinium chloride gives rise to intermediate folded pentamers and the further increase on the concentration of the denaturant produces unfolded monomers. Both equilibria are reversible, allowing the use of different protocols for the generation of polyvalent chimeras. Thus, mixing different chimeras at step 1 [see Fig. 4(A)] would render polyvalent

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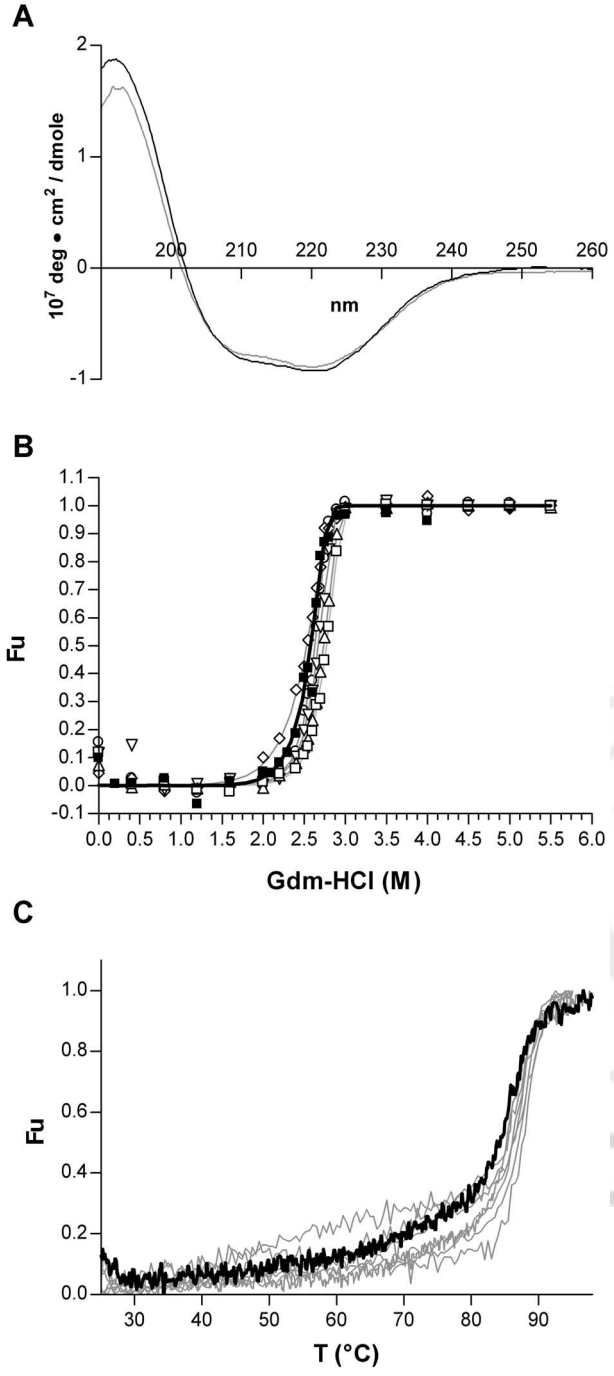

Fig. 3. **A**: The circular dichroism far UV spectra of BLS (black trace) and the chimera BLS-OMP31 (gray trace) are superimposable, showing that the chimeras have the same overall secondary structure of BLS. **B**: The guanidinium denaturation curves of BLS (black trace) and five of the generated chimeras show no significant differences on the stability of the chimeras as compared to the wild-type enzyme. **C**: Thermal denaturation of BLS (Black trace) and the generated chimeras monitored by circular dichroism at 222 nm.

decameric chimeras with each pentamer decorated with a different peptide. In contrast, if the mixing begins at step 2, a complex stochastic combination of polyvalent chimeras would be formed. Experimental testing of this hypothesis showed that this is the case: BLS-OMP31 and BLS-KETC1 chimeras, differing in the length and isoelectric point of the inserted peptides (see Table I) were unfolded, mixed in equimolar concentrations and then refolded. The chimeras mixed at step 1 only formed three species after refolding, as can be seen by anion-exchange chromatography, SDS-PAGE, and native gel analyses [see Fig. 4(B,D) for details]. The presence of only one intermediate species indicates that only folded pentamers carrying different peptides have been combined. In contrast, the chimeras mixed at step 2 generated heterogeneous polyvalent chimeras with intermediate mobility in anion-exchange chromatography [Fig. 4(C)], revealing the presence of decamers with different proportions of the two monomers (see the SDS-PAGE and isoelectrofocusing analyses for details). The bell-shaped chromatogram reveals that the mixed chimeras were produced following random association of monomers into decamers. In line with this analysis, the predominant species appear to be those that contain between four to six copies of BLS-OMP31 monomer per decamer.

These results indicate that BLS can be engineered and manipulated to carry two different peptides in two different ways. One in which the two peptides are distributed randomly and the other in which each face of the spool-like shaped BLS is decorated with a different peptide. This second strategy would be the choice because the local density and spatially-order array of the peptides are important to elicit strong immune responses.

