Acrosome Reaction in Spermatozoa from the Amphioxus Acrosome Reaction in Branchiostoma belcheri (Cephalochordata, Chordata)

Authors: Morisawa, Sachiko, Mizuta, Takanobu, Kubokawa, Kaoru, Tanaka, Hiroyuki, and Morisawa, Masaaki

Source: Zoological Science, 21(11) : 1079-1084

Published By: Zoological Society of Japan

URL: https://doi.org/10.2108/zsj.21.1079

ZOOLOGICAL SCIENCE **21**: 1079–1084 (2004)

Acrosome Reaction in Spermatozoa from the Amphioxus *Branchiostoma belcheri* (Cephalochordata, Chordata)

Sachiko Morisawa[1]*, Takanobu Mizuta[2], Kaoru Kubokawa[2], Hiroyuki Tanaka[3] and Masaaki Morisawa[3]

[1]*Department of Anatomy, St. Marianna University, School of Medicine, 2-16-1 Sugao, Miyamae, Kawasaki 216-8511, Japan*
[2]*Ocean Research Institute, 1-15-1 Minamidai, Nakano, Tokyo 164-8639, Japan*
[3]*Misaki Marine Biological Station, 1024 Koajiro, Misaki, Miura 238-0225, Japan*

ABSTRACT—The formation of an acrosomal process at acrosomal exocytosis in spermatozoa of the amphioxus was described in the present report for the first time. A non-reacted acrosome was located in front of the nucleus, where a cup-shaped acrosomal vesicle covered a conical accumulation of subacrosomal material. When naturally spawned spermatozoa were treated with a calcium ionophore, ionomycin, the acrosomal vesicle opened at the apex and an acrosomal process was projected. The process exhibited a filamentous structure. The reaction followed the mode typically seen in marine invertebrates. These observations suggest that the features and function of the acrosome of amphioxus, whose position is on the border between invertebrates and vertebrates, reflect their ecological adaptation and phylogenic position.

Key words: acrosome reaction, ionomycin, spermatozoa, amphioxus, Cephalochordata

INTRODUCTION

Amphioxus, which is found widely in the world, has attracted much interest. This organism occupies an important position in the phylogeny of the animal kingdom as a key species in discussions on the relationship between invertebrates and vertebrates in Chordata. Much attention has been paid to this species in the fields of physiology and developmental biology, and a large amount of evidence has also been accumulated in the field of reproductive biology. Amphioxus embryogenesis has been extensively studied (Conklin, 1932, 1933; Yan, 1999), and the fine structures of amphioxus spermatozoa (Baccetti *et al*., 1972; Holland and Holland, 1989a; Jamieson, 1984; Lin *et al*., 1987), growing oocytes (Aizenstadt *et al*., 1990; Holland and Holland, 1991), and mature eggs (Holland and Holland, 1989b, 1992) have been investigated.

The acrosome reaction in spermatozoa, the first step in the interaction between gametes, is an important process for fertilization and has been studied in many animal species (Dan, 1967). Two reaction modes have been described: one in marine invertebrates and the other in vertebrates. In invertebrates, an acrosomal process is projected at the apex of the spermatozoa upon acrosomal exocytosis; in vertebrates, on the other hand, acrosomal exocytosis occurs at many points, without the formation of an acrosomal process. Electron microscopic observations have shown that non-reacted testicular spermatozoa of genus *Branchiostoma* consist of a head containing an acrosome, a midpiece containing a C-shaped or circular mitochondrion, and a tail containing an axoneme with a 9+2 structure (Baccetti *et al*., 1972; Holland and Holland, 1989a; Jamieson, 1984; Lin *et al*., 1987). Based on these observations, the formation of an acrosomal process during acrosomal exocytosis was suspected in amphioxus. However, the actual acrosome reaction has not been previously described in amphioxus.

Here, we describe the acrosome reaction that occurs in the amphioxus, *Branchiostoma belcheri*, and describe the fine structures of non-reacted and reacted acrosomes in detail.

