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REVIEW ARTICLE

Expression systems of human β-defensins: vectors, purification and biological activities

L. L. Corrales-Garcia · L. D. Possani · G. Corzo

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Abstract Human beta-defensins are 2–5 kDa, cationic, microbicidal peptides, which represent the first-line host defense against several Gram-negative and Gram-positive bacteria, fungi and viruses. They contain a conserved disulfide-bridge pattern of three pairs of intramolecular cystine bonds. The well-known public health problem related with the growing number of multiresistant bacteria has driven research to look for novel antibiotics, such beta-defensins and a feasible way to produce them. Heterologous expression of beta-defensins could be one way to generate large quantities of beta-defensins for clinical research; however, heterologous expression of beta-defensins has some biochemical problems, such toxicity toward the host cell, peptide degradation by proteolytic cell enzymes, size, folding constrains and low recombinant peptide yields. In this communication, several heterologous systems for producing human beta-defensins are reviewed.

Keywords Antibiotic · Bacteria · Defensin · Heterologous expression · Antimicrobial · Peptide

L. L. Corrales-Garcia · L. D. Possani · G. Corzo
Departamento de Medicina Molecular y Bioprocesos, Instituto de Biotecnología, Universidad Nacional Autónoma de México, UNAM, Apartado Postal 510-3, 61500 Cuernavaca, Morelos, México

G. Corzo (✉)
Institute of Biotechnology, UNAM, Av. Universidad 2001, Cuernavaca, Morelos 62210, Mexico
e-mail: corzo@ibt.unam.mx

Introduction

Antimicrobial peptides represent the first-line host defense against pathogens. They act as part of non-specific response of multi-cellular organisms, among which are plants, insects, birds, amphibians, lower eukaryotes and mammals (Bensch et al. 1995; Hancock and Chapple 1999; Hancock and Diamond 2000; Hancock 2001; Schneider et al. 2005; Aerts et al. 2007), but they cannot recognize specific antigens. One of the first antimicrobial peptides found by Fleming was lysozyme (Fleming 1922) and since then much work has been performed to find new and strong antimicrobial peptides. A considerable amount of antimicrobial cationic peptides has been reported during the last 25 years. Some antimicrobial peptides have common characteristics, such as short length (15–47 amino acid residues) and high basic net charge, having at least four lysines and/or arginine residues, as is the case of human β-defensins. Among the antimicrobial peptides, the defensins constitute a significant peptide family. They have been found broadly in plants, insects, reptiles, birds and mammals (Lehmann et al. 2002; Lehrer and Ganz 2002; Zasloff 2002). They have been identified directly in different tissues and organs or by cloning and genome analysis. Mammalian defensins are divided into three groups, α-defensins, β-defensins and θ-defensins, differing in their disulfide-bridge pattern and number of cationic charges. To date, six human α-defensins (HNP-1-4, HD-5 and HD-6) (Lehrer 2004) and 31 human β-defensins (HBD1-31) have been reported and all of them are considered part of the immune system (Schutte et al. 2002). Apart from being short-chain peptides with a net positive charge, their tridimensional structures are tightly folded by three disulfide bridges. Defensins exhibit a broad antimicrobial activity spectrum and usually show low

susceptibility to generate bacterial resistance (Ganz 2002, 2003; Song et al. 2009).

