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PERIRECEPTION IN OLFACTION: MOLECULAR MASS SIEVING BY AESTHETASC SENSILLAR CUTICLE DETERMINES ODORANT ACCESS TO RECEPTOR SITES IN THE CARIBBEAN SPINY LOBSTER *PANULIRUS ARGUS*

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Summary

The responsiveness of chemoreceptor neurons depends on a combination of perireceptor and receptor events. Olfactory neurons of crustaceans are packaged into distinctive cuticular sensilla called aesthetascs. The cuticle of aesthetascs is thin and permeable, even though it does not contain any obvious surface pores or pore tubules. This suggests that this 'spongy' aesthetasc cuticle may act as a molecular sieve that restricts large odorant molecules from entering the sensilla and binding to the olfactory neurons. We examined whether this is so for the aesthetasc cuticle of the Caribbean spiny lobster *Panulirus argus*. We used a chromatographic column packed with aesthetasc cuticle and connected to a flow-through ultraviolet spectrophotometer to measure the elution times of ultraviolet-absorbent molecular mass markers between 165 and 2×10^6 Da. Molecules larger than approximately 8.5 kDa had similar elution times, indicating that they did not penetrate the cuticle. Molecules smaller than 8.5 kDa had longer elution times that were directly and inversely proportional to their molecular mass. These results suggest that aesthetasc cuticle excludes molecules larger than 8.5 kDa from having access to the olfactory receptor neurons. We conclude that the molecular sieving capacity of the aesthetasc cuticle of *P. argus* is a perireceptor mechanism that is a critical determinant of the types of molecules capable of stimulating its olfactory receptors.

Key words: spiny lobster, *Panulirus argus*, aesthetasc, olfaction, olfactory receptor, chemoreception, chemical senses, Crustacea, arthropod, perireception.

Introduction

Several factors are important in determining what odorants stimulate an olfactory system. One is the odorant-binding properties of receptor molecules on the membrane of dendrites of olfactory neurons (Buck, 1996; Sengupta *et al.* 1996). Perireceptor events, which are events that occur in the extracellular space surrounding the receptor neurons, are also critical in determining what odorant molecules are active (Pelosi, 1996).

Some perireceptor events are a consequence of the fact that chemoreceptor neurons often operate in a harsh external environment. Terrestrial vertebrates reduce the exposure of their olfactory neurons to desiccation by maintaining a layer of mucus over the olfactory epithelium (Joshi *et al.* 1987). Arthropods, which have a cuticular exoskeleton, fight desiccation by packaging their receptors into sensilla. These sensilla are fine elaborations of the cuticle with a central lumen containing the dendrites of chemosensory neurons. The sensilla are filled with lymph (Kaissling and Thorson, 1980; Gleeson *et al.* 1993; Kijima *et al.* 1995), and the sensillar cuticle is permeable enough to allow movement of odorants into the receptor environment (Altner and Prillinger, 1980; Heimann, 1984). But such an organization of chemoreceptor neurons in vertebrates and arthropods causes its own problems. For terrestrial animals such as vertebrates and insects, the odorant molecules, which are usually lipophilic volatiles, must move into the lipophobic mucus or sensillar lymph in which they are minimally soluble. For arthropods, the sensillar cuticle must be permeable enough to allow passage of odorants without being so permeable as to allow desiccation.

The solutions to these problems are the realm of perireceptor events, and they are important determinants of what types of molecules can activate chemoreceptor cells. In terrestrial species, odorant- and pheromone-binding proteins in the mucus or sensillar lymph bind volatile and lipophilic odorant molecules and deliver them to or away from chemoreceptor proteins located on the neuronal membrane (Pelosi, 1996). Odorant- and pheromone-binding proteins show specificity in their binding properties and thus can limit or exclude certain odorants from reaching the receptors (Vogt and Riddiford, 1981; Pevsner *et al.* 1990; Hérent *et al.* 1995; Pelosi, 1996;

