

Purification and Characterization of a Novel Chemorepellent Receptor from *Tetrahymena thermophila*

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Abstract. Chemosensory transduction and adaptation are important aspects of signal transduction mechanisms in many cell types, ranging from prokaryotes to differentiated tissues such as neurons. The eukaryotic ciliated protozoan, *Tetrahymena thermophila*, is capable of responding to both chemoattractants (O'Neill et al., 1985; Leick, 1992; Kohidai, Karsa & Csaba, 1994, 1995) and chemorepellents (Francis & Hennessey, 1995; Kuruvilla, Kim & Hennessey, 1997). An example of a nontoxic, depolarizing chemorepellent in *Tetrahymena* is extracellular lysozyme (Francis & Hennessey, 1995; Hennessey, Kim & Satir, 1995). Lysozyme is an effective chemorepellent at micromolar concentrations, binds to a single class of externally facing membrane receptors and prolonged exposure (10 min) produces specific chemosensory adaptation (Kuruvilla et al., 1997). We now show that this lysozyme response is initiated by a depolarizing chemoreceptor potential in *Tetrahymena* and we have purified the membrane lysozyme receptor by affinity chromatography of solubilized *Tetrahymena* membrane proteins. The solubilized, purified protein is 42 kD and it exhibits saturable, high affinity lysozyme binding. Polyclonal antibodies raised against this 42 kD receptor block the in vivo lysozyme chemoresponse. This is not only the first time that a chemoreceptor potential has been recorded from *Tetrahymena* but also the first time that a chemorepellent receptor has been purified from any unicellular eukaryote.

Key words: Chemorepellent receptor — Signal transduction — Electrophysiology — Affinity Chromatogra-

phy — Polyclonal antibodies — Chemosensory adaptation — Lysozyme

Introduction

Chemosensory transduction is an important feature of many cell types, ranging from prokaryotes (Alder, 1987; Parkinson, 1988; Koshland, 1988) to unicellular eukaryotes such as *Paramecium* (Van Houten, 1978, 1990, 1991; Francis & Hennessey, 1995; Hennessey et al., 1995), *Tetrahymena* (O'Neill et al., 1985; Leick, 1992; Leick et al., 1994; Kohidai et al., 1994, 1995; Kuruvilla et al., 1997) and differentiated eukaryotic cells such as neurons (Messersmith et al., 1995; Tamada, Shirasaki & Murakami, 1995). Many cells, including *Paramecium*, *Tetrahymena* and developing neurons have the capacity to respond to both chemoattractants and chemorepellents. In neurons, for instance, a single messenger molecule, netrin-1, serves as a chemoattractant to commissural neurons while having a repulsive effect on trochlear motor axons (Colamarino & Tessier-Lavigne, 1995). Recently, a chemorepellent receptor for the repellent semaphorin III has been characterized from neuronal cells and identified as neuropilin (He & Tessier-Lavigne, 1997; Kolodkin et al., 1997).

The general model for integration of sensory information in *Paramecium* (and presumably *Tetrahymena*) is similar to the model for neuronal decision making. In general, a "typical" neuron integrates excitatory and inhibitory inputs in terms of depolarizing and hyperpolarizing somatic receptor potentials. If a summed somatic depolarization reaches threshold, the decision is made to fire an action potential. Similarly, *Paramecium* transduce all known sensory information as changes in the somatic (body) membrane potential. Somatic receptor potentials have been recorded in response to mechanosensory (Machemer, 1985), thermosensory (Hennessey, Saimi & Kung, 1983) and chemosensory (Van