The β-defensins are cationic peptides showing three strands of antiparallel β-sheets that provide a compact small structure (Schneider et al. 2005) and also posses an α-helix at the N-terminal region (Hoover et al. 2000, 2001; Schibli et al. 2002; Hoover et al. 2003; Dhople et al. 2006) (PDB codes for HBD1, HBD2 and HBD3 are 1IJU, 1FD3, 1KJ6, respectively). Usually contain 38–47 amino acid residues with a molecular weight from 4 to 5 kDa. They are highly cationic at neutral pH due to a high number of lysine and arginine residues (Schöder and Harder 1999). They all contain a triple antiparallel β-sheet stabilized by three disulfide bridges (Schneider et al. 2005) made between six cysteine residues. Figure 1 shows representative structures of human β-defensins. A large list of human β-defensin genes was uncovered using computational techniques and the human genome available (Schutte et al. 2002). High-resolution structure of human β-defensins 1, 2 and 3 have been elucidated using X-ray crystallography and NMR spectroscopy (Hoover et al. 2000; Schibli et al. 2002); however, there is still much to be learning about the structure–function relationship of human β-defensins. Similar to other antimicrobial peptides, the principal function of β-defensins is to control bacteria and fungi growth and development by affecting their surrounding membrane surface, such as the case of HBD2 and HBD3 (Harder et al. 2001) or by acting into phagolysosomes (HBD1) (Schöder and Harder 1999). They are molecules involved in the innate immunity defense system in mammals and their main purpose is to protect the organisms and their systems against microorganism invasion. Up to date, several reports have demonstrated the chemoattractant activity of β-defensins (Dhople et al. 2006; Wong et al. 2007); however, other reports such as Hoover et al. (2003) have confronted this idea only if the β-defensin is not folded correctly. As more β-defensins will be experimentally assayed and their positive or negative chemoattractant activities will be established. Furthermore, pathogenic bacteria seem to have a low possibility to become resistant to defensins (Song et al. 2009). Human defensins are endogenous antibiotics with broad bactericidal activity toward Gram-positive and Gram-negative bacteria, not only affect a few pathogenic fungi (Schneider et al. 2005; Schwaab et al. 2009), capsulate viruses and protozoa (Mathews et al. 1999; Zasloff 2002; Schneider et al. 2005; Schwaab et al. 2009). Since 1995, at least four β-defensins have been characterized in humans (Lehmann et al. 2002; Schwaab et al. 2009). Human defensins exert antimicrobial activity against Gram-positive and Gram-negative bacteria, such as *Mycobacterium tuberculosis*, *Treponema pallidum*, *Chlamydia trachomatis*, *Listeria monocytogenes* (Porter et al. 1997), *Staphylococcus aureus* (Wu et al. 2003), *Escherichia coli* and *Pseudomonas aeruginosa* (Harder et al. 1997; Zasloff 2002; Schneider et al. 2005; Xu et al. 2006a; Schwaab et al. 2009), yeast such as *Candida albicans* (Harder et al. 1997; Zasloff 2002) and other fungi. They also can affect enveloped viruses (Harder et al. 1997; Porter et al. 1997; Zasloff 2002; Schneider et al. 2005; Xu et al. 2006a; Schwaab et al. 2009). It is possible that β-defensins have appeared as alternative antimicrobial agents and could be used in therapeutics, food preservative and novel antibiotic drugs.

The heterologous expression system of β-defensins has several biochemical problems, such as toxicity toward the host cell, peptide degradation by proteolytic cell enzymes and their small size (Valore and Ganz 1997). Moreover, β-defensins are not easily detectable. Therefore, understanding the mechanisms and activities of the β-defensins have been hampered by the lack of appropriate expression systems, which could produce significant amounts and effective biological quality of these types of antimicrobial peptides. The possible production of human β-defensins by means of genetic engineering would allow obtaining a sufficient amount of peptide for complementary studies and assays. It would be important for control of resistant pathogen bacterial infections, but would allow conducting additional studies on their molecular interactions and antimicrobial mechanisms, as well on their eventual use in public health care. In addition, β-defensins are not expected to induce use-dependent resistance microorganisms; thus, becoming a perfect natural antibiotic. Therefore, the purpose of this communication is to revise the state of the art on the study of human β-defensins concerning their expression systems and biological activities of the recombinant products.

Heterologous expression of β-defensins

The prominent public health problem related with the growing number of multi-resistant bacteria motivated further research on the subject, leading to the discovery of natural existing peptide that could be used in solving the problem. A few years ago, the ways of obtaining these small cationic peptides were (1) isolation from the host organism, which requires large amounts of material from the source with the problem of having very low yields of the interest peptide; and (2) chemical synthesis of peptides, which generate high costs and low yields because of the in vitro folding steps. Therefore, genetic engineering became a great strategy to produce large amounts of interesting cationic peptides with low cost, to produce possible variants using site-directed mutagenesis, and to elucidate the antimicrobial mechanisms and to improve the final yields.

