Calcium Ion Regulation of Chirality of Beating Flagellum of Reactivated Sea Urchin Spermatozoa

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ABSTRACT   Near an interface, sea urchin spermatozoa swim almost in circles. The direction is usually clockwise at the lower surface of a coverslip and counterclockwise at the upper surface of a glass slide, when viewed from above. Examination of demembranated spermatozoa has shown that Ca^{2+} regulates the direction of the circular motion of spermatozoa reactivated with adenosine triphosphate (ATP). This finding suggests that Ca^{2+} changes the chirality of the three-dimensional bending waves of sperm flagella.

INTRODUCTION

It has long been known that spermatozoa yaw (rotation of a spermatozoon about the axis perpendicular to the beating plane) in a constant direction at the lower surface of a coverslip or the upper surface of a glass slide, although some deviations from this consistency have been reported (Ishijima and Hamaguchi, 1992). We have observed that some sea urchin spermatozoa occasionally change the direction of their roll (rotation of a spermatozoon about its longitudinal axis) when attached vertically to a coverslip at the tip of their head and roll about their longitudinal axis (Ishijima, et al., 1992). This suggests that the direction of yawing at the interface is also lax because the change in direction of the rolling motion of spermatozoa about the longitudinal axis will cause a change in direction of yawing at the interface (Ishijima and Hamaguchi, 1992).

The intracellular concentration of Ca^{2+} is a possible candidate for a factor that regulates the direction of yaw, because Ca^{2+} induces changes in the ciliary (Naitoh and Kaneko, 1972) or flagellar (Brokaw, et al., 1974; Brokaw and Simonick, 1977; Gibbons, 1980) beating pattern and conformational changes in the isolated doublet microtubules (Miki-Noumura and Kamiya, 1976). The effect of Ca^{2+} on the yawing motion of sea urchin spermatozoa has been examined using demembranated spermatozoa.

MATERIALS AND METHODS

Japanese sea urchin spermatozoa, *Hemicentrotus pulcherrimus*, were demembranated and reactivated by several methods. The method of Brokaw et al. (1974) yielded the reactivated spermatozoa with a beat frequency of 29.7 Hz (SD = 3.3; n = 35), at pCa = 6, when the beat frequency of live spermatozoa was 38.3 Hz (SD = 4.6; n = 41). Thus, we chose to use the method of Brokaw et al. (1974) to give the same Ca^{2+} response of the Japanese sea urchin spermatozoa as that of the Californian sea urchin spermatozoa. Concentrated sea urchin spermatozoa were obtained by removing the gonad and placing it in a plastic culture dish (35 × 10 mm) kept in a refrigerator until use. Approximately 200 µl of the spermatozoa was diluted with 400 µl of cold 0.5 M NaCl. Approximately 1.5 µl of the sperm suspension obtained was placed in a well of a 24-well tissue culture plate containing 0.5 ml of extraction solution containing 0.15 M KCl, 2 mM tris(hydroxymethyl)aminomethane (Tris) buffer, 2 mM dithiothreitol, 0.5 mM EDTA, 0.04% (v/v) Triton X-100, 2 mM $MgSO_4$, and 5 mM $CaCl_2$, pH 8.0. The suspension was then stirred gently for approximately 30 s, after which time 5 µl of the mixture was transferred to a well containing 0.5 ml of reactivation solutions consisted of 0.15 M KCl, 20 mM Tris buffer, 2 mM dithiothreitol, 2 mM EDTA, or [ethylenebis(oxyethylenenitrilo)]tetraacetic acid (EGTA), 2% (w/v) polyethylene glycol, and various concentrations of $CaCl_2$, $MgSO_4$, and ATP, pH 8.0 (Table I, Brokaw et al. (1974)). For observations and recording of reactivated sperm motility, an aliquot (100 µl) of the sperm suspension was transferred to the observation chamber. All procedures and observations were carried out at 23°C. Stability constants used for calculation of Ca^{2+} concentrations were not adjusted for temperature, because their logarithmic values at 23°C differ by less than 2% from those at 16°C, at which Brokaw et al. (1974) performed their experiments.
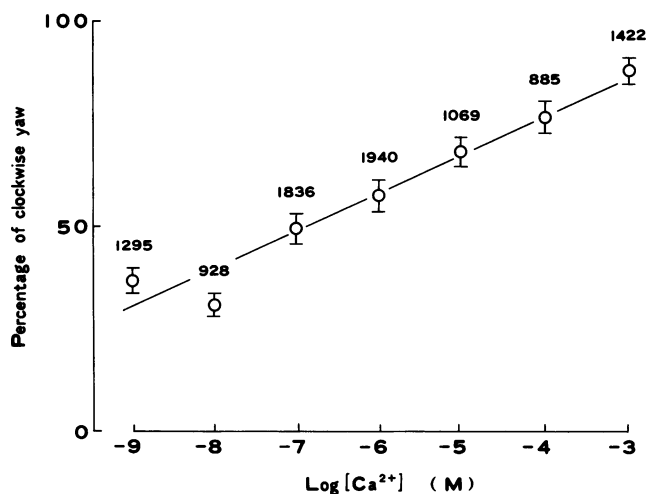