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Houten, 1990, Hennessey et al., 1995) stimuli. A threshold depolarizing receptor potential triggers a ciliary, Ca^{++} -based action potential and causes backward swimming in *Paramecium* (Eckert, 1972) and *Tetrahymena* (Onimaru, Ohki & Naitoh, 1980). This is the basis of the "avoidance reaction" bioassay used in these ciliates to study depolarizing chemorepellents such as lysozyme (Francis & Hennessey, 1995; Hennessey et al., 1995; Kuruvilla et al., 1997). The general model in *Paramecium* is that hyperpolarizing chemoattractants cause faster forward swimming while chemorepellents cause depolarization and backward swimming (if the depolarization is above a threshold value) (Van Houten, 1990). In this manner, these ciliates integrate chemosensory information in terms of the somatic membrane potential and translate it into a behavioral response. Although it has been assumed that the related ciliate *Tetrahymena* has similar electrophysiological responses (Onimaru et al., 1980), no receptor potential has previously been recorded from these cells. Lysozyme is a chemorepellent in the ciliates *Paramecium* (Hennessey et al., 1995) and *Tetrahymena thermophila* (Kuruvilla et al., 1997) at low (μM) concentrations and it is a secretagogue in *Paramecium* (Hennessey et al., 1995) at higher (mM) concentrations. Lysozyme is also a secretagogue in serosal mast cells (Mousli et al., 1994) but it is not a secretagogue in *Tetrahymena*. It has been proposed that lysozyme reception may be involved in predator recognition, avoidance and defense (Harumoto, 1994).

Lysozyme exerts its chemorepellent effects in the micromolar range, with a K_D of 6.28 μM in *Paramecium* and 6.62 μM in *Tetrahymena*, respectively (Kim, Kuruvilla & Hennessey, 1997; Kuruvilla et al., 1997). The EC_{50} of lysozyme in a behavioral assay is 0.01 μM in both ciliates under identical calcium conditions (Kim et al., 1997; H.G. Kuruvilla, unpublished data). Lysozyme binding activates a novel receptor-operated somatic Ca^{++} conductance to cause the depolarizing receptor potential in *Paramecium* and these effects are independent of the polysaccharide hydrolysis activity of lysozyme (Hennessey et al., 1995). *Paramecium* show reversible behavioral and electrophysiological adaptation to lysozyme that involves decreases in both the number of surface [^3H]-lysozyme binding sites and in the amplitude of the lysozyme-induced depolarization (Kim et al., 1997).

Tetrahymena are also capable of behavioral adaptation to micromolar concentrations of lysozyme over a 10-min time period (Kuruvilla et al., 1997). As in *Paramecium* (Kim et al., 1997) chemosensory adaptation to lysozyme in *Tetrahymena* is accompanied by a large decrease in the number of functional surface [^3H]-lysozyme binding receptors (Kuruvilla et al., 1997).

While some attractant receptors have been partially purified from *Tetrahymena thermophila*, such as the insulin receptor (Christopher & Sundermann, 1995), a che-

morepellent receptor has never been isolated from *Tetrahymena* or from any other unicellular eukaryote studied to date. In this study, we describe the purification and partial characterization of the lysozyme receptor from *Tetrahymena thermophila* in an effort to further elucidate the mechanisms involved in chemosensory transduction and adaptation to repellents such as lysozyme.

Materials and Methods

CELL CULTURES

T. thermophila B, serotype H3, a generous gift from N.E. Williams (University of Iowa), was used throughout this study. For behavioral studies, cells were grown for 48 hr in the axenic medium of Dentler (1988) without addition of antibiotics. For protein purification work, cells were grown for 72 hr in the same medium. All cell cultures were incubated on a rotary shaker at 25°C during growth.

CHEMICALS AND SOLUTIONS

Behavioral bioassays for lysozyme avoidance were carried out in a buffer containing 10 mM Trizma base, 0.5 mM MOPS, 50 μM CaCl_2 , pH 7.0 at 25°C. Electrophysiology and [^3H]-lysozyme in vivo binding assays were carried out at 25°C in a buffer containing 1 mM CaCl_2 , 1 mM MOPS, pH 7.2.

[^3H]-lysozyme was synthesized by methylation of commercial grade lysozyme with [^3H]- NaBH_4 as described by Means and Feeney (1969). Briefly, hen egg lysozyme was purified by size exclusion chromatography on a G-50 Sephadex column. Lysozyme was reconstituted in 1 mM CaCl_2 , 1 mM MOPS, pH 7.2 to a final concentration of 10–100 mg/ml and added to 5 mCi [^3H]- NaBH_4 on ice with constant stirring. This solution was incubated on ice for 60 min with 0.5 $\mu\text{l/ml}$ formaldehyde (37% w/v, Fisher) added every 5 min during the first 25 min for a total of five additions. After methylation, lysozyme was purified away from unreacted [^3H]- NaBH_4 by size exclusion chromatography on G-50 Sephadex. The specific activity of the purified product was 0.175 Ci/mmol. All other compounds were supplied by Sigma Chemical (St. Louis, MO) unless otherwise noted.