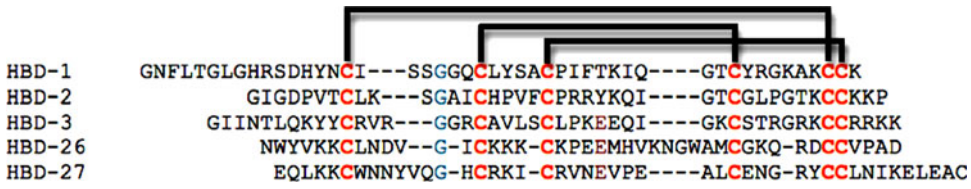

Fig. 1 Linear structures and alignment of human β-defensins

The production of heterologous proteins have been studied for years, established and developed in different kind of expression systems, such as prokaryotes, yeast, plants and viruses (Terpe 2006). In recent years, genetic engineering has become one of the most attractive methods to obtain recombinant proteins from microorganisms, due to their fast growth, high product density with a low cost substrate, well characterized genetic and mutant host strains and cloning vectors availability (Terpe 2006). Table 1 summarizes most of the studies on the heterologous expression of β-defensins. However, the rational choice of appropriate promoter system and host for production of a specific interest protein is difficult and requires much attention and thoughts (Terpe 2006). Indeed, selection of *E. coli* strains with low proteolytic activity and the use of carefully designed plasmids to prevent the toxic activity of β-defensins is decisive for obtaining cationic antimicrobial peptides (Fang et al. 2002). Among the important steps taken towards a rational design for successful expression of recombinant β-defensins are the choice of different fusion protein hybrids which protect from protein degradation inside the cell, and prevents the antimicrobial activity of the peptide against the host cell, but allows in identifying the hybrid produced in the expressing system (Piers et al. 1993). Finally, this fusion protein can be released by specific proteolytic action, leaving no extra amino acids on the N- and C-terminal regions (Piers et al. 1993; Terpe 2006; Xu et al. 2006a).

Basic to the successful recombinant production of β-defensins is to choose a system that permits a correct folding of the peptide, with accurate disulfide-bridge formation, assuring in this way the effectiveness of the antimicrobial action (Estrada et al. 2007). Hence, it is necessary to evaluate and consider the potential of some vectors and expression systems because many bacterial systems are not able to conduct post-transcriptional modifications on proteins, thus, some of the bacterial expression systems could not be appropriate for the production of heterologous proteins. This would require the implementation of additional steps for solving the problem. Obtaining recombinant proteins in the format of inclusion bodies inside the bacterial cell, selection of alternative hosts such as yeast, insects, plants (Terpe 2006) would offer a feasible solution for β-defensin production. In the following sections, several expression systems of β-defensins will be revised.

Bacteria

To study β-defensin mechanisms and antibacterial activity, it is imperative to produce high amounts of active isoforms. One of the best expression systems studied used as host strain to produce human β-defenses is *E. coli*. It is used both in the subcloning as in the expression of proteins. Nevertheless, it is important to consider molecular biology troubles to overcome when *E. coli* is used as a host strain, such as preferential codon usage and survival of strain when an antimicrobial molecule is produced. Codon optimization improves defensins production (Peng et al. 2004b). In most cases, the sequence of the mature defensin gene is obtained from information deposited in public data base, such as NCBI; chemically synthesized and inserted in a cloning plasmid, such as pBluesript II SK (+) (Cipáková et al. 2004), pGEM-T (Promega®) (Fang et al. 2002; Chen et al. 2005; Huang et al. 2006; Xu et al. 2006b; Song et al. 2009; Vargues et al. 2009). The constructed gene is then expressed using plasmids like: pLMM (Cipáková et al. 2004), pET-32 (+) (Stratagene®) (Peng et al. 2004a; Huang et al. 2006; Xu et al. 2006a, b; Huang et al. 2008; Song et al. 2009) and pET-28 (+) (Stratagene®) (Fang et al. 2002; Vargues et al. 2009). For most cases, the product is expressed in the format of hybrids, that is, the defensin gene is fused to a carrier protein.

