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Fu-Lin E. Chu
Virginia Institute of Marine Science

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FU-LIN E. CHU

Virginia Institute of Marine Science, School of Marine Science
The College of William and Mary, Gloucester Point, Virginia 23062, USA

Abstract.—Natural humoral components have been discovered and described in hemolymph from several marine bivalve species including eastern oyster *Crassostrea virginica*, blue mussel *Mitilus edulis*, northern quahog *Mercenaria mercenaria*, softshell *Mya arenaria*, and Pacific oyster *Crassostrea gigas*. These hemolymph components are enzymes of lysosomal origin, agglutinins, lectins, hemolysin, and antimicrobial substances. These components are proteins or glycoproteins found in the serum, hemocytes, or both. The exact relationship of these substances to the internal defense of marine bivalves against parasites and pathogenic microorganisms is not known. Lysosomal enzymes seem to have a double role, defense and nutrition. The free- and cell-bound lectins and agglutinins are believed to serve as recognition factors for the attachment of nonself particles to the phagocytes. The nature of bivalve hemolymph components appears to be innate and nonspecific. It has been suggested that the elevation in titer of enzymes of lysosomal origin in bivalve hemocytes and hemolymph after antigenic challenge is the acquired "humoral" protection produced by the animal. As yet, no experimental evidence has been obtained to support this idea. Whether humoral factors can be acquired in oysters and other marine bivalves needs further investigation. The specificity and the function of humoral factors relating to internal defense remain to be determined. Several mechanisms have been hypothesized for humoral defense in invertebrates. In these hypothesized mechanisms, recognition sites presented on the hemocytes are proposed for the events of phagocytosis, encapsulation, hypersynthesis and release of lysosomal enzymes.

In recent years, the question of immune defense mechanisms in some economically important bivalve molluscs has attracted much attention and generated substantial controversy. Though humoral defense of marine bivalves has not been studied as extensively as cellular defense (see Feng 1988, this volume), the humoral factors in bivalves and their exact relationship to the internal defense of the animals against parasites and pathogenic microorganisms are of interest. The understanding of bivalve humoral factors and their function in defense might allow us to manipulate their resistance or tolerance to diseases. In this chapter, the literature concerning humoral defense factors in marine bivalves and the possible roles of humoral factors in defense against diseases is reviewed and discussed.

Lysosomal Enzymes

Lysozyme and Lysosomal Enzymes

Lysozyme activity has been documented in many invertebrate species (e.g., McDade and Tripp 1967a, 1967b; Feng 1974; Cheng et al. 1975). The bacteriolytic enzyme, lysozyme, is a basic protein with a molecular weight of approximately 15,000 daltons; its characteristics are (1) ability to lyse the bacterium *Micrococcus lysodeikticus* with the release of amino sugars and (2) stability at low pH and high temperature but becoming inactive at high pH especially at high temperatures (Jolly 1967). Lysozyme activity was first reported in the hemolymph and mantle mucus of the eastern oyster *Crassostrea virginica* by McDade and Tripp (1967a, 1967b). Lysozyme or lysozymelike substances have subsequently been described in softshell *Mya arenaria* (Cheng and Rodrick 1974), northern quahog *Mercenaria mercenaria* (Cheng et al. 1975), and blue mussel *Mytilus edulis* (Hardy et al. 1976; McHenery and Birkbeck 1982).

The enzymatic activity of lysozymes found in these bivalve species resembles avian lysozyme in their ability to lyse bacteria and in sharing some biochemical properties (Cheng and Rodrick 1974; Rodrick and Cheng 1974) As with egg-white lysozyme, the lytic activity on *Micrococcus lysodeikticus* is salt-dependent, relatively heat-stable, and very sensitive to changes in ionic concentration. However, Feng (1974) reported that the oyster lysozyme differs from egg-white lysozyme in electrophoretic mobility, molecular weight, isoelectric point, and association with acidic proteins.

Bivalve lysozymes are active not only against *M. lysodeikticus* but also against other species of bacteria including *Bacillus subtilis, B. megaterium, Escherichia coli, Gaffkya tetragina, Proteus vulgaris, Salmonella pullorum,* and *Shigella sonnei* (Cheng and Rodrick 1974; Rodrick and Cheng 1974).

Lysozyme activities were found in both the serum and hemocytes of eastern oyster, softshell, and northern quahog. Except for northern quahog, lysozyme activity was greater in serum than in cells (Cheng and Rodrick 1974; Rodrick and Cheng 1974; Cheng et al. 1975).