BEHAVIORAL ASSAYS

The chemorepellent behavioral assay was the same as previously described for *Paramecium* (Hennessey et al., 1995) and *Tetrahymena* (Kuruvilla et al., 1997). In this bioassay, individual cells were transferred to a well (0.5–1.0 ml) containing a test solution and observed under a dissection microscope to determine the occurrence of avoidance reactions (AR) within the first few seconds after transfer. Ten cells were individually scored for avoidance (+ or –) for each trial. The mean \pm SD was calculated for three trials and expressed as "Percent Cells Showing Avoidance Reactions".

PREPARATION OF A LYSOZYME AFFINITY COLUMN

CN-Br activated Sepharose (Pharmacia) was hydrated and activated according to the manufacturer's instructions. Hen egg lysozyme (Sigma) was then added in the ratio of 35 mg lysozyme/gram dry Sepharose. Lysozyme was allowed to cross-link to the matrix for 1 hr

at room temperature in a buffer containing 1 mM CaCl₂, 1 mM MOPS, pH 7.2. The matrix was then washed with 1 mM neomycin to remove excess lysozyme, washed three times with buffer, and poured.

PURIFICATION OF THE LYSOZYME RECEPTOR

The lysozyme receptor was purified from whole, wild-type *Tetrahymena thermophila* (about 10ml packed cells). Cells were harvested and washed three times in normal wash buffer (1 mM CaCl₂, 1 mM MOPS, pH 7.2). They were then extracted in normal wash buffer containing 1% Triton X-114 at 4°C for 1 hr. The cell extract was then spun at 100,000 × *g* at 4°C for 1 hr to remove particulates. The solubilized cell extract (about 10 ml) was then applied to a 10 ml lysozyme affinity column, which was washed with 10 volumes of buffer containing 1% Triton X-114, and then eluted with 1.0 mM neomycin sulfate in the same buffer. Fractions were then dialyzed and frozen prior to conducting soluble binding assays. Prior to SDS-PAGE treatment, fractions eluted from the column with neomycin were pooled, heated to 30°C to precipitate the Triton X-114, and spun in a TOMY capsule microcentrifuge at 2400 rpm for 5 min. The lysozyme receptor partitioned into the lower (detergent) phase, consistent with it being an integral membrane protein. Fractions were then precipitated with CHCl₃ and MeOH, dried, and reconstituted in 1× SDS-loading buffer, pH 6.8.

SOLUBLE BINDING ASSAYS

Lysozyme receptor was purified and dialyzed as described above. Buffer (1 mM CaCl₂, 1 mM MOPS, 1% Triton X-114, pH 7.2) and [³H]-lysozyme were added. Samples were removed for scintillation counting. This volume represents [bound + free]. The remainder of the sample was heated to 30°C to precipitate the Triton X-114 and the receptor-containing fraction was collected as the detergent (lower) phase. Samples were then centrifuged in a TOMY capsule microcentrifuge at 2400 rpm for 5 min and an aliquot removed for scintillation counting. This volume represents the amount of [free] ligand. The value of [bound] ligand was determined by the equation [bound + free] - [free] = [bound]. K_D and B_{max} were found using Scatchard analysis (Scatchard, 1949).

DENATURING GEL ELECTROPHORESIS

Denaturing (SDS) discontinuous gel electrophoresis was conducted as described by Ausubel et al. (1987). Electrophoresed proteins were detected either by silver staining as described by De Moreno, Smith & Smith (1991) or by staining with the ISS Pro Blue system according to the manufacturer's instructions.

