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Shear-Stress Dependence of Dinoflagellate Bioluminescence

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Abstract. Fluid flow stimulates bioluminescence in dinoflagellates. However, many aspects of the cellular mechanotransduction are incompletely known. The objective of our study was to formally test the hypothesis that flow-stimulated dinoflagellate bioluminescence is dependent on shear stress, signifying that organisms are responding to the applied fluid force. The dinoflagellate *Lingulodinium polyedrum* was exposed to steady shear using simple Couette flow in which fluid viscosity was manipulated to alter shear stress. At a constant shear rate, a higher shear stress due to increased viscosity increased both bioluminescence intensity and decay rate, supporting our hypothesis that bioluminescence is shear-stress dependent. Although the flow response of non-marine attached cells is known to be mediated through shear stress, our results indicate that suspended cells such as dinoflagellates also sense and respond to shear stress. Shear-stress dependence of flow-stimulated bioluminescence in dinoflagellates is consistent with mechanical stimulation due to direct predator handling in the context of predator-prey interactions.

Introduction

Bioluminescence is a cosmopolitan phenomenon in the world's oceans, visible in breaking waves (Stokes *et al.,* 2004), ships' wakes (Rohr *et al.,* 2002), and around swimming animals (Rohr *et al.,* 1998). The most common sources of bioluminescence in near-surface waters are dinoflagellates, which at high concentrations can highlight moving objects (Rohr *et al.,* 1998; Latz and Rohr, 2005). Dinoflagellate bioluminescence is believed to serve an antipredation function by reducing predator grazing (Esaias and Curl, 1972; White, 1979) through disruption of feeding behavior (Buskey and Swift, 1983; Buskey *et al.,* 1985). Dinoflagellate bioluminescence can also act as a "burglar alarm" to attract secondary visual predators to a primary predator, thereby reducing grazing pressure on the bioluminescent dinoflagellate (Mensinger and Case, 1992; Abrahams and Townsend, 1993; Fleisher and Case, 1995). Additionally, dinoflagellate bioluminescence is stimulated by swimming animals and may serve as a luminescent "minefield" for prey, which—highlighted by stimulated light—attract visual predators (Young, 1983). Bioluminescence stimulated by swimming animals (Mensinger and Case, 1992; Rohr *et al.,* 1998) or through direct manipulation of a cell by a predator (Buskey *et al.,* 1985) is predicted on the basis of response thresholds of flow stimulation that are experimentally determined using fully characterized flow fields (Latz *et al.,* 1994, 2004; Latz and Rohr, 1999; von Dassow *et al.,* 2005). Understanding the hydrodynamic conditions that stimulate dinoflagellate bioluminescence helps in elucidating the biomechanics involved in its adaptive value as an antipredation behavior.

Studies using fully characterized flow fields such as simple Couette flow and fully developed pipe flow have identified relationships between stimulation of bioluminescence and levels of shear stress. The response threshold for flow stimulation occurs in laminar flows with shear stresses of 0.02–0.3 N m^{-2}, depending on dinoflagellate species, with *Lingulodinium polyedrum* showing the highest threshold (Latz *et al.,* 1994, 2004; Latz and Rohr, 1999). These threshold shear-stress levels are several orders of magnitude greater than those found in the ocean interior, indicating that luminescent reserves would not be depleted for typical oceanic ambient flows (Latz *et al.,* 1994; Rohr *et al.,* 2002), and they are consistent with flow stimulation due to breaking waves and swimming animals. Above this threshold,

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average bioluminescence intensity increases as a function of shear stress as a greater proportion of a population of cells is stimulated. When the flow field is maintained at a constant shear stress, light intensity decays exponentially as a function of shear stress (Latz *et al.,* 1994). The decay rate represents the probability of flashing, *i.e.,* the proportion of the stimulable population per unit time. For *L. polyedrum,* the decay rate is about 0.001 s^{-1} near the response threshold and 0.01 s^{-1} at a shear stress of 1.4 N m^{-2}. Thus both bioluminescence intensity and decay rate are proportional to the shear stress present in the flow.