MATERIALS AND METHODS

Adult amphioxus, *B. belcheri*, were caught by dredging in the Enshu-Nada Sea in Aichi prefecture, Japan, at the end of June (the breeding season for this species). Females and males were kept in a laboratory tank at 25°C without circulating seawater under natural light conditions until the males and females began to exhibit spawning behavior under culture conditions (Mizuta and Kubokawa, 2004). On an early evening in August, a male began to exhibit

* Corresponding author: Tel. +81-44-977-8111;
FAX. +81-44-977-8133.
E-mail: s2mori@marianna-u.ac.jp

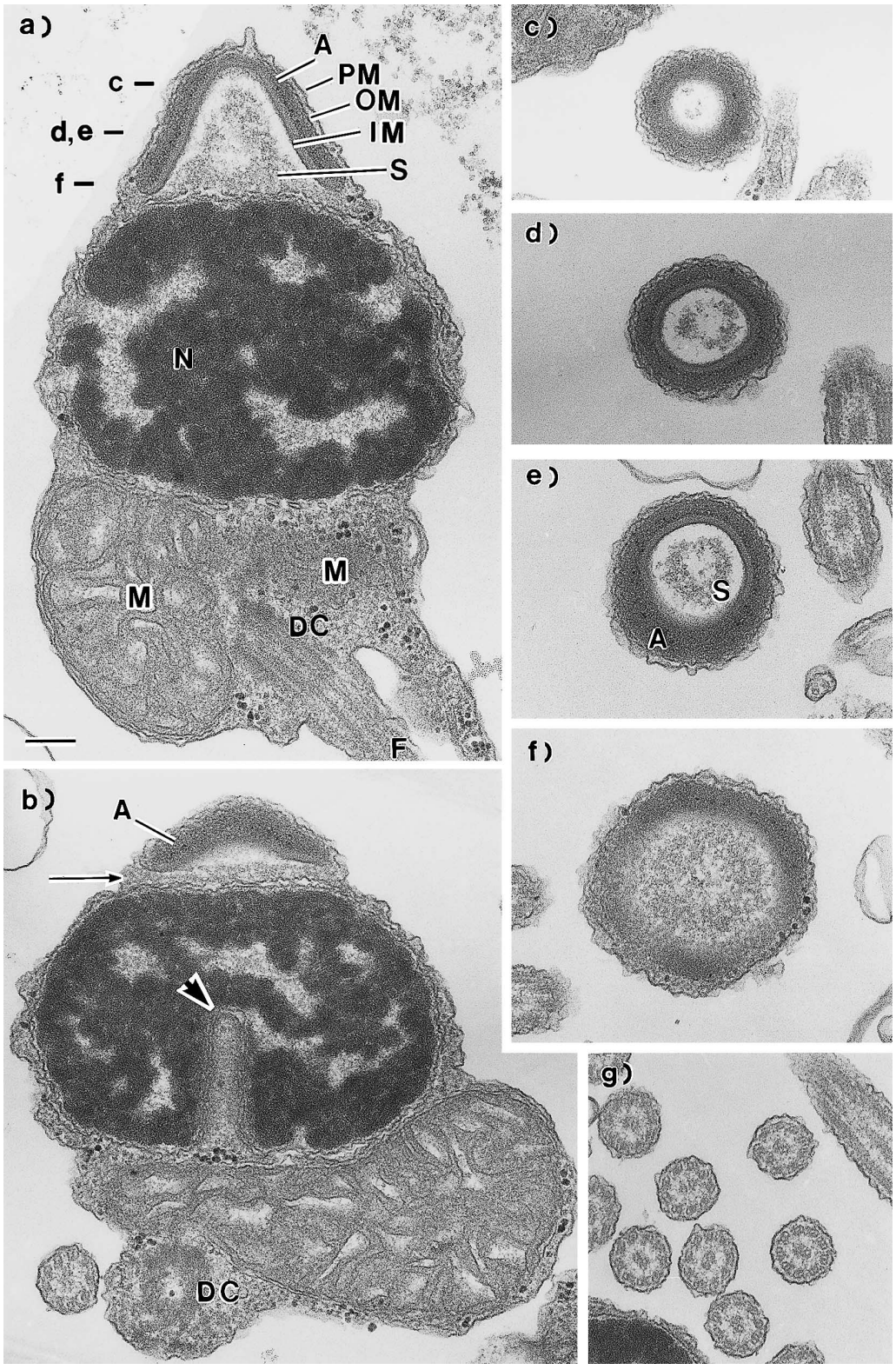

Fig. 1. Transmission electron micrographs of testicular spermatozoa from the amphioxus, *Branchiostoma belcheri*. (a) A longitudinal section of the head region. The acrosomal vesicle (A) shows several layers of material, covering the subacrosomal material (S). (b) A longitudinal section of the head, showing the nuclear indentation (arrowhead) and extension of the subacrosomal material (arrow). (c, d, e, f) Cross sections of the acrosomal region. Each figure represents a section at the level of the labels c-, d-, e- and f- indicated in figure (a), respectively. Note the presence of acrosomal materials of various electron densities and the unstructured subacrosomal material. (g) Cross sections of flagella. DC, distal centriole; F, flagellum; IM, inner acrosomal membrane; M, mitochondria; N, nucleus; PM, plasma membrane; OM, outer acrosomal membrane. Bar, 200 nm (a), common in (a–g).

spawning behavior and swam up from the sand bed of the tank. The male was caught in a net, placed in a small dish containing seawater, and allowed to spawn. The released spermatozoa were then collected. Testicular spermatozoa were also collected by the dissection of a ripe testis.

The released spermatozoa were concentrated in a small amount of seawater by centrifugation at 6000 r.p.m. for 1 minute at room temperature. One volume of the sperm suspension was mixed with 4 volumes of artificial seawater (ASW) (462 mM NaCl, 9 mM KCl, 10 mM $CaCl_2$, 48 mM $MgCl_2$, and 10 mM HEPES, at a pH of 8.5) with or without ionomycin (Sigma Chemical, MO, USA) at a concentration of 100 μM, and the samples were left for 3 minutes at room temperature. Ionomycin treated- and untreated-spermatozoa and spermatozoa from the dissected testis were fixed in 1% glutaraldehyde in ASW and post-fixed with 1% osmium tetroxide in a 0.1 M phosphate buffer (pH 7.4).