PROTEIN SEQUENCING

Partial amino acid sequencing of the lysozyme receptor was done by Dr. John Leszyk of the Worcester Foundation (Boston, MA). Prior to sequencing, the protein was purified, electroblotted to nitrocellulose and stained with 0.1% amido blue. The 42 kD band was excised, dissolved in DMSO, dried, and subjected to endoproteinase Lys-C cleavage. Cleavage products were purified by reverse-phase HPLC prior to sequencing.

POLYCLONAL ANTIBODY PRODUCTION

Polyclonal antibodies to the *Tetrahymena* lysozyme receptor were produced in rabbits by Lampire Biological Laboratories (Pipersville, PA).

Lysozyme receptors were purified from whole *Tetrahymena* extract as previously described, run on SDS-PAGE, and electroblotted to nitrocellulose membrane. Membranes were stained with 0.1% Ponceau S (Harlow & Lane, 1988), and the 42 kD protein was excised. The protein-containing strip was destained in water, dissolved in DMSO, and the denatured antigen used for polyclonal antibody production.

WESTERN BLOTTING

Western blotting was performed as described by Harlow and Lane (1988). Briefly, SDS-polyacrylamide gels were electroblotted to Immobilon PVDF transfer membrane (Millipore, Bedford, MA) and blocked overnight in a solution of 3% bovine serum albumin (BSA in Tris-buffered saline (TBS). After washing the membrane in TBS, the primary antibody was diluted 1:100 into 3% BSA and allowed to react with the membrane for 2 hr. After washing the membrane in TBS, goat anti-rabbit secondary antibody (alkaline phosphatase conjugate) was diluted 1:2,000 into 3% BSA and reacted for 1 hr. The membrane was developed using Sigma Fast™ BCIP/NBT substrate tablets.

ELECTROPHYSIOLOGY

Standard one-electrode whole cell membrane potential recordings were similar to previously reported procedures in *Paramecium* (Hennessey et al., 1995; Satow & Kung, 1979; Hennessey & Kung, 1987). The recording buffer contained 1 mM Ca²⁺, 1 mM MOPS, pH 7.2. Membrane potentials were displayed on a digital oscilloscope and retained on a chart recorder during continuous bath perfusion at a rate of about 20.0 ml/min. The recording bath had a volume of about 1 ml. Solutions were changed by switching valves connected to different solutions without changing the flow rate of the perfusion system.

Results

SDS-PAGE analysis of the purified *Tetrahymena* membrane extract revealed a single band with an apparent molecular weight of 42 kD (Fig. 1A). This result contrasts with findings in *Paramecium*, in which purification of the lysozyme receptor yielded a single protein band of approximately 58 kD (Fig. 1C).

Receptor binding assays (Fig. 2) showed that [³H]-lysozyme binding to the solubilized receptor was saturable, and had a K_D of 0.2 μM. This was lower than the previously published *in vivo* value of 6.62 μM (Kuruvilla et al., 1997). Using this soluble binding assay, we were able to calculate a specific activity, or amount of lysozyme bound/μg total protein, and thereby determine the purification factor for the receptor. Based on this method, we calculated a 29.6-fold purification of the receptor in the purified fraction relative to the whole cell extract control and a 0.37% recovery (Table 1).

Preliminary amino acid sequencing of a 19 amino acid fragment obtained by endoproteinase Lys-C cleavage of the purified receptor gave a sequence of GGNC-SACDAGTSTPAAQTK. This sequence showed no significant homologies with any known receptors or protein classes when searched in the SWISS-PROT database.

