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Species-specific sequences of abalone lysin, the sperm protein that creates a hole in the egg envelope

(*Haliotis*/fertilization/gamete recognition proteins)

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ABSTRACT **Abalone eggs are contained within a rigid, elevated vitelline envelope through which the sperm must pass before reaching the egg cell membrane. Abalone spermatozoa possess an acrosomal protein called lysin that creates a hole in the egg vitelline envelope by a nonenzymatic mechanism. Lysins from two species of abalone, termed pink and red, which share the same habitat, exhibit species specificity in the dissolution of isolated egg envelopes. Cloning and sequencing the cDNAs for pink and red abalone lysins reveal transcript lengths of ≈660 nucleotides. The open reading frames of 465 (pink) and 462 (red) nucleotides show a 13% difference. The 3′ untranslated regions before the poly(A) tails are 170 (pink) and 165 (red) nucleotides long and differ from each other by about 7%. The protein sequences show nearly identical signal sequences of 18 amino acids for both lysins. The mature protein is 137 amino acids in the pink abalone and 136 in the red abalone; the two mature lysins differ in 29 of 137 amino acids (21%). The most variable region, which may account for lysin's species specificity, is at the NH_2 terminus, where 11 of the 15 amino acids differ between the two species. Predictions of secondary structure indicate that both lysins contain four homologous amphiphilic α-helices.**

In animals the fusion of sperm and egg during fertilization is preceded by several events mediated by gamete recognition proteins. These events include the adhesion of sperm to the egg envelope, the induction of the acrosome reaction by components of the egg's extracellular matrix, and penetration of the sperm through the egg envelope. When cross-species fertilization is attempted, blocks to these events can be demonstrated depending on the particular species combinations being studied. Such blocks to cross-species fertilization can be readily demonstrated in congeneric species of marine invertebrates that share the same habitat, have overlapping breeding seasons, and release their gametes to the seawater where fertilization occurs. Knowledge of the proteins (and their genes) that mediate the species-specific events of sperm–egg interaction is important for understanding the molecular mechanisms of fertilization. It will also be important in understanding the evolution of the mechanisms that prevent successful sperm–egg interaction and ultimately create barriers to reproduction between species.

Abalone are large archeogastropod molluscs of the genus *Haliotis*, eight species of which occur on the Pacific Coast of North America. The abalone egg is enclosed in an elevated glycoproteinaceous vitelline envelope (VE; ≈1 μm in thickness) through which the spermatozoon must pass before it can fuse with the egg. During fertilization the abalone spermatozoon attaches to the VE, its acrosome granule opens, and two proteins of M_r ≈16,000 and ≈18,000 are released onto the VE. A hole 3 μm in diameter appears in seconds in the VE to allow the 1-μm (diameter) sperm to pass through this extracellular barrier (1). The M_r 16,000 protein (hereafter called lysin) can be purified from seawater after exocytosis of the acrosome (1); this protein alone will dissolve the egg VE, which is why it is termed lysin. The M_r 18,000 acrosomal protein, when purified from lysin by ion-exchange chromatography, has only about 2% of the lytic activity of an equivalent weight of lysin (V.D.V., unpublished). Although its role in abalone fertilization remains unknown, the M_r 18,000 protein does not appear to function in lysis of the VE.

Lysin is not an enzyme; when it dissolves egg VEs no covalent bonds are broken (1, 2). Lysin has a pI of about 9, is not glycosylated, is readily soluble, and behaves as a globular protein on gel filtration (M_r ≈17,000; ref. 1). Its biological action to dissolve VEs is not affected by cations or sulfhydryl reagents. However, lysin binds avidly to silicone- or paraffin-coated glass. Lysin interacts with liposomes made of phosphatidylcholine to release entrapped carboxyfluorescein, and it also induces the fusion of negatively charged liposomes (30). These characteristics show that lysin is truly an amphiphilic protein. Our goal is to determine the mechanism of action of lysin to define the structural basis for the species-specific dissolution of egg VEs by lysin. As a first step we have cloned and sequenced the cDNAs for lysin from two species of abalone.¶ The region of greatest difference in amino acid sequence between these two lysins is at their NH_2 termini.