Other lysosomal enzymes were also reported to be present in hemolymph of eastern oyster and northern quahog (Cheng and Rodrick 1975; Cheng 1976). These lysosomal enzymes are β-glucuronidase, alkaline and acid phosphatases, lipase, aminopeptidase, and amylase. All of these enzymes, except amylase, were also found in hemocytes (Yoshino and Cheng 1976). Antimicrobial substances have also been detected in tissue extracts of oysters and clams (Li 1960). These substances were able to inhibit growth of bacteria. The nature and origin of these substances are unknown.

Function and Origin of Lysosomal Enzymes

The biological roles of lysosomal enzymes in molluscan bivalves are not completely understood. They are generally presumed to be involved with host defense and digestion. Lysozymes from eastern oyster, softshell, and northern quahog are active against several species of bacteria. The β-glucuronidase from northern quahog was found to hydrolyse mucopolysaccharides which are found in bacteria cell walls. It has been speculated (Cheng 1983a, 1983b, 1983c) that lysosomal enzymes were inducible and served as a form of "acquired" humoral immunity in the animal. The elevated level of lysosomal enzymes in the serum fraction, when oyster and clam hemocytes were challenged with bacteria, is thought to represent a type of humoral defense mechanism against invading microorganisms (Cheng et al. 1975; Cheng et al. 1978; Cheng and Butler 1979).

Beside the suggested role of lysozyme in defense, evidence provided by McHenery et al. (1979) indicates that its primary function is digestion. The tissue distribution of lysozyme in more than 30 species of bivalve molluscs was studied, including the marine species *Chlamys opercularis, Tellina tenuis,* softshell, and blue mussel. In most cases, the style and the digestive gland contained more enzyme than the blood and body fluid. McHenery et al. (1979), therefore, concluded that (1) the function of lysozyme is associated with digestion and degradation of bacteria for nourishment of the animal, (2) lysozyme concentration is related to the proportion of bacteria utilized as food, and (3) host defense is secondary to that of nutrition.

Lysosomal enzymes from different marine bivalves share similar biochemical and bacteriolytic properties. The roles of lysosomal enzymes as agents of defense and digestion are probably equally important. Digestion and defense could actually occur at the same time. Because bacteria are one of the foods of filter feeders such as marine bivalves, a substance that is able to hydrolyze bacteria would fulfill the purposes of both defense and digestion. Perhaps digestion and defense are more distinct in the minds of investigators than in the life of the animal.

The origin of lysosomal enzymes is postulated to be in the lysosomes of granular hemocytes (Rodrick and Cheng 1974). The release of lysosomal enzymes into the serum is a consequence of hemocyte "degranulation" during phagocytosis (Rodrick and Cheng 1974; Cheng and Yoshino 1976a, 1976b; Foley and Cheng 1977; Cheng and Butler 1979). Cheng and his associates have demonstrated that lysosomal enzymes could be induced experimentally by in vitro exposure of molluscan hemocytes to bacteria or in vivo challenge with bacteria or bacteria lipids (Cheng et al. 1975; Cheng and Yoshino 1976a, 1976b; Cheng et al. 1977, 1978; Cheng and Butler 1979). For example, when northern quahog hemocytes were exposed to *Bacillus megaterium,* the level of lysozyme in the serum fraction rose. It was postulated that the elevated levels of serum lysosomal enzymes (e.g., lysozyme, aminopeptidase, acid phosphatase) are due to the hypersynthesis and release of these enzymes in the phagocytosing hemocytes in response to the bacterial challenge. The experimental induction of lysosomal enzymes will be discussed further in the section, Induction of Humoral Factors.

Agglutinins and Lectins

Serum agglutinins are common components of bivalve hemolymph. Among the various humoral factors, agglutinins have received much attention because of their ability to agglutinate vertebrate erythrocytes and bacteria in vitro.

Hemagglutinin and Bacterial Agglutinin

Naturally occurring substances that agglutinate red blood cells (hemagglutinins) are known to be widely distributed among plants and animals. Such substances in plants are called lectins (carbohydrate-binding proteins), and they agglutinate cells or materials by glycosyl moieties (Sharon and Lis 1972). The specificity of plant lectins varies with the plant species (Boyd 1962). Natural hemagglutinins have been found in the body and

seminal fluids of numerous invertebrates, including several marine bivalves (Tyler 1946; Cheng and Sanders 1962; Cushing et al. 1963; Johnson 1964; Boyd and Brown 1965; Cohen et al. 1965; Tripp 1966; Li and Fleming 1967; Brown et al. 1968; Hardy et al. 1976, 1977b).