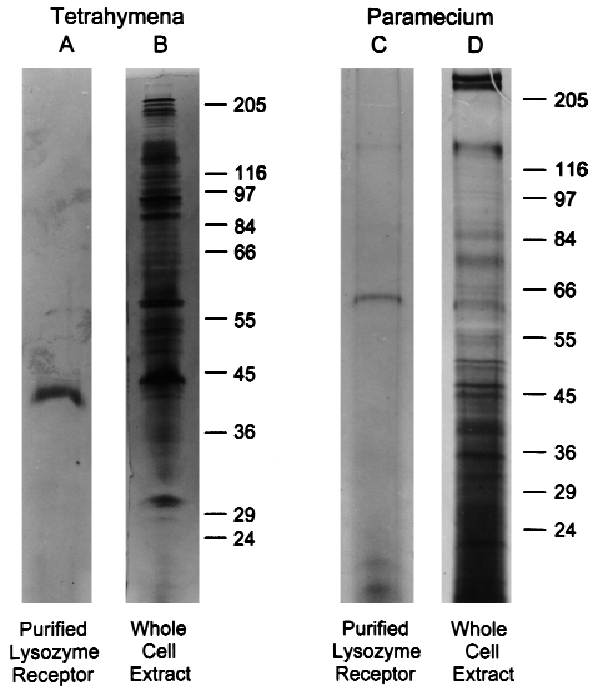


Fig. 1. The solubilized, purified lysozyme receptor is seen as a 42 kD protein in *Tetrahymena* (A) and a 58 kD protein in *Paramecium* (C) by SDS-PAGE. The lysozyme receptor was purified from Triton X-114 detergent extracted whole cell membrane proteins as described in Materials and Methods. The starting material (whole cell membrane protein extract) shows many protein bands in both *Tetrahymena* (B) and *Paramecium* (D) extracts. Molecular weight markers are included for comparisons. Proteins were stained with silver and each lane contained about 10 μ g protein.

Polyclonal antibodies produced against the receptor protein recognized a 42 kD protein in both whole cell extract and purified receptor preparations on a Western Blot (Fig. 3). These antibodies also effectively eliminated the behavioral avoidance response of *Tetrahymena* to 10 μ M lysozyme at a 1:100 dilution while control cells (under these buffer conditions) showed 100% avoidance (Fig. 4). As seen in Fig. 4, the EC_{50} of this antibody in *Tetrahymena* (concentration of antibody at which 50% of cells no longer showed lysozyme avoidance) was a 1/500 antibody dilution. The immune serum caused some avoidance reactions in *Tetrahymena*, so cells were adapted to the antibody for 5–10 min before addition of the chemorepellent. This antibody effect was quite specific because responses to another chemorepellent, GTP (20 μ M) were not affected by the antibody. However, this *Tetrahymena* antibody did not recognize any *Paramecium* proteins in an ELISA assay and it had no effect on lysozyme-induced avoidance reactions (*data not shown*).

Electrophysiological experiments showed a large, transient depolarization of whole *Tetrahymena* in response to lysozyme in the presence of 1 mM Ca^{++} (Fig.

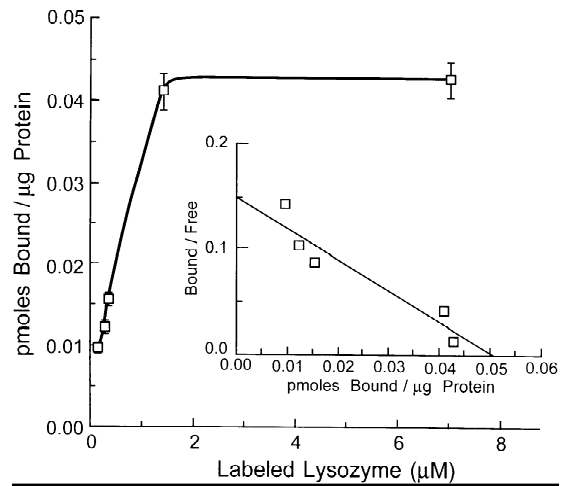


Fig. 2. In vitro binding assays using the solubilized, purified lysozyme receptor from *Tetrahymena* show that binding is saturable. The amount of bound [3 H]-lysozyme increased in a concentration-dependent manner until saturation. When these data were displayed as a Scatchard plot (inset), the K_D was found to be 0.2 μ M ($R = 0.784$).