In these studies, bioluminescence response is typically reported as a function of shear stress because the flow fields are dominated by shear. For example, in pipe flow the response is characterized as a function of wall shear stress, which is readily calculated on the basis of flow rate and pressure drop, and represents maximum shear values across the pipe radius (Latz and Rohr, 1999). Similarly, in Couette flow the shear stress levels are readily calculated on the basis of chamber dimensions and angular velocity (Latz *et al.,* 1994). In working with a nozzle flow field that was dominated by flow acceleration, Latz *et al.* (2004) demonstrated that bioluminescence stimulation was associated with regions of high fluid deformation due to shear stress and not to acceleration. Thus there is consistent evidence from several independent flow fields that the bioluminescence response of dinoflagellates is associated with high shear conditions.

However, despite the convention of describing bioluminescence in terms of shear stress, it has not been experimentally validated that the response is shear-stress dependent. Shear-stress dependence implies that dinoflagellates respond directly to the fluid force acting on the cell, which may be equivalent to the force experienced when the cell is directly manipulated by a predator. Shear-stress dependence has been reported in other cell types, most notably mammalian endothelial cells, where responses such as cell alignment, pseudopod formation, and vascular dilation caused by nitric oxide release have all been shown to be shear-stress dependent (Davies, 1995). Unlike planktonic dinoflagellates, endothelial cells are attached to a substrate. When shear stress is applied to an attached cell, deformation is proportional to the applied shear stress (Davies, 1995).

It is unknown whether dinoflagellate bioluminescence is shear-stress dependent. Dinoflagellates may respond differently than endothelial cells because they are in suspension and experience translation and rotation in addition to deformation. One approach for studying shear-stress dependence is through direct manipulation of the viscosity of the cell growth medium (Vogel, 1994). By definition for a viscous fluid, the shear stress is the product of the fluid shear and viscosity:

$$\tau = \mu\gamma \quad (1)$$

where τ is the fluid shear stress (N m^{-2}), μ is the fluid dynamic viscosity (kg m^{-1} s^{-1}), and γ is the fluid rate of strain, hereafter referred to as shear rate (s^{-1}). Thus for a fluid sheared at a constant rate, the resulting shear stress will proportionally increase with an increase in viscosity. If bioluminescence is not dependent on shear stress, there should be no difference in the flow response of cells exposed to low-viscosity and high-viscosity conditions. On the other hand, if the response is shear-stress dependent, then a higher viscosity condition will result in a higher shear-stress level and thus an increase in the intensity and decay rate of bioluminescence. This approach has been successfully used to investigate shear-stress dependence in attached cells such as endothelial cells (Malek and Izumo, 1992; Ando *et al.,* 1993; Masuda and Fujiwara, 1993; Hutcheson and Griffith, 1996) and osteoblasts (Reich *et al.,* 1990; McAllister and Frangos, 1999; Bakker *et al.,* 2001). The only evidence for shear-stress dependence in a suspended organism is a study with the protist *Tetrahymena pyroformis,* in which cell disruption was shear-stress dependent (Midler and Finn, 1966).

We used simple Couette flow and the manipulation of fluid viscosity to test the hypothesis that dinoflagellate bioluminescence is shear-stress dependent. Our findings that support this hypothesis are (1) bioluminescence intensity increases with increasing shear stress, and (2) bioluminescence decay rate increases as shear stress increases.

Materials and Methods

Cultures

Cultures of *Lingulodinium polyedrum* (Stein) Dodge 1998 strain HJ, originally collected from the Scripps Pier, La Jolla, California, were grown in half-strength f/2 medium minus silicate (Guillard and Ryther, 1962) at 20 ± 1.5 °C in an environmental chamber on a 12:12 h light:dark cycle. Cultures were also grown in half-strength f/2 medium with addition of the polysaccharide dextran (Fisher Scientific, Pittsburgh, PA,, cat. no. BP1580-100) at a concentration of 1.5% (w:v) (500,000 m.w.) or 1% (2,000,000 m.w.) to achieve a final relative kinematic viscosity 2 times that of seawater. Dextran is nontoxic to dinoflagellates (Legrand and Carlsson, 1998) and has been used in studies on other cell types to increase the viscosity of growth media (Johnson *et al.,* 1998; McAllister and Frangos, 1999). Viscosity conditions greater than twice that of seawater were not considered because they resulted in a decrease in net population growth.

