Enhanced expression of tandem multimers of the antimicrobial peptide buforin II in Escherichia coli by the DEAD-box protein and trxB mutant

Abstract The tandem multimeric expression of various peptides has been explored by many researchers. However, expression levels have usually not been proportional to the degree of multimerization. To increase the expression level in Escherichia coli of tandem multimers of a cationic antimicrobial peptide, buforin II, fused to an anionic peptide, we studied the effect of the DEAD-box protein and the trxB mutant on the expression of tandem multimers. An expression vector with a tac promoter was more effective in directing multimeric expression than one with a T7 promoter. The expression level of large multimers was substantially increased with the tac promoter, possibly through stabilization of long transcripts by synchronization of transcription and translation. Coexpression of the DEAD-box protein, an RNA-binding protein, with the T7 expression system increased the expression level of multimers, especially large multimers, due to protection of the long RNA transcripts. In addition, the use of the trxB mutant also enhanced the expression level of tandem multimers, which contain two cysteine residues at both ends of the monomeric unit. It seems that disulfide bonds formed in the multimers in the trxB mutant might help efficient charge neutralization for inclusion body formation of the multimers, resulting in enhancement of expression. Our results show that the expression of multimers can be improved through the stabilization of the long transcripts by the DEAD-box protein or the expression, under an oxidizing environment, of the trxB mutant in which covalent cross-links through disulfide bonds facilitate inclusion body formation of the multimeric fusion peptide.

Introduction

Many experiments have been carried out to express various peptides as multimers in Escherichia coli as a means towards their efficient mass production (Gigova et al. 1989; Jonasson et al. 2000; J.H. Lee et al. 1996, 1998a, b, 1999; N. Lee et al. 1984; Lennick et al. 1987; McPherson et al. 1992; C.J. Park et al. 1998; Rainegeaud et al. 1996; Schulz et al. 1987; Shen 1984; Stahl et al. 1990). Even though expression of peptides has been significantly improved by tandem-multimeric expression, expression levels are not always directly proportional to the degree of multimerization, especially with multimers of long length (Gigova et al. 1989; Lee et al. 1998a, 1999; C.J. Park et al. 1998; Rainegeaud et al. 1996). In our previous study on the multimeric expression of the basic antimicrobial peptide buforin II fused to an acidic peptide (Lee et al. 1999), we also found that the expression level decreased as the degree of multimerization increased. Two possible reasons for the decreased expression of large multimers were postulated: (1) the instability of T7 transcripts (Chevrier-Miller et al. 1994; Iost and Dreyfus 1994, 1995; Iost et al. 1992; Lewicki et al. 1993) may cause the low level expression of multimers, and (2) the charge interactions enhanced by covalent disulfide bonds in the multimeric peptide may not be stably established due to the reducing environment in the cytoplasm of E. coli, resulting in less efficient neutralization.

The T7 promoter has been widely used for the expression of foreign genes in E. coli because of its strong transcription ability. However, it has been shown that mRNA and rRNA transcribed by T7 RNA polymerase are relatively unstable compared to transcripts derived from the host RNA polymerase. This is a result of the fast movement of T7 RNA polymerase during transcription, which is also asynchronous to translation (Chevrier-Miller et al. 1994; Iost and Dreyfus 1994, 1995; Iost et al. 1992;
Lewicki et al. 1993). This asynchronous transcription and translation caused the decreases in both protein expression and production of active rRNAs. Iost and Dreyfus (1994) reported that coexpression of the E. coli DEAD-box protein, the product of the deaD gene (Toone et al. 1991), greatly increased the expression of β-galactosidase under the control of the T7 promoter. The DEAD family of RNA-dependent ATPases is a ubiquitous class of proteins involved in many facets of RNA metabolism (Chuang et al. 1997; Raynal and Carpousis 1999; Schmid and Linder 1992; Vanzo et al. 1998; Wassarman and Steitz 1991). Although proteins of this family are involved in very diverse cellular functions, they are all thought to share in common an RNA helicase activity, which has been inferred from their ability to bind and dissociate RNA duplexes in an ATP-dependent manner. Since its ability to directly bind to RNA makes it a good candidate for an RNA-protecting molecule for unstable transcripts produced by the T7 expression system (Iost and Dreyfus 1994), we assessed the effect of the DEAD-box protein on the expression of multimers in this system.

For the multimeric expression of buforin II, the basic antimicrobial peptide is fused to an acidic peptide counterpart to neutralize the positive charge of the antimicrobial peptide, which binds strongly to DNA and RNA resulting in cell death (C.B. Park et al. 1998). During the construction of the fusion peptide, cysteine residues were introduced at both ends of the acidic peptide to facilitate the charge interaction. However, the effect of cysteine residues was negligible because the cytoplasm of E. coli is a highly reducing environment, which prevents formation of disulfide bonds (Cornelis 2000). Several researchers reported that a thioredoxin reductase (trxB) mutant strain induces stable disulfide bonds in the cytoplasm (Bessette et al. 1999; Derman and Beckwith 1995; Derman et al. 1993; Proba et al. 1995; Schneider et al. 1997). Therefore, we also examined the effect of covalent cross-links through inter- or intramolecular disulfide bonds on the expression of multimers of the fusion peptide in the cytoplasm using the trxB-deficient E. coli.

**Materials and methods**

Strains, vectors and enzymes

E. coli strain XL1-Blue (Stratagene, La Jolla, Calif.) was used as a host for subcloning; strains BL21(DE3) and AD494(DE3) (Novagen, Madison, Wis.) were used for gene expression. E. coli cells were grown in LB medium at 37°C and 50 µg/ml ampicillin was added for plasmid-containing cells. Vector pUC-deaD (Iost and Dreyfus 1994) containing a deaD gene, was kindly provided by Dr. S. Y. Lee, Korea Advanced Institute of Science and Technology. Restriction enzymes and modifying enzymes were purchased from New England Biolabs (Beverly, Mass.) and used according to the recommendations of the supplier. A mini-scale preparation of vector DNA was carried out using the alkaline lysis method (Maniatis et al. 1982) and large quantities of vector DNA were prepared by the PEG precipitation method (Sambrook et al. 1989). Other recombinant DNA techniques were as described by Maniatis et al. (1982) and Sambrook et al. (1989).

Construction of expression vectors containing the multimeric MMIS-buforin II fusion peptide genes under the control of a tac promoter

For the expression of the antimicrobial peptide buforin II in E. coli, the basic antimicrobial peptide buforin II was fused to the acidic peptide (MMIS), which was derived from the magainin intervening sequence by adding a cysteine residue on each end. The monomer encoding the fusion peptide is shown in Fig. 1A. Multimeric MMIS-buforin II fusion genes were constructed using the method described in our previous report (Lee et al. 1998b). The multimers MBn, where n means the number of monomeric units in each multimer, were cloned into vector pET21c, resulting in pET21c-MBn (Fig. 1B). The expression vector pETACC, in which the region containing the T7 promoter was replaced by a fragment containing the tac promoter, was constructed as follows. pET21c was digested with XbaI and the 5.44-kb vector fragment was treated with Klenow fragment and digested with BglII, producing a 5.38-kb vector fragment. The 5.44-kb vector fragment was treated with Klenow fragment and digested with BglII, producing a 5.38-kb vector fragment prepared above, resulting in vector pETACC. The expression vectors pETACC-MBn (Fig. 1B) were constructed by cloning the BamHI-HindIII fragments containing multimeric peptide genes, which were isolated from the expression vectors pET21c-MBn, into pETACC digested with BamHI and HindIII.