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Aquaculture 191 (2000) 13–21

Aquaculture

www.elsevier.nl/locate/aqua-online

Beta glucan binding protein and its role in shrimp immune response

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Received 1 March 2000; accepted 9 May 2000

Abstract

Despite their relatively short life and assumed lesser complexity, crustaceans have mechanisms to detect foreign matter. In particular, they appear to recognize common characteristics present in bacteria and fungi, such as lipopolysaccharides (LPS) and β-glucans. Although these microbial components can directly activate defensive cellular functions such as phagocytosis, melanization, encapsulation and coagulation, plasma recognition proteins amplify these stimuli. Beta glucan binding protein (BGBP) reacts with β-glucans and the glucan–BGBP complex induces degranulation and the activation of prophenoloxidase (proPO). This protein is present in all crustaceans studied so far and is highly conserved. Together with LPS-binding agglutinin, BGBP stimulates cellular function only after its reaction with LPS or β-glucans, resembling the secondary activities of vertebrate antibodies.

Keywords: Penaeus; Immunity; Crustacea; Glucan

1. Introduction

An essential component of immunity is a mechanism for surveillance, by which an organism can detect foreignness or the presence of “non-self” molecules. A good non-self recognition system should also stimulate defensive responses, including those mediated by cells. In vertebrates, the immune defense includes adaptive memory, specific immunoglobulins and specialized cells, as well as non-specific response through phagocytic cells and NK cells.

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0044-8486/00/$ - see front matter
PII: S0044-8486(00)00416-6

Eventhough an immune response against specific epitopes and/or immunoglobulins has not been detected in invertebrates, they are able to recognize and destroy invading microorganisms or parasites. Proteins involved in the recognition process of cell wall components from microorganisms, such as lipopolysaccharide (LPS) and β-1,3-glucans (BG), have been found in invertebrates. However, these proteins are unable to destroy foreign matter, and a cellular activity, mainly phagocytosis, is required (Ratcliffe et al., 1985). Consequently, opsonic factors stimulating phagocytic activity should be important in invertebrates, but few factors able to recognize microbial components and enhance the phagocytic rate have so far been reported. Examples of opsonic factors recognizing LPS include the LPS-binding proteins from the cockroach *Periplaneta americana* (Jomori and Natori, 1992), the crayfish *Pacifastacus leniusculus* (Kopácek et al., 1993) and the yellowleg shrimp *Penaeus californiensis* (Vargas-Albores et al., 1993a). Other defence systems can be also activated by LPS such as the horseshoe crab coagulation cascade (Liang et al., 1985; Muta et al., 1991) and the prophenoloxidase (proPO) activating system of arthropods (Ashida and Yamazaki, 1990; Söderhäll et al., 1990).

2. ProPO system and recognition proteins

In shrimp, as in all crustaceans, a dark pigmented spot appears after an animal is injured. This is due to the action of phenoloxidase (PO), which promotes hydroxylation of phenols and oxidation of *o*-phenols to quinones, necessary for the melanization process observed in response to foreign intruder in the hemocoele and during wound healing (for review, see Johansson and Söderhäll, 1989; Ashida and Yamazaki, 1990; Söderhäll, 1992; Söderhäll et al., 1994). Quinones are subsequently transformed, by a non-enzymatic reaction, to melanin and often deposited around encapsulated objects, in hemocyte nodules and at sites of fungal infections in the cuticle. Although a direct antimicrobial activity has been described for melanin and its precursors (Nappi and Vass, 1993), the production of reactive oxygen species such as superoxide anions and hydroxyl radicals during the generation of quinoids (Song and Hsieh, 1994; Nappi et al., 1995) also has an important antimicrobial role. In addition, biological reactions such as phagocytosis, encapsulation and nodulation are also activated.

PO (E.C. 1.14.18.1) is present in the hemolymph as an inactive pro-enzyme called proPO. The transformation from proPO to PO involves several reactions known as the proPO activating system. This system is specifically activated by BG (Söderhäll and Unestam, 1979; Ashida et al., 1983; Smith and Söderhäll, 1983; Vargas-Albores, 1995; Vargas-Albores et al., 1996, 1997), bacterial cell walls (Ashida et al., 1983; Rowley and Rahmet-Alla, 1990) and LPS (Söderhäll and Häll, 1984; Hernández-López et al., 1996; Gollas-Galván et al., 1997). The proPO activating system is considered a constituent of the immune system and is probably responsible, at least in part, for the non-self recognition process of the defence mechanism in crustaceans and insects (Söderhäll, 1982, 1992; Ashida and Söderhäll, 1984; Ratcliffe, 1985; Ratcliffe et al., 1985, 1991; Johansson and Söderhäll, 1989, 1992; Ashida and Yamazaki, 1990; Söderhäll et al., 1990, 1994; Lanz et al., 1993). Furthermore, the proPO system has been proposed as