Another agent that has been used in previous studies to increase viscosity (Podolsky and Emlet, 1993) is the synthetic compound polyvinylpyrrolidone (PVP). Attempts were made to grow cultures in half-strength f/2 medium with addition of PVP-360 (360,000 m.w., SigmaAldrich Chemical Co. St. Louis, MO) at a concentration of 0.75%

(w:v) to achieve a final relative kinematic viscosity 2 times that of seawater. Although cultures did not grow in 2× viscosity medium enhanced with PVP, short-term treatment had no effect on chemically stimulated bioluminescence capacity ($t_{14} = -0.204$; $P = 0.842$). Thus short-term PVP treatment was used to increase viscosity for bioluminescence tests even though cells could not be pre-acclimated to the PVP conditions during population growth as they were for the dextran treatment.

There was no effect of dextran or PVP on fluid density of the seawater media ($F_{27,2} = 1.213$, $P = 0.313$). Based on the relationship between $\mu = \rho\nu$, where ν = kinematic viscosity ($m^2\ s^{-1}$), at similar densities the dynamic viscosity is directly proportional to the kinematic viscosity. Thus viscosity was determined on the basis of relative kinematic viscosity, which was measured with an Ostwald U-tube viscometer (Fisher Scientific, cat. no. 13–695) at 20 °C. The recorded travel times of media with dextran or PVP were compared to that of seawater medium to obtain kinematic viscosity. The kinematic viscosity (ν) of seawater at 20 °C is $1.047 \times 10^{-6}\ m^2 s^{-1}$ (Vogel, 1994). Thus, two kinematic viscosities were used in this study: $1.047 \times 10^{-6}\ m^2 s^{-1}$, representing untreated seawater media, and $2.094 \times 10^{-6}\ m^2 s^{-1}$, representing 2 times viscosity.

Experimental apparatus

Cultures were exposed to steady laminar shear using simple Couette flow. Simple Couette flow is stable (Coles, 1965) and has been used previously to expose dinoflagellates to steady shear conditions (Thomas and Gibson, 1990, 1992; Latz *et al.,* 1994; Juhl *et al.,* 2000, 2001; Juhl and Latz, 2002). This type of flow is generated in chambers by rotating the outer cylinder while keeping the inner cylinder stationary. These conditions produce a nearly linear velocity gradient in the seawater-filled gap between the two cylinders, resulting in a nearly constant shear across the gap.

The test chambers were identical to those used by Latz *et al.* (1994). The chamber dimensions were as follows: inner radius (r_i) = 23 mm; outer radius (r_o) = 25.5 mm; length (l) = 330 mm; gap volume = 130 ml. The gap width of 2.5 mm is 70 times larger than the 35-μm diameter of *L. polyedrum* cells. The ratio of gap width to outer cylinder radius of 0.1 assured laminar flow and minimized flow instabilities (Taylor, 1936; van Duuren, 1968). The flow field at the tested rotation rates was always laminar (Latz *et al.,* 1994).

For testing, a chamber was enclosed in an integrating light chamber. The outer cylinder was rotated using a DC servomotor (Silvermax) under computer control. Bioluminescence was detected using a photon-counting Electron Tubes model P10232 photomultiplier fitted with a Uniblitz electronic shutter (Vincent Associates). The number of photons emitted by the dinoflagellates was measured by the photomultiplier and expressed as photons per 10 millisecond integrations on the basis of radiometric calibration with an Optronics Laboratories model 310 multispectral source.

Flow field

The flow field within the flow chambers is easily quantified. Characteristics of the flow field, such as velocity and shear stress, can be determined at any point within the gap. Couette flow is characterized by a linear velocity gradient across the gap:

$$u(r) = \omega_o r_o^2 (r^2 - r_i^2)/(r_o^2 - r_i^2) r \qquad (2)$$

where ω is the angular velocity ($\omega = 2\pi N/60$, where N is the rotational speed in rpm) and r is the radial position within the gap (Schlichting, 1979).

