The Velocity of Microtubule Sliding: Its Stability and Load Dependency

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It is now well understood that ATP-driven active sliding between the doublet microtubules in the sperm axoneme generates flagellar movement. However, much remains to be learned about how this movement is controlled. Detailed analyses of the flagellar beating of the mammalian spermatozoa revealed that there were two beating modes at a constant rate of microtubule sliding: that is, a nearly constant-curvature beating in nonhyperactivated spermatozoa and a nearly constant-frequency beating in hyperactivated spermatozoa. The constant rate of microtubule sliding suggests that the beat frequency and waveform of the flagellar beating are independently regulated. Comparison of the sliding velocity of several mammalian and sea urchin sperm flagella with their mechanical property clarified that the sliding velocity of the microtubule was determined by the stiffness of the flagellum at its base, and that its relationship was expressed by a logarithmic equation that is similar to the classical force-velocity equation of the muscle contraction. Data from sea urchin spermatozoa also satisfied the equation, suggesting that the same microtubule sliding system functions in both the mammalian and echinoderm spermatozoa. Cell Motil. Cytoskeleton 64: 809–813, 2007.

Key words: force-velocity equation; hyperactivation; mode conversion; outer dense fibers; stiffness; sperm flagella

INTRODUCTION

Various types of experiments have carried out for understanding the regulatory mechanism of the flagellar beating of spermatozoa [Okuno and Hiramoto, 1976; Gibbons et al., 1987; Brokaw, 1989; Shingyoji et al., 1995]. However, whether the beat frequency and waveform of the flagellar beating are dependently or independently regulated has not been determined, although these parameters of the flagellar beating of echinoderm spermatozoa were presumed to be independently regulated [Gibbons, 1975]. It is also unknown as to which parameter of microtubule sliding produces the change in the beating pattern of the sperm flagella.

Mammalian spermatozoa are particularly suitable material for investigating the regulatory mechanism of the flagellar beating because they alter their beating in the male and female reproductive tracts [Ishijima and Mohri, 1985, 1990; Yanagimachi, 1994]. In the male reproductive tract, the spermatozoa gradually acquire their motility and change their beating pattern while passing through the epididymis. In the female reproductive-
tive tract, they remarkably change their motility during the capacitation, i.e., so-called hyperactivation.

Detailed analyses of the flagellar beatings of monkey, golden hamster, and Suncus spermatozoa before and after hyperactivation were carried out using high-speed video microscopy and digital image processing [Ishijima et al., 2002, 2006; Ohmuro and Ishijima, 2006; Kaneko et al., 2007]. These analyses revealed the essential changes in the sperm and flagellar movements during hyperactivation and the microtubule sliding mechanism underlying these changes. Based on these results, these fundamental features of the flagellar beating of mammalian spermatozoa were outlined, then the sliding-filament mechanism underlying these features was clarified, and finally, a characteristic equation of the flagellar beating, such as the classical force-velocity equation of muscle contraction, was defined.

**BIPHASIC CHARACTERISTIC OF FLAGELLAR BEATING**

Spermatozoa incubated in a capacitation medium changed their motility with incubation time; namely, the spermatozoa swimming in relative straight paths immediately after incubation changed their swimming to circular paths, followed by a nonprogressive whiplash or figure-of-eight movement [Ishijima et al., 2002, 2006; Ohmuro and Ishijima, 2006]. These changes in sperm swimming were due to the change in the flagellar beating; that is, the spermatozoa swimming in circular paths beat rapidly with fairly symmetrical flagellar bends, the spermatozoa swimming with circular paths beat slowly with rather asymmetrical flagellar bends, and the spermatozoa executing the figure-of-eight movement beat rather slowly with large bends at the base of the flagellum [Ishijima et al., 2002, 2006]. Detailed examination of the curvature of the flagellar bends revealed that the curvature of the flagellar bends at the base of the flagellum increased with the incubation time while that of the flagellar bends in the other regions of the flagellum only slightly changed. Concomitantly, their beat frequency remarkably decreased. These changes in the flagellar beating during the hyperactivation are common among the golden hamster, monkey, mouse, and Suncus spermatozoa, although these spermatozoa have different morphologies and movement characteristics. Thus, large bends at the flagellar base and a low beat frequency seem to be essential and common features during the hyperactivation (below). Typical examples of these changes in the golden hamster and monkey spermatozoa are shown in Fig. 2. The flagellar beating changes in a biphasic manner; that is, a nearly constant-curvature beating in the nonhyperactivated sperm flagella and a nearly constant-frequency beating in the hyperactivated sperm flagella. Therefore, there is a mode conversion of the flagellar beating of the mammalian spermatozoa.