FIGURE 1   Percentage of spermatozoa yawing clockwise at the upper surface of the sperm suspension. Results from four to seven experiments were averaged. Vertical bars represent standard deviations. Number of spermatozoa measured are shown for each distribution. The line is a weighted least square regression line, given by $P = 113.2 + 9.2\ c$, where P is percentage of spermatozoa yawing clockwise and c is the concentration of Ca^{2+} in logarithms. The concentration of Ca^{2+} ion at which the number of spermatozoa yawing clockwise is equal to that of spermatozoa yawing counterclockwise is 1.4×10^{-7} M.

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The circular motion of the reactivated spermatozoa at the upper surface of the sperm suspension was recorded using a Nikon Optiphot microscope equipped with a dark-field condenser, a 10 × objective and 10 × eyepieces. Asymmetry of the bending waves of the reactivated spermatozoa was checked with 40 × objective. The recording system is described elsewhere (Ishijima and Hamaguchi, 1992). The direction of the yawing motion of the reactivated spermatozoa was determined from the images in the video monitor by playing back the tape recorded for at least 10 s. The curvature of the swimming path made by the reactivated spermatozoa was measured by finding a circle fitting the trace of the head of reactivated spermatozoa among many template circles.

RESULTS AND DISCUSSION

When reactivated sea urchin spermatozoa were placed in an observation chamber, they gathered at the upper and lower surface of the sperm suspension and within a few minutes began to move in characteristic circular paths. The direction of the circular motion of the reactivated spermatozoa at an interface depended upon the Ca^{2+} concentration in the reactivation solution. Closer examination showed that the per-

centage of the reactivated spermatozoa revolving clockwise at the upper surface of the sperm suspension gradually increased with the Ca^{2+} concentration (Fig. 1); whereas, the percentage of reactivated spermatozoa revolving counterclockwise gradually decreased with increasing Ca^{2+} concentration. At 1.4×10^{-7} M Ca^{2+}, the number of spermatozoa revolving clockwise and counterclockwise was equal (Fig. 1).