The mean shear stress (τ) within the gap is the product of dynamic viscosity (μ) and mean shear ($\delta u/\delta r$):

$$\tau = \mu(\delta u/\delta r) = \mu[(2\omega_o r_o r_i)/(r_o^2 - r_i^2)]. \qquad (3)$$

The outer cylinder was rotated at 600 rpm, providing laminar flow (Latz *et al.,* 1994). This rotation speed produced a mean shear rate (γ) of 600 s^{-1} and a mean shear stress (τ) of 0.6 N m^{-2} for the 1× viscosity treatment. For the 2× viscosity treatment, the identical rotation speed produced a shear stress of 1.2 N m^{-2}. These shear levels were chosen because they are above the threshold needed to stimulate bioluminescence in *L. polyedrum* (Latz *et al.,* 1994, 2004).

The outer cylinder was initially accelerated over 8 s to the maximum rotation speed. This time was chosen because it is longer than the 6-s time for diffusion of momentum across the gap for this flow chamber (Latz *et al.,* 1994), as determined from the equation:

$$t = d^2/v \qquad (4)$$

where d(cm) is the gap width (Schlichting, 1979). Transient high shears during acceleration phase are avoided when the acceleration phase is longer than the time for diffusion of momentum. The rotation of the cylinder was then kept steady for 10 s, a duration sufficient to observe decay in bioluminescence intensity (Latz *et al.,* 1994).

Experimental protocol

To avoid depleting bioluminescence during handling, preparation for all experiments was done at the end of the light phase when bioluminescence is not excitable (Biggley *et al.,* 1969). The cultures were diluted with the appropriate test solution (filtered seawater for the 1× viscosity treatment, or 2× viscosity dextran and PVP solution for the 2× viscosity treatment), to obtain a cell concentration of 1000 cells ml^{-1} in a volume of 600 ml. Diluted cultures were loaded directly into the flow chambers and sealed. They were then acclimated in the dark at room temperature (~20

°C) in a horizontal orientation to promote a more even cell distribution along the length of the chamber. For testing, chambers were mounted at a 45° angle to maintain lubrication of the top chamber bushing. Testing was performed 3 h into the dark phase, when levels of stimulated bioluminescence are high (Biggley *et al.,* 1969).

Data analysis

Values are stated as means with standard deviations. Mean bioluminescence intensity (photons s^{-1}) was determined using the average of five intensity measurements immediately after the beginning of the steady phase (at time 8 s). The plot of intensity (photons s^{-1}) *versus* time (s), between 8 and 13 s, was then fitted with a least-squares exponential regression. The slope of the regression was taken as the best estimate of decay rate (s^{-1}) of light intensity in each chamber. To determine the response threshold, the intercept of the least-squares power regression of intensity *versus* shear stress was calculated for where the regression reached background light levels. Statistical tests were performed using Statview software (SAS Institute, Inc.). The data were tested for statistical significance using an unpaired Student's t test or one-way ANOVA with $P = 0.05$ as the criterion for significance.

Results

Bioluminescence increased during the acceleration phase and decreased exponentially during the steady phase (Fig. 1). The response threshold for bioluminescence stimulation occurred at 0.241 ± 0.1 N m^{-2} ($n = 14$) for the 1× (control) treatment, 0.157 ± 0.1 N m^{-2} ($n = 9$) for the 2× viscosity dextran treatment, and 0.194 ± 0.1 N m^{-2} ($n = 8$) for the 2× viscosity polyvinylpyrrolidone (PVP) treatment. These values were not significantly different from each other ($F_{28,2} = 1.732$, $P = 0.195$) and are consistent with results from previous studies using high concentrations (65–1100 cells ml^{-1}) of *Lingulodinium polyedrum* (Latz *et al.,* 1994; Latz and Rohr, 1999). Light emission reached its maximum at the end of the acceleration phase in the 1× (Fig. 1A) and 2× (Fig. 1B) viscosity treatments. During the steady phase, when shear was held constant at 600 s^{-1}, the bioluminescence intensity decreased exponentially in the 1× (Fig. 1C) and 2× (Fig. 1D) viscosity treatments.