For the transmission electron microscopy (TEM) studies, the samples were dehydrated in an alcohol series and embedded in epoxy resin through propylene oxide. Ultrathin sections were stained with uranyl acetate and lead citrate. For the scanning electron microscopy (SEM) studies, the samples were dehydrated and critical-point dried. Observations were performed using JEM 1200 (JEOL Co. Ltd, Tokyo, Japan) and S-4800 (Hitachi High-Technologies, Tokyo, Japan) electron microscopes.

RESULTS

The spermatozoa from the testis of *B. belcheri* consisted of a head (containing an acrosome and a nucleus), a midpiece, and a tail (Fig. 1). The acrosome was cone-shaped and located in front of the nucleus. An acrosomal vesicle overlay a conical accumulation of subacrosomal material that was unstructured, with a somewhat diffused aspect of moderate electron density (Fig. 1a–f). The subacrosomal material was extended into a thin layer between the peripheral edges of the acrosomal vesicle and the nucleus (Fig. 1b). The nucleus was almost spherical in cross section and subovoid in longitudinal section. The nucleus faced the acrosome with its anterior plate, or shallow cavity, while it had a deep posterior indentation that was invaded by relatively electron-dense material (Fig. 1b). The midpiece was short and contained, possibly, a single mitochondrion. In the tail extending diagonally to the acrosome-nucleus axis, an axoneme with a 9+2 structure was simply surrounded by the plasma membrane (Fig. 1g). These TEM features are similar to those reported previously in *Branchiostoma* (Baccetti *et al*., 1972; Holland and Holland, 1989a; Jamieson, 1984; Lin *et al*., 1987). In addition, the present study revealed several distinct features. The acrosomal vesicle was thinner in the center region than in the side region and contained several layers of materials with different electron densities (Fig. 1a–f). A material with a low electron density was located on the inner side, facing the subacrosomal material, and an electron-denser layer occupied the outer side, which appeared to be thick in the lateral region. Between them, the accumulation of another electron dense material was observed. In some sections of the midpiece, the centrioles were surrounded by a mitochondrion, one side of which was wider than the other (Fig. 1a, b). In the SEM studies, the head-midpiece region of intact spermatozoa was estimated to be about 1.8 μm long and 1.2 μm wide at the nucleus level, and the length of the tail, including the