MATERIALS AND METHODS

Gametes, Cell Fractions, and VE Dissolution Assay. Pink abalone (*Haliotis corrugata*) and red abalone (*Haliotis rufescens*) eggs and sperm were spawned from adults, and egg VEs were isolated, radioiodinated, and washed as described (1). The VEs were stored indefinitely at 4°C in filtered seawater containing 1 mg of bovine serum albumin per ml and 0.05% sodium azide. A preparation of impure lysin was made by homogenization of a 50-μl frozen sperm pellet in 1 ml of seawater, centrifugation of the homogenate at 20,000 × *g* for 20 min, dilution of the homogenate to 0.5 mg of protein per ml in seawater, and storage in 0.5-ml samples at −70°C. The major proteins in this sperm extract were lysin and the M_r 18,000 acrosomal protein (1). For each assay point, 100 μl of lysin was diluted 50% per dilution in a row of ten 1.5-ml Eppendorf tubes in seawater containing 1 mg of bovine serum albumin per ml. Ten microliters of ^{125}I-labeled VE suspension (≈10^5 cpm) was added and the tube was agitated on a rotary table for 30 min (23°C). The tubes were microcentrifuged 2

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Abbreviation: VE, abalone egg vitelline envelope.

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¶The sequences reported in this paper have been deposited in the GenBank data base (accession nos. M34388 for red abalone lysin and M34389 for pink abalone lysin).

min at 10,000 × *g* and 55 μl of the supernatant was removed. Seven milliliters of scintillation fluid was added and the cpm were determined. The remaining 55 μl, representing both soluble and insoluble ^{125}I-VE cpm, was dissolved by addition of 50 μl of 10% $NaDodSO_4$ and similarly assayed for radioactivity. Protein assays (4) used bovine serum albumin as a standard.

Molecular Biology. To isolate total RNA, ripe testes were dissected from male abalone and cut into 2-mm pieces (4°C). Ten-gram portions were homogenized in 200 ml of solution D (5) in a Waring blender using the exact ratios of the other additions to solution D as published (5). Total RNA was stored in 80% ethanol at −20°C. The poly(A)$^+$ fraction of RNA was isolated on oligo(dT)-cellulose (Collaborative Research) and stored in 2-μg samples in 80% ethanol at −20°C (6).

cDNA was made from 2 μg of poly(A)$^+$ RNA using an Amersham cDNA synthesis kit and oligo(dT) as primer. λgt11/cDNA libraries were constructed with an Amersham λgt11 kit using all of the cDNA from 2 μg of poly(A)$^+$ RNA. Phage λgt11 were plated on Y-1090 *Escherichia coli* and plaque lifts on nitrocellulose filters were screened with a 1:5000 dilution of the rabbit antiserum to red abalone lysin, followed by detection with an alkaline phosphatase-conjugated goat anti-rabbit IgG as given in the Bio-Rad Lambda-Lift detection kit (Bio-Rad). The rabbit antibody to chromatographically purified M_r 16,000 red abalone lysin (7) was a gift of Anita Fridberger (University of Uppsala). Positive plaques were isolated and the phage plaque was purified by an additional screen. Phage DNA was isolated and digested with *Kpn* I and *Sac* I (Stratagene) and the size of the insert was determined. The longest inserts were cloned into pBluescript (KS+ and KS−; Stratagene) following the manufacturer's directions. M13KO7 phage was used to generate single-strand DNA (8) for dideoxy chain-termination sequencing using a Sequenase 2 kit (United States Biochemical). Both strands of two independently picked λgt11 plaques of each species were sequenced. For Northern analysis a full-length probe was made to the red abalone lysin cDNA sequence using a Gene Amp kit (Perkin–Elmer/Cetus), pBluescript DNA containing a red abalone lysin cDNA insert, and oligonucleotide primers at both extreme ends of the lysin sequence. The amplified product was labeled with [^{32}P]dCTP to a specific activity of 3 × 10^8 cpm/μg using a BRL random-primed DNA labeling system (BRL). Two micrograms each of red and pink abalone testis poly(A)$^+$ RNA was resolved on a 1% agarose/formaldehyde gel, and the standard Northern transfer, hybridization, washing, and autoradiography procedures followed. A wash in 0.9 M NaCl/90 mM sodium citrate/0.1% $NaDodSO_4$ at 37°C for 30 min removed all the nonspecific background.