The 2× viscosity treatment for the same protocol resulted in a higher intensity of bioluminescence (Fig. 2). Mean intensity for the 2× viscosity dextran treatment was significantly different ($t_{16} = -2.383$; $P = 0.030$) and 71% higher compared to the 1× viscosity treatment. Mean intensity for the 2× viscosity PVP treatment was also significantly different ($t_{14} = -2.68$; $P = 0.018$) and 52% higher compared to the 1× viscosity treatment.

Exponential decay rate also increased for the 2× viscosity treatment (Fig. 3). Mean decay rate for the 2× viscosity dextran treatment was significantly different ($t_{18} = 3.59$; $P = 0.003$) and 34% higher compared to the 1× viscosity treatment. Mean decay rate for the 2× viscosity PVP treatment was significantly different ($t_{14} = 4.69$; $P < 0.001$) and 40% higher compared to the 1× viscosity treatment.

Discussion

Previous studies of dinoflagellate bioluminescence stimulated in shear flows use the convention of expressing bioluminescence stimulation as a function of the fluid shear stress in the experimental flow field (Latz *et al.,* 1994, 2004; Latz and Rohr, 1999). For example, in fully developed pipe flow the response to flow stimulation is best characterized as a function of maximum shear stress at the wall (Latz and Rohr, 1999; Latz *et al.,* 2004). This convention has been used without verification of whether the flow stimulation is actually mediated by a force due to fluid viscosity. In our study, we assessed shear-stress dependence by increasing viscosity using dextran and polyvinylpyrrolidone (PVP), treatments that had no effect on bioluminescence capacity. At a constant shear rate, an increase in viscosity increased both bioluminescence intensity and exponential decay rate. Mean intensity in the 2× viscosity dextran and PVP treatments was 71% and 52% higher, respectively, compared to the 1× viscosity treatment. Decay rates were 34% and 40% higher in the 2× viscosity dextran and PVP treatments, respectively, compared to the 1× viscosity treatment. Because the magnitudes of both bioluminescence intensity and decay rate are proportional to fluid shear stress (Latz *et al.,* 1994), our results support the hypothesis that dinoflagellate bioluminescence is shear-stress dependent. This finding indicates that cells responding to sufficiently high shear levels present in laminar or turbulent flows are being stimulated by the fluid force.

The shear-stress dependence of dinoflagellate bioluminescence is relevant in the context of interactions between the dinoflagellates and their predators. The bioluminescence serves an antipredation function by reducing predator grazing (Esaias and Curl, 1972; White, 1979) through disruption of feeding behavior (Buskey and Swift, 1983; Buskey *et al.,* 1985), acting as a burglar alarm (Mensinger and Case, 1992; Fleisher and Case, 1995), and providing a luminescent minefield that highlights swimming animals (Hobson, 1966; Young, 1983; Rohr *et al.,* 1998). Shear-stress dependence indicates that dinoflagellates respond directly to the fluid force present in the experimental shear flow—a force that may be equivalent to that experienced by a cell in the presence of a swimming animal or when directly manipulated by a predator. The response threshold is greater than typical oceanic levels of shear stress (Latz *et al.,* 2004; von Dassow *et al.,* 2005), with the adaptive value of being high enough to prevent repeated stimulation by environmental