Changes in the flagellar bends during the hyperactivation mainly occurred at the base of the flagellum, while the other regions of the flagellum only had limited involvement [Ohmuro and Ishijima, 2006], suggesting that the basal regions of the flagellum play a special role in generating a flagellar bend. In fact, a comparison of the shear angle of the flagellar bends of the hyperactivated spermatozoa with that of the nonhyperactivated spermatozoa revealed that the large bends at the flagellar base of the hyperactivated spermatozoa were induced by an increase in the total length of the microtubule sliding [Ohmuro and Ishijima, 2006]. An important role of the basal regions in the ciliary movement has been emphasized by Kinoshita and Kamada [1939]. Recently, several studies analyzing the microtubule sliding have also suggested that the microtubule sliding at the flagellar base are different from that in the other regions of the flagellum [Shingyoji et al., 1995; Woolley and Vernon, 2002].

**STABILITY OF MICROTUBULE SLIDING VELOCITY**

The maximum shear angle was found to be proportional to the beat period (the reciprocal of the beat frequency) (Fig. 3), suggesting that the maximum shear angle and beat period, in the other words, the waveform and frequency of flagellar beating, are dependently regulated. However, the waveform and frequency of flagellar beating behave almost independently over a fairly wide range (Fig. 2), because the curvature of flagellar bends was proportional to the maximum shear angle [Ohmuro and Ishijima, 2006]. Since the product of the shear angle and beat frequency is proportional to the sliding velocity of the microtubule, the sliding velocity of the microtubule is proportional to the product of the shear angle and beat frequency. Therefore, the sliding velocity of the microtubule is proportional to the product of the shear angle and beat frequency.
bule remained constant before and after hyperactivation within each species, although among the monkey, golden hamster, and Suncus spermatozoa, the values of the sliding velocity were entirely different [Fig. 3; Ishijima et al., 2006]. The constant nature of the sliding velocity of the cilia and flagella has been previously pointed out [Hiramoto, 1974; Oiwa and Takahashi, 1988].

LOAD DEPENDENCY OF MICROTUBULE SLIDING VELOCITY

The velocity of the microtubule sliding of the sperm flagella in each species remained constant before and after the hyperactivation because of the balanced decrease in the beat frequency and increase in the maximum shear angle [Ohmuro and Ishijima, 2006; Kaneko et al., 2007]. However, the value of the sliding velocity was apparently different among the golden hamster, monkey, and Suncus spermatozoa [Kaneko et al., 2007]. The sliding velocity of the monkey, golden hamster, and Suncus spermatozoa decreases in this order, whereas the diameter of their flagellum increases in this order (Fig. 4). This suggests the classical force-velocity relationship of the muscle contraction; that is, the higher the load, then the slower the sliding velocity. To test this hypothesis, the rate of the maximum shear angle of the monkey, golden hamster, and Suncus spermatozoa was plotted versus the moment of inertia of the cross-section of the base of their flagellum. The moment of inertia, which is
Fig. 4. Load-velocity relationship of flagellar beating of the spermatozoa. The rate of the maximum shear angle (sliding velocity) of the flagellar beating of the monkey (■), golden hamster (○), Suncus (●), and sea urchin spermatozoa (□) is plotted as a function of the moment of inertia of the accessory fibers and the axoneme. The moment of inertia is calculated using computer software (C-net Co., Kochi, Japan) and images of the cross-section in the proximal mid-piece (shown above each data). The regression line for the monkey, golden hamster, and Suncus spermatozoa (the solid line) is given by \( v = -4.0 \times \ln I - 9.2 \) and that for these mammalian and sea urchin spermatozoa (the dotted line) is \( v = -3.7 \times \ln I - 7.2 \), where \( v \) is the rate of the maximum shear angle and \( I \) is the moment of inertia. The bar in cross section is 0.5 \( \mu \)m. [Reproduced from Kaneko et al., 2007].

The rate of maximum shear angle and moment of inertia of the sea urchin spermatozoa is also plotted in Fig. 4. These data approximately lie on the line extrapolated from the mammalian spermatozoa data, meaning that there is no fundamental difference in the performance of the microtubule sliding between the mammalian and echinoderm spermatozoa. In other words, the same microtubule sliding system functions in both the mammalian and echinoderm spermatozoa.

Mammalian spermatozoa have accessory fibers such as outer dense fibers and satellite fibers [Fig. 4; Kaneko et al., 2007]. The role of these structures has not been defined well although several hypotheses have been suggested; that is, they determine the degree of transverse movement of the sperm flagellum [Phillip, 1972] and they strengthen the flagellum to protect sperm from the shear forces during ejaculation [Baltz et al., 1990]. As mentioned above, the hyperactivation was a mode conversion from a constant-curvature beating to a constant-frequency beating. We propose that the accessory fibers enable this beating mode conversion by reducing the sliding velocity from a high value of sperm flagella lacking the accessory fibers to a low value of mammalian spermatozoa having them, and then the beat frequency decreases to a low value required for the hyperactivated flagellar beating by increasing the total length of the microtubule sliding as mentioned above. This is probably the main reason why echinoderm sperm flagella do not show a dramatic change in the flagellar beating, such as hyperactivation [Brokaw, 1989].

REFERENCES


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