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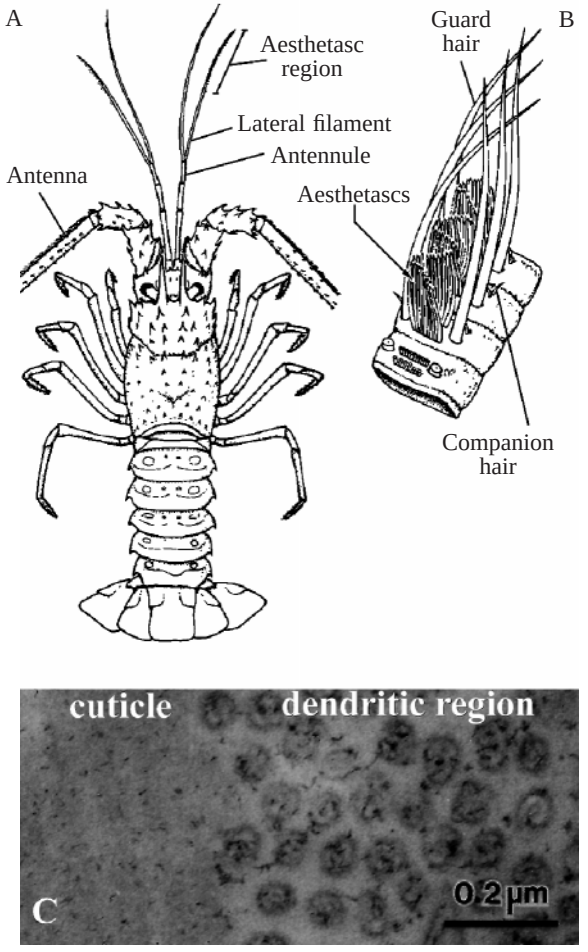

Fig. 1. Antennules and aesthetascs of *Panulirus argus.* (A) The antennules (Fig. 1a from Grünert and Ache, 1988). (B) A higher-magnification view of the aesthetasc region of the antennular lateral filament (Fig. 1b from Grünert and Ache, 1988). Each aesthetasc from *P. argus* is 0.8 mm long, and its diameter is 20 µm at the base, 27 µm in the bulbous region and 15–20 µm over most of its length. The diameter of its cuticle is 2.5–3.0 µm at the base, 1.5 µm in the bulbous region and 0.8–1.0 µm in the more distal regions. (C) A transmission electron micrograph of an aesthetasc sensillum exposed to lanthanum chloride before sectioning (Fig. 5j from Grünert and Ache, 1988). Note the dark staining in the cuticle and on the dendrites within the lumen of the sensillum, demonstrating that lanthanum (molecular mass 139 Da) penetrated the cuticle. Note also a lack of pore tubules in the cuticle. Scale bar, 0.2 µm. These figures are from Grünert and Ache (1988) and are used with permission from Springer-Verlag.

Steinbrecht, 1996). Arthropod sensilla usually have a series of passageways, pores and tubules through which chemicals pass to reach the sensory neurons (Altner and Prillinger, 1980; Keil, 1982; Altner *et al.* 1983; Schmidt, 1989; Popov *et al.* 1994). The width of these pore tubules is usually 10–40 nm or more in insects and ticks (Keil, 1982; Hess and Vlimant 1982; Popov *et al.* 1994) and 100–200 nm in crustaceans (Risler, 1977; Altner *et al.* 1983; Hamilton *et al.* 1985; Schmidt, 1989). Since 100 kDa proteins typically have diameters of less than 10 nm (Fasman, 1975), the pore tubules should allow passage of most chemical stimuli, certainly that of volatile compounds which are usually less than 300 Da.

An unusual and distinctive type of sensillum is the aesthetasc sensillum, which is an anatomically distinct class of sensillum located on the lateral branch of the first pair of antennae (antennules) of a phylogenetically diverse group of crustaceans (Fig. 1) (for reviews, see Heimann, 1984; Hallberg *et al.* 1992; see also Laverack, 1964; Laverack and Ardill, 1965; Ghiradella *et al.* 1968; Snow, 1973; Guse, 1979; Heimann, 1979; Bauchau and Passelecq-Gérin, 1984; Spencer and Linberg, 1986; Tierney *et al.* 1986; Grünert and Ache, 1988; Johansson and Hallberg, 1992; Gleeson *et al.* 1996). Aesthetascs are characterized as having a thin cuticle, lacking a terminal pore or pore tubules and being densely innervated only by chemoreceptors. Each aesthetasc is typically divided into two regions. The proximal region has a thick, laminar, non-permeable cuticle. The distal region constitutes most of the length of the aesthetasc and is where the cilia of the olfactory neurons, with their receptors and other transduction elements, are located (Blaustein *et al.* 1993; Hatt and Ache, 1996). The cuticle in this distal region is thin (e.g. 0.8-1.0 µm in the spiny lobster *Panulirus argus*: Grünert and Ache, 1988), non-laminar and 'spongy' (i.e. with spaces but no obvious surface pores or pore tubules) (Fig. 1C). Chemicals, at least dyes with molecular masses less than 500 Da such as Crystal Violet and lanthanum, have been shown to enter the aesthetascs only in this distal region (Ghiradella *et al.* 1968; Snow, 1973; Heimann, 1984; Tierney *et al.* 1986; Grünert and Ache, 1988; Gleeson *et al.* 1996) (Fig. 1C). Odorants move quickly through this cuticle, enabling aesthetasc olfactory neurons to respond within 300 ms of an odorant reaching the aesthetasc surface (Michel and Ache, 1992).