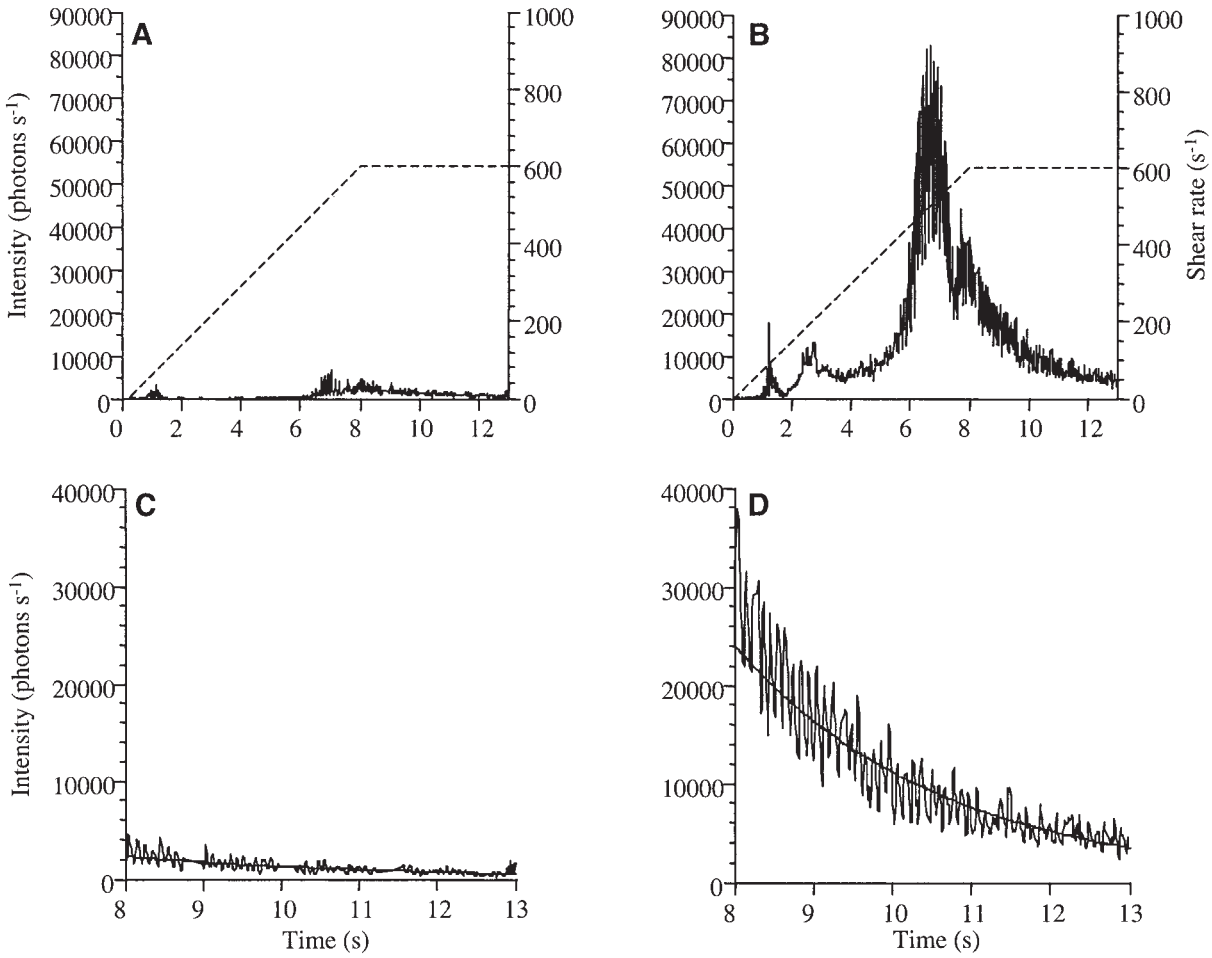

Figure 1. Representative bioluminescence time series for the dinoflagellate *Lingulodinium polyedrum* exposed to different viscosities using the polysaccharide dextran. Shear was increased over 8 s to 600 s^{-1}, after which shear rate was held constant for 5 s. The solid lines represent the intensity of light emission as a function of time for a population of *L. polyedrum* at a concentration of 1000 cells ml^{-1}. For (A) and (B) the dotted line represents the shear rate as a function of time. For (C) and (D) the curved line represents the least-squares exponential regression of light intensity *vs.* time. (A) Light intensity in 1× viscosity treatment (control). (B) Light intensity in 2× viscosity treatment. (C) Exponential decay of bioluminescence intensity in 1× viscosity treatment ($y = 6.016 \times 10^5 * e^{-0.309x}$). (D) Exponential decay of bioluminescence in 2× viscosity treatment ($y = 5.252 \times 10^5 * e^{-0.385x}$).

flows and the unnecessary depletion of energetically costly chemicals involved in the light reaction.