Fusion proteins

All these designs rely on different fusion proteins bound to the peptide of interest, working as a carrier of the defensin peptide. The function of the carrier protein is to protect the small cationic peptides against proteolytic degradation (Piers et al. 1993). In addition, the fusion system provides several advantages (1) they protect the hybrid when proteases are exported to the external environment, (2) the carrier protein might show affinity for a specific ligand, which enables easy purification not only might prevent antimicrobial peptide toxicity against the host cell and (3) the carrier protein can finally be released by specific proteolytic action, leaving no extra amino acids on the C- or N-terminal regions. Among the fusion proteins are glutathione *S*-transferase (GST) (Piers et al. 1993; Wang et al. 2004; Li-Gang et al. 2007); the presence of histidine tails, which permits an easy purification of the hybrid protein;

Table 1 Different molecular systems of beta-defensins expression

	HBD-1	HBD-1	HBD-2	HBD-2	HBD-2
Clone plasmid	pBluesript II SK (+)	pBluesript II SK (+) and pPIC9 K	pGEM-T	pGEM-T	pGEM-shBD2
Strains (Cloning)	*E. coli* TG1	*E. coli* TG1	*E. coli* TG1	*E. coli* DH5a	*E. coli* DH5a
Expression plasmid	pLMM	pVT103L	pET-32 (+)	pET-28a (+)	pIVEX2.4c-TrxA-shBD2
Expression strain	*E. coli* AD202	*S. cerevisiae* strain AH22	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	Free-cell system (CECF)
Fusion protein	LMM	None	TrxA	N-term + 35 aa + 6-His	TrxA (from pET32)
Promoter	Lac	ADH1	T7/Lac	T7/Lac	T7
Culture conditions	37°C up to 0.7–0.8 OD	28°C, 250 rpm; 120 h up to 0.5 OD	37°C, 250 rpm; up to 0.7 OD	30°C, 200 rpm up to 0.4 OD	30°C, 990 rpm, 24 h
Inductor	100 μM IPTG	None	0.8 mM IPTG	0.8 mM IPTG	None
Expression time	8 h	No	8 h	4 h	None
Induction temperature	37°C	No	37 and 28°C	37°C	None
Yield	1 mg HBD1/6 g cell (dry weight)	55 μg/L	346 mg/L 28°C; 235 mg/L 37°C	DNS	2 mg/mL CECF
6×-His	No	No	Yes	Yes	Yes
Protein obtained	Soluble	Soluble	Soluble	Soluble	Soluble
Folding data	None	None	None	None	None
Biological activity	*E. coli* clinical isolates	*E. coli* ML35-p (dose 150 ng)	*E. coli* K12D31 >5 μg/mL	DNS	*E. coli* D31
Culture medium	LB	YPD (for cell growth) and TSB (antimicrob)	LB, MBL	2×YT	Kit for cell-free systems
Reference	Cipáková et al. 2004	Cipáková and Hostinová 2005	Xu et al. (2006a, b)	Fang et al. 2002	Chen et al. (2005)