Hemagglutinins were found in tissue extracts and in the hemolymph of blue mussel (Brown et al. 1968; Hardy et al. 1976), eastern oyster (Tripp 1966; McDade and Tripp 1967a, 1967b; Acton et al. 1969), and Pacific oyster *Crassostrea gigas* (Hardy et al. 1977a, 1977b). These hemagglutinins could agglutinate more than one type of human erythrocyte and the erythrocytes of many vertebrate species. Some marine bivalve agglutinins are heterogenous, i.e., they are not group specific (e.g., agglutinins from Pacific oyster and northern quahog). They agglutinated cells other than red blood cells (e.g., algae, vertebrate sperm, and bacteria). Bacterial agglutination has been observed only in Pacific oyster and northern quahog (Arimoto and Tripp 1977; Hardy et al. 1977a, 1977b); hemolymph from eastern oyster did not agglutinate bacteria (Tripp 1966). Because marine bivalves live in an environment full of bacteria, it is not known why other bivalves lack bacterial agglutinins. Perhaps investigation to date has been too limited to detect them.

Lectins

Two serum and one cell-bound lectin have been discovered in the eastern oyster (Vasta et al. 1982; Cheng et al. 1984). Each of these serum lectins was shown to have a distinct serological agglutination specificity. The hemocyte-bound lectin was able to agglutinate certain vertebrate erythrocytes (Vasta et al. 1982; Cheng et al. 1984). It showed only one of the two serological activities found in the serum lectins and did not appear to be associated with a specific subpopulation of oyster hemocytes (Cheng et al. 1984).

Nature and Biochemical Properties of Agglutinins

Hemagglutinins and bacterial agglutinins from marine bivalves showed strong biochemical and biological similarity. Hemagglutinins and bacterial agglutinins are all lectinlike glycoproteins, bearing divalent or multivalent receptors for certain specific carbohydrate determinants. They appear to have a common structure consisting of small identical subunits with molecular weights of 20,000–21,000 daltons. The bacterial agglutinin of northern quahog was found to be composed of subunits, each with a molecular weight of approximately 21,000 daltons (Arimoto and Tripp 1977). The oyster hemagglutinin was composed of identical subunits with molecular weights of approximately 20,000 daltons (Acton et al. 1969) and contained fewer protein components than human immunoglobulin. These subunits are usually linked by noncovalent bonds, and each subunit has a carbohydrate binding site. Hemagglutination and bacterial agglutination can be inhibited by saccharides. Bacterial agglutination of northern quahog hemolymph was partially inhibited by *N*-acetyl-D-glucosamine, D-glucosamine, *N*-acetyl-D-galactosamine, and D-fucose (Arimoto and Tripp 1977). Agglutination of human red cells by eastern oyster hemolymph was completely inhibited by galactosamine, *N*-acetylglucosamine, and *N*-acetylgalactosamine, and partially inhibited by glucosamine. *N*-Acetyl-D-galactosamine and *N*-acetylglucosamine inhibited the agglutination of human erythrocytes A by an extract from butter clam *Saxidomus giganteus* (Johnson 1964). Bovine salivary gland glycoprotein inhibited hemagglutination by the hemolymph of Pacific oyster (Hardy et al. 1977b).

Marine bivalve agglutinins are reported to be nondialysable and are inactivated by temperatures of 65–70°C. Calcium ions are required for agglutination activity and contribute to the heat stability of the molecule (Johnson 1964; Tripp 1966; McDade and Tripp 1967a, 1967b; Arimoto and Tripp 1977). A more detailed account of biochemical properties of agglutinins is presented elsewhere in this volume (Olafsen 1988).

Functional Role of Agglutinins and Lectins

There is confusion among the terms, lectin, agglutinin, and opsonin, even though they are different by definition. But in reality, we still cannot distinguish agglutinins from lectins in many invertebrate species, including bivalves. Agglutinins have some characteristics of lectins. It is likely that many of the agglutinins found in bivalves are lectins, and both can function as opsonins. The terminology of agglutinins and opsonins may only represent various functions of the same protein.

The role of agglutinins and lectins in molluscs has been discussed in an immunological context (Hardy et al. 1977b; Lackie 1980; Warr 1981; Vasta et al. 1982; Cheng et al. 1984; Coombe et al. 1984). The following possible roles of agglutinins and lectins in defense mechanisms are suggested.

(1) Agglutinins and lectins inactivate bacteria or parasites by agglutination. The inactivation

may lead to two defense events: lysis of bacteria by extracellular enzymes (e.g., lysozyme) and subsequent phagocytosis and encapsulation of agglutinated particles. As described earlier, hemagglutination and bacteria agglutination by hemolymph, tissue extract, or both of marine bivalves have been well documented.