The ultrastructure of the aesthetasc cuticle suggests that it may act as a molecular sieve by preventing large molecules from reaching the chemoreceptor neurons. In this study, we have explored the molecular filtering properties of the aesthetasc cuticle of the Caribbean spiny lobster *Panulirus argus*. We used a chromatographic column packed with aesthetasc cuticle and connected to a flow-through ultraviolet spectrophotometer to measure the elution times of ultraviolet-absorbent molecular mass markers between 165 and 2×10^6 Da. We found that molecules above approximately 8500 Da were too large to diffuse into the aesthetasc cuticle, and molecules smaller than this were chromatographically separated according to their molecular mass. These results suggest that the molecular sieving capacity of aesthetasc cuticle is a critical determinant of which molecules can stimulate the olfactory receptors.

Materials and methods

Chemicals

Eleven chemicals with molecular masses between 165 and 2×10^6 Da were tested as molecular mass markers. They are listed in Table 1, along with their abbreviation, molecular mass, the concentration tested and the number of times each

Table 1. *Molecular masses and concentrations of compounds for which elution times were measured*

Name	Abbreviation	Molecular mass (Da)	Concentration (mg ml^{-1})	*N*
Dextran blue (D4772)	BD	2×10^6	5.0	3
Bovine serum albumin (A8531)	BSA	66×10^3	5.0	2
Carbonic anhydrase (C7025)	CA	29×10^3	2.5	3
Cytochrome *c* (C7150)	CC	12 400	2.5	3
Ubiquitin (U6253)	Ubiq	8557	5.0	3
Aprotinin (A3886)	Apro	6500	5.0	2
Insulin chain A (I1633)	InsA	2532	5.0	2
Angiotensin II fragment 3-8 (A3950)	AngII	775	10.0	2
Riboflavin (R4500)	Ribo	376	5.0	3
L-Tryptophan (T8659)	Trp	204	0.2	3
L-Phenylalanine (P8324)	Phe	165	8.3	3

The number in parentheses below each compound name is its Sigma catalogue number.

N, number of times each compound was tested in the chromatographic column.

was run on the column. All molecular mass markers were mixed in artificial sea water (ASW) at a pH of 8.1, with the following composition (in mmol l^{-1}): 423 NaCl, 9.0 KCl, 9.27 $CaCl_2$, 22.9 $MgCl_2$, 25.5 $MgSO_4$ and 2.15 $NaHCO_3$ (Cavanaugh, 1964). Tris buffer (pH 7.8) had the following composition (in mmol l^{-1}): 50 KCl, 10 Tris base, 320 sucrose, 12.9 $CaCl_2$, 23.1 $MgCl_2$ and 25.6 $MgSO_4$.

Collection of antennules

Lateral filaments of the antennules of Caribbean spiny lobsters, *Panulirus argus* (Latrielle), were collected from commercial fish houses in the Florida Keys, USA. Lateral filaments were excised and immediately placed in tubes filled with ice-cold Tris buffer, with approximately 100 antennules per tube. The tubes were kept on dry ice or in a freezer at –80 °C until they were transported to Atlanta. There they were stored in a freezer at –80 °C until used.

Preparation of aesthetasc cuticle

The antennules from one tube were thawed and placed in Tris buffer. The guard hairs and companions hairs, which are located on either side of the rows of aesthetascs (Fig. 1B), were manually removed from the antennule by scraping with a scalpel, and these hairs were discarded. The aesthetascs were then carefully removed and placed in Tris buffer on ice. To separate the cuticle from the cellular material, the aesthetascs were homogenized manually using a glass–glass homogenizer (Kontes). This mixture was sonicated five times for 5 s each on setting 35 of a Fisher sonic dismembrator (model 300) with a 4 mm titanium tip. This tissue was centrifuged at 6000 ***g*** for 10 min, resulting in a pellet (P1) containing aesthetasc sensillar cuticle and a supernatant (S1) containing the dendritic membrane of the olfactory neurons (Gentilcore *et al.* 1996). The P1 fraction was frozen. P1 fractions approximately 5000 antennules were thawed, combined, mixed in approximately 10 ml of ASW, sonicated again using the same procedure as above, and added to a chromatographic column as described below.