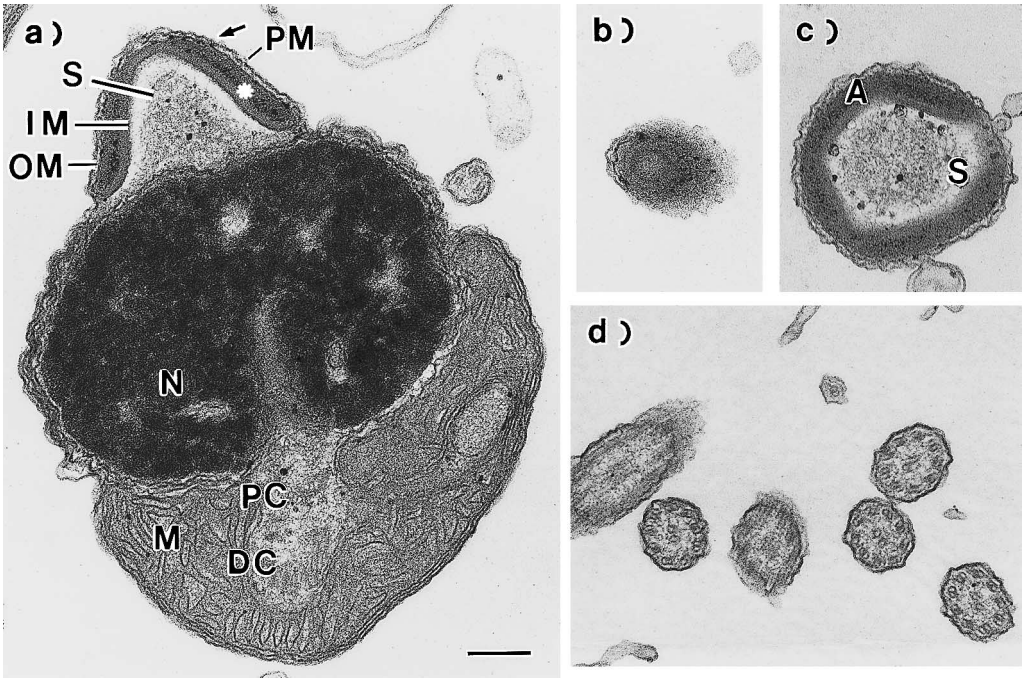

Fig. 2. Transmission electron micrographs of spawned spermatozoa from *B. belcheri*. (a) A longitudinal section of the head region. Layered acrosomal materials (*) and the proximal centriole (PC) of triplets are shown. (b) A cross section of the acrosomal region at the level of the small arrow indicated in Fig. (a). (c) A cross section of the acrosomal region near the base. (d) Cross sections of flagella. For the labels, see the legend to Fig. 1. Bar, 200 nm (a), common in (a–d).

end piece, was roughly 35 μm long (Fig. 4).

The acrosomal region of the spawned motile spermatozoa was generally similar to that of the testicular immotile spermatozoa (Fig. 2). Some sections of the midpiece exhibited a single circular mitochondrion encircling the centriole. When the spawned spermatozoa were treated with ionomycin at a concentration of 80 μM (final) in ASW at a pH of 8.5, the plasma membrane and the underlying outer acrosomal membrane fused at the very apical point, and an acrosomal process projected through the opening (Fig. 3). At early stages, a collar-like structure derived from the fusion of the outer acrosomal and plasma membranes encircled the base of the process (Fig. 3a–d). The core of the process seemed to be derived from the subacrosomal material, being covered by a membrane that was continuous with the plasma membrane via the inner and outer acrosomal membranes. Thin filaments were observed in the core (Fig. 3e). The acrosomal materials seemed to disappear at an early stage of exocytosis. At later stages, the collar-like structure disappeared and only the projected acrosomal process was visible (Fig. 3f). Judging by the image in Fig. 3f, a well-projected acrosomal process was 70–80 nm thick and longer than 2 μm.

A SEM image of the acrosome reaction is shown in Fig.

