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Concentration-independent mechanics and structure of hagfish slime

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ABSTRACT

The defense mechanism of hagfish slime is remarkable considering that hagfish cannot control the concentration of the resulting gel directly; they simply exude a concentrated material into a comparably "infinite" sea of water to form a dilute, sticky, cohesive elastic gel. This raises questions about the robustness of gel formation and rheological properties across a range of concentrations, which we study here for the first time. Across a nearly 100-fold change in concentration, we discover that the gel has similar viscoelastic time-dependent properties with constant power-law exponent ($\alpha = 0.18 \pm 0.01$), constant relative damping $\tan\delta = G''/G' \approx 0.2{-}0.3$, and varying overall stiffness that scales linearly with the concentration ($\sim c^{0.99\pm0.05}$). The power-law viscoelasticity (fit by a fractional Kelvin-Voigt model) is persistent at all concentrations with nearly constant fractal dimension. This is unlike other materials and suggests that the underlying material structure of slime remains self-similar irrespective of concentration. This interpretation is consistent with our microscopy studies of the fiber network. We derive a structure-rheology model to test the hypothesis that the origins of ultra-soft elasticity are based on bending of the fibers. The model predictions show an excellent agreement with the experiments. Our findings illustrate the unusual and robust properties of slime which may be vital in its physiological use and provide inspiration for the design of new engineered materials.

Statement of Significance

Hagfish produce a unique gel-like material to defend themselves against predator attacks. The successful use of the defense gel is remarkable considering that hagfish cannot control the concentration of the resulting gel directly; they simply exude a small quantity of biomaterial which then expands by a factor of 10,000 (by volume) into an "infinite" sea of water. This raises questions about the robustness of gel formation and properties across a range of concentrations. This study provides the first ever understanding of the mechanics of hagfish slime over a very wide range of concentration. We discover that some viscoelastic properties of slime are remarkably constant regardless of its concentration. Such a characteristic is uncommon in most known materials.

1. Introduction

Many species of animals have evolved mechanisms for defending themselves against predators. One such strategy utilized by the deep-sea dwelling hagfishes involves the production of a large volume of a gel-like material, commonly known as hagfish slime or defense gel. Hagfish slime is known to clog the gills of fish predators and threaten them with suffocation [1,2]. It is different from many other animal secretions in the sense that its rheological complexity is vital to its function, unlike many other secretions where typically the toxicity/chemical deterrence of the secretion is important e.g. in several arthropods [3,4], molluscs [5–8]and mammals [9,10]. Rheological complexity is also known to influence the physiological performance of several other animal secretions e.g. snail slime [11], frog saliva [12] and coral mucus [13]. Interestingly, unlike most other secretions that are ejected/secreted from an animal's body, hagfish slime is not fully formed until outside the body of the animal in the surrounding water. A very small quantity of biomaterial (exudate) is secreted from hagfish slime glands (Fig. 1A-B), which then expands in sea water achieving expansion ratios up to 10,000 [14]. This process occurs

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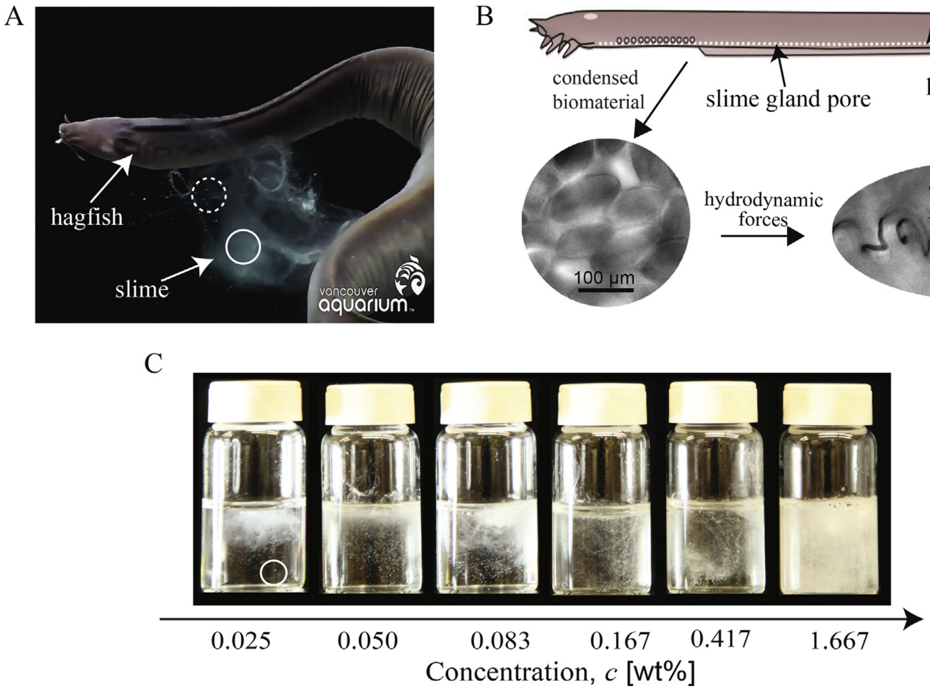