Table 1. Purification of the lysozyme receptor from *Tetrahymena thermophila*

Total protein in whole cell extract	27.2 mg
Total protein recovered from purified fraction	0.100 mg
Percent recovery (based on total mg protein)	0.37%
Lysozyme binding capacity of whole cell extract	16,271 cpm
Lysozyme binding of purified fraction	1,774 cpm
Percent recovery (based on binding activity)	10.9%
Specific activity of whole cell extract (cpm bound/mg protein)	598.2
Specific activity of purified protein (cpm bound/mg protein)	17,743
Fold purification	29.6

The lysozyme receptor was solubilized with Triton X-114 and purified by affinity chromatography on a lysozyme-Sepharose column. The specific activity of the final purified fraction, expressed as cpm [3 H]-lysozyme bound/mg protein, was 17,743 cpm/mg. This represented a 30-fold purification.

5). Three different cells are shown for each lysozyme concentration tested (Fig. 5) to show the variability in the type of response seen. The transient nature of this response, coupled with the identification of the receptor involved, identifies this as a true chemoreceptor potential. Cells gave a maximal transient response at 1.0 μ M lysozyme (Fig. 5C, Table 2) but cells perfused with 10.0 μ M lysozyme did not readily return to their resting membrane potential unless reperfused with buffer (*data not shown*). The lysozyme-induced depolarizations were dramatically reduced by the presence of the polyclonal antibodies. In the presence of these antibodies (1:100), 1.0 μ M lysozyme elicited transient depolarizations of only 1.9 ± 0.9 mV ($n = 7$).

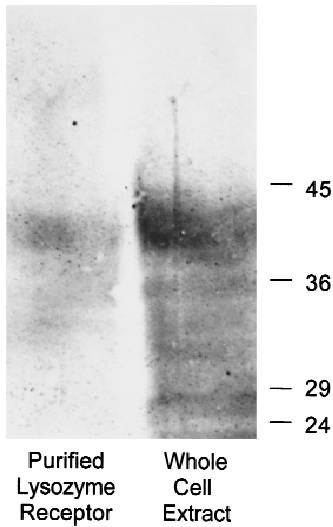


Fig. 3. The Western blot of the purified *Tetrahymena* lysozyme receptor showed major reactivity with the 42 kD protein. Polyclonal antibodies raised against the receptor were used to probe a blot of either the purified receptor (Lane 1) or a whole cell extract (Lane 2).

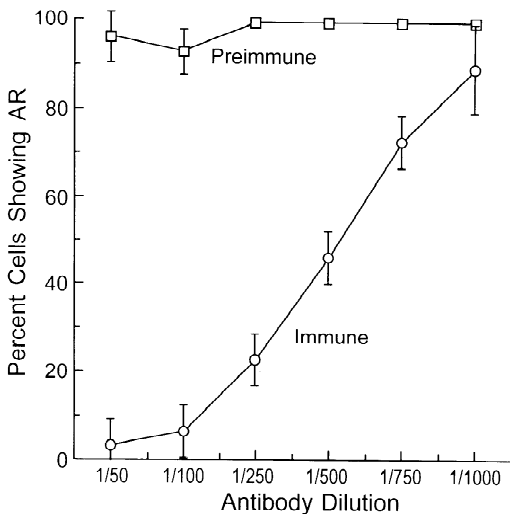


Fig. 4. Polyclonal antibodies raised against the lysozyme receptor of *Tetrahymena* effectively blocks their lysozyme avoidance behavior. Avoidance reactions to 10 μM lysozyme are completely inhibited at antibody concentrations of 1:100. The EC_{50} (effective concentration to reduce the response by 50%) of this antibody is 1:500. Experiments were conducted in a buffer containing 1 mM Ca^{2+} , 1 mM MOPS, pH 7.2.

Discussion

The data presented here are consistent with the hypothesis that lysozyme acts as a chemorepellent in *Tetrahymena thermophila* by binding to an externally facing membrane-bound protein that is 42 kD in molecular weight (Fig. 1A). Binding triggers a depolarizing tran-