	HBD-2	HBD-2	HBD-2	HBD-3	HBD-3 -4
Clone plasmid	DNS	pGEM-T easy	pGEM-T	pGEM-T	pET32-shBD3 pET32-shBD4
Strains (cloning)	*E. coli* TG1	*E. coli* Top 10	*E. coli TG1*	*E. coli* DH5α	*E. coli* DH5α
Expression plasmid	pET-32a (+)	pET-28a (+) {pET-31b (+)}	pET-28a (+) y pGEX-4T-2	pET-32a (+)	pIVEX2.4c
Expression strain	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	Free-cell system
Fusion protein	TrxA	Keto-steroid isomerase (KSI)	GST and multiple HBD2 genes	No	trxA from pET32; GFP from pBluescript-GFP
Promoter	T7/Lac	T7/Lac	T7/Lac	T7/Lac	T7
Culture conditions	37°C, 250 rpm up to 0.4 OD	37°C, 200 rpm up to 0.5 OD	30°C, 200 rpm; up to 0.4 OD	37°C, 200 rpm; up to 0.6 OD	30°C, 300 rpm, 3 h
Inductor	0.8 mM IPTG	1 mM IPTG	0.8 mM IPTG	0.6 mM IPTG	DNS
Expression time	8 h	6 h	4 h	4 h	DNS
Induction temperature	25 and 28°C	37°C	37°C	37°C	DNS
Yield	1.3 g/L batch de 10L	HBD-2 oxidized 1–2 mg/L	DNS	1 g/L protein and 23% HBD3	trx-HBD-3: 390 trx-HBD-4: 385 GFP-HBD-3: 310 GFP-HBD-4: 320 μg/mL
6×-His	Yes	Yes	Yes	Yes	Yes (in pIVEX2.4c)
Protein obtained	Soluble	Inclusion bodies, guanidine 6 M	Inclusion bodies	Inclusion bodies	Soluble
Folding data	None	Yes	None	None	None
Biological activity	None	*E. coli* K12 MG1655; *P. aeruginosa* (PAO1), *S. aureus* (ATCC 25923), *C. albicans* (J2922) >5 μg/mL	No	*S. aureus* ATCC 25923 (0.195 g/mL); *E. coli* ATCC 25922 >25 g/mL	No

Table 1 continued

	HBD-2	HBD-2	HBD-2	HBD-3	HBD-3 -4
Culture medium	LB y MBL (Jar fermenter)	2×YT	LB	LB (for cell growth) and brain–heart infusion for antimicrobial activity	Kit for cell-free systems
Reference	Peng et al. (2004a, b)	Vargues et al. (2009)	Wang et al. (2004)	Song et al. (2009)	Chen et al. (2007)

	HBD-3	HBD-3	HBD-4	HBD-5-6	HBD-26-27
Clone plasmid	pGEX-KG	pGEM-T	pGEM-T	pDrive	pDrive
Strains (cloning)	*E. coli* Top 10	*E. coli* TG1	*E. coli* TG1	*E. coli* NovaBlue	*E. coli* NovaBlue
Expression plasmid	pGEX-KG- HBD-3	pET-32a (+)	pET-32a (+)	pET-32a (+)	pET-32a (+)
Expression strain	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)	*E. coli* BL21(DE3)
Fusion protein	GST	TrxA	TrxA	TrxA	TrxA
Promoter	Lac	T7/Lac	T7/Lac	T7/Lac	T7/Lac
Culture conditions	Up to 0.3–0.5 OD	32°C, 250 rpm up to 0.4 OD	37°C; up to 9 OD	32°C; 220 rpm up to 10 OD	32°C; up to 10 D.O
Inductor	0.5 mM IPTG	0.5 mM IPTG	0.4 mM IPTG	0.4 mM IPTG	0,4 mM IPTG
Expression time	DNS	6 h	6 h	4 h	4 h
Induction temperature	DNS	DNS	34°C	DNS	4 h; 32°C
Yield	DNS	trx-HBD-3: 0.99; trx-HBD-4: 0.09 g/L	0.689 g/L	0.38 g HBD5/L; 0.36 g HBD6/L	0,31 g/L HBD26; 0,31 g/L HBD27
6×-His	No	Yes	Yes (between Trx and hBD4)	Yes (between Trx and hBD)	Yes
Protein obtained	Soluble	Soluble	Soluble	Soluble	Soluble
Folding data	None	None	None	None	None
Biological activity	*S. aureus* (ATCC25923) 12.5 μg/mL	*E. coli* K12D31	*E. coli* K12 D31, *P. aeruginosa* ATCC 27853	*E. coli* K12	*E. coli* K12, *S. aureus* and *S. cerevisiae*
Culture medium	LB	LB y MBL (jar fermenter)	LB, MBL (for expression), Muller–Hinton (antimicrobial)	LB, MBL (for expression), Muller–Hinton (antimicrobial)	LB, MBL (for expression), Muller–Hinton (antimicrobial)
Reference	Li-gang et al. (2007)	Huang et al. (2006))	Xu et al. (2006a, b)	Huang et al. (2008)	Huang et al. (2009)