(2) Serum agglutinins and lectins serve as opsonins to link receptors with similar glycosyl moieties on the surface of nonself particles and hemocytes. There is evidence for the opsonic effect of purified agglutinins from the Pacific oyster on the phagocytosis of bacteria (Hardy et al. 1977b). Various studies have also demonstrated opsonic activity of molluscan serum both in vitro (Tripp and Kent 1967; Prowse and Tait 1969; Anderson and Good 1976; Arimoto and Tripp 1977; Hardy et al. 1977a; Sminia et al. 1979; Van der Knapp et al. 1982) and in vivo (Renwrantz and Mohr 1978, Renwrantz 1981), although in some cases the evidence could be considered circumstantial (Coombe et al. 1984).

(3) Agglutinins and lectins attach to the hemocytes and function as cell surface recognition factors to bind nonself particle bearing appropriate glycosyl moieties. A cell membrane-associated lectin has been found in hemocytes of eastern oyster (Vasta et al. 1982). Lectin-binding receptors have also been detected at the hemocyte surface of the marine bivalves eastern oyster (Yoshino et al. 1979; Cheng et al. 1980) and blue mussel (Renwrantz et al. 1985), and of gastropods bloodfluke planorb *Biomphalaria glabrata* (Schoenberg and Cheng 1980; Yoshino 1981), swamp lymnaea *Lymnaea stagnalis* (Sminia et al. 1981), and escargot *Helix pomatia* (Renwrantz and Cheng 1977). These animals possess binding sites on the hemocytes for various lectins.

Sialic acids are suggested as the binding sites of serum lectins in Pacific oyster and of membrane-associated lectins in eastern oyster, although the configuration of lectin molecules should be taken into consideration (Hardy et al. 1977b; Cheng et al. 1984). Lectin-binding activity of hemocytes was found to be temperature-dependent (Yoshino et al. 1979) and relied on the presence of carbohydrates such as glucose, fructose, mannose, galactose, *N*-acetylneuraminic acid, and *N*-acetylgalactosamine (Renwrantz and Cheng 1977).

The second and third suggested roles are the ones best supported by the evidence. They describe adhesion of nonself particles and hemocyte through the binding of serum or cell-associated agglutinins–lectins to nonself particles and to phagocytes. The serum and cell-bound lectins–agglutinins mediate the processes of phagocytosis and encapsulation of foreign materials.

Hemolysin

Hemolytic factor (hemolysin) was found in northern quahogs (Graham 1968; Anderson 1981) and blue mussels (Hardy et al. 1976; Feng and Barja 1986). The lytic factor described by Graham (1968) from hemolymph and shell liquor lysed erythrocytes of many vertebrate species. This lysin was heat-labile (inactivated at 47°C for 30 min), nondialysable, could not be absorbed by erythrocytes, and its activity was dependent on calcium ions. Hemolysin from northern quahog was further investigated by Anderson (1981). Hemolytic activity was detected in both sera and in hemocytes and hemolymph of northern quahog and could be induced experimentally. Anderson's results differed from those of Graham in erythrocyte specificity, reactivity at temperature optima for hemolytic reaction kinetics, and absorption and inactivation of hemolytic activity by homologous erythrocytes. The hemolymph of blue mussels was examined by Hardy et al. (1976); human erythrocytes of type O were lysed at pH 7–9 and an optimum temperature of 4°C.

The exact role of hemolysin is not defined, and the mechanism for hemolysis is unknown. The capability to lyse erythrocytes from species of distant phylogenetic origin does not have practical importance for bivalve defense. If hemolysin is a rudimentary complement system in bivalves, why is it restricted only to northern quahogs and blue mussels?

Induction of Humoral Factors

In marine bivalves, the humoral factors, agglutinins, lectins, and enzymes of lysosomal origin appear to be innate and nonspecific. Attempts have been made to induce lysosomal enzymes in bivalves. An increase of lipase activity has been demonstrated in the serum and hemolymph cells of softshells that were injected with heat-killed *Bacillus megaterium* (Cheng and Yoshino 1976b).

Similarly, levels of aminopeptidase and lysozyme were increased in eastern oyster hemocytes and northern quahog serum after in vitro exposure to bacteria (Cheng et al. 1975; Yoshino and Cheng 1976). No data exist on the induction of lysozyme in oysters or clams in vivo. Messner and Mohrig (1969) failed to produce this effect in the freshwater mussel *Anodonta anatina*.