RESULTS

Dissolution of VE by Lysin. The species specificity of red and pink abalone lysins to dissolve isolated ^{125}I-labeled VE was determined (Fig. 1). Pink abalone lysin dissolves both pink and red abalone VE, with significant differences (specificity) seen only in the part of the curve below 313 ng of lysin per assay. Fifty percent dissolution of pink VE occurs at 450 ng of pink abalone lysin and of red abalone VE at 565 ng of pink abalone lysin (Fig. 1 *Left*). The linear plot obtained when a geometric increase in nanograms of pink abalone lysin is plotted against percent solubilized pink abalone VE is supportive evidence for a nonenzymatic mechanism of the dissolution process. However, red abalone lysin (Fig. 1 *Right*) is very specific in its dissolution of only red abalone VE (50% solubilization at 156 ng per assay); it is essentially unable to dissolve pink abalone VEs at these and even 10-fold higher concentrations.

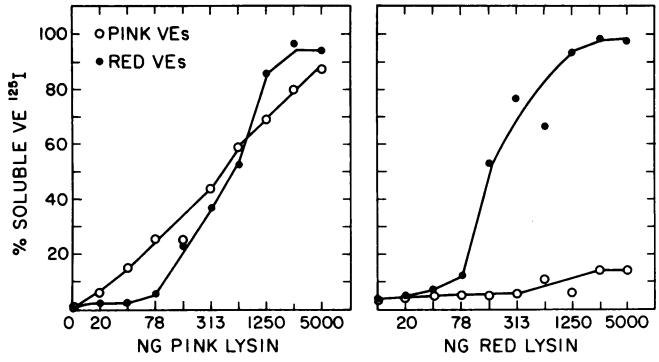

FIG. 1. Dissolution of isolated ^{125}I-labeled VEs by abalone sperm lysin. Pink abalone sperm lysin dissolves both pink and red abalone VE; however, red abalone lysin dissolves only red abalone VE. Each point is the average of two or three determinations. Each point (horizontal axis) is a doubling of lysin protein.

Cloning and Sequencing of cDNAs Coding for Pink and Red Abalone Lysin. The abalone spermatozoon has an enormous acrosome granule (1, 9), and we had previously shown that lysin is the most abundant protein in the cell (1). The rabbit antibody to chromatographically purified lysin (7) reacts monospecifically with only the lysin band on Western immunoblots of whole abalone sperm (not shown). *In vitro* translation of the total poly(A)$^+$ RNA, followed by immunoprecipitation, shows lysin to be a major protein product of translation, indicating that the mRNA coding for lysin is a major fraction of the total poly(A)$^+$ RNA of abalone testes. We also found that the antibody to red abalone lysin cross-reacts with pink abalone lysin (data not presented).

Screening λgt11 phage libraries of testis cDNA with the antibody to red abalone lysin identified 24 positive red abalone plaques (R1–R24) and three pink abalone plaques (P1–P3). Although we had used the *Eco*RI site of λgt11 for cloning, we could not excise inserts from λgt11 DNA with *Eco*RI due to loss of one or both restriction sites. We therefore used *Kpn* I and *Sac* I to digest the λgt11 DNA and looked for a shift upward in the 1% agarose gel of the 2.08-kilobase λgt11 *Kpn* I/*Sac* I fragment. The shift upward of this fragment, indicating the presence of abalone cDNA, ranged from about 50 to 700 nucleotides. The longest inserts of two independently picked phage plaques from the red abalone cDNA library (R13, R14) and two from the pink abalone library (P1, P3) were cloned into pBluescript and single-stranded DNA was generated. All four clones contained the entire coding region for lysin.

Comparison of the nucleotide sequences (Fig. 2) shows 79% similarity between the two lysins. The previous determination by Edman degradation of the amino acid sequence of red abalone lysin (7) had established that nucleotide 55, the first C of the codon CGC, codes for the NH_2-terminal arginine of the secreted protein. Three ATG codons occur 5′ to this arginine. The ATG numbered 1 is assigned as the initiation codon. This yields a signal sequence of 54 nucleotides (18 amino acids) and an open reading frame of 465 nucleotides for pink abalone lysin and 462 abalone for red lysin cDNA. The ATG numbered 1 also fulfills Kozak's criterion for initiation sequence motifs in having an adenine at position −3 (10). None of the four clones extend more than 15 nucleotides upstream from the ATG numbered 1. The sequences of the lysin cDNAs of the two species in this short 5′ untranslated region are identical and do not contain stop codons or regulatory sequence motifs.