The change in yawing direction at the interface is due to a change in the direction of the rolling motion of spermatozoa about their longitudinal axis, as previously expected by Gray (1955) and shown by Ishijima and Hamaguchi (1992). The rolling direction is hence regulated by Ca^{2+} concentration as well. The difference in rolling direction between live mammalian and sea urchin spermatozoa, which was previously reported by Ishijima et al. (1992), can hence be explained by a difference in intracellular Ca^{2+} concentration of these spermatozoa. The percentage of mammalian spermatozoa rolling clockwise was less than 43% (Ishijima et al., 1992), which
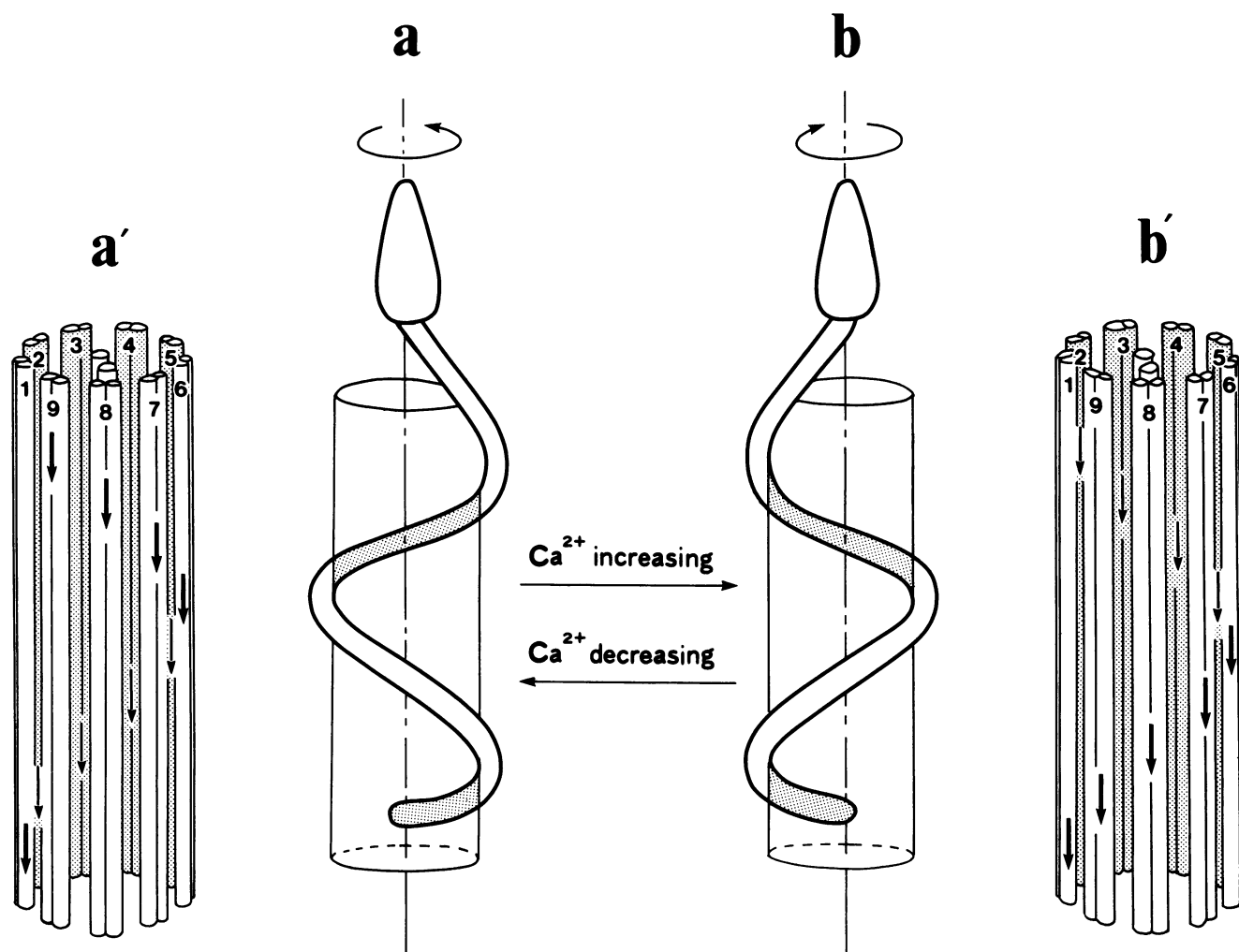

FIGURE 2 Schematic diagram showing the change in the chirality of three-dimensional bending waves of reactivated sea urchin sperm flagella. Sea urchin sperm flagella beat with left-handed flattened helicoidal waves at Ca^{2+} concentrations below 1.4×10^{-7} M (a); whereas, they beat with right-handed waves above this concentration (b). Arrows in a and b show the direction of roll of the spermatozoa caused by the flagellum. a' and b' are hypothetical diagrams producing different chirality (a and b), which show the localized sliding traveling from the base of the axoneme toward the tip along it and simultaneously about the axoneme (arrows).

corresponds to a Ca^{2+} concentration of less than 2.3×10^{-8} M as evaluated using the weighted least square regression line in Fig. 1; whereas, the percentage of sea urchin and starfish spermatozoa rolling clockwise was greater than 69% (Ishijima et al., 1992), which corresponds to a Ca^{2+} concentration of greater than 1.6×10^{-5} M evaluated using the same line. These values of the Ca^{2+} concentration agree with the experimental data of intracellular Ca^{2+} concentration; less than 4×10^{-7} M in mammalian spermatozoa (Mahanes et al., 1986; Babcock and Pfeiffer, 1987; Simpson and White, 1988) and greater than 2×10^{-6} M in sea urchin spermatozoa (Schackmann and Chock, 1986).

If it is accepted that the direction of the rolling motion is determined by the Ca^{2+} concentration, then the chirality of the bending waves of reactivated sea urchin sperm flagella may also depend on the Ca^{2+} concentration. The direction of the rolling motion of sea urchin spermatozoa about their longitudinal axis is most likely determined by the sense of the flattened helicoidal waves (Fig. 2, a and b, Gray (1962), Hiramoto and Baba (1978), Ishijima et al. (1992)).

We suggest that the sperm flagellum beating with flattened helicoidal waves occurs as follows. Three-dimensional helical waves are generated by localized sliding between doublet microtubules, sliding which propagates from the base of the flagellum toward the tip along the axoneme and at the same time spreads about the axoneme (Fig. 2, a' and b', Costello (1973), Hiramoto and Baba (1978)). In this case, the localized sliding between doublet microtubules is successively transmitted in the order of doublet microtubules 1, 2, 3, etc. or 1, 9, 8, etc. (Fig. 2, a' and b'). The helical waves formed by the localized sliding are modified into flattened helicoidal waves by the central complex of the axoneme (Ishijima et al., 1988) and the bridge between the doublet microtubules 5 and 6 (Costello, 1973). The sliding transmitted in the sequence of doublet microtubules 1, 2, 3, etc. forms left-handed waves; whereas, the sliding in the sequence of doublet microtubules 1, 9, 8, etc. forms right-handed waves (Fig. 2); the chirality of the bending waves is changed by transmitting the localized sliding between doublet microtubules about the axoneme in an alternative direction. Reversals of the direction of the localized sliding transmitted about the axoneme can be caused by bidirectional strokes of the dynein arms, although no data have yet been reported. Two other possibilities of controlling the axonemal conformation by the central pair and the doublet microtubules are probably excluded by the observation that the chirality of these structures is insensitive to the Ca^{2+} concentration (Miki-Noumura and Kamiya, 1979; Kamiya, 1982).

The function of changing the chirality of the beating flagellum or the direction of sperm rotation in the life of a spermatozoon is unclear. However, when the direction of sperm rotation changes, a spermatozoon can quickly and effectively change its direction of movement. Therefore, the change in the chirality of the beating flagellum is probably responsible for changing the direction of progression for certain needs, such as during chemotaxis. The observations that the spermatozoa escaped from the interface by changing their directions of yaw (Ishijima and Hamaguchi, 1992) and the
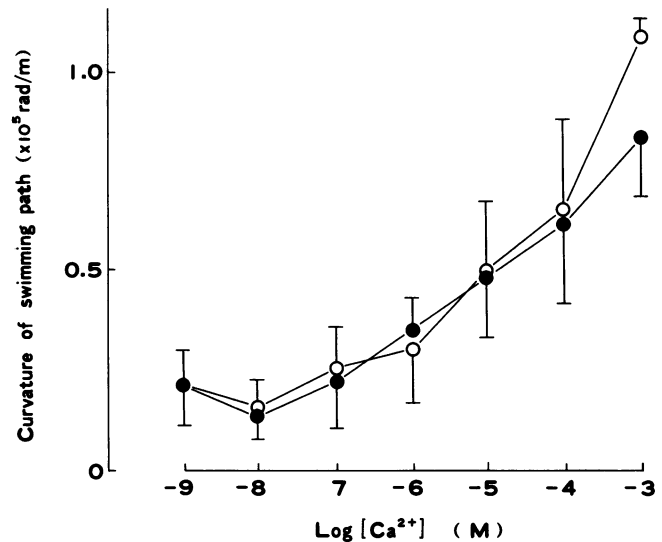

FIGURE 3   Changes in curvature of the swimming path of the reactivated sea urchin spermatozoa with Ca^{2+} in the reactivation solution. (O) the spermatozoa yawing clockwise at the upper surface of the sperm suspension, when viewed from above. (●) the spermatozoa yawing counterclockwise at the upper surface of the sperm suspension. Results from two to four experiments were averaged. Vertical bars represent standard deviations. Approximately 20 to 60 spermatozoa were measured.

spermatozoa quickly changed their swimming directions during chemotactic response (Cosson, 1990) may support this idea.

The curvature of the swimming path made by each reactivated spermatozoon depended upon the concentration of Ca^{2+} of the reactivation solution (Fig. 3). This effect of Ca^{2+} on the curvature of the swimming path is similar to that previously reported for Californian sea urchin spermatozoa (Brokaw et al., 1974; Okuno and Brokaw, 1981), implying that the sperm axoneme from Japanese sea urchins responds to Ca^{2+} in a manner similar to that of the Californian species. Since there was only a slight difference in the response to Ca^{2+} between the reactivated spermatozoa yawing clockwise and those yawing counterclockwise, the response to Ca^{2+} of the curvature of the swimming path was different from that of the direction of the yawing motion of the spermatozoa (Fig. 3); different regulations of Ca^{2+} underlie the two phenomena, curvature of the swimming path and direction of the yawing motion.

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