Fig. 1. Hagfish slime is produced *ex vivo* with an uncontrolled concentration. (A) Hagfish producing slime in a large tank of sea water. Since the exudate is released into a large volume of water, its concentration-dependent properties become vital for its functional requirements. Even in the released slime the concentration can vary as apparent from the image (dashed circle corresponds to low concentration, solid circle corresponds to high concentration) (image adapted with permission from Vancouver Aquarium YouTube channel). (B) Hagfish (image adapted [16] with permission) locally eject a small quantity of biomaterial from specialized slime pores near the site of stimulus. Thread cells and mucus are major components of the secreted biomaterial, which under hydrodynamic forces produce slime within a fraction of a second. The fibrous component in slime comes from the Gland Thread Cells that are ovoids of coiled intermediate filament-based fiber (arrowhead) and unravel into long fibers (arrow) when mixed in sea water. These fibers have a diameter of 1–3 μm and have length up to 10–15 cm [19]. (C) Hagfish slime samples of varying exudate concentrations (wt%) prepared in the lab by mixing fresh exudate with sea water. Samples with apparent concentration 0.025 wt% and 0.050 wt% have significant sol fraction (circle) in them while samples with concentration 0.083 wt% or greater appear to have a sample spanning network with negligible sol fraction.

within a fraction of a second (≈ 100–400 ms) [1,2]. Exudate contains two primary components: thread cells and mucous vesicles [15]. A thread cell is made up of an intermediate filament-based fiber coiled into an ovoid ($\sim 150\,\mu$m) [15]. Mucous vesicles (~ 5–$7\,\mu$m) are glycoprotein containing membrane-bound vesicles. Once the exudate is ejected into the sea water, advective mixing causes thread cells to unravel into long fibers [1], up to 10–15 cm in length and 1–3 μm in diameter, and mucous vesicles swell and rupture [16], to set up a fibrous network [14]. The fiber intermediate-filaments are composed of α and γ proteins [17,18] and undergo an $\alpha \rightarrow \beta$ transition at large strains [19]. The composition of hagfish mucus resembles that of mucin glycoproteins but the relative composition of components, namely: protein, carbohydrates, sulfate and lipids, is different [20]. Recent work focused on exploring the effect of the relative concentration of mucus and thread cells [21,22] found that mucus is essential for slime formation although its detailed role in slime production and functioning remain to be discovered. Several other works have explored the roles of organic and inorganic ions and compounds on slime production [16,23].

Recent work [24–26] on the mechanics of hagfish slime suggests that this material has predominantly elastic properties with a very low elastic modulus(~ 0.01 Pa). A very narrow range (0.01–0.08 wt%) of concentration was considered in these studies. However, hagfish exudate is ejected into a comparably infinite sea of water and the animal itself cannot directly control the concentration of the resulting network nor its mechanical properties. It is thus vital to understand how the concentration of slime affects its properties and the resulting performance of this material. A recent work [27], focused on water retention in slime over the concentration range of (0.005–0.1) wt%, but many questions about the mechanics are yet to be answered.

In this paper, we focus on the viscoelastic shear rheology of this gel over two orders of magnitude variation in its concentration – a much wider range compared to our own prior work [24] and those of others [25,26]. We found that irrespective of concentration, the gel has similar viscoelastic time-dependent properties with constant power law exponent ($n = 0.18 \pm 0.01$), a constant ratio $\tan\delta = G''/G'$, and varying overall stiffness that scales exactly linearly with the concentration ($\sim c^{0.99\pm0.05}$). The power-law creep compliance, with an exponent that is independent of concentration, suggests an underlying self-similar structure at all concentrations. We consider constitutive modeling to understand the time-dependent material properties and find that a three-parameter fractional Kelvin-Voigt model has an excellent agreement with the linear creep response for all concentrations. We gain insights into the structure–property relationship based on the mechanics of an individual fiber and develop a scaling estimate for the bulk response that agrees with our experimental observation.

2. Materials and methods

2.1. Sample preparation

Pacific hagfish (*Eptatretus stoutii*) were maintained at the University of Guelph as previously described [22]. All housing, feeding, and experimental conditions were approved by the University of Guelph Animal Care Committee (Animal Utilization Protocol 2519). Hagfish were anesthetized before the extraction of exudate. Electrical stimulation near the slime glands resulted in the secretion of the exudate locally near the site of stimulation [22]. The exudate was collected directly from multiple fish using a spatula, stored in a microcentrifuge tube at 10 °C (no buffer or