invertebrate counterpart of the vertebrate complement system since it can be activated by BG (Söderhäll, 1982; Smith and Söderhäll, 1983; Leonard et al., 1985; Vargas-Albores et al., 1993b), has a cascade reaction, and involves proteinases (Aspán et al., 1990; Söderhäll, 1992; Söderhäll et al., 1994). However, other than these similarities, no direct lytic activity of the proPO system has been detected.

Although LPS or β-glucans can activate directly the horseshoe crab coagulation cascade (Liang et al., 1985; Muta et al., 1991) and the proPO activating system of arthropods (Söderhäll, 1982, 1992; Söderhäll et al., 1990; Vargas-Albores et al., 1993b; Yoshida et al., 1996), the presence and participation of plasma recognition proteins can amplify this activation. Several recognition proteins with affinity to bacterial or fungal cell walls have been reported in arthropods and other invertebrates, but they are not related to immunoglobulins, either structurally (see below) or functionally. In general, recognition proteins are expressed constitutively and they are present in the plasma or cell surface before invasion of foreign objects. However, a peptidoglycan recognition protein has recently been detected in the moth *Trichoplusia ni* after bacterial challenge (Kang et al., 1998). In shrimp, a 180-kDa LPS-binding agglutinin has been reported as a multivalent carbohydrate-binding agglutinin that, besides its bacterial agglutination ability, increases the phagocytic rate (Vargas-Albores, 1995). A second protein involved in the recognition of microbial products and the activation of cellular functions is the beta glucan binding protein (BGBP). It is apparently monovalent and does not induce agglutination, but enhances proPO system activation.

3. BGBP

BGBP has been described as a component of the immune system in arthropods and has been purified from two insects: *Blaberus craniifer* (Söderhäll et al., 1988) and *Bombyx mori* (Ochiai and Ashida, 1988), and three crustaceans: the freshwater crayfish *Pac. leniusculus* (Duvic and Söderhäll, 1990), the yellowleg shrimp, *Pen. californiensis* (Vargas-Albores et al., 1996) and the white shrimp *Penaeus vannamei* (Vargas-Albores et al., 1997). This protein appears to be widely distributed among crustaceans, conserving most of its antigenic properties, since a monospecific polyclonal antiserum against *Pac. leniusculus* BGBP can recognize BGBP in different crustaceans including the freshwater crayfishes *Astacus astacus* (Duvic and Söderhäll, 1993), *Procambarus clarkii* (Duvic and Söderhäll, 1993), marine crab *Carcinus maenas* (Thörnqvist et al., 1994) and several shrimp species (Vargas-Albores et al., 1996, 1997). In addition, antibodies prepared against the purified yellowleg shrimp BGBP (Vargas-Albores et al., 1996) clearly detected a 100-kDa protein in plasma from *Pen. vannamei* and *Penaeus stylirostris*.

BGBP molecular size is also very similar among crustaceans. In *Pro. clarkii* and *Pac. leniusculus* BGBP is a unique protein with molecular mass of 100 kDa, but in other freshwater crayfish (*A. astacus*), two bands were recognized by the anti-BGBP (Duvic and Söderhäll, 1993). However, this result was explained as heterogeneity of glycosylation that might give rise to molecular masses of 95 and 105 kDa. BGBP from the marine crustacean *C. maenas* is a 110-kDa protein also capable of inducing direct phagocytic

stimulation (Thörnqvist et al., 1994). From *Pen. californiensis* plasma, BGBP was purified by using anti-crayfish BGBP antibodies as a 100-kDa monomeric protein (Vargas-Albores et al., 1996) that extends the proPO activating system. Similar molecular mass has been described for *Pen. stylirostris* and *Pen. vannamei* BGBP, the latter one purified by immobilized laminarin and the first one isolated by low ionic strength precipitation (Vargas-Albores et al., 1997). Such as in other crustaceans BGBPs, the protein isolated from *Pen. californiensis*, *Pen. vannamei* and *Pen. stylirostris* are glycosylated with sugar residues containing mannose or glucose and *N*-acetyl glucosamine as determined by a positive reaction with concanavalin A and wheat germ agglutinin.

