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Deployment of hagfish slime thread skeins requires the transmission of mixing forces *via* mucin strands

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SUMMARY

Hagfishes are benthic marine protovertebrates that secrete copious quantities of slime when threatened. The slime originates as a two-component glandular exudate comprised of coiled bundles of cytoskeletal intermediate filaments (thread skeins) and mucin vesicles. Holocrine secretion of the slime into seawater results in the rapid deployment of both fibrous and mucin components, resulting in about a liter of dilute slime. Deployment of the thread skeins involves their unraveling in a fraction of a second from a 150 μm-long ellipsoid bundle to a thread that is 100× longer. We hypothesized that thread skein deployment requires both vigorous hydrodynamic mixing and the presence of mucin vesicles, both of which are required for whole slime deployment. Here we provide evidence that mixing and mucin vesicles are indeed crucial for skein unraveling. Specifically, we show that mucin vesicles mixed into seawater swell and elongate into high-aspect ratio mucin strands that attach to the thread skeins, transmit hydrodynamic forces to them and effect their unraveling by loading them in tension. Our discovery of mucin strands in hagfish slime not only provides a mechanism for the rapid deployment of thread skeins *in vivo*, it also helps explain how hagfish slime is able to trap such impressive volumes of seawater *via* viscous entrainment. We believe that the deployment of thread skeins *via* their interaction with shear-elongated mucins represents a unique mechanism in biology and may lead to novel technologies for transmitting hydrodynamic forces to microscale particles that would typically be immune to such forces.

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Key words: mucus, animal defense, biomechanics, *Myxine glutinosa*.

INTRODUCTION

Hagfishes are known to secrete copious quantities of slime when threatened. The slime, which can comprise 3–4% of the animal's total body mass, originates in epidermally derived slime glands that line the ventro-lateral sides of the animal (Downing et al., 1981; Fernholm, 1981; Fudge et al., 2005). The slime glands contain large quantities of two cell types that develop from undifferentiated cells lining the periphery of the slime gland (Fernholm, 1981; Spitzer et al., 1984). The first are ellipsoid-shaped cells known as gland thread cells (GTCs), which are entirely unique to hagfish (order *Myxinoidei*) (Newby, 1946). Within the GTC a highly condensed coil (or 'skein') of cytoskeletal elements known as 'intermediate filaments' is assembled in preparation for secretion (Downing et al., 1981; Fernholm, 1981; Newby, 1946; Spitzer et al., 1984) (Fig. 1). The second type of cells are gland mucous cells (GMCs), which produce large numbers of membrane-bound mucin vesicles that contain mucin-like glycoproteins (Fernholm, 1981; Fudge et al., 2005; Leppi, 1968; Spitzer et al., 1984). Contraction of the musculature surrounding the slime glands causes the expulsion of these two slime constituents into the surrounding environment (Fernholm, 1981). Ejection from the slime gland liberates both the GTCs and GMCs of their plasma membranes (Fernholm, 1981).

While much is known about the structure, biochemistry and physical properties of the two slime components (Downing et al., 1981; Fernholm, 1981; Fudge et al., 2005; Fudge et al., 2009; Koch et al., 1991a; Koch et al., 1991b; Koch et al., 1994; Spitzer et al., 1984), relatively little is known about the mechanisms involved in the deployment of the slime. In most slimy secretions, deployment involves exocytosis of mucin granules from goblet cells and their subsequent hydration and swelling (Verdugo, 1991). By contrast, hagfish slime is formed *via* the forceful ejection of a two-component exudate from specialized slime glands. Mixing of the exudate with seawater is known to result in the very fast (about 100 ms) maturation of the slime *in vivo* (Lim et al., 2006). Furthermore, slime maturation *in vivo* results in a large volume of whole slime (about 900 ml in *Eptatretus stoutii*) that is believed to discourage attacks by gill-breathers such as fishes (Lim et al., 2006). What is not known is how the mucin vesicles swell and rupture, how the thread skeins unravel and how the two components interact (if at all) during the transformation of the thick exudate to a mass of watery and fibrous slime.

Newby proposed that contact of the slime exudate with seawater causes spontaneous and explosive slime formation (Newby, 1946). However, more recent work has shown that slime formation is not explosive but requires convective mixing of the exudate with seawater (Fudge et al., 2005; Koch et al., 1991a; Koch et al., 1991b; Lim et al., 2006). In addition, the disruption of mucin networks with the disulfide-cleaving compound dithiothreitol (DTT) is known to drastically reduce the volume of mature slime formed and delay the formation of mature slime *in vitro* (Fudge et al., 2005; Koch et al., 1991b). These experiments suggest that the mucins play a role in whole slime formation, although their connection to thread skein unraveling and the requirement for convective mixing is still unclear.