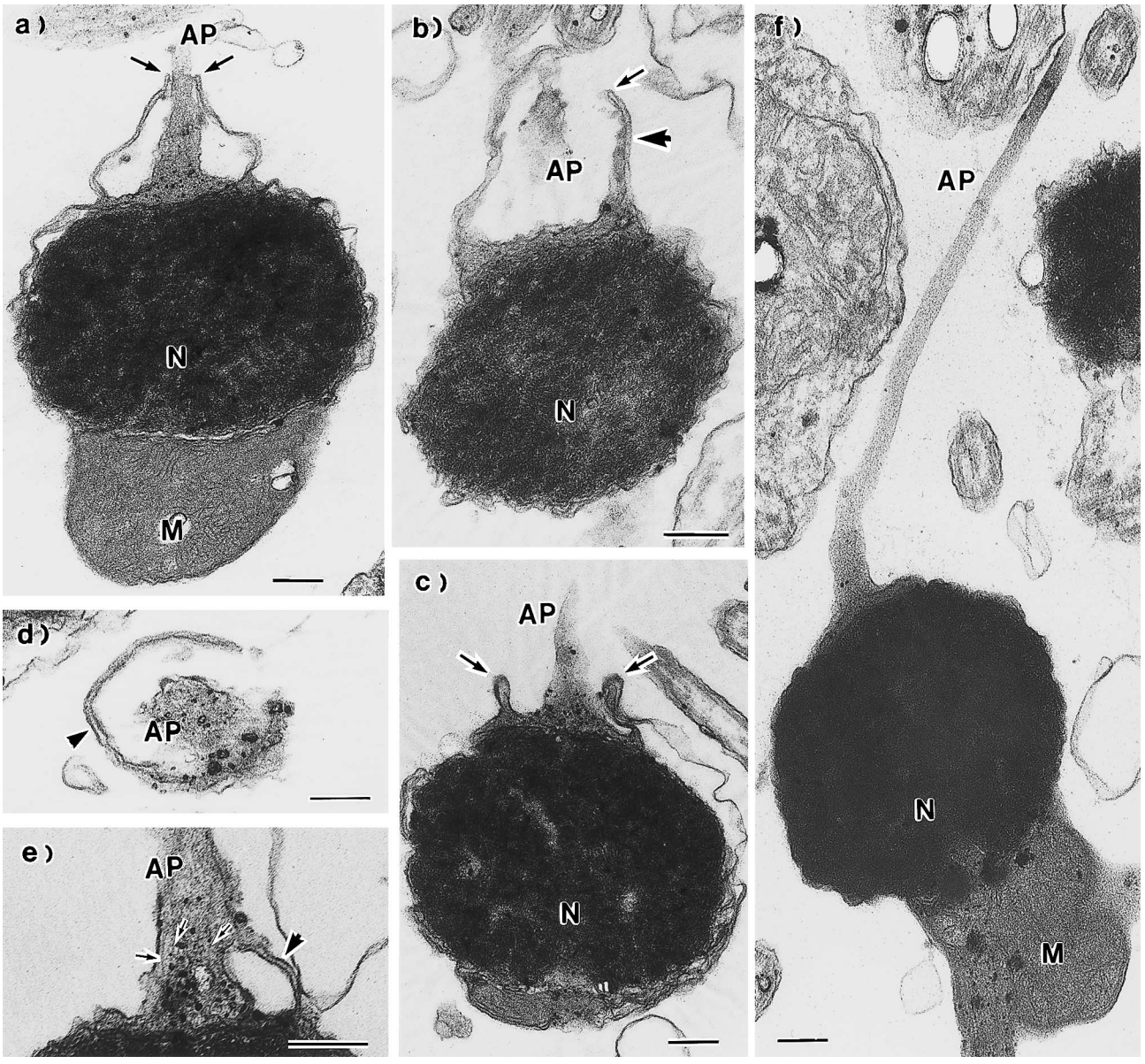

Fig. 3. Transmission electron micrographs of an acrosome reaction in spermatozoa from *B. belcheri*. (a) Acrosomal opening (arrow) and projection of the acrosomal process (AP). (b, c) Disappearance of the collar-like structure (arrowhead) that had appeared as a consequence of the fusion between the plasma and outer acrosomal membranes. (d) A cross section near the base of the process. Arrowhead, collar-like structure. (e) Filaments in the process (small arrow). Arrowhead, collar-like structure. (f) Acrosomal process extending from the sperm apex. AP, acrosomal process; arrow, rim of the acrosomal opening. For other labels, see the legend to Fig. 1. Bar, 200 nm.