Like other invertebrates, molluscs are incapable of synthesizing antibodies (immunoglobulins). Cheng (1981, 1983b) hypothesized that the elevated lysosomal enzymes found in marine bivalves are inducible "protective" humoral factors. The increased levels of lysosomal enzymes in the cell and serum after in vivo or in vitro bacterial exposure are believed to be the consequence of hypersynthesis of enzymes in the cells and their subsequent release to the sera. These soluble molecules may play a role in acquired resistance. For example, the elevated level of serum aminopeptidase may alter the surface protein of secondarily introduced parasites and thus act as a form of acquired humoral immunity. Acquired immunity, however, is characterized by its specificity, adaptive nature (i.e., capacity to learn), and irreversibility. The specificity of lysosomal enzymes has not been elucidated, and thus far, response to antigenic challenge appears to be nonspecific. The production of lysosomal enzymes can be stimulated, but their formation cannot be "induced" since these lysosomal enzymes are innate. Moreover, as stated by Feng and Barja (1986), elevated levels of hemolymph enzymes could also be interpreted as pathological manifestations rather than defense responses. Feng and Canzonier (1970) found that hemolymph lysozyme levels decreased in eastern oysters infected with *Haplosporidium nelsoni* (MSX), but it increased in oysters infected with *Bucephalus* sp.

Lysozyme activity has been reported higher in winter than in summer, and it exhibits a great variation among individual oysters (S. Y. Feng, Marine Sciences Institute, University of Connecticut, personal communication; Chu, unpublished data). Lysozyme activity was detected only in a few (0–10%) of the eastern oysters maintained in ambient estuarine water (York River, Virginia) during the summer (Chu, unpublished data).

All of these data leave some questions unanswered. What is the real role of lysozyme in diseases oysters? If lysozyme is an inducible "protective" agent, why did its level drop when the oysters were infected with MSX (Feng and Canzonier 1970), and why is it absent during the summer? Why does the hemolymph lysozyme level vary so much individually within the same population? Is this variation related to age or to the physiological and nutritional status of the animal? Is seasonal variation due to changes of environmental temperature and salinity?

Attempts to increase oyster hemagglutinin titers by challenging with erythrocytes were unsuccessful (Acton et al. 1969; Feng 1974). Hardy et al. (1977a) found that exposure of Pacific oysters to bacteria stimulated an increase in the titer of human hemagglutinin. Anderson (1981) also reported that hemolysin could be induced by both red blood cell injection and experimental wounding.

In studying the chemical and physical properties of cell-free hemolymph of "normal" eastern oysters and those infected with *Bucephalus* sp. and *H. nelsoni* (MSX), Feng and Canzonier (1970) found quantitative changes in some of the hemolymph proteins of infected oysters. They suggested that these changes were evidence of host humoral responses to infection. However, the study was performed on naturally infected oysters. The observed changes in hemolymph protein could be caused by environmental effects rather than by infection.

Hypothesized Humoral Defense Mechanisms

There are several humoral defense mechanisms proposed for invertebrates. These hypothesized mechanisms are applicable also to molluscs.

(1) Lysosomal enzymes act as defense molecules against invading microorganisms and abiotic foreign particles. These lysosomal enzymes are inducible and serve as a form of acquired humoral immunity in the animal. This mechanism was hypothesized and discussed by Cheng (1983a, 1983b, 1983c). Cheng (1983b) proposed three recognition sites for the occurrence of hypersynthesis and release of lysosomal enzymes, the cell surface, the nuclear membrane, and the lysosomal surface. The operational sequence of this mechanism is shown schematically in Figure 1. Sites on the surfaces of hemocytes recognize the challenging agent. Signals pass from the cell membrane recognition sites to those on the nuclear membrane that receive the transcytoplasmic messenger. This sequence of events results in the synthesis of enzymes on ribosomes and their eventual release from the lysosomes as a consequence of dégranulation.

(2) Agglutinins and lectins are humoral recognition factors which may also act as opsonins. They may form a molecular link between hemocytes and the foreign object or attach to the hemocytes and act as cell-bound recognition molecules. This hypothesized mechanism explains the phenomena of self–nonself discrimination in invertebrates very well, although the exact nature of this discrimination is not yet known. Cheng et al. (1984) postulated that, during phagocytosis and encapsulation, lectins facilitate attachment be-