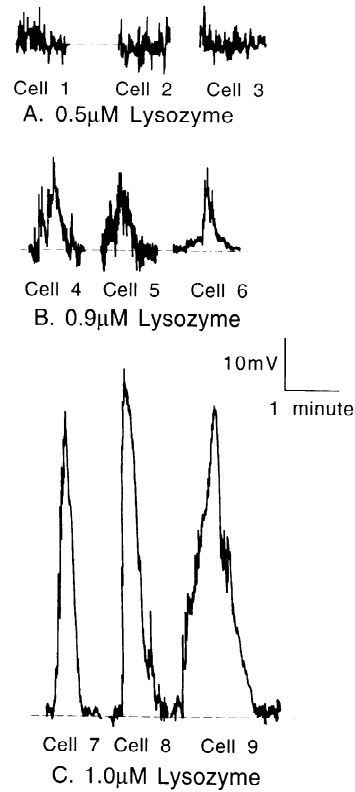


Fig. 5. Lysozyme produces graded receptor potentials in *Tetrahymena*. Triplicate representative traces are shown from whole cell intracellular electrophysiological recordings of *Tetrahymena* in either 0.5 μM (A), 0.9 μM (B) or 1.0 μM (C) lysozyme. Each trace is from a different cell. Cellular membrane potentials were recorded in a buffer containing 1 mM Ca^{2+} , 1 mM MOPS, pH 7.2. In each trace, lysozyme was added at the beginning of the recording trace.

Table 2. Concentration dependence of *Tetrahymena* electrophysiological response to lysozyme

[Lysozyme, μM]	$V_{m_{\text{rest}}}$ (mV)	ΔV_m (mV)	Maximal V_m (mV)	N
10.0	-39.3 ± 4.0	46.0 ± 5.2	6.6 ± 1.5	3
1.0	-44.5 ± 8.5	50.1 ± 4.9	5.5 ± 8.7	9
0.90	-46.0 ± 5.4	12.8 ± 6.6	-38.2 ± 9.49	5
0.75	-47.0 ± 6.5	0	-47.0 ± 6.5	3
0.50	-44.0 ± 4.0	0	-44.0 ± 4.0	3
0.10	-36.0 ± 6.2	0	-36.0 ± 6.2	3

The lysozyme-induced receptor potential is a transient, concentration-dependent depolarization. The resting membrane potential ($V_{m_{\text{rest}}}$), change in the measured membrane potential (ΔV_m) and maximal membrane potential reached at the peak of the receptor potential (Maximal V_m) are shown for a number of individual cells at each concentration of lysozyme. Maximal responses were seen above 1.0 μM lysozyme.

sient receptor potential (Fig. 5) which causes action potentials and consequent avoidance reactions. In *Paramecium*, this receptor potential is calcium-dependent (Hen-

nessey et al., 1995), but its ionic dependency has not yet been documented in *Tetrahymena*. However, since depolarizations are seen in solutions containing calcium alone, it is likely that the ionic dependency is similar to that of *Paramecium*.

Polyclonal antibodies that recognize the purified receptor as a 42 kD protein on a Western blot (Fig. 3) inhibit both the electrophysiological and behavioral responses of *Tetrahymena* to lysozyme. Since these antibodies have no effect on the GTP response, they do not cause a general loss of chemorepellent responses. The specific *in vivo* effectiveness of these antibodies supports the conclusion that the 42 kD protein is an externally facing lysozyme receptor. Preliminary immunofluorescence studies (unpublished observations) also support the external orientation of this receptor but further studies with a purified antibody preparation are necessary to solidify this conclusion. Obtaining a higher titer antibody would also aid in the quantification of the effects on the behavioral and electrophysiological responses to lysozyme.

The *Tetrahymena* lysozyme receptor appears to be significantly different than the *Paramecium* lysozyme receptor. The 42 kD *Tetrahymena* receptor protein is considerably smaller than the 58 kD protein purified from *Paramecium* under the same conditions (see Fig. 1). Also, the *Tetrahymena* antibody has no effect on lysozyme-induced avoiding reactions in *Paramecium* and does not recognize any *Paramecium* proteins in an ELISA assay (*data not shown*).