Scanning electron microscopy

A sample of the P1 fraction used in the chromatographic column was visualized using scanning electron microscopy. The sample was pipetted onto a stub, dried and sputter-coated with gold/palladium (Desk II sputter coater, Denton). The cuticle was examined at a voltage of 5 kV using a Leica S420 scanning electron microscope.

Chromatographic column and ultraviolet spectrophotometry

A chromatographic column (0.7 cm internal diameter, 10.0 cm length and 4.0 ml total volume) was gradually filled with the slurry of aesthetasc cuticle prepared as described above. The packed column was connected to a constant-pressure reservoir, or Mariotte flask (Determann, 1969; Brewer *et al.* 1974), filled with ASW. A pump was not used to deliver ASW, since preliminary experiments showed that the pressure of a pump, even at its lowest setting, caused such compression of the aesthetasc bed that the ASW flow virtually stopped. We allowed the system to run until the aesthetasc cuticle bed stabilized (4.0 cm height, or 1.8 ml total volume) and a constant flow rate was achieved (0.253±0.005 ml h^{-1}; mean ± S.E.M., N=10 measurements throughout the experiment). The experiments were performed in a cold room at 4 °C.

Each molecular mass marker was added to the column by uncoupling the tube from the Mariotte flask, carefully adding 100 µl of the marker to the top of the column, allowing the marker to move completely into the aesthetasc bed (which usually required approximately 1 h), carefully adding 1 ml of ASW to the top of the column, then re-attaching the tube to the Mariotte flask.

The output of the column was connected to a flow-through ultraviolet spectrophotometer (BioRad model EM-1) coupled to a chart recorder. This allowed continuous monitoring of the absorbence of the elutant at 280 nm.

All molecular mass markers (Table 1) except ubiquitin, insulin chain A and angiotensin II were tested in random order. At least one of the repeat trials for each compound was

separated from the others. After the results from these eight compounds revealed that the inflection point in the graph of molecular mass *versus* ultraviolet absorbence was in the molecular mass range between cytochrome *c* and tryptophan, ubiquitin, insulin chain A and angiotensin II were purchased and subsequently tested in random order. Thus, markers were not tested in the order of their molecular mass.

Data analysis

Our method of defining the molecular filtering capacity of aesthetasc cuticle was similar to that used in calibrating a column in filtration chromatography (Determann, 1969). Filtration chromatography separates molecules on the basis of differences in their molecular mass, and such separation is largely independent of the charged state and solubility of the molecules. In filtration chromatography, a column is packed with a porous material (e.g. Sephadex, Sephacryl, Sepharose or, in our case, 'sephaesthetasc') and washed with a solvent into which solutes (such as molecular mass markers) are added. Only the solvent (with its dissolved solute) that resides between the porous material flows and advances through the column; solvent and solute in the pores are in diffusion equilibrium with the stationary phase and do not advance in the column. Fractionation is based on the principle that molecular mass markers of different sizes show differential diffusion into these pores. Molecules larger than the pores cannot diffuse into them, so they pass through the column in the solvent between the packing material and consequently move rapidly through the column. Molecules small enough to diffuse into the pores move through the column more slowly. The pore size of the material is determined by comparing the ratio V_e/V_0 for the molecular mass markers, where V_e is the elution volume of the sample and V_0 is the void volume and is determined as the elution volume for an extremely large marker that cannot move through the pores. Molecules that do not move into the pores will have V_e/V_0 values of 1.0. Molecules that move into the pores will have V_e/V_0 values greater than 1.0. This value is proportional to the size of the molecule: the smaller the molecule, the greater the value of V_e/V_0. The value of V_e/V_0 for any compound is essentially independent of the size of the column, the concentration of the marker and the flow rate (Determann, 1969).

In our experiments, the value of V_e was measured as the volume of elution collected from the time that the 100 µl of marker completely moved into the cuticle bed and the ASW flow from the Mariotte bottle was turned on, to the time of peak absorbence. The value of V_0 was determined using Dextran Blue.