DNS data not shown

thioredoxin fusion protein (Trx) (Peng et al. 2004a; Chen et al. 2005; Huang et al. 2006; Xu et al. 2006b; Huang et al. 2008, 2009) which helps the disulfide-bridge formation and facilitates accurate folding process. In addition, Trx interact with human β-defensins and shades their antimicrobial action (Huang et al. 2006) avoiding the interaction with bacterial membrane, thus protecting the host cells from cationic peptide activity (Piers et al. 1993). Other constructions have used: green fluorescent protein (Chen et al. 2007); light meromyosin (Cipáková et al. 2004) and ketosteroid isomerase (Vargues et al. 2009). In short, the use of defensins joined with carrier proteins has become a possible method to reduce toxicity, increase yield and improve biological activity by facilitating the correct disulfide pairing.

Induction, purification, folding and biological activity

The induction of the protein of interest is certainly related to the plasmid characteristics chosen, but alternatively it is convenient to consider the use of a strong promoter to easily regulate the expression of the desired product. The molecular design details of expression vector can be crucial to the level of protein production (Bass and Yansura 2000). Upstream of the HBDs mature sequences, it is usually suggested to use a histidine tag and an enterokinase recognition site, which will be important for protein purification using affinity chromatography and for releasing the desired peptide from the hybrid protein. Proper peptide folding is then required for effective antimicrobial activity against pathogenic bacteria (Lehmann et al. 2002).

Limitations and yield

One of the main limitations in the expression of β-defensins is the amount of active product. Although some reports have claimed a large production of β-defensins (Peng et al. 2004a, b; Song et al. 2009), they have failed to prove the correct folding and activity of such antimicrobial peptides (see Table 1).

Yeast and fungi

The use of the yeast *Saccharomyces cerevisiae* as host for the production of β-defensins offers several advantages. It does not require induction for expression (Cipáková and Hostinová 2005) and it does not need the use of cleavage factors, nor uses additional systems for proper folding. Therefore, the effective biological activity of the antimicrobial peptide could be used at the end of the expression.

Strains and vectors

The most use strain has been *S. cerevisiae* AH22 (*MAT*a *leu*2-3 *leu*2-112 *his*4-519 can1^{R}) ATCC 38626 (Cipáková and Hostinová 2005). The yeast plasmid pVT103L (6.3 kbp) confers the capability of growing in media lacking leucine and the plasmid marker Leu^{+} exhibits stability during mitotic cell division. The deficient *leu*2-d gene of the pVT103L vector confers a high copy number to this plasmid in yeast.

Induction, purification, folding and biological activity

It has been demonstrated that the expression in yeast of the defensin HBD1 by genetic engineering was successful. Expressed and purified recombinant protein demonstrated bacterial killing against *E. coli* ML35p as low as 150 ng dose, and the effect was shown to be peptide concentration-dependent (Cipáková and Hostinová 2005). This β-defensin was purified by CM Sepharose and it was not subject to any additional folding process. The positive result in antimicrobial activity was correlated with the correct peptide folding (Cipáková and Hostinová 2005).

Limitations and yield

During the production of HBD1, Cipáková and Hostinová (2005) found that the amount of secreted β-defensin obtained by liquid fermentation, 250 rpm, 28°C in YPD media for 120 h, was dropping along the culture time, from 55 μg/l at 48 h to 9 μg/l at 120 h. The diminished product yield was correlated with the low stability of HBD1 at 28°C and proteolytic activity inside the fermentation media more than with the inhibition of *S. cerevisiae* growth due to β-defensin expression. Indeed, they showed that the transformants secretes into the medium and stands the presence of HBD1 (Cipáková and Hostinová 2005).