Bioluminescence is but one physiological response to flow by planktonic organisms. The escape jump of copepods and protists such as ciliates and flagellates (Kiørboe *et al.,* 1999; Jakobsen, 2001, 2002) occurs in laminar flow conditions with fluid rate-of-strain values some 2 orders of magnitude less than that stimulating dinoflagellate bioluminescence. These flow conditions, present within the feeding current of predators, would allow organisms to escape predator capture by triggering a jump. Similar low levels of fluid strain in laminar flow inhibit the population growth of some dinoflagellates (Thomas and Gibson, 1990; Juhl *et al.,* 2000, 2001; Stoecker *et al.,* 2006). It is as yet unknown whether flow-induced escape behavior and growth inhibition are shear-stress dependent and mediated through the fluid force acting on the organism.

Shear-stress dependence validates the ability of suspended cells, such as dinoflagellates, to sense and respond to fluid shear stress. Shear-stress dependence is present in attached cells such as vascular endothelial cells (Malek and Izumo, 1992; Hutcheson and Griffith, 1996), which respond directly to fluid shear stress through a complex signal transduction pathway (Davies, 1995). In endothelial cells, shear stress decreases the viscosity of the plasma membrane (Haidekker *et al.,* 2000), causing conformational changes in guanosine triphosphate (GTP)-binding proteins (G proteins) embedded in the plasma membrane (Gudi *et al.,* 1998). The

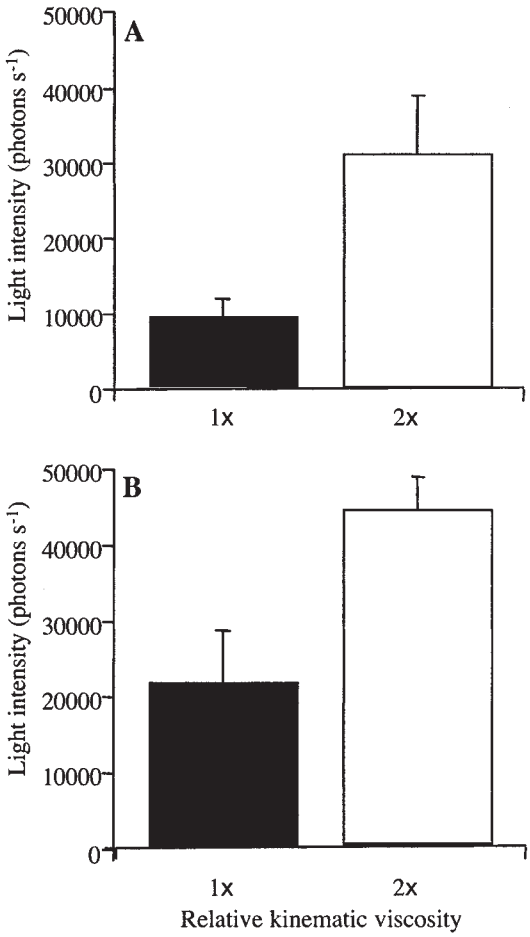

Figure 2. Effect of viscosity on bioluminescence of the dinoflagellate *Lingulodinium polyedrum* stimulated at a shear rate of 600 s^{-1}. Mean bioluminescence intensity was determined using the average of 5 intensity measurements immediately after the beginning of the steady phase at a time of 8 s. Light intensity is expressed as a function of relative kinematic viscosity. Bars represent 1 standard error of the mean. (A) Effect of dextran treatment. Mean intensity for the 2× viscosity dextran treatment was significantly different and 71% higher compared to the 1× viscosity treatment. (B) Effect of polyvinylpyrrolidone (PVP) treatment. Mean intensity for the 2× viscosity PVP treatment was significantly different and 52% higher compared to the 1× viscosity treatment.

activated G proteins bind GTP, initiating a complex signaling pathway that leads to changes in cell morphology and physiology (Davies, 1995; Wang and Thampatty, 2006).