The overall yield of the unfolding, mixing, and refolding procedure is of about 30–50%, revealing the feasibility of the strategy. All the process can be conducted starting from a mixture of urea-solubilized inclusion bodies (results not shown), facilitating the application of the strategy to large-scale production of mixed chimeras.

Immunogenicity of BLS-OMP31 and BLS-OMP31-KETc1

The chimera BLS-OMP31 contains a 27-residues long peptide comprising a putative extracellular exposed loop of the OMP31 outer membrane protein of *Brucella melitensis*.[34] Immunization of a rabbit with BLS-OMP31 induced a good anti-OMP31 antibody response [Fig. 5(A)] and after **F5** three or four immunizations, a serum titer of 1/25,600 was achieved. The elicited anti-peptide antibodies recognize the native membrane protein, as demonstrated by the reactivity of the serum against rough *Brucella melitensis* whole bacteria (not shown). Sera from mice immunized with the BLS-OMP31 chimera, either in the presence or absence of adjuvant, also showed strong anti-OMP31 reactivity. In all cases, the insertion of the OMP31 peptide did not affect the immunogenicity of the carrier protein (not shown). The intrinsic immunogenicity of BLS as a carrier would be an advantage since the adjuvant effect on the inserted peptides allows the use of the chimeric proteins in absence of adjuvants.

The BLS-OMP31-KETc1 mixed chimera obtained from step 1 was also tested for its immunogenicity. Figure 5(B) shows that mice immunized with the mixed chimera elicit

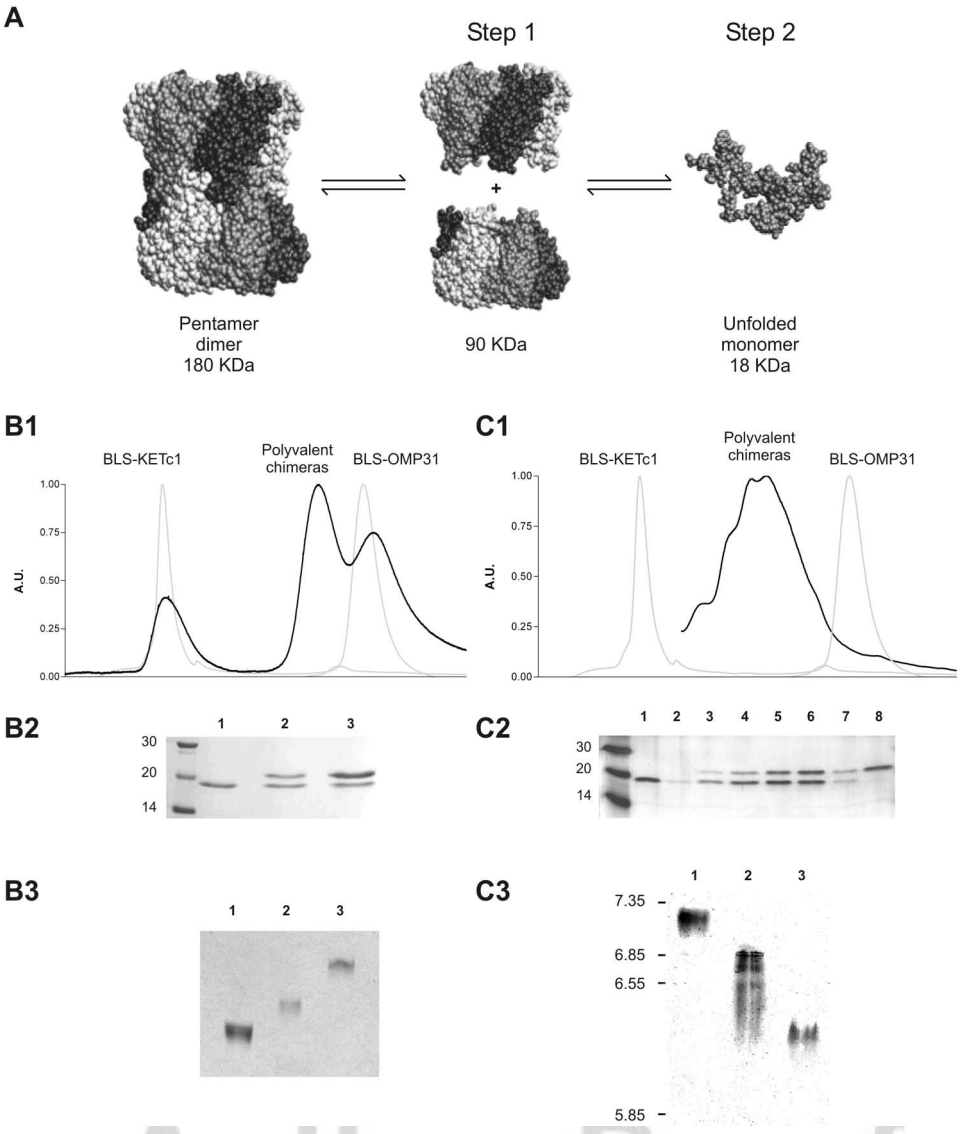

Fig. 4. Generation of polyvalent chimeras. **A**: Schematic outline of the mechanism of dissociation and unfolding of BLS by guanidinium chloride. Polyvalent chimeras (see below) were alternatively generated by mixing BLS-OMP31 and BLS-KETc1 at step 1 (folded pentamers) or step 2 (unfolded monomers) of the reversible equilibria. **B**: Purified BLS-OMP31 and BLS-KETc1 chimeras were unfolded to step 1, mixed and refolded by dialysis. Each chimera was also unfolded and refolded by itself as control. Refolded decameric proteins were purified by gel filtration in a Superdex 200 column for further analysis by anion-exchange chromatography. **B1**: MonoQ chromatograms generated by runs of the different preparations are superimposed. Grey trace: BLS-KETc1 and BLS-OMP31 controls; black trace: mixed chimeras generated at step 1. **B2**: SDS-PAGE analysis of the run of the mixed chimeras. Lanes 1 to 3 are the three peaks observed after MonoQ analysis. Note that peaks 1 and 3 coelute with pure BLS-KETc1 and BLS-OMP31, respectively. Peak 2 shows an equimolar stoichiometry corresponding to a mixed homogeneous chimera. **B3**: Native gel analysis demonstrating the formation of only one homogeneous species of mixed chimera after mixing at step 1. Lanes 1 to 3 are the same samples analyzed in B2. **C**: Polyvalent chimeras generated at step 2. Procedures and controls as described in B. **C1**: MonoQ chromatograms generated by runs of the different preparations are superimposed. Grey trace: BLS-KETc1 and BLS-OMP31 controls; black trace: mixed chimeras generated at step 2. **C2**: SDS-PAGE analysis of the run of the mixed chimeras. **Lane1**: refolded BLS-KETc1; 2-7. Reconsituted polyvalent decamers (peaks overlapped). Note the increasing proportion of LS-OMP31 in the peaks. 8. Refolded LS-OMP31. LMW: Low Molecular Weight markers (KDa). **C3**: Coomassie-stained isoelectric focusing gel demonstrating the formation of proteins of intermediate mobility after mixing at step 2. BLS-KETc1 (**lane 1**) and BLS-OMP31 (**lane 3**) run with isoelectric points of 7.20 and 6.35, respectively. **Lane 2**: Refolded polyvalent decamers (before MonoQ separation) run with pIs between 6.35 and 6.85, demonstrating the heterogeneity of the polyvalent chimeras. Position of pI standards are shown.

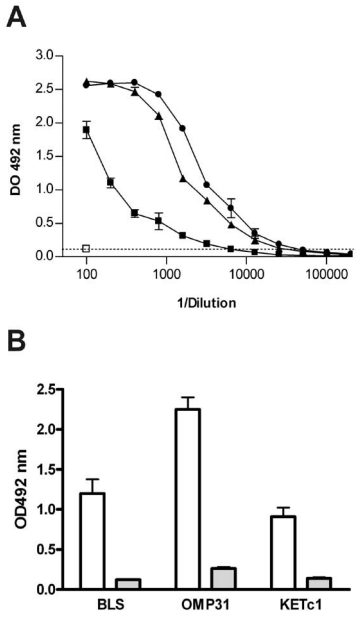

Fig. 5. Immunogenicity of LS-OMP31. **A**: Rabbit sera obtained after the second (filled squares), third (filled triangles) and fourth (filled circles) immunization with BLS-OMP31 were titrated by ELISA using recombinant OMP31 as antigen. Serum from a rabbit immunized with BLS was used as negative control (open square and dashed line). **B**: Sera from mice immunized with the BLS-OMP31-KETc1 mixed chimera from step 1 was assayed against BLS, OMP31, and KETc1 peptides at a dilution 1/100 (open bars). The mean value plus standard deviation of duplicates is plotted. As a control, a serum from a nonimmunized mouse at the same dilution was assayed (grey bars).

a strong antibody response against the two inserted peptides, indicating that BLS as a scaffold is able to present simultaneously two different peptide epitopes to the immune system. Again, the decoration of the protein with two different peptides does not interfere with the immune response against the scaffold protein BLS.

CONCLUSION

We describe in the present work a flexible and versatile system to develop new immunogens based on the remarkable quaternary order and stability of BLS. The ease to engineer and manipulate BLS derivatives opens the possibility to fully exploit this system as a new tool for vaccine design. Experimental testing of the usefulness of the generated chimeras is underway. Preliminary results show that BLS is a potent scaffold for vaccine design. This system would be also useful for the polymeric display of protein domains, given the compact folding and high thermodynamic stability of the decameric BLS. The ability to control the stoichiometry of the presented epitopes in polyvalent chimeras will be used to assay the relationship between epitope repetitivity and immunogenicity. This system might also prove useful in the field of nanobiomaterials by virtue of its flexibility, predefined geometry, and low production costs.

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