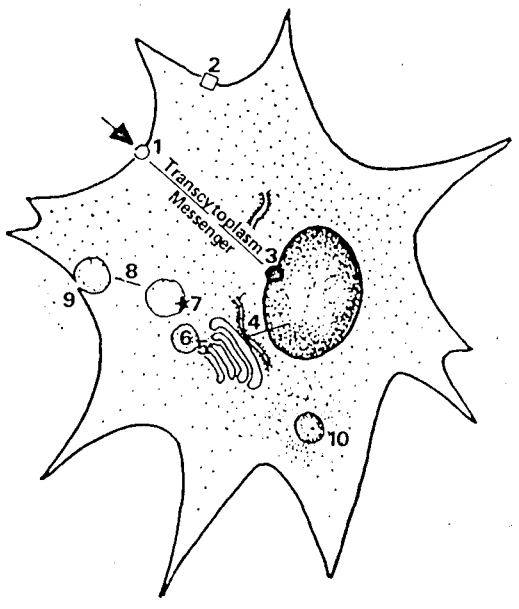

FIGURE 1.—Diagram explaining hypersynthesis of lysosomal enzymes and their subsequent release to serum. (After Cheng et al. 1984.) Surface sites (e.g., 1 and 2) on the cell membrane recognize stimulating agents and send a transcytoplasmic messenger to recognition sites (3) on the nuclear membrane. Enzyme synthesis on ribosomes (4) is directed by a deoxyribonucleic acid–messenger ribonucleic acid–transfer ribonucleic acid sequence, and lysosomal enzymes are packaged by the Golgi apparatus (5) into lysosomes (6). Activated recognition sites (7) on lysosomal membranes trigger lysosome migration (8). Degranulation of lysosomes (9) releases lysosomal enzymes to the serum. Lysosomes may also be destabilized to release lysosomal enzymes into the cytoplasm (10).

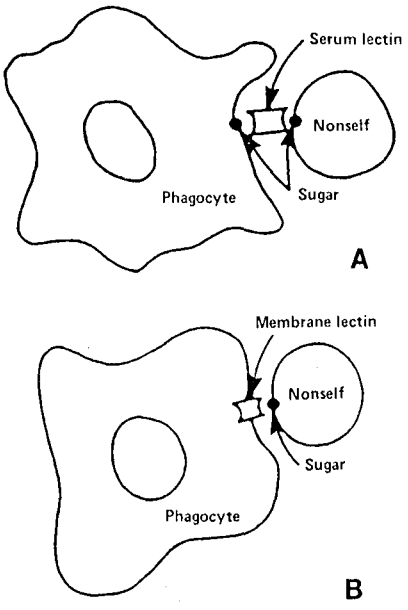

FIGURE 2.—Diagrams showing positions of lectins and sugars that would facilitate attachment between nonself particles and a phagocyte. (After Cheng et al. 1984.) **A.** A serum lectin links the phagocyte and nonself particle by appropriate sugar moieties. **B.** A nonself particle binds to a membrane lectin with compatible sugar moieties.

tween cell and nonself material through the binding of appropriate sugar moieties (Figures 2, 3). Coombe et al. (1984) proposed a different model for the mechanism of self–nonself discrimination (Figure 4) based simply on a specific recognition of self. Self-reactivity is controlled by the recognition of self through a self-marker (H) and a recognition structure on the phagocyte (anti-H). Self-recognition is hypothesized to control phagocytosis in the absence or presence of an opsonin. In the absence of opsonin, the selection of nonself particles could occur either by means of a cell-surface receptor or through nonspecific means, e.g., a physical attraction. In the presence of opsonin, phagocytosis is initiated by the binding of opsonized nonself cells or particles. In both cases, any cell or particle to which the phagocyte becomes attached would be ingested unless self is specifically recognized. Olafsen (1988) suggested the presence of multiple heterogenous receptor sites on lectins, some for nonself particles and some for potential phagocytes (self). He contends that self-receptors must be masked to avoid premature binding of the agglutinin with hemocytes, but these are unmasked after a nonself particle is bound to the agglutinin.

(3) Self–nonself discrimination in invertebrates is based on recognition of carbohydrate determinants by dissolved or cell-bound oligomers of glycosyl-transferases. The model (Figure 5) hypothesized by Parish (1977) is also of interest. Five proteins (transferases) with different sugar binding properties are present in the organism. These five transferases act as the subunits of the recognition factors and are synthesized and secreted by hemocytes into the hemolymph. They randomly associate into hexamers by a catalyzed protein which has an acceptor site on the hemocyte surface. The inclusion of this additional protein gives cytophilic and opsonic properties to the recognition factors.

All of these hypothesized defense mechanisms propose the occurrence of recognition (or binding)

