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Variability in the bioluminescence response of the dinoflagellate *Pyrocystis lunula*

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Abstract

Light emission in dinoflagellates is induced by water motions. But although it is known that mechanical stimulations of these organisms trigger the bioluminescent response, the exact mechanism that involves some cell membrane excitations by fluid motions is not yet fully understood and is still controversial. We show in this experimental study that the accelerated shear flow, created by abrupt rotations of one or both co-axial cylinders of a Couette shearing chamber excites the light emission from cultured dinoflagellates *Pyrocystis lunula*. Following our first results published earlier that state that pure laminar shear does not excite the main bioluminescent response in dinoflagellates, our present experiments show that both shear and acceleration in the flow are needed to trigger the bioluminescent response. Besides, the probability to stimulate this bioluminescent response under acceleration and shear is deduced from the response curves. This response follows a Gaussian distribution that traduces a heterogeneity in individual cell thresholds for the stimulation of bioluminescence in a dinoflagellate population. All these results will have a repercussion in the possible applications of dinoflagellate bioluminescence in flow visualizations and measurements. Moreover, this study opens a new way in studying mechanically-induced stimulus thresholds at the cell level.

Keywords: Accelerated shear flow; Bioluminescence threshold; Dinoflagellate

1. Introduction

In the marine world, luminous trails behind ships or any other moving object is well known and has been observed and reported since antique times. This light emission is produced by living organisms and is called bioluminescence. In surface waters, these phenomena are often due to dinoflagellates which are micro-organisms that belong to the phytoplankton community. These organisms are often responsible for luminous trails observed around moving ships, dolphins and breaking waves (Rohr et al., 1998, 2002; Stokes et al., 2004). Light is emitted in dinoflagellates as a rapid flash of approximately 100 ms in duration. The latency between the stimulus and the response is less than 20 ms (Hickman et al., 1980; Widder and Case, 1981). The total stimulable light varies from 10^8 photons $cell^{-1}$ in *Gonyaulax* sp. (Seliger et al., 1969) to 10^9 for *P. noctiluca* and *P. fusiformis* (Swift et al., 1985) and the energy is centered

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around 473–478 nm (Swift et al., 1973; Hastings and Morin, 1991). Although it is known that a mechanical stimulation of these single cell organisms triggers a bioluminescent response, the exact mechanism that involves the interaction between the cell membrane and the flow around the organisms is not yet fully understood and deserves a deeper analysis.

Mechanical stimuli of living cells induce a large variety of cellular responses in which cell membrane excitations trigger complex biochemistry reactions. Examples of such mechanotransductions include the growth and maintenance of muscle cells (Burkholder, 2003), morphological changes of red blood cells (Kuzman et al., 2003), macromolecules synthesis in the case of chondrocytes (Mabasheri et al., 2001) or reorientation (Wang et al., 2001) or vascular remodeling (Lehoux and Tedgui, 1998) in the case of endothelial cells subjected to cycling or pulsatile stretching. Among all these studies, some authors have recently emphasized the role of the stretching velocity of the cells (or the stretching frequency in the case of cycling forcing) and not only on the amplitude of the applied stretching (Wang et al., 2001; Burkholder, 2003; Bao et al., 1999).

The exact mechanism of bioluminescence in dinoflagellates is not yet completely known and it is currently believed that a mechanical stimulus induces cell membrane deformations which in turn activate a vacuole membrane action potential and consequently activate the entry of hydrogen ions into some sub-cellular organelles called scintillons (Fogel and Hastings, 1972). Then a series of biochemical reactions involving the catalytic oxidation of a specific photo-protein (luciferin) releases some energy in the form of blue light flashes.

Although Latz et al. (1994) showed that a constant shear can induce bioluminescence of dinoflagellates, several more recent studies have shown that massive light emissions are obtained when the cells experience temporal changes in the mechanical constraints rather than constant values of these parameters (Stolzenberg et al., 1995; Anderson et al., 1988; Blaser et al., 2002; Cussatlegras and Le Gal, 2004). In particular, bioluminescence induced by transient Couette flows between two cylinders has been the subject of several analyses. It was first shown in Cussatlegras and Le Gal (2004) that when the rotation rate of the outer cylinder of the shearing chamber is abruptly changed, an intense bioluminescence response is triggered. This first experiment, realized on a population of *Pyrocystis noctiluca*, was then followed by two independent similar experimental studies. Cussatlegras and Le Gal (2005) studied the light emission of *Pyrocystis lunula* whereas Von Dassow et al. (2005) considered the bioluminescence of dinoflagellate *Lingulodinium polyedrum*. The main results of both studies concern the bioluminescent response during ramps of increasing rotation rate of the outer cylinder, i.e. during developing Couette flow as called by Von Dassow et al. (2005). In both cases, the role of the temporal change of shear (or the shearing velocity) was shown to be essential to trigger the bioluminescence response. Moreover, Von Dassow et al. (2005) explained the faculty that possess dinoflagellates not to emit light at weak changes of shear by a desensitization process.

In the same spirit of the studies performed on the role of the stretching velocity on diverse cell responses, our experiment demonstrates the need of acceleration (or temporal velocity changes) in the bioluminescence response of dinoflagellates. As shown in a previous series of experiments (Cussatlegras and Le Gal, 2004), stationary homogeneous shear flows do not trigger the main bioluminescent response of dinoflagellates. Hence we performed and reported here on two sets of experiments using a Couette shearing chamber to tests the effect of 1) different shear values with a given acceleration and 2) different acceleration values with a given shear. These measurements show respectively that shear and acceleration both drives the dinoflagellate population bioluminescent response. Hence, a change of shear is necessary to trigger bioluminescence. The results of our study are thus important in the understanding of plankton bioluminescence itself, but more generally contribute to the knowledge of mechanical stimulation of living cells reactions via membrane deformations and mechano-sensitive receptors.

2. Material and methods

2.1. Organisms

P. lunula (Schütt) is a common oceanic species of dinoflagellates. Our cultures were obtained from the CCMP, Bigelow (strain 731) and grown in enriched f/2 media (Guillard and Ryther, 1962) in a culture chamber maintained at 20 °C+/−2 °C on a 12:12 Light : Dark cycle. Subjective day always started at 00:00 and subjective night at 12:00. Experiments were always done in the middle of the dark phase when the level of emitted light was maximum, according to the rhythmic cycle of stimulated and spontaneous bioluminescence in dinoflagellates (Sweeney and Hastings, 1957; Colepicolo et al., 1993; Latz and Lee, 1995). Fig. 1A shows the elongated shape of *P. lunula* organisms whose size is approximately 50 μm in diameter and 200 μm in length. In order to use always cultures in the