Comparison of the two sequences shows there are 59 nucleotide differences in the open reading frame of 462 nucleotides (≈13%). The stop codon (TGA) is followed by 170 nucleotides in the pink abalone lysin and 165 in the red

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P TAC AAG ATG AAG CTG TTG GTG CTT TGC CTT TTT GCG ATG ATG GCG ACA TTG GCT GTG TCG
R ... ... ... ... ... ... ... ... ... A.. ... ... ... ... ... ... ... ..A A.. ..A

P CGC CAC CGC TTC CGT TTT ATT CCA CAT AAG TAT ATA CGC AAG GAG TTT GAA GTT GCA CTG
R ... AG. T.G CAT TA. G.. GAA ..C --- ... .T. T.. AA. ... .CT ... ... ... ... ...

P AAG GTA GAG ATC ATT GCC GGA TTC GAT AGA ACG CTG GTC AAA TGG CTT AGA GTT CAT GGC
R ... ... C.. ... ... ..T ... ... ... ... GG. ... ... ... ... ... ... ... ... ...

P GGA CGT TTG AGT ACA GTT CAG AAG AAG GCG CTG TAC TTC GTC AAT AGA CGT TAC ATG CAG
R A.. ACA ... TC. ..C ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

P ACC CAC TGG CAG AAT TAC ATG CTC TGG ATA GTG AGG AAG ACC GAT GCA CTG GGC AGA CCT
R ... ... ... GC. ... ... ... ... ... ... AAT .A. ... .T. ... ... ... ... ... A..

P CCA GTT GTT GCT GAC TAC AGT CGG TTA GGT GCC GAA ATT GGA AGA CGT ATT GAT ATG GCA
R ... ... ... .G. ... ... .CA ... ... ... ... ... ... ... ... ... ... ... ... ..T

P TAT TTT TAC AAC TTC CTG AAC GGG AGG AAC ATG ATC CCA AAA TAT CTG CCA TAC ATG GAG
R ..C ... ... G.. ... ... ..A .AT .A. ... ... ... ... ... ... ... ... ... ... ..A

P GAA ATT AAT AGA ATG CGC CCG GCA GAT GTC CCA GTT GCA AAC AGA GGC AAG TGAAACCAACG
R ... ... ... ... ... ... ... ... ... ... ... AA. T.. .TG ... ... ... ....C.T.

P AAGAAGAAAGCTGCATGCACCCTGCATGTCAACAGATACAGAAAACATCGTCCGTCAAACACGAGGAGCTGTGACAGCA
R .....C....T.....G.....----.....G.....A.....C..

P TCCATCCCAACATGGGTTGGCTATTTGTTGCACGATT-TTAACACAGAAAGATTGTACATTC
R ...-.---.....T....A..G.....

P CAGAATAAATAGATTTACT--A20
R .....ACA20
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FIG. 2. Comparison of cDNA sequences coding for pink (P) and red (R) abalone lysins. Dashes are for alignment and dots denote identity. In addition to λ forward and reverse primers (Promega), oligonucleotides corresponding to 5′→3′ positions −6 to +9 and 318–336 and to the complementary strand 5′→3′ positions of 120–100, 263–241, and 633–613 were used as sequencing primers. The initiation ATG, stop TGA, and poly(A) consensus sequence AATAAA are underlined. The CGC (underline) at position 55 is the NH_2-terminal arginine of mature lysin (7).

abalone lysin of 3′ untranslated sequence before the termination of the cDNA by the poly(A)$^+$ tail. There are 13 nucleotide differences out of ≈165 (7.9%) in the 3′ untranslated portions of the two cDNAs. The poly(A) consensus sequence AATAAA is 16 (pink) and 18 (red) nucleotides upstream from the start of the poly(A)$^+$ tail. The longest lysin cDNAs we have sequenced are about 670 nucleotides.

The fact that the 5′ untranslated region of all four cloned cDNAs does not extend more than 15 nucleotides upstream from the assigned initiation codon indicates that the 5′ untranslated region of the lysin transcript is either very short or, alternatively, contains secondary structure that blocks full-length cDNA synthesis. A Northern analysis was performed to establish the approximate length of the mRNA. Analysis of pink and red abalone testes poly(A)$^+$ RNA using an amplified full-length red abalone lysin cDNA probe yielded a single band of hybridization of ≈660 nucleotides for both species (Fig. 3). We tentatively conclude from these data that the mRNA coding for lysin has a relatively short 5′ untranslated region and poly(A)$^+$ tail.