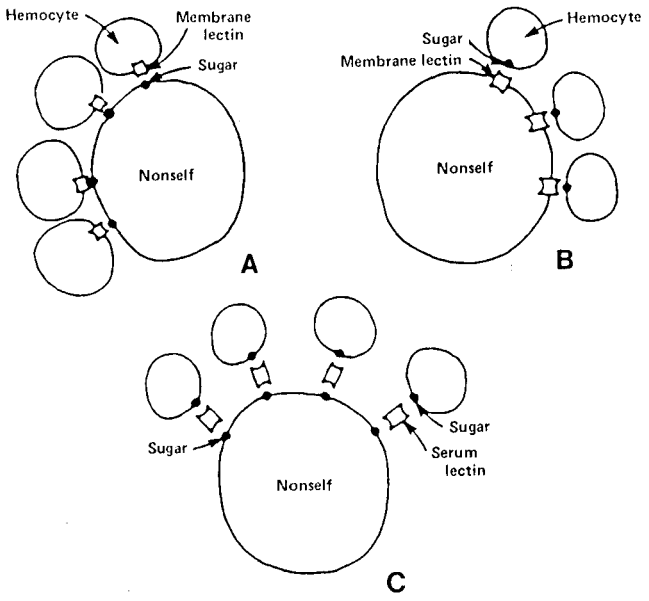

FIGURE 3.—Diagrams showing positions of lectins and sugars that would facilitate encapsulation of nonself particles. (After Cheng et al. 1984.) **A.** Hemocytes attach to nonself particles through the binding of membrane-associated lectins and compatible sugars on the surface of nonself particles. **B.** Nonself particles bind to hemocytes bearing lectin-binding receptors. **C.** Serum lectin links hemocytes and nonself particles together with appropriate sugar moieties.

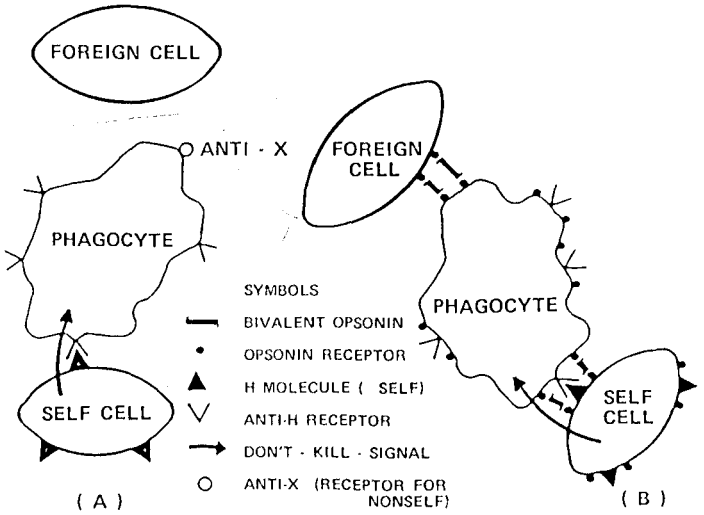

FIGURE 4.—A model proposed to explain phagocytosis by invertebrate cells. Self-reactivity is controlled by the recognition of self through a self-marker (H) and a recognition structure on the phagocyte (anti-H). Self-recognition is proposed to control phagocytosis in the absence of an opsonin (**A**) or when phagocytosis is initiated by the binding of opsonized cells (**B**). In both cases it is envisaged that any cell or particle to which the phagocyte becomes attached would be ingested unless the self is specifically recognized. (After Coombe et al. 1984.)

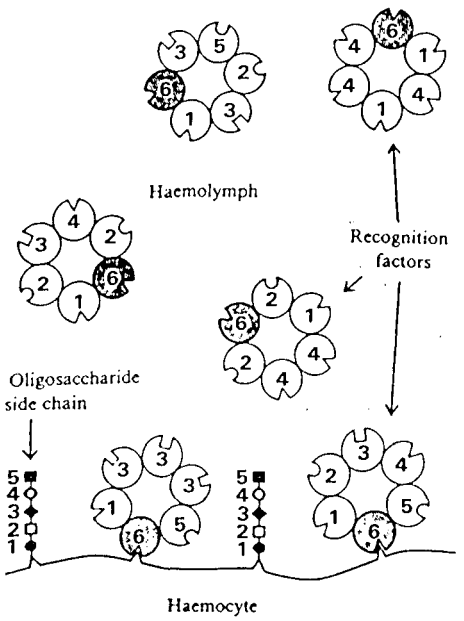

FIGURE 5.—Possible model for the recognition factors in invertebrates which augment the phagocytosis of foreign substances. Various monosaccharides on the hemocyte surface, which form oligosaccharide side chains, are represented by small circles, diamonds and squares numbered 1–5. Each monosaccharide is inserted in the oligosaccharide by a specific glycosyltransferase (notched circles 1–5). Thus, transferase 2 binds to monosaccharide 1 and catalyses the attachment of monosaccharide 2, transferase 3 binds to monosaccharide 2 and facilitates the attachment of monosaccharide 3, and so on. The transferases randomly associate into recognition factors (hexamers), a process of polymerization that is initiated by an additional protein (6). Protein 6 has an acceptor site on the hemocyte surface which enables the recognition factors to be cytophilic for hemocytes. (After Parish 1977.)

sites on the hemocytes. As mentioned previously, recent studies have revealed that cell-bound lectins and sites (receptors) that are able to bind more than one kind of lectin are present on molluscan hemocytes. Hemocyte lectin receptors of eastern oysters include concanavalin A, albumin gland extracts of escargot and grovesnail *Cepaea nemoralis*, and wheat germ agglutinin (Yoshino et al. 1979; Cheng et al. 1980). Those of blue mussels include wheat germ agglutinin, *Ricinus* 60 and 120, and agglutinin from albumin gland extracts of escargot (Renwrantz et al. 1985). The second mechanism implies the direct interaction of agglutinins–lectins and cellular components. However, very little is known about the interaction mode of agglutinin–lectin and the phagocytic cells at the molecular level. Although there is still no evidence for secretion of glycosyltransferases into serum, Parish (1977) made an important point that enzymes of several specificities should be available to extend the range of recognition possible.