The flow-sensing mechanotranduction process in luminescent dinoflagellates—eukaryotic protists that are most prevalent as suspended cells—appears to have features similar to those of attached mammalian vascular endothelial cells. Light emission is presumably mediated by physical deformation of the cell due to fluid forces acting on it, with the response threshold (Latz *et al.,* 1994; Latz and Rohr, 1999) being similar to shear-stress levels in venous and arterial blood flow in mammalian systems (Berthiaume and Frangos, 1993; Davies, 1995). As in endothelial cells, shear stress increases the fluidity of the cell membrane in dinoflagellates (Mallipattu *et al.,* 2002). This may also activate G proteins, because treatment with the G protein inhibitor GDPβS inhibits light emission (Chen *et al.,* 2007). Although the signaling pathway is not yet known, one step involves an increase in Ca^{2+} levels in the cytosol through the release of intracellular stores (von Dassow and Latz, 2002). The Ca^{2+} flux leads to the generation of an action potential in the internal vacuole membrane (Eckert, 1965; Widder and Case, 1981), which results in the flux of protons from the vacuole into the cytoplasm. The acidification of the cytoplasm activates the luminescent chemistry, resulting in light emission (Wilson and Hastings, 1998). The entire signaling process takes 15–20 ms (Eckert, 1965; Widder and Case, 1981; M.I. Latz *et al.,* unpubl.).

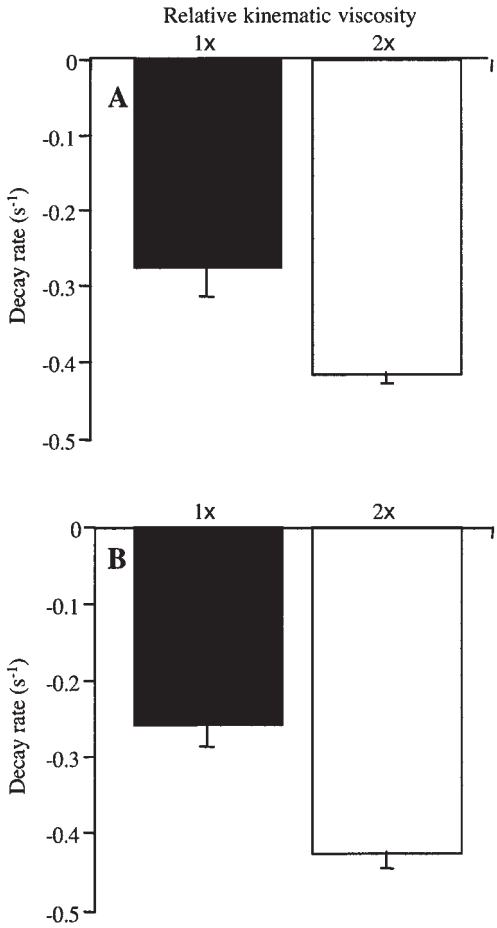

Figure 3. Effect of viscosity on exponential decay of bioluminescence of the dinoflagellate *Lingulodinium polyedrum* exposed to a steady shear rate of 600 s^{-1} for 5 s. Decay rate (s^{-1}) is expressed as a function of relative kinematic viscosity. Bars represent 1 standard error of the mean. (A) Effect of dextran treatment. Decay rate was significantly different and 34% higher in the 2× viscosity dextran treatment compared to the 1× viscosity treatment. (B) Effect of polyvinylpyrrolidone (PVP) treatment. Mean decay rate was significantly different and 40% higher in the 2× viscosity PVP treatment compared to the 1× viscosity treatment.

Thus even though dinoflagellates represent some of the most basal eukaryotes, they apparently possess flow-sensing capabilities similar to those of higher eukaryotes such as mammalian cells. There are many examples in biology of the evolution of sophisticated systems in mammalian cells from simpler eukaryote systems. Ancestral globin, for example, was most likely an oxygen-utilizing enzyme that gave rise, through minor structural changes, to hemoglobin as atmospheric O_2 became available (Moens *et al.,* 1996). Receptor-mediated signal transduction systems may also have evolved before development of the Metazoa and are used by the ciliates *Paramecium, Stentor,* and *Tetrahymena* to locate food, for example (Marino *et al.,* 2001). G proteins have been identified in these species and are most likely involved in signal transduction (Forney and Rodkey, 1992; Marino *et al.,* 2001). G proteins are present in dinoflagellates (Tsim *et al.,* 1996) and may be involved in the signaling pathway for the mechanical stimulation of bioluminescence (Chen *et al.,* 2007). Our study provides further evidence for the similarity in mechanisms of flow sensing between dinoflagellates and mammalian endothelial cells, suggesting that mechanotransduction originated in simple eukaryotes and has been evolutionarily conserved.

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