The purified *Tetrahymena* receptor shares characteristics with the previously published *in vivo* studies (Kuruvilla et al., 1997). For instance, binding is saturable and appears to result from a single population of sites (Fig. 2). The *in vitro* K_D for the receptor is 0.2 μM , compared with 6.62 μM *in vivo*. There are many possible reasons for this discrepancy, including the fact that many properties of membrane proteins are changed once solubilized into detergents. In the *in vivo* assays, there was a large amount of nonspecific binding, which had to be subtracted. In the soluble assay, no nonspecific binding was detected, eliminating this potential source of error in terms of K_D determination. Our recent purification data (Fig. 1A, Table 1) supports the earlier suggestion of a single receptor population (Kuruvilla et al., 1997) by showing that the receptor appears as a single, 42 kD band on SDS-PAGE.

The partial amino acid sequence obtained from internal sequencing gave no relevant information about the nature of this receptor protein. This could be because either this is a unique receptor or it is an area of a known receptor that is not conserved. However, it is possible that this sequence could be used to make oligonucleotide probes for cloning, and the full-length clone could then be sequenced. This information could prove to be more

valuable than that obtained from the 19-amino acid fragment. Cloning this receptor would also enable the employment of techniques such as gene knockouts (Gaertig et al., 1994) to assay directly the role of this gene in *Tetrahymena* lysozyme avoidance.

The antibody generated against the lysozyme receptor recognized the 42 kD protein to which it was raised when it was used to probe a Western blot (Fig. 3). However, when probing a lane containing whole cell extract, other lower molecular weight bands were detected in addition to the band of interest (Fig. 3). In addition, a 1:100 dilution of this antibody was required to completely eliminate *Tetrahymena* lysozyme avoidance (Fig. 4). These data indicate that while this antibody is a useful tool with which to study the lysozyme receptor *in vivo*, it is a relatively low-titer antibody. A higher titer antibody would facilitate immunolocalization studies, allowing us to determine whether adaptation occurs through receptor-mediated endocytosis or whether secondary modification of the receptor is involved.

Electrophysiological studies (Fig. 5, Table 2) show that the *Tetrahymena* response to lysozyme, like that of *Paramecium* (Hennessey et al., 1995; Kim et al., 1997), is the result of a transient depolarization. This is the first chemoreceptor potential ever described in *Tetrahymena*. The depolarization in response to 1.0 μM lysozyme is very large, averaging about 50 mV under buffer conditions of 1.0 mM external calcium (Fig. 5C, Table 2). This depolarization is similar in character, though almost twice as large, as that seen in *Paramecium* to 0.1 μM lysozyme under the same external calcium conditions (Kim et al., 1997). Since this is the first recorded chemoreceptor potential from *Tetrahymena*, we have no other electrophysiological responses to compare it with in this organism. However, we have recently found that a 24 amino-acid cyanogen bromide fragment of lysozyme (which we call CB₂) is also a chemorepellent and produces similar transient depolarizations in *Tetrahymena* at a concentration of 1.0 μM (H.G. Kuruvilla and T.M. Hennessey, *in preparation*). This behavioral response to CB₂ is also blocked by the polyclonal antibody to the 42 kD receptor (*unpublished observation*). The concentration dependence of the lysozyme-induced depolarizations in Table 2 appears to be rather abrupt, but since this is the first chemoreceptor potential ever measured in *Tetrahymena*, more detail may emerge as our recording procedures become more refined.

In conclusion, these data are consistent with the hypothesis that the lysozyme response in *Tetrahymena* is mediated by a 42 kD-protein which is related to a transient, depolarizing receptor potential. This is first description of a chemoreceptor potential in *Tetrahymena*. Although chemorepellent receptors have been well described in prokaryotic chemosensory transduction studies (Alder, 1987; Koshland, 1988; Parkinson, 1988) and

in developing neurons cells (He & Tessier-Lavigne, 1997; Kolodkin et al., 1997), this lysozyme receptor is the first repellent receptor to be purified from any free swimming, unicellular eukaryote. Although it is not known whether this is a specific and dedicated lysozyme receptor, lysozyme is a convenient agonist for purification and characterization of this receptor. If lysozyme is the intended endogenous ligand for this receptor, it may play a role in defensive behavior in this organism (Harumoto, 1994). This response to an intercellular ligand may be an evolutionary precursor to the more specialized cell-cell communication mechanisms seen in multicellular organisms.

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