The goal of this study was to explore possible mechanisms of thread skein unraveling, or more specifically, to explain how a 150 μm thread bundle elongates to 150 mm in a fraction of a second. We hypothesized that there are two main requirements for thread

Fig. 1. Differential interference contrast (DIC) image of partially unraveled thread skein in seawater illustrating their coiled structure.

skein unraveling: (1) thread skein deployment is dependent on the rate of mixing within the water column, and (2) mucin vesicles are required for proper thread skein deployment. These hypotheses predict that increasing the mixing rate will increase the degree of thread skein unraveling, and disruption of the mucin network will inhibit thread skein unraveling. We tested these predictions using a number of *in vitro* unraveling experiments, as well as fluorescent microscopy to observe the behaviour of mucin vesicles in response to flow. Here we present data that support both of these hypotheses and propose a new model for the mechanism of hagfish slime deployment.

MATERIALS AND METHODS

Experimental animals

Specimens of Atlantic hagfish (*Myxine glutinosa* Linnaeus) were collected from the Huntsman Science Centre in St Andrews, New Brunswick, Canada. At the University of Guelph, animals were maintained in 2000 l tanks at the Hagen Aqualab in artificial seawater (34‰, 10°C). *Myxine glutinosa* were fed squid once per month to satiety (University of Guelph Animal Care Protocol 05R154).

Animal anesthesia and slime collection

A stock anesthetic solution was prepared by combining clove oil (Sigma-Aldrich, Oakville, ON, Canada; stock# C8392) and 99% ethanol in a 1:9 ratio. The stock solution was added to artificial seawater to yield a final clove oil concentration of 63.5 mg l^{-1}. Hagfish anesthesia was conducted by placing specimens in 8 l buckets filled with 3.5 l of 34‰ artificial seawater (Coralife, Energy Savers Unlimited, Inc., Carson, CA, USA). Anesthesia buckets were placed on ice and monitored to maintain a temperature of 10±0.5°C.

Collection of the slime exudate from anesthetized hagfish was carried out following a modified protocol from Fudge et al. (Fudge et al., 2003). Following anesthesia, individual hagfish were placed ventral side up on a chilled dissection tray lined with a seawater-moistened cloth. After rinsing an area surrounding a number of adjacent slime gland pores with deionized water, the area was blotted dry with Kimwipes (Kimberly-Clark Corporation, Irving, TX, USA). Using a GRASS SD9 electronic stimulator (Grass Instruments, Quincy, MA, USA) and custom stimulation wand, the hagfish were stimulated (60 Hz, 18 V) in the dried area to produce a small puddle of fresh exudate. This exudate was collected immediately prior to experimentation using a micropipette.

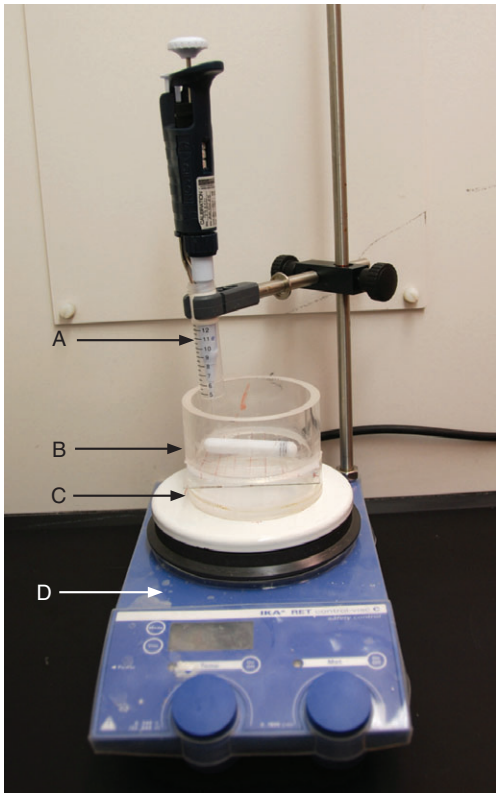

Fig. 2. Thread skein unraveling experimental apparatus. (A) Micropipette insertion guide, (B) experimental chamber with stir bar, (C) spacer and chamber alignment guide, (D) stir plate with feedback speed control.