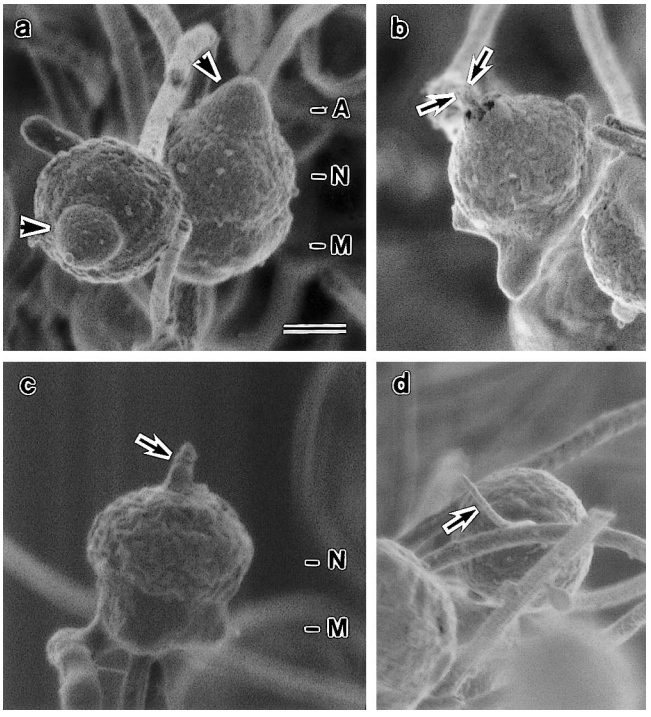

Fig. 4. Scanning electron micrographs of an acrosome reaction in spermatozoa from *B. belcheri*. (a) The head-midpiece region of intact spawned spermatozoa. Note the cone-shaped acrosome (arrowhead). (b) An early stage of the acrosome reaction. The acrosomal process (arrow) extends through the acrosomal opening: cf. Fig. 3 (b, c). (c, d) Acrosomal process (arrow) projecting from the sperm apex. A, acrosome; M, midpiece; N, nucleus. Bar, 0.5 μm (a), common in (a–d).

4. A cone-shaped intact acrosome was clearly observed at the apex of ionomycin-untreated spermatozoa (Fig. 4a). The surface of the acrosome was less wavy than that of the nuclear region. In ionomycin-treated spermatozoa, the acrosome disappeared, and a projected acrosomal process was visible (Fig. 4b–d). A short process was sometimes surrounded by a collar-like structure (Fig. 4b). These images supported the TEM observations described above.

DISCUSSION

During the exocytosis of the acrosomal vesicle in marine invertebrates, such as annelids (Takashima and Takashima, 1963), bivalves (Niijima and Dan, 1965), horseshoe crab (Andre, 1963), and echinoderms (Dan, 1967), the plasma and outer acrosomal membranes fuse at the apex and an acrosomal process is produced (Dan, 1967). Acrosomal exocytosis in vertebrates, such as amphibians (Yoshizaki and Katagiri, 1982), birds (Okamura and Nishiyama, 1978), and mammals (Yanagimachi and Noda, 1970), occurs at many points by vesiculation of the two membranes, resulting in the exposure of the perforatorium, which is not followed by the formation of a process. Spermatozoa of the neopterygii group of teleosts lack an acrosome (Jamieson, 1991). In other chordates, often called protochordates, the mode of acrosome reaction differs among species: a process is formed during the exocytosis at the apex in *Oikopleura dioica*, an appendicularian urochordate (Holland *et al*., 1988); in the spermatozoa of *Ciona intestinalis*, an ascidian, acrosomal exocytosis occurs through the vesiculation of the two membranes, and a process is not formed (Fukumoto, 1990). In basal vertebrates, the spermatozoa of lampreys follow the mode seen in invertebrates, and the long organelle originally found extending through the nucleus is expelled in the form of a rod (Jaana and Yamamoto, 1981). Spermatozoa of the sturgeon, *Acipenser transmontanus*, also form acrosomal processes, which appear to originate from the nuclear channels and the subacrosomal region (Cherr and Clark, 1982). Recent studies in hagfish, a member of the most primitive vertebrate group, showed an acrosome reaction with intermediate features between the mode in invertebrates and the one in vertebrates: an acrosomal process containing actin projects, deriving from the amorphous subacrosomal material, and the alignment of vