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A Novel Expression System for Recombinant Peptide as Inclusion Body

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Introduction

The production of large numbers of targets is a key step in three-dimensional structural studies on peptides and proteins. For example, NMR studies using stable isotope-labeled recombinant peptides/proteins are among the most powerful means of studying peptide and protein structures. Many protein expression systems have been established to produce recombinant proteins. Moreover, a wide variety of strategies have been developed to improve the yield of recombinant proteins. One such strategy, fusion protein systems have generally been used to improve protein folding and solubility, yielding biologically active products. However, these approaches are not always successful. In particular, it is very difficult to produce target peptides and proteins that are lethal to host cells and/or are easily degraded in soluble form.

We present here a novel method of facilitating the expression level in *E. coli* of a recombinant peptide that is difficult to express in conventional production systems. We demonstrated that coexpression of the aggregation-prone protein remarkably enhanced the target peptide's expression level. It seems that overexpression of the partner protein protects the target peptide from proteolytic degradation by forming insoluble inclusion bodies, thus accounting for the higher observed yields. Importantly, this method can isolate the target peptide from the partner protein by simple affinity chromatography, and it never requires chemical or enzymatic cleavage of the fusion protein. These advantages make our new method sufficiently effective and cost-efficient for large-scale production.

Results and Discussion

In the present study, we chose two kinds of proteins as aggregation-prone partners and four kinds of proteins/peptides as expression targets (Table 1). Lysozyme and α-lactalbumin appear to have evolved from a common ancestral protein, as evidenced by the similarity of their amino acid sequences and three-dimensional structures as well as by the high conservation of disulfide bridges. Some studies clearly showed that these two proteins each form a large amount of inclusion body on overexpression in *E. coli*. Interestingly, the isoelectric points of these two proteins are remarkably different despite their sequential similarity. Thus, we selected these proteins (human α-lactalbumin (HLA) and human lysozyme (HLZ)) as good models of aggregation-prone partners in order to confirm the effect of the charge of the partner protein on the overexpression of target peptides/proteins.

ABF, one of our targets, is a small antimicrobial peptide consisting of 67 residues stabilized with four intramolecular disulfide bridges derived from the nematode *C. elegans*. In the case of overexpression in *E.*

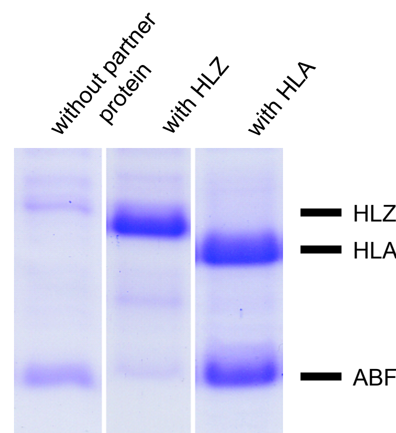

Fig. 1. SDS-PAGE analysis of the partner protein's effect on ABF overexpression.

Table 1. Proteins and peptides in this work.

Protein / Peptide	Origin	M. W. (Da)	pI	Comments	Reference
α-lactalbumin (HLA)	human	14,031	4.8	partner protein	1.
lysozyme (HLZ)	human	14,700	9.3	partner protein	1.
antibacterial factor (ABF)	*C. elegans*	6,999	9.1	target peptide	2.
antibacterial factor (ASABF)	*A. suum*	7,420	9.1	target peptide	2.
fibroin modulator binding protein (FMBP -1)	*B. mori*	24,714	9.7	target protein	3.
hypothetical peptide (PHS001)	*P. horikoshii*	8,744	4.1	target peptide	4.

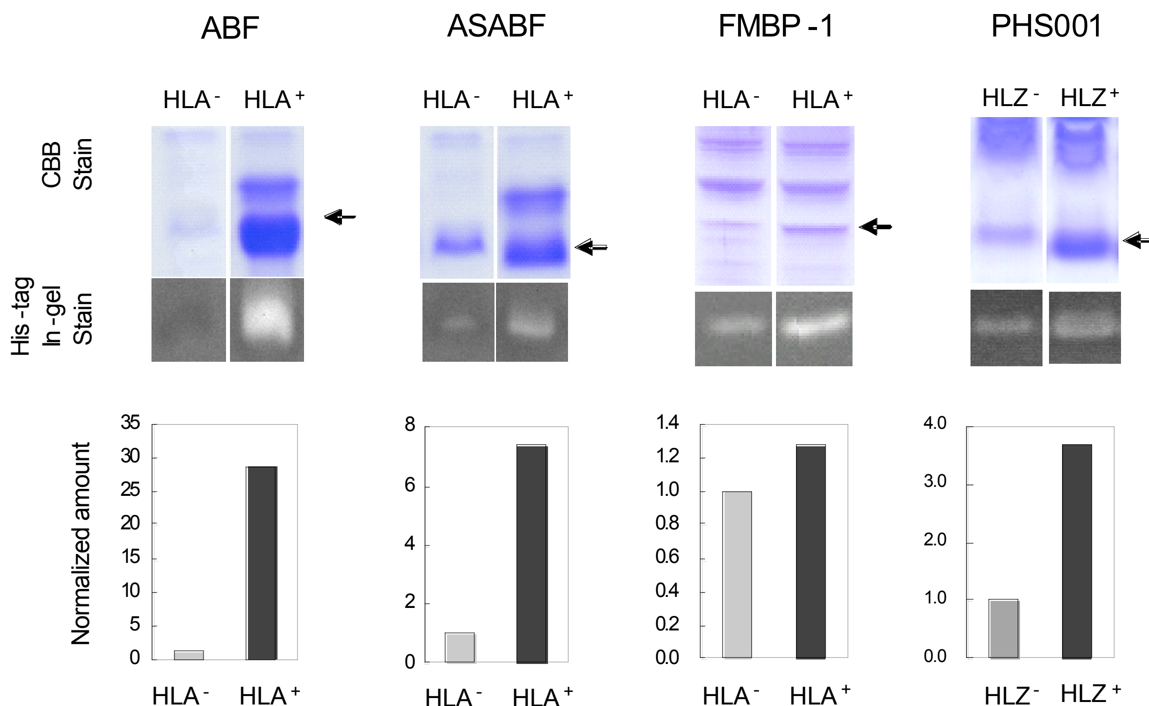

Fig. 2. Comparison of amount of overexpressed inclusion body of target peptides.

coli by using pET vector without partner protein, ABF was hard to synthesize even in insoluble form, probably because of proteolysis (Fig. 1). In the case of coexpression of ABF and HLZ, the amount of expressed ABF was significantly reduced although HLZ was clearly overexpressed as an inclusion body. In contrast, ABF was produced effectively as an inclusion body in the case of coexpression with HLA. This suggested that the isoelectric point of a target molecule is one of the most important factors in the selection of a partner protein.

Figure 2 compares the amounts of overexpressed inclusion body of target peptides with His-tag with the use of the coexpression method. In the cases of ABF and ASABF, coexpression of HLA clearly enhanced overexpression of the target peptides. Unfortunately, in the case of FMBP-1, the effect of HLA coexpression is limited. Because the isoelectric point of PHS001 is low, we utilized HLZ as the coexpression partner for this peptide and succeeded in enhancing productivity.

One merit of our method is that the purification of targets is easy. It is well known that the inclusion body is a relatively pure protein and is usually produced as one large, dense particle. Thus, purification of the inclusion body is very easy. In addition, in our coexpression method, the process of separating a target peptide from a partner protein is also easily achieved by using ion-exchange chromatography, because of the opposite charge. After the refolding procedure, we obtained a large amount of active-form ABF, and a stable isotope-labeled ABF for NMR studies was also successfully produced by this method (Fig. 3).

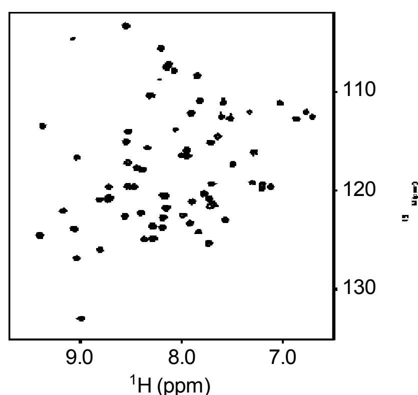

Fig. 3. HSQC spectrum of ABF.

Acknowledgments

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