Results

Aesthetasc cuticle

Scanning electron microscopy showed that the P1 fraction used to pack the chromatographic column had a variety of lengths and shapes (Fig. 2). These included various lengths of tubular-shaped sections of broken aesthetasc sensilla and some non-tubular pieces of cuticle (Fig. 2). Consequently, while the aesthetasc cuticle met one criterion for being a good packing material for a chromatographic column (that the material has a rigid structure), it did not meet a second criterion (uniform size and shape) (Determann, 1969). Nonetheless, we had consistent results with the column, as described below.

Molecular mass chromatography and ultraviolet spectrophotometry

Four examples of ultraviolet spectrographs are shown in Fig. 3 for insulin chain A, cytochrome *c* and L-tryptophan. The shapes of the peaks varied for different markers (compare Fig. 3A, 3C and 3D) but were similar for different runs of the same marker (compare Fig. 3A and 3B for insulin chain A). The absorbence peak in these and other spectrographs was clear; the height-to-width ratio for all compounds (except riboflavin, see below) was 0.47±0.06 (mean ± S.E.M.). Some spectrographs showed a small absorbence early in the record (see ** in Fig. 3). When present, this absorbence peak appeared approximately 1 h after the Mariotte flask had been connected to the column and well before the absorbence maximum, and its height was always much smaller than the absorbence maximum. This absorbence peak was probably an artifact resulting from the slight disturbance of the cuticle bed caused by the introduction of ASW, which may have resulted in the release of a small amount of marker that had been trapped from previous runs.

The void volume V_0 (i.e. the elution volume V_e for Dextran Blue) was 1.20 ml. V_e/V_0 values for all molecular mass markers greater than 8.5 kDa (i.e. Dextran Blue, cytochrome *c*, carbonic anhydrase, bovine serum albumin, ubiquitin) were very similar, approximately 1.0 (Fig. 4). This indicates that molecules larger than 8.5 kDa did not diffuse into the spongy cuticle but rather moved through the column between the pieces of cuticle.

Molecules smaller than 8.5 kDa (aprotinin, insulin chain A, angiotensin II, tryptophan, phenylalanine) had V_e/V_0 values greater than 1.0 (Fig. 4). In addition, these markers showed a chromatographic effect. Smaller molecules had higher V_e/V_0 values than larger molecules, such that for molecules smaller than 8.5 kDa there was a significant inverse and linear relationship between molecular mass and retention time on the column.

Riboflavin was not included in Fig. 4 because its elution from the column differed from that of all the other markers in two ways. Compared with the other markers, its peak height-to-width value was exceptionally low (0.036±0.008, mean ± S.E.M., N=3) and its V_e/V_0 value was very high (4.58±0.23). These results suggest that this compound was partitioned on the column on the basis of factors other than molecular mass.

Discussion

Our results using aesthetasc sensillar cuticle from the lateral filaments of the antennules of the Caribbean spiny lobster *Panulirus argus* in ultraviolet column chromatography have