Amino Acid Sequence and Secondary Structure. Translation of the cDNA sequences into protein (Fig. 4) shows that the proposed signal sequences of 18 amino acids for each lysin agree well with von Heijne's predictions for eukaryotic signal sequences (11) in having one positive charge at its NH_2 terminus (lysine at −17), followed by the exclusively hydrophobic core (−15 to −6), which is followed by the COOH-terminal (−5 to −1) portion containing two neutral amino acids (serine and threonine). Comparison of the two signal sequences shows them to be extremely conserved.

Mature pink abalone lysin beginning at the arginine numbered 1 (7) is 137 amino acids in length (M_r 16,320), whereas red abalone lysin, lacking the histidine at position 9, is 136 residues (M_r 16,083) (Fig. 4). There are no cysteines and no sites for N-linked glycosylation in either mature protein. A GenBank search shows no homology to any known protein. Both lysins are very basic, highly charged proteins, red lysin having a net charge of +16 and pink lysin +19. The amino acid sequence of red abalone sperm lysin shown in Fig. 4 differs in seven positions from the previously reported sequence (7). This earlier work was extremely valuable in establishing the NH_2-terminal sequence of lysin (7). The 18-amino acid signal sequence plus the 136-amino acid sequence of mature red abalone lysin would have an M_r of about 18,000, which leads to the speculation that the M_r 18,000 protein released with lysin from the acrosome (1) might be unprocessed lysin. However, gas-phase sequencing of the M_r 18,000 acrosomal protein shows that the first 41 amino acids are unrelated to lysin (V.D.V., unpublished).

The major differences at the NH_2 terminus of lysin may be the reason for the observed species specificity of lysin's

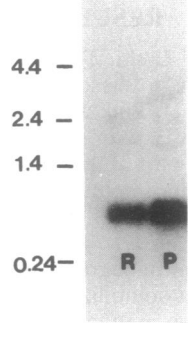

FIG. 3. Northern blot analysis using an amplified full-length red abalone lysin cDNA as a random-primed labeled probe. A single band of hybridization of ≈660 nucleotides in length was detected in 2 μg of poly(A)$^+$ RNA isolated from pink (P) and red (R) abalone testes. Sizes are given in kb.

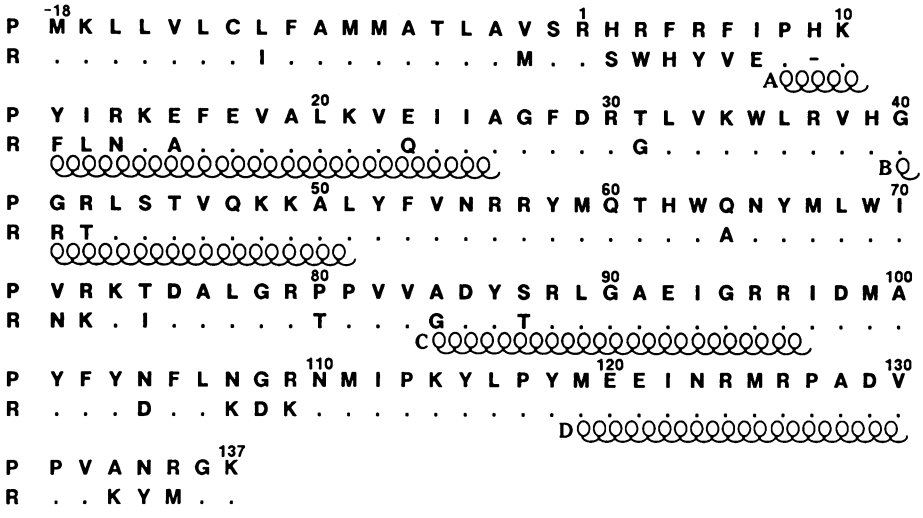

FIG. 4. Comparison of amino acid sequences of pink and red abalone lysin using the single-letter amino acid code. Dots denote identity. The R labeled no. 1 marks the NH_2-terminal arginine of mature lysin (7). The dash at position 9 in the red lysin sequence is for alignment. The predicted α-helices (A–D) common to both sequences are shown as coils. The previously reported red lysin sequence determined by Edman degradation contained seven errors in amino acid placement and differed substantially in placement of predicted α-helices (7).

biological activity (Fig. 1). Counting the histidine at position 9 of the pink abalone lysin, there is a total of 29 differences (21%) between the two mature lysins. The greatest variation between the two occurs between positions 2 and 15, where 11 of the amino acids are different. Most of these differences are not conserved changes. The amino acid composition for pink abalone lysin between positions 2 and 15 is seven positively charged, five hydrophobic, one neutral, and one negatively charged residue. The composition from positions 2 to 14 for the red abalone lysin is three positively charged, six hydrophobic, three neutral, and one negatively charged residue. Thus, the NH_2 termini of the two lysins differ dramatically in spite of overall great similarity of both protein and DNA sequences.