Thread skein unraveling

To quantify the effect of convective mixing on the deployment of thread skeins we constructed a custom thread skein unraveling chamber. This chamber was used in a series of experiments to test the degree to which hagfish slime threads unravel in response to 'natural' hydrodynamic forces that might be experienced during a predator's attack. The thread cell unraveling chamber was constructed from 9.1 cm outer diameter (o.d.) clear Plexiglas pipe cut to a length of 3.9 cm, and fixed to a 10.1 cm × 8.25 cm (0.1 cm thick) glass plate with silicone sealant to create a water-tight chamber. A 0.9 cm × 0.9 cm grid pattern was etched into the bottom side of the glass plate to aid in thread skein counting (Fig. 2).

Four thread skein unraveling experiments were conducted using this chamber design. The first set of experiments (N=6) was conducted to test the prediction that increasing the rate of mixing will increase the degree of thread skein unraveling in the whole slime. In these experiments the chamber was filled with 75 ml of chilled (10°C) artificial seawater (34‰) and aligned on a section of Plexiglas piping (diameter=9.1 cm, length=2.5 cm) on an electronic stir plate (RET control viscosity C IKAMAG® safety control; Mandel, Guelph, ON, Canada). This precise stir plate enabled us to maintain a constant stirring rate at each of the mixing regimes. Unraveling experiments were conducted over six different mixing regimes ranging from 0 r.p.m. to 500 r.p.m. – in increasing

increments of 100 r.p.m.. A 5.1 cm magnetic stir bar was used to create the desired mixing forces in the chamber. Each trial was conducted using 0.5 µl of fresh slime exudate, which was collected from the anesthetized hagfish following stimulation with a micropipette. The exudate was then introduced into the chamber, following removal of the stir bar, by placing the micropipette into a custom insertion guide and ejecting the exudate into the seawater. The guide ensured consistent placement of the slime into the chamber (1.5 cm from inner wall of the chamber, 1 mm deep). Preliminary trials showed that the stir bar interacted with the slime exudate in a way that made it difficult to measure the number of skeins that failed to deploy. This was primarily a result of physical interaction of the stir bar with the exudate in the form of entanglement of threads around it as well as shearing of pelleted skeins as the stir bar continued to rotate on the bottom of the chamber. Thus, while removing the stir bar was somewhat disruptive to the flow in the chamber, we are confident that the exudate was subjected to real differences in the level of hydrodynamic forces among the six mixing rates used. After water movement had ceased, the chamber was moved to a microscope stage (Nikon SMZ 1500, Nikon Canada, Mississauga, ON, Canada) that was chilled to 10°C using a water recirculation/chiller unit. Chilling was necessary to maintain a consistent environment for the formed slime, as well as to prevent condensation and mitigate convective currents caused by heat given off by the microscope lamp. The condensed thread skeins that pelleted out of solution after each mixing regime were counted using a monochrome digital camera (Q-imaging Retiga 1300, Surrey, BC, Canada) and Open Lap 3.5.1 software (Improvision, Waltham, MA, USA) on a computer. Counting the number of condensed thread skeins on the bottom provided an inverse measure of the degree of thread bundle unraveling in the whole slime. Data were converted to the percentage of the number of skeins that pelleted at 0 r.p.m..

The second set of experiments (N=6) involved a similar experimental protocol; however, 75 ml of 10 mmol l^{-1} DTT solution (Sigma-Aldrich; CAT. #BP172-25), prepared in 34‰ artificial seawater at 10°C, was used. This experiment was aimed at addressing the prediction that an intact mucin network is necessary for thread skein unraveling. The third set of stir-plate experiments (N=6) were conducted using 75 ml of 200 mmol l^{-1} β-mercaptoethanol solution (MP Biomedicals, Solon, OH, USA; CAT. #194705) prepared in 34‰ artificial seawater at 10°C. This experiment was performed at the 0 r.p.m. and 400 r.p.m. mixing regimes to test whether increasing the rate of mixing could compensate for the complete disruption of the mucin network in facilitating thread skein unraveling.

The fourth experiment was designed to test whether a viscous solution could rescue skein unraveling in the presence of 200 mmol l^{-1} β-mercaptoethanol. These unraveling trials were conducted in a viscous 2% polyethylene oxide (MW 900,000 Da) in seawater solution with a viscosity approximately 150× higher than that of seawater. Quantification of skein unraveling could not be carried out in these trials because the condensed skeins did not pellet out in the viscous solution but the results were clear nonetheless.

Visualization of mucin vesicles under shear flow

Preliminary experiments focused on optimizing the fluorescent tagging of ruptured mucin vesicles by testing a variety of fluorescein-labeled lectin dyes (Vector Laboratories Lectin Kit; Vector Laboratories, Inc.; Burlingame, CA, USA; CAT. #FLK-2100). Visualization of the ruptured mucins was superlative using a lectin soybean agglutinin dye diluted 500× in 10°C artificial seawater (34‰). Using this dye solution we tested the response of mucin vesicles to shear flow conditions in order to gain insight into the role of mucins during thread skein deployment. Experiments were conducted using microscope slide flow-through chambers constructed from 3 mm × 1 mm × 1 mm glass slides and 18 mm × 18 mm × 0.17 mm cover glass (Fisher Scientific, Ottawa, ON, Canada). By focusing on mucin vesicles that adhered to the glass slide we could image the mucin vesicles and visualize their response to shear flow. Hagfish slime used in this experiment was collected, as detailed above, but placed into a 10°C stabilization solution (0.9 mol l^{-1} sodium citrate, 0.1 mol l^{-1} PIPES) modified from Spitzer et al. (Spitzer et al., 1988).

In preparation for experimentation, the chamber was first loaded with the stabilization solution by adding a small 50 µl drop near one opening to the chamber. After allowing capillary action to pull this drop into and fill the chamber, a small volume (50 µl) of mucin vesicle suspension was placed near the same opening to the chamber. Using small pre-cut filter paper (Whatman #1, Maidstone, Kent, UK), the sample was pulled into the chamber by drawing from the opposite opening. A 50 µl drop of artificial seawater containing the lectin dye was placed adjacent to the flow-through opening. The dye solution was drawn through the chamber to observe the response of the mucin vesicles to flow. Images were captured using a Nikon 90i Eclipse epifluorescent microscope with a FITC filter cube, Nikon Intensilight (C-HGFIE) and a cooled monochrome Q-Imaging EX*i* 12-bit camera. NIS Elements AR software (Nikon Instruments, Inc., Melville, NY, USA) was used for image capture and analysis.

Visualization of thread skein unraveling

In order to visualize the mechanism of thread skein deployment, a small 5 ml chamber (6 cm × 4 cm) was made on a 10.1 cm × 8.25 cm (0.1 cm thick) glass plate using a Liquid Blocker super PAP pen (Daido Sangyo Co. Ltd., Tokyo, Japan). This chamber was filled with 10°C artificial seawater and situated on a chilled (10°C) microscope stage. A syringe pump (Harvard 33; Holliston, MA, USA) with a 10 ml B-D Glass syringe and 0.6 mm inner diameter (i.d.) flexible tubing with a 0.2 mm × 2.0 mm (i.d.) Vitrotube tip (Fiber Optic Center, Inc; CAT. #3520) were used to create suction flow within the chamber. Experiments were conducted using 0.1 µl of fresh exudate collected using a micropipette and placed on the glass surface within the seawater chamber. A flow rate of 200 µl min^{-1} and monochrome Q-Imaging EX*i* 12-bit camera were used to record the exudates' response to flow at a rate of 21 frames per second.

Visualization of whole slime network

In order to visualize the formed slime network, 0.2 µl of fresh slime exudate was placed into 2 ml of artificial seawater containing the fluorescein-labeled lectin soybean agglutinin dye (500:1). The slime was established by agitating the 2 ml microcentrifuge tube up and down five consecutive times. The whole slime was then poured onto a glass slide in preparation for visualization. A Nikon 90i Eclipse microscope with a Nikon Intensilight (C-HGFIE) and Q-Imaging monochrome 12-bit camera were again used to visualize the network in both fluorescence and differential interference contrast (DIC).

Statistical analysis

A two-way analysis of variance (ANOVA) analysis was conducted on the 100 r.p.m. to 500 r.p.m. data to determine whether the degree of thread skein unraveling was significantly affected by mixing rate, and whether the use of DTT significantly affected the degree to which the thread skeins unraveled. The 0 r.p.m. percentage data were

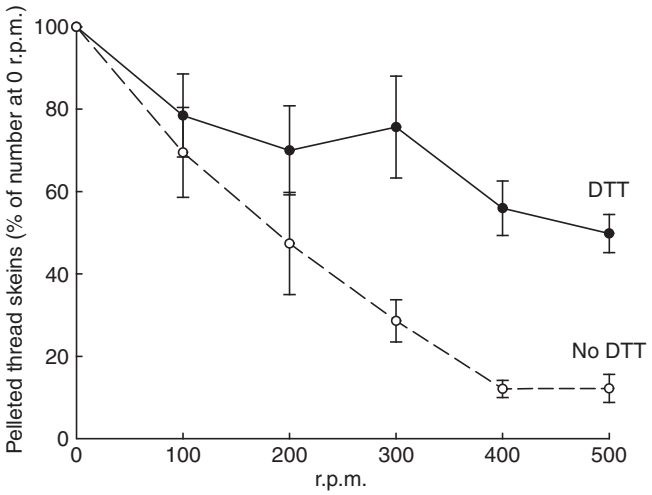

Fig. 3. Mean (±s.e.m.) number of condensed thread skeins as a function of mixing rate expressed as the percentage of the number of skeins at 0 r.p.m.. These data provide an inverse measure of the degree of thread skein unraveling in the whole slime. Solid line indicates data for trials in which mucins were disrupted with 10 mmol l^{-1} dithiothreitol.

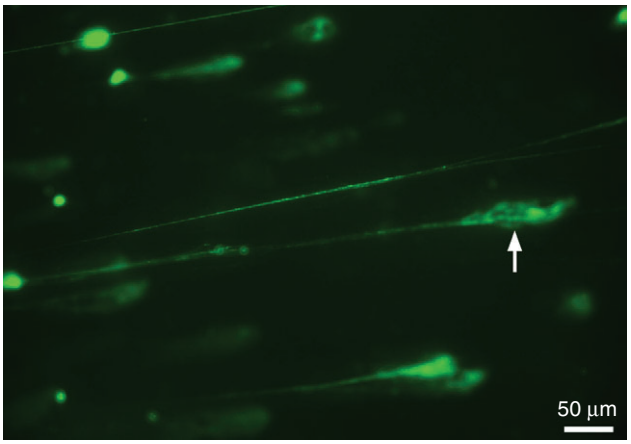

Fig. 4. Fluorescent tagging of mucins using a fluorescein-labeled lectin dye illustrates the formation of mucin strands from elongated mucin vesicles exposed to shear in a flow-through chamber. Arrow indicates an aggregation of ruptured mucin vesicles that have begun elongating to form strands.

not used in this analysis because the values for each individual hagfish were all 100% by definition. A two-tailed *t*-test assuming equal variance was conducted to determine whether a significant difference existed between the two mixing regimes (0 r.p.m. and 400 r.p.m.) in the presence of β-mercaptoethanol.

RESULTS

Thread skein unraveling

Two-way ANOVA analysis revealed significant main effects of mixing rate ($P<0.001$) and the presence of DTT ($P<0.001$) but no significant interactive effect ($P=0.169$). The results are consistent with the prediction that increasing the mixing rate significantly increases the degree of thread skein unraveling in the whole slime (Fig. 3). As such, this implicates convective mixing as a necessary requirement for thread skein unraveling and ultimately whole slime formation. The number of condensed thread skeins as a proportion of the total number of skeins at 0 r.p.m. appears to level off between 400 r.p.m. (5.08±2.07%) and 500 r.p.m. (8.31±3.39%) in the no DTT treatment (Fig. 3). This suggests that a certain fraction of skeins are resistant to unraveling, at least in exudates expressed from anesthetized hagfish. The data also support the hypothesis that thread skein unraveling is significantly affected by the presence of the mucin network during slime maturation. Furthermore, our attempts to completely disrupt the mucin network with high concentrations of β-mercaptoethanol revealed no significant differences ($P=0.697$) between the number of condensed threads at 0 r.p.m. (497±79) and 400 r.p.m. (459±51). The lack of unraveling in the presence of high concentrations of β-mercaptoethanol, even at high mixing rates suggests that convective mixing is necessary but not sufficient to affect thread skein unraveling, at least at the mixing rates we investigated. These data also underscore the importance of mucin vesicles to the unraveling of thread skeins in hagfish slime. We carried out further unraveling trials in the presence of 200 mmol l^{-1} β-mercaptoethanol and a viscous 2% polyethylene oxide solution in seawater to test whether the mucins aid in unraveling by increasing the local viscosity around thread skeins. At a mixing rate of 400 r.p.m., we saw no evidence of skein unraveling in this solution, but we could not quantify our results due to the fact that the condensed skeins were not able to pellet out of the highly viscous polyethylene oxide solution. We conducted a similar trial in the absence of 200 mmol l^{-1} β-mercaptoethanol and also found no evidence of unraveling, which suggests that increased viscosity actually inhibits thread skein unraveling.

Mucin strand formation, thread cell unraveling and whole slime structure

Fluorescent-labeling of ruptured mucin vesicles demonstrated that the vesicles readily elongate into strands when subjected to flow in the experimental chamber. Fig. 4 shows the formation of mucin strands from swollen mucin vesicles within the flow-through chamber. Exposure of the condensed slime exudates to flow created by the syringe pump apparatus revealed three important observations: (1) swollen mucin vesicles elongate in response to flow to form mucin strands, (2) the elongated mucin strands attach to the thread skeins, and (3) the mucin strands transduce hydrodynamic forces directly to the thread skeins and initiate unraveling by pulling them apart (Fig. 5) (see Movie 1 in supplementary material). Fluorescent imaging of the whole slime network provided a first-ever view of the interaction between mucin strands and threads in slime produced from un-stabilized exudate. Images taken with differential interference contrast (DIC) depict the whole slime network (Fig. 6A), while the same image viewed in fluorescence highlights the complexity of mucin and thread interactions in the whole slime (Fig. 6B).

DISCUSSION

Thread skein unraveling

The results from these experiments provide strong evidence for the importance of vigorous mixing in facilitating thread skein unraveling in hagfish slime. These results are consistent with previous findings that demonstrated the effects of mixing on whole slime formation (Fudge et al., 2005; Lim et al., 2006). These studies demonstrated that hagfish slime exudate released into still seawater does not explosively mature into the whole slime and requires significant mixing forces. Our results presented here indicate that the importance

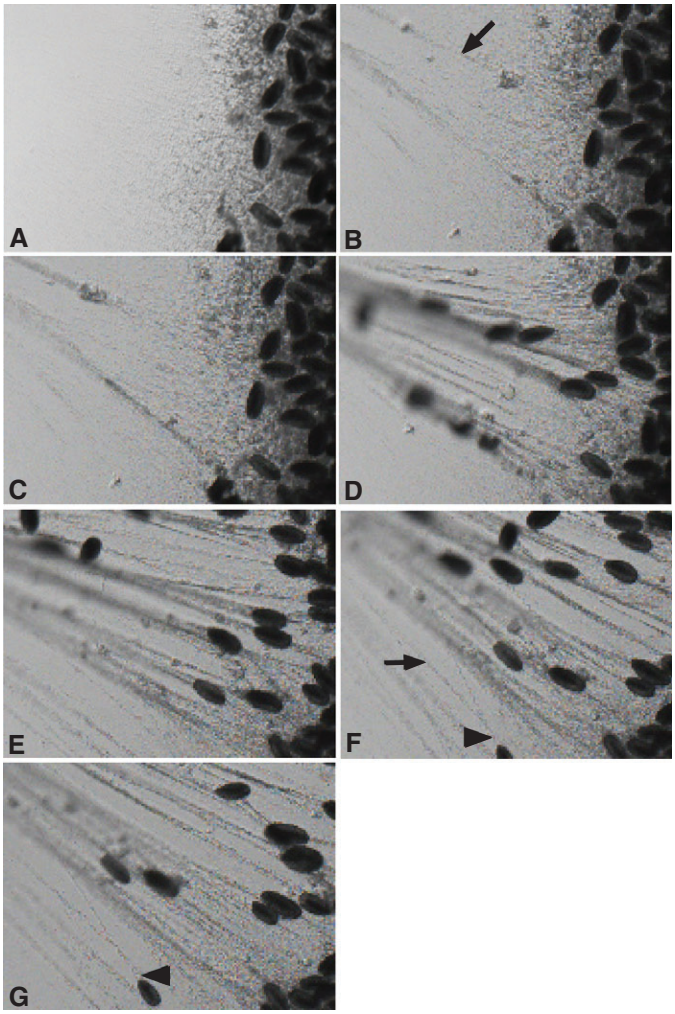

Fig. 5. Slime exudate exposed to flow created by a syringe pump. (A) Condensed exudate puddle prior to flow. Note the condensed thread skeins on the far right and the mucin vesicle boundary layer to the left. (B,C) Mucin strands and chains begin to form as flow is initiated. (D,E) Mucin strand attachment to thread skeins and movement of thread skeins. (F,G) Unraveling of thread skeins initiated. Arrowheads indicate an unraveling thread skein, arrows indicate aggregations of mucin strands.

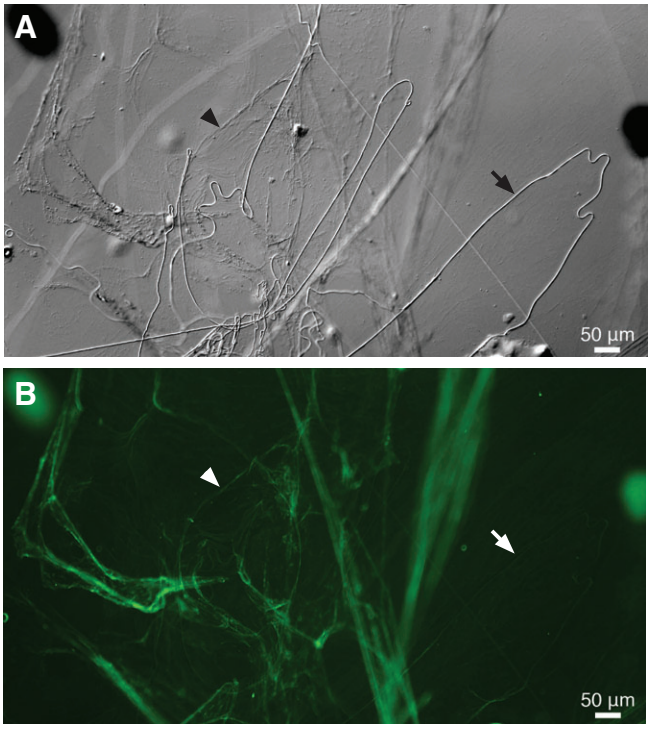

Fig. 6. Whole slime formed in seawater containing the fluorescent lectin dye (A) Differential interference contrast (DIC) image of whole slime network depicting unraveled threads and mucin strand network. Arrowhead indicates mucin strands connecting slime threads, and the arrow indicates a slime thread. (B) Same image viewed in fluorescence highlights the complexity of the mucin network.

of mixing on whole slime formation is at least in part due to the unraveling of individual thread skeins.

Previous studies found that the disulfide cleaving compound DTT has two main effects on hagfish slime maturation – it reduces the mass of whole slime formed *in vitro*, and it delays the time required for slime maturation (Koch et al., 1991b; Fudge et al., 2005). Our data indicate that high concentrations of disulfide cleaving compounds completely abolish thread skein unraveling and even low concentrations of these compounds significantly reduce the degree of thread skein unraveling *in vitro*. These results suggest that mucins are absolutely crucial for skein unraveling and furthermore suggest that DTT effects on whole slime are mediated at least in part by their effects at the level of thread skein unraveling. The data in Fig. 3 suggest that higher rates of mixing can compensate for disruption of the mucin network with DTT; however, the concentration used (10 mmol l^{-1}) in these experiments was probably not sufficient to completely disrupt the mucin networks (Koch et al., 1991b), especially over the timescale of unraveling. The use of high concentrations (200 mmol l^{-1}) of the more highly diffusible β-mercaptoethanol, however, abolished unraveling completely, even at high mixing rates. These data strongly suggest that an intact mucin network is absolutely crucial for thread skein deployment.

A new model of hagfish slime deployment and structure

An early model of hagfish slime deployment proposed that thread skeins are packaged under pressure within the GTCs (Newby, 1946). According to this hypothesis, contact with seawater causes an osmotic swelling of the GTC and rupture of the membrane, followed by a rapid expansion and unraveling of the thread driven by a release of internal pressure. Newby also proposed that the role of the mucins is simply to act as a fluid vehicle for the thread skeins. The discovery that the GTCs in hagfish slime are liberated of their plasma membranes during release of slime from the gland (Fernholm, 1981) cast doubt on Newby's hypothesis. More recent models of hagfish slime deployment propose that the initiation of thread skein unraveling is dependent on seawater-induced swelling of thread skeins as well as physical perturbation of the skeins themselves (Fernholm, 1981; Koch et al., 1991a). The model that we put forth here differs from these previous models in that it directly implicates the mucin vesicles in the process of thread skein unraveling and demonstrates their crucial importance in slime deployment. We also saw no evidence that thread skein swelling precedes unraveling over the time course of natural slime maturation.

Visualization of mucins with fluorescently labeled lectins revealed that mucin vesicles exposed to shear stresses in a flow chamber elongate into high aspect ratio mucin strands that readily attach to threads and thread skeins. Previous models suggested that the mucins

remain as coherent structures after they swell and rupture (Fudge et al., 2005; Koch et al., 1991b). Based on these observations, as well as direct observations of fresh slime exudates mixed with seawater under the microscope, we propose a new mechanism of hagfish slime deployment in which elongated mucin strands transmit mixing forces to thread skeins to initiate their unraveling. According to this 'mucin transmission hypothesis,' hagfish slime deployment occurs *via* the following sequence of events: (1) expulsion of the slime exudate into convectively mixing seawater results in the swelling and subsequent elongation of mucin vesicles to form mucin strands, (2) these elongated mucin strands attach to the thread skeins, (3) the mucin strands transmit the hydrodynamic forces of mixing to the thread skeins, thereby initiating unraveling, and (4) entanglement of the threads and mucin strands results in the complete unraveling of thread skeins. The whole slime is therefore a highly complex network of mucin strands, slime threads and seawater.

The results of this study also provide a new model for whole slime structure in which thin mucin strands interact with unraveled slime threads to yield a complex network capable of the viscous entrainment of water described by Fudge et al. (Fudge et al., 2005) (Fig. 6A,B). Previous studies depicted swollen mucin vesicles decorating the slime threads (Fudge et al., 2005; Koch et al., 1991a). According to these models, the viscous entrainment of seawater within fully formed slime was accomplished by confining water to channels between entangled slime threads covered with swollen mucin vesicles. The findings of this study expand our understanding of how the mucins and threads slow the flow of water by forming a complex network of mucins and threads in which water can be entrained.

Our results raise interesting questions about how exactly the mucin vesicles bring about thread skein unraveling. Videomicroscopy (Fig. 5) (see Movie 1 in supplementary material) provided compelling evidence that the mucin strands are capable of exerting elastic tensile forces on thread skeins but another possibility is that mucins facilitate the transfer of shear stresses to the skeins by 'viscosifying' the seawater around them. To test this idea, we carried out an experiment to see if high viscosity solutions can rescue the abolition of skein unraveling by β-mercaptoethanol. Not only did a viscous solution of high molecular weight polyethylene oxide in seawater not rescue skein unraveling at high mixing rates (400r.p.m.), it had an inhibitory effect, as unraveling did not occur in a subsequent trial in which β-mercaptoethanol was not present.

A deeper understanding of how mucins transmit hydrodynamic forces to the thread skeins will require knowledge of the mechanical properties of swollen mucin vesicles. While little is known about hagfish slime mucin material properties, chemical analyses suggest that they may have distinct physical properties. Typical vertebrate mucins contain up to 85% carbohydrate by dry mass but hagfish mucins contain only 12% (Salo et al., 1983). It is possible that the properties of the mucins have been shaped by selection to optimize their ability to transmit hydrodynamic forces to thread skeins. For example, mucins must be compliant enough to elongate into strands when exposed to shear or elongational flow (in spite of being considerably smaller than thread skeins), and yet the strands that form must be robust enough to exert tensile forces on the skeins that can initiate their unraveling.

These results suggest that viscous transfer of shear forces to thread skeins is not sufficient to effect their unraveling. Furthermore, viscous solutions are likely to be inhibitory for skein unraveling because of their ability to dissipate turbulent energy before it can reach the microscale inhabited by the skeins. By contrast, soft, swollen mucin vesicles are susceptible to shear and extensional flow, which can elongate the vesicles into high aspect ratio elastic strands that can attach to the skeins and increase their effective size. We propose that these mucin strands are capable of transmitting hydrodynamic forces to the skeins and exerting tensile forces on them that effect their unraveling. More generally, the mechanism described above may be valuable for industrial processes in which hydrodynamic mixing forces are transmitted to suspended particles that are typically immune to such forces due to their small size.

CONCLUSIONS

In this study, we provide evidence that convective mixing and intact mucin networks are required for proper deployment of thread skeins in hagfish slime. We also provide evidence for a new mechanism of hagfish slime deployment in which elongated mucin vesicles transmit hydrodynamic forces to the skeins and facilitate their unraveling. Our work provides a new model for hagfish slime structure and also provides novel insights into the evolution of the slime, suggesting that the evolution of thread skeins could not have preceded the appearance of the mucin vesicles in hagfish ancestors. The mechanisms described here may be of use for industrial processes in which hydrodynamic forces are transmitted to microscale particles that are typically immune to mixing forces.

LIST OF ABBREVIATIONS

DIC	differential interference contrast
DTT	dithiothreitol
GMCs	gland mucous cells
GTCs	gland thread cells

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