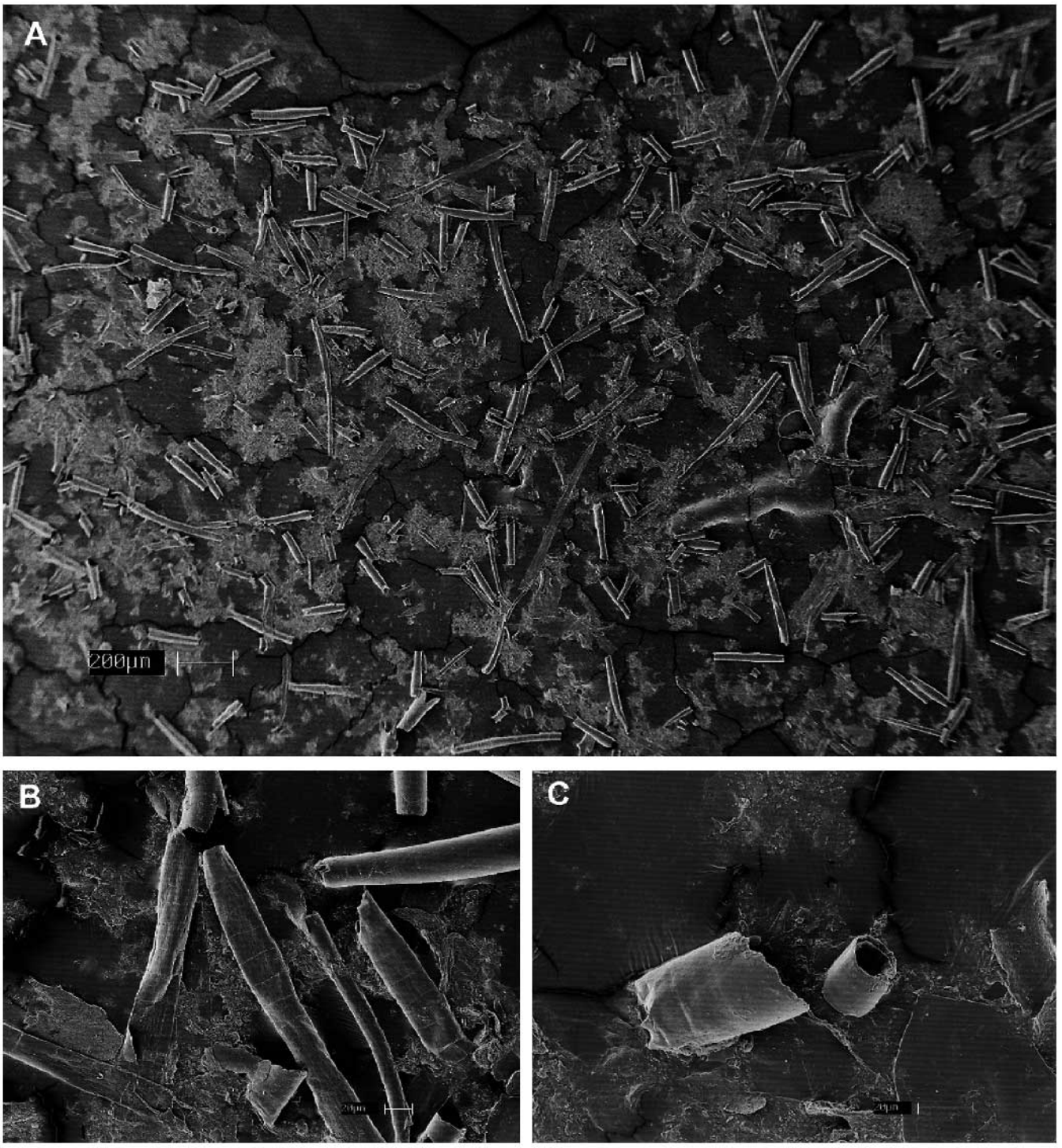

Fig. 2. Scanning electron micrographs of aesthetasc cuticle from the P1 fraction used to pack the chromatographic column. (A) Low-magnification image showing the diversity of different shapes and sizes of the pieces of aesthetasc cuticle used in the column, including tubular pieces of different lengths and non-tubular pieces seen as debris in the salt layer coating the stub. Scale bar, 200 µm. (B) High-magnification view showing long tubular sections of aesthetasc cuticle. Scale bar, 20 µm. (C) High-magnification view showing short tubular sections of aesthetasc cuticle. Scale bar, 20 µm.

shown that this spongy cuticle effectively excludes molecules larger than 8.5 kDa from diffusing into or out of the sensillar lymph that surrounds the olfactory receptor neurons. In addition, the movement of permeant molecules through the cuticle is chromatographic, with the movement of molecules being proportional to their molecular mass.

The limited permeability of the aesthetasc cuticle of *P. argus* should affect the sensitivity of its olfactory organ and,