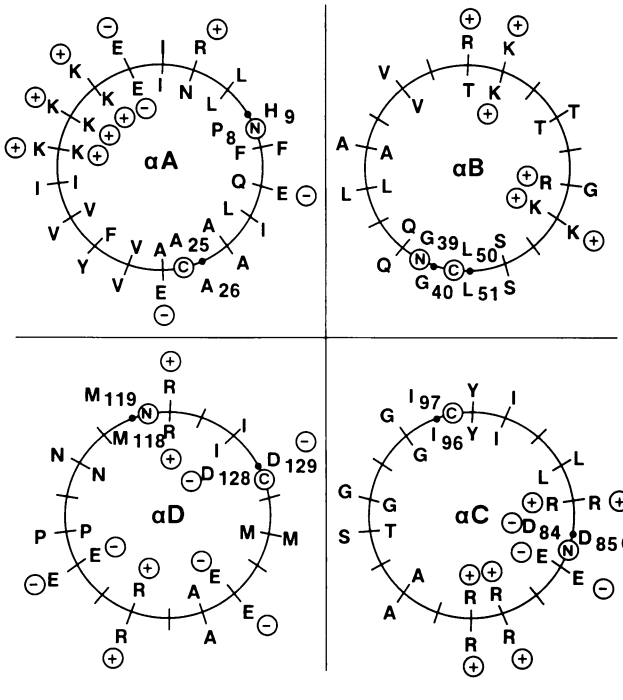

FIG. 5. Amphiphilic α-helices of lysin shown as helical wheels. Pink abalone sequences are shown on the outside of the wheels, and red abalone sequences are on the inside. Each perpendicular line is 20° of arc. Contiguous residues are 100° apart. NH_2 to COOH progression is counterclockwise; the termini of each helix are marked circle N or C. In helix A the red abalone sequence is shifted one position for best alignment.

Analysis for secondary structure in both lysin sequences (12) predicts four α-helices common to both lysins (Fig. 4). Wheel diagrams of these helices (Fig. 5) show that A, B, and C are highly amphiphilic. All three plot near the boundary of globular and surface-acting proteins in a hydrophobic moment plot (13). The most amphiphilic is helix C, with a hydrophobic moment of 0.75. The average hydrophobic moment of the four red lysin helices is 0.46, meaning that lysin resembles apolipoproteins in intrinsic amphiphilicity (14).

Three distinct physical properties of lysin are consistent with the prediction that the molecule contains amphiphilic α-helices. (*i*) The circular dichroism spectrum of ion-exchange-purified red abalone lysin indicates an α-helical content of 56% (data not shown). (*ii*) Evidence for the presence of basically charged amphiphilic α-helices in red abalone sperm lysin comes from the observation that the purified protein binds to calmodulin-Sepharose in the presence of Ca^{2+} and is eluted by addition of a Ca^{2+} chelator (V.D.V., unpublished). Such behavior is a characteristic of proteins containing positively charged amphiphilic α-helical domains (15). (*iii*) The x-ray diffraction pattern of single crystals of red abalone lysin (16) exhibits strong reflections at d spacings consistent with the presence of α-helices aligned nearly parallel to one crystal axis (17).

DISCUSSION

Species-Specificity of Abalone Fertilization. Fig. 1 suggests that red abalone sperm cannot penetrate the pink abalone VE, and thus fertilization in this cross would be impossible. However, hybrid embryos can be produced from both possible crosses of these two species by addition of excess sperm (18). Although carefully controlled quantitative data are unavailable on cross-fertilizations, it is reported (18) that the easiest cross between these two species occurs using red abalone eggs and pink abalone sperm. The discrepancy between the data in Fig. 1 and the laboratory demonstration of hybrid fertilization in both directions between these two species may be due to relatively small quantities of lysin being evenly distributed over the entire VE in the assay, whereas during exocytosis of the acrosome a large quantity of lysin is deposited on only a small area of the VE and a hole

of only 3 μm (three sperm diameters) is formed (see figures in ref. 1).