Summary

Humoral substances with agglutinating, opsonic, lytic, and antimicrobial activities have been discovered and described in several bivalve species, including eastern oyster, Pacific oyster, northern quahog, and softshell (Table 1). These humoral components may play a significant role in host defense. Some of the humoral factors (e.g., lysozyme, β-glucuronidase, hemolysin) can lyse bacteria and erythrocytes of several vertebrate species. Some (e.g., agglutinins and lectins) are thought to mediate cellular defense mechanisms. The nature of these humoral components appear to be innate and nonspecific rather than acquired. Although there are indications in the literature that directly and indirectly point to induction of humoral response in oysters and other bivalve species, firm evidence that the inducible humoral response is both specific to challenged antigen and memorized has yet to be presented.

Mechanisms hypothesized to interpret the phenomena of self–nonself discrimination in invertebrates involve the interaction of humoral components and phagocytic cells. Agglutinins and lectins have been found in molluscs, including oysters, clams, and mussels and have been designated "recognition factors" (or receptors) and "opsonic factors" for foreign particles. These substances are both free in the hemolymph and bound to the cell membrane. Although the dissolved recognition factor(s) in the serum and the cell-bound molecule(s) are distinct, they are serologically related. It is suggested that a specific recognition of more than one kind is involved in phagocytosis. The molecular mode of these humoral components is not clear, but existing hypotheses are discussed by Olafsen (1988). Similarly, the origin, specificity, and precise role of the lysosomal enzymes in humoral defense need further investigation. Lack of reliable information makes it difficult to construct a general scheme for humoral defense in marine bivalves. Nevertheless, the discovery of cell-associated lectins in marine bivalves may open the door for studies of host and parasite interaction at the molecular level. The hypothesized humoral mechanisms presented in this chapter offer a basis for experimentation as well as direction for future research.

TABLE 1.—Humoral components of host defense in some marine bivalves.

Humoral component	Species	Source	Activity	Reference[a]
Lysozyme	Eastern oyster	Hemocytes and serum	Lyse bacteria, inducible	(4, 10)
	Northern quahog	Hemocytes and serum	Lyse bacteria, inducible	(5)
	Softshell	Hemocytes and serum	Lyse bacteria	(3)
	Blue mussel	Body fluid and hemolymph	Lyse bacteria	(7)
Aminopeptidase	Eastern oyster	Hemocytes and serum	Exopeptidase	(4)
	Northern quahog	Hemocytes and serum	Exopeptidase	(4)
β-Glucuronidase	Eastern oyster	Hemocytes and serum	Hydrolyze mucopolysaccharides	(13)
	Northern quahog	Hemocytes and serum	Hydrolyze mucopolysaccharides	(13)
Antimicrobial factors	Eastern oyster	Tissue extract and juice	Inhibit bacteria growth	(9)
	Northern quahog	Tissue extract and juice	Inhibit bacteria growth	(9)
Hemagglutinin	Pacific oyster	Serum	Agglutinate vertebrate erythrocytes	(10)
	Eastern oyster	Hemocytes and serum	Agglutinate vertebrate erythrocytes	(10, 11, 12)
Bacterial agglutinin	Pacific oyster	Serum	Agglutinate bacteria, fish sperm, and algae	(8)
	Northern quahog	Serum	Agglutinate bacteria and algae	(2)
Hemolysin	Blue mussel	Hemocytes and serum	Lyse mammalian erythrocytes and human lymphocytes	(7)
	Northern quahog	Serum and shell liquor	Lyse mammalian and avian erythrocytes, inducible	(1, 6)

[a](1) Anderson (1981)
(2) Arimoto and Tripp (1977)
(3) Cheng and Rodrick (1974)
(4) Cheng and Rodrick (1975)
(5) Cheng et al. (1975)
(6) Graham (1968)
(7) Hardy et al. (1976)
(8) Hardy et al. (1977a, 1977b)
(9) Li (1960)
(10) McDade and Tripp (1967a, 1967b)
(11) Tripp (1966)
(12) Vasta et al. (1982)
(13) Yoshino and Cheng (1976)

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