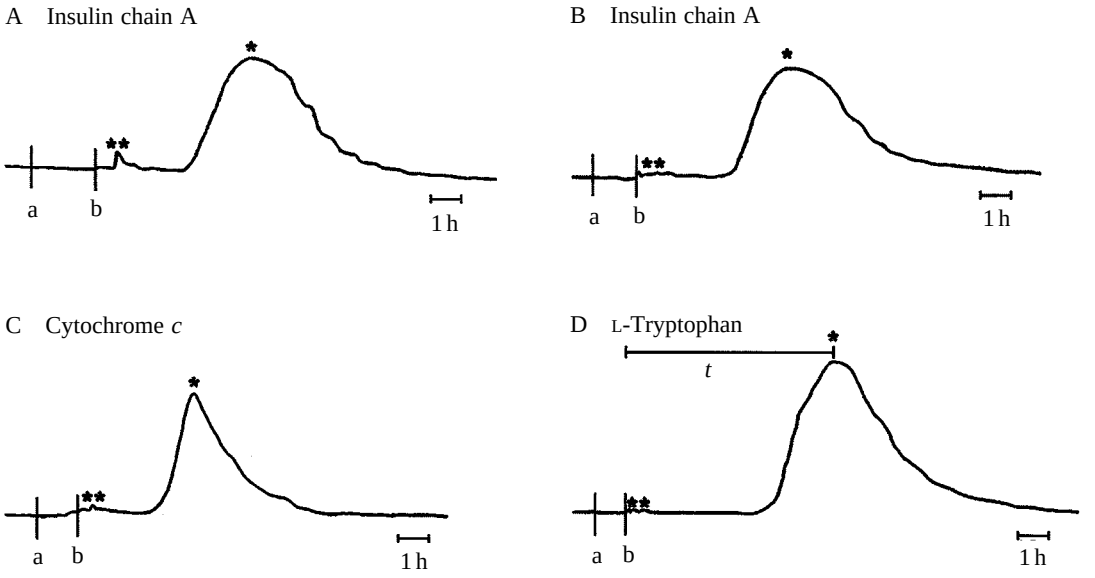

Fig. 3. Examples of ultraviolet spectrographs for (A,B) insulin chain A, (C) cytochrome *c* and (D) L-tryptophan. The times when the marker was introduced (a) and the ASW flow from the Mariotte flask was initiated (b) are marked. * marks the peak absorbence and ** marks an artifact caused by mechanical disturbance upon introduction of ASW (see text for explanation). For L-tryptophan, the elution time *t* was 8.3 h. The value of V_e/V_0, (where V_e is the elution volume of the sample and V_0 is the void volume) for tryptophan was calculated to be (8.3 h)(0.0253 ml h^{-1})/(4.8 h)(0.253 ml h^{-1}) or 1.73.

consequently, olfactory-driven behaviors such that only compounds smaller than 8.5 kDa are stimulatory. The most direct evidence supporting this idea comes from an electrophysiological study of the responses of lateral antennular chemoreceptive neurons to eight different extracts and body fluids of potential food before and after molecular mass fractionation by ultrafiltration (Ache *et al.* 1976). They found that the molecular mass fraction containing molecules smaller than 10 kDa was much more effective than the fraction containing molecules larger than 10 kDa and was as potent as the full extract. They also reported that the fraction containing molecules larger than 10 kDa had some activity, albeit minor. There are two likely explanations for this observation in the light of our finding that aesthetasc cuticle is completely impermeable to compounds larger than 10 kDa. The first explanation is that low molecular mass compounds may not have been completely removed by ultrafiltration, as was acknowledged by Ache *et al.* (1976). The second is that the fraction containing molecules larger than 10 kDa stimulates lateral antennular chemoreceptors that do not innervate the aesthetascs, which are known to exist (Laverack, 1964; Schmidt and Ache, 1996). Behavioral studies of the relative activity of molecular mass fractions have not been performed for *P. argus.* However, the low molecular mass fraction of food extracts has been shown to be the most effective behavioral feeding stimulant for a number of crustacean species, including the clawed lobster *Homarus americanus* (Derby, 1984; Daniel and Bayer, 1987), the shrimp *Palaemonetes pugio* (Carr, 1978) and an amphipod (Meador, 1989). Exceptions are known (Carr and Gurin, 1975; Carr, 1978; Zimmer-Faust *et al.* 1984), but the sensilla mediating these behaviors may not be aesthetascs and may not have the same permeability as aesthetascs of *P. argus*.

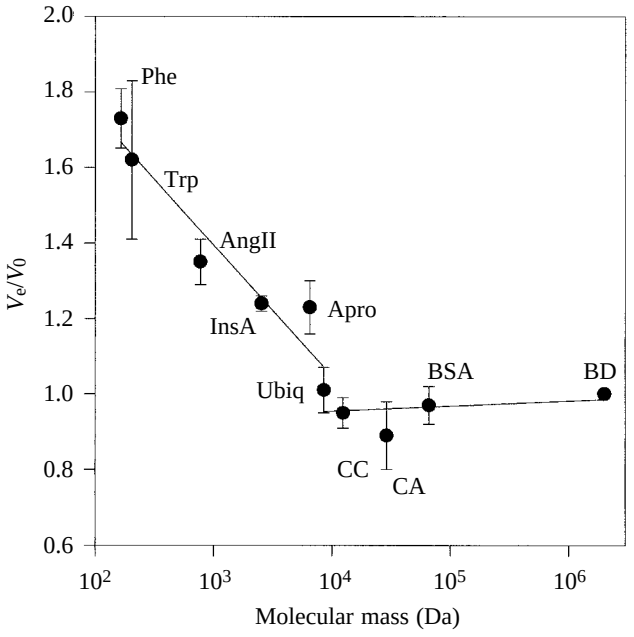

Fig. 4. Molecular mass sieving by aesthetasc cuticle. The figure shows mean values ± S.E.M. for V_e/V_0 for different molecular mass markers. Two linear regression lines are fitted to the data, with an intersection at about 8.5 kDa. The regression equation for the compounds larger than 8.5 kDa was y=0.014x+0.900 (r^2=0.074, P=0.48). The regression equation for the compounds smaller than 8.5 kDa was y=−0.347x+2.436 (r^2=0.919, P=0.0008). See Table 1 for information about the marker abbreviations, and see the text for an explanation of the figure.

Many of the stimulatory low molecular mass compounds for the antennular chemoreceptors of *P. argus* have been identified through biochemical, electrophysiological and behavioral studies (Derby and Ache, 1984; Carr, 1988; Derby *et al.* 1989; Michel *et al.* 1993; Lynn *et al.* 1994; Olson and Derby, 1995). Especially prominent are receptors for amino acids (e.g. taurine, glutamate, alanine, glycine), nucleotides (adenosine 5′-monophosphate, adenosine 5′-triphosphate), quaternary ammonium compounds (betaine) and ammonium itself. Since aesthetasc chemoreceptors in *P. argus* and other species are used for detecting chemicals from a distance and mediate searching behavior (Reeder and Ache, 1980; Devine and Atema, 1982), their molecular filtering properties help establish that high molecular mass compounds tend not to be used for these types of behaviors.

Why should spiny lobsters or other crustaceans have chemoreceptive sensilla that restrict the entry of larger chemical stimuli? Since all aesthetascs are alike in having a thin cuticle without pore tubules (Heimann, 1984; Hallberg *et al.* 1992), this may be a conserved feature of crustaceans. The molecular mass sieving property of the aesthetascs of each species has been influenced by several factors, depending on the animal's life history and selection pressures. On the one hand, a thin, highly porous cuticle has the advantage of allowing rapid entry of odorants of all sizes. On the other hand, if the cuticle is too porous, the sensillar neurons would be highly susceptible to the loss of functionally important molecules that might be secreted into the sensillar lymph (e.g. enzymes, odorant-binding proteins) and to desiccation in some environments (Ghiradella *et al.* 1968). For example, semi-terrestrial species, which are more susceptible to desiccation, have thick aesthetasc cuticle compared with aquatic species (Ghiradella *et al.* 1968). For some species in some environments, biologically important chemicals may not include large molecules. In this case, there would not be selection pressure driving increased porosity of sensilla. The porosity of the aesthetasc cuticle of a species is determined by the relative importance of such influences.

Our discovery of molecular mass filtering by *P. argus* aesthetasc cuticle adds a new member to the perireceptor events in this sensillum that are known to shape the animal's olfactory sensitivity. After odorants enter the sensillar lymph and bind to receptors to initiate transduction (Michel and Ache, 1992; Olson and Derby, 1995; Hatt and Ache, 1996), they can be functionally eliminated by enzymes such as ectonucleotidases that degrade the odorants into inactive or less active forms (Trapido-Rosenthal *et al.* 1987, 1990; Gleeson *et al.* 1992), by pumps that internalize them (Trapido-Rosenthal *et al.* 1987, 1990; Gleeson *et al.* 1987) and by antennular flicking that removes them from in and around the sensilla (Schmitt and Ache, 1979; Moore *et al.* 1991). Another way that the aesthetasc cuticle may affect odorant movement into its lumen is by ion-exchange chromatographic effects or adsorption. For example, there might be ionic or van der Waals interactions between the cuticle and odorant due to their respective charges or molecular structures (Determann, 1969). Such events may be explain the high V_e/V_0 values for riboflavin.

All of these perireceptor events can shape the type, quantity and timing of arrival of chemicals in the receptor environment. Together, the perireceptor and receptor events determine the output of the aesthetasc's olfactory neurons and thus the signals sent to the olfactory processing centers in the brain.

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