The fossil record of the genus *Haliotis* is poor, with scant representatives resembling modern species being known from the Miocene (19). The time of divergence of pink and red abalone species remains unknown. However, careful quantitation of the haploid DNA content of their sperm shows a difference of 10%, the red abalone value being 1.8 pg, whereas the pink abalone is 2.0 pg (20). Currently, ≈80 species of the genus *Haliotis* are distributed worldwide. In order to understand the molecular evolution of a sperm protein involved in fertilization, additional lysin sequences from other species are needed. Lysin provides an excellent genetic marker for examining the evolutionary divergence of a gamete recognition protein in congeneric species of a marine invertebrate.

Gamete Recognition Proteins. Preliminary evidence exists supporting the hypothesis that invertebrate gamete recognition proteins such as abalone sperm lysin and sea urchin sperm bindin (21–23) might be subject to relatively rapid evolutionary change and may be important in the process of speciation. For example, the amino acid sequence of a VE lysin (M_r 13,800) from sperm of another archeogastropod, *Tegula pfeifferi*, has been determined (2). The *Tegula* sequence shows no amino acid sequence homology to abalone lysin. However, the *Tegula* lysin also acts by a nonenzymatic mechanism, and predictions of its secondary structure suggest that it also contains four α-helices, at least two of which are amphiphilic (2). Thus, although these two genera of archeogastropods have diverged to the point of showing no homology at the level of primary structure of their lysins, the secondary structure and mechanism of action of their lysins may be highly conserved.

Besides abalone sperm lysin, the only other marine invertebrate gamete recognition protein known at the sequence level is sea urchin sperm bindin (21–23). Bindin is an acrosomal protein that attaches the sperm to the egg VE and shows species specificity in interacting with the VE (24, 25). The published partial amino acid sequence comparison of bindins from two congeneric sea urchin species shows regions of major differences (23, 26). Further support for the rapid evolution of bindin comes from a recent study of the tropical Pacific sea urchin *Echinometra mathaei* (S. R. Palumbi and E. C. Metz, personal communication). This study shows that on the basis of mitochondrial DNA and single-copy nuclear DNA, this single species is actually four separate species. The average genetic difference in these two types of DNA among the four groups is only 1–2%, making them the closest related urchin congeners. However, in cross-gamete mixtures among these four groups, the eggs do not fertilize, and the block appears to be the failure of sperm to attach to eggs. The hypothesis is presented that rapid change of the bindin gene (and also the egg surface bindin receptor) may be a major factor in the process of speciation in *Echinometra*.

Structure and Mode of Action of Lysin. Based on the predictions of secondary structure and the experimental data, we propose that the α-helices of lysin associate to form a four-helix bundle (27) with the hydrophobic helical surfaces facing each other and the charged surfaces facing the aqueous environment. Lysin's behavior to act as a globular protein on gel filtration, dissolve a structure (the VE) held together by hydrophobic interaction, render neutral liposomes leaky, fuse negatively charged liposomes, and bind to paraffin is consistent with an amphiphilic helix bundle model for this sperm protein.

We suspect that lysin dissolves VEs by acting as a competitor for the hydrophobic bonds holding the VE fibers together (1). Evidence for such hydrophobic bonds comes from the fact that VEs readily dissolve in 30% isopropyl alcohol, 80% ethanol, 100% dimethyl sulfoxide, 100% acetic acid, 4 M guanidine hydrochloride, 0.5% sarkosyl, and 1 M NaSCN (1). We hypothesize that when lysin contacts the VE, liposomes (3), or paraffin, the α-helices rotate, presenting their hydrophobic surfaces to the hydrophobic environment as observed for intrinsic membrane proteins (28). In other words, the nonenzymatic mechanism of actin of lysin may require unfolding of the protein.

Lysin is of biological interest for several reasons. In terms of fertilization, the morphological events of abalone fertilization closely resemble those of mammals (1). The mechanism used by mammalian sperm to penetrate the egg zona is unknown (29). Vertebrate and invertebrate sperm–egg interactions exhibit species specificity; lysin sequences provide a partial explanation for this specificity in one group of animals. In terms of cell biology, lysin not only fuses membranes but it also represents a unique mechanism used by one cell to penetrate the extracellular matrix of another cell. There are many examples in normal development, regenerative growth, and disease processes where cells pass through extracellular matrices. Lysin-like proteins may be found to be involved in such processes.

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