The composition analysis revealed that the amino acid content of shrimp BGBP is nearly identical to the homologue from the freshwater crayfish, *Pac. leniusculus*. In general, all the amino acids have similar concentrations in the compared BGBPs. Asx and Glx are the most abundant amino acids. Neutral residues such as Ala and Gly are present in moderate concentrations, whereas Met and His concentrations are lower (Table 2). When the known *N*-terminal amino acid sequence of crustacean BGBPs (*Pen. californiensis*, *Pen. vannamei* and *Pac. leniusculus*) are compared, high homology is observed. Crayfish BGBP was recently cloned and sequenced (Cerenius et al., 1994), but until now no other crustacean or insect BGBP complete sequence has been available. Eventhough amino acid sequence comparison among BGBP can only be done with the *N*-terminus of the protein, the sequence appears to be highly conserved between shrimp and crayfish (Fig. 1). There are only three different residues between white and yellowlegs shrimp BGBPs and notably all of them are substituted by conserved amino acids. In addition, there are 14 of 25 residues identical and eight conservative replacements among the three BGBPs. Interestingly, when compared with crayfish BGBP, residue number seven is different only in yellowlegs shrimp and residue 24 is different in white shrimp BGBP. Overall, the homology is higher considering the conservative replacements of the changed residues. If this region corresponds to a specific domain important for the protein function or is representative of the entire protein conservation, remains to be determined once the complete sequence of more BGBPs are obtained. Although this type of proteins has been found in insects (Ochiai and Ashida, 1988; Söderhäll et al., 1988), major differences were noted when these were compared with crustacean BGBP, including differences in molecular masses and amino acid composition (Tables 1 and 2). *Bom. mori* BGBP has a molecular mass of 62 kDa (Ochiai and

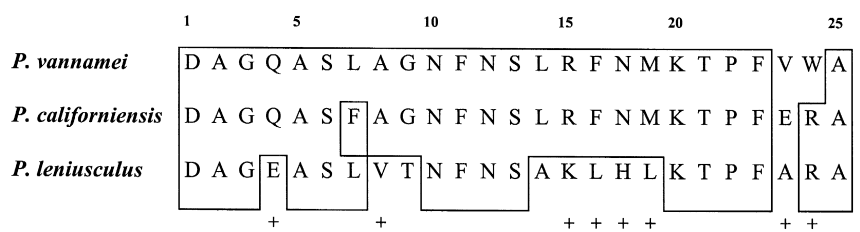

Fig. 1. Alignment of *Pen. vannamei* (Vargas-Albores et al., 1997), *Pen. californiensis* (Vargas-Albores et al., 1996) and *Pac. leniusculus* (Duvic and Söderhäll, 1990) BGBP *N*-terminal sequences. Identical sequences are boxed. Plus signs indicate conservative replacement between white shrimp and crayfish.

Table 1
BGBP from insects and crustaceans

Organism	Molecular weight (kDa)	Reference
Bla. craniifer	91	(Söderhäll et al., 1988)
Bom. mori	62	(Ochiai and Ashida, 1988)
Pac. leniusculus	100	(Duvic and Söderhäll, 1990; Cerenius et al., 1994)
A. astacus	95–105	(Duvic and Söderhäll, 1993)
Pro. clarkii	100	(Duvic and Söderhäll, 1993)
C. maenas	110	(Thörnqvist et al., 1994)
Pen. californiensis	100	(Vargas-Albores et al., 1996)
Pen. vannamei	100	(Vargas-Albores et al., 1997)
Pen. stylirostris	100	(Vargas-Albores et al., 1997)

Ashida, 1988), significantly smaller than crustacean BGBP, which range from 95 to 110 kDa. In *Bla. craniifer*, BGBP was reported as a 91-kDa protein that under reducing conditions splits to subunits of 63 and 52 kDa (Söderhäll et al., 1988), but such dimeric structure has not been reported in crustaceans. Recently, a LPS- and glucan-binding protein involved in the activation of proPO was identified in the earthworm *Eisenia foetida* (Beschin et al., 1998). The protein was cloned and its amino acid sequence shows similarity with sea urchin glucanase, *L. polyphemus* factor G and a gram-negative bacteria-binding protein from *Bom. mori*, but not with BGBP from insects or crus-

Table 2
Amino acid composition of BGBP from insect and crustaceans: *Bla. craniifer* (Söderhäll et al., 1988), *Bom. mori* (Ochiai and Ashida, 1988), *Pac. leniusculus* (Duvic and Söderhäll, 1990), *Pen. vannamei* (Vargas-Albores et al., 1997) and *Pen. californiensis* (Vargas-Albores et al., 1996)