Plant cells

The first defensin found in plants was γ-threonine, which was isolated from wheat and barley grains in the 1990s. Later on, it was demonstrated that the same defensin was present in all plant kingdom. Plant defensins are active molecules acting against phytopathogenic fungi (such as *Botrytis cinerea*) (Aerts et al. 2007). Seeking to demonstrate if human defensins could confer protection against fungus attack, the plant species *Arabidopsis thaliana* was used as a recombinant system to obtain human β-defensin 2. The bacteria *Agrobacterium tumefaciens* GV3101 was transformed with the vector pCMPG7057 harboring HBD2-coding sequence. HBD2 was coupled to a leader peptide

DmAMP1, which is part of plant defensins, to go throughout the plant secretory system to obtain finally a correct disulfide bond formation. The presence of the transgene HBD2 in transformant plants was confirmed using PCR analysis with HBD2-specific primers to amplify the region of the inserted gene. After *A. thaliana* growth, the recombinant protein was obtained from the transgenic leaves. It was proved that the over expression of HBD2 conferred resistance against fungal infection (Li et al. 2003; Aerts et al. 2007). Therefore, it is possible to produce HBD2 and other defensins using plants as expression systems for obtaining higher quantities of the antimicrobial peptides.

Phage/baculoviruses

Variants of human β-defensin 1 containing different amino acid chain lengths (36, 39 and 42 residues) were produced using an insect cell/baculovirus protein expression system through ligation of inserts into the BacPAK9 transfer vector (Clontech). The vector was transformed and amplified in *E. coli* XL-1 blue, then co-transfected with the transfer vector as the defective baculovirus into *Spodoptera frugiperda* Sf21 insect cell line. Finally, viable recombinant baculoviral clones were harvested from culture medium after co-transfection. Individual viral clones were selected using a plaque assay (Porter et al. 1997; Valore et al. 1998). Additional work reported the use of the nuclear polyhedrosis virus *Autographa californica*, which generates baculoviral polyhedrin (Polh) that protects virus particles from biochemical or physical degradation. Polyhedrin also keeps virus infection potential, as a fusion partner during recombinant protein obtained in *E. coli* expression systems and leads to the formation of inclusion bodies (Wei et al. 2005). These phage systems could be used to express β-human defensins against pathogenic Gram-negative as Gram-positive bacteria (Valore et al. 1998; Wei et al. 2005), but there are still issues to be solved, such as establishing whether the recombinant peptides produce in insect cells posses the adequate folding system, despite its large possibilities of the disulfide-bridge formation. Nevertheless, it is important to consider that HBD1 and HBD2 can fold to form native disulfide-bridge pairing structures in vitro, whereas HBD3 generates a complex mixture of products under similar conditions. It is possible that the higher number (one-third of the peptide) of positive charges in HBD3 may have contributed to impede the correct native-like folding (Wu et al. 2003).

Free-cell systems

Wall and cell membrane disruption, pore formation, lost of internal homeostatic equilibrium and cell death are related to possible human defensins activities. To avoid these damages in the protein expressing cell, a cell-free system could be used as an alternative for producing harmful molecules (Chen et al. 2007). This in vitro system might work in the absence of cells, since all the required transcription and protein translation machinery is still present for the production of proteins (Chen et al. 2006, 2007).

Concluding remarks

Finding the correct folding conditions is an important issue for heterologous expression of proteins, particularly for specific disulfide-bridge formation that allows the recombinant molecules to be biologically active. Table 1 shows several expression systems for the production of β-defensins, including the production of possibly folded three disulfide-bridged human β-defensins by heterologous means. Unfortunately, there are poor demonstrations of correct folding of the peptides. The validation of the results is mainly based on the biological activity (Fang et al. 2002; Cipáková et al. 2004; Peng et al. 2004a; Chen et al. 2005; Cipáková and Hostinová 2005; Huang et al. 2006; Xu et al. 2006a, b; Chen et al. 2007; Li-Gang et al. 2007; Huang et al. 2008; Song et al. 2009). Additional reports have claimed to have express successfully three disulfide-bridged β-defensins, but they have not demonstrated the correct folding and the biological activity of the product.

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