Amino acid	Insects		Crustaceans		
	Bla. craniifer	*Bom. mori*	*Pac. leniusculus*	*Pen. vannamei*	*Pen. californiensis*
Ala	5.00	6.5	5.74	6.53	7.14
Arg	1.04	4.1	5.00	4.83	5.91
Asx	9.84	11.0	12.22	12.36	12.87
Cys	1.04	0.5	0.54	nd	nd
Glx	20.80	9.0	11.41	10.95	10.19
Gly	20.32	11.1	7.52	8.92	10.22
His	2.53	1.4	3.17	1.79	1.50
Ile	1.70	5.9	6.03	6.08	5.90
Leu	2.75	7.4	7.51	8.86	8.04
Lys	7.25	6.7	6.84	6.37	6.66
Met	0.57	1.0	1.18	0.95	0.79
Phe	1.20	5.3	5.55	5.34	5.21
Pro	2.81	7.8	3.20	3.56	3.14
Ser	15.62	6.1	7.70	8.44	9.01
Thr	3.74	4.4	6.23	6.31	5.91
Tyr	1.29	4.2	3.37	2.98	2.79
Val	2.85	5.7	6.78	5.62	4.55

nd, not determined.

taceans. Unfortunately, molecular descriptions of other stimulating proPO system arthropod BGBPs are not yet available.

4. Mechanism of action

The mechanism of action of invertebrate recognition proteins appears to be similar to vertebrate antibodies where, after reaction with the antigen, the immunoglobulin can activate cellular functions (degranulation or phagocytosis) or plasma complement (C). While hemolymph agglutinins can react with the hemocyte surface, this reaction only occurs after the agglutinin has reacted with the sugar-containing particle. Otherwise, hemocyte aggregation could occur in vivo. To enhance phagocytosis, a previous reaction of the agglutinin with a target cell is necessary, which covers the target cell surface, leading to an easier attack by the phagocytic cell. Most probably, when the agglutinin reacts with non-self material, the molecule undergoes structural changes that allow its reaction with hemocyte membranes. On the other hand, although this has not been studied in shrimp, in several invertebrates including *Mytilus edulis* (Renwrantz and Stahmer, 1983), *Crassostrea virginica* (Vasta et al., 1984) and *Lymnaea stagnalis* (van der Knaap et al., 1981), agglutinins have been found in hemocyte membranes. The participation of these membrane-bound agglutinins in phagocytosis cannot be denied and could be explained as the presence of membrane-bound receptors that can recognize "foreignness".

Shrimp BGBP appears to be a constitutive plasma protein that after binding to β-glucans reacts with hemocyte surface and stimulates the release of hemocytic granules. The contents of the granules become activated in presence of plasma Ca^{2+}, leading to PO activity. Obviously, the activating reaction does not occur solely by the presence of BGBP, although its interaction with hemocyte membrane could exist.

In shrimp, both LPS (Vargas-Albores et al., 1993a; Maheswari et al., 1997) and β-glucans (Vargas-Albores et al., 1996, 1997) binding proteins are present as possible recognition proteins. Besides differences in specificity, microbial products are recognized by proteins showing common characteristics. Both kinds of proteins are normally present in serum and its cellular receptors have been described and cloned (Duvic and Söderhäll, 1992; Johansson et al., 1999). In addition, they appear to have two biologically active sites: one for the microbiological component, and other for the circulating cells. The second active site seems to be only available after reaction with LPS or β-glucans, respectively. The case of LPS-binding agglutinin is clear, because this protein is, at least, divalent which is a basic requirement for the induction of agglutination. Normally, the agglutinin does not induce hemocyte agglutination, but when the agglutinin reacts with an LPS-containing particle it is capable of reacting with the hemocyte surface and increasing the phagocytic activity (Vargas-Albores, 1995). In the same manner, BGBP per se is unable to induce release and activation of the proPO system, but the protein–glucan complex is able to react with the circulating cells and increase the effect of glucans on the proPO system (Barracco et al., 1991; Johansson and Söderhäll, 1992; Vargas-Albores, 1995; Vargas-Albores et al., 1996). Thus, these recognition proteins are capable of activating cellular activities only after reaction with

the microbial carbohydrates (LPS peptidoglycans or glucans). This is analogous to vertebrate antibodies, which promote phagocytosis and activation of the C system after they have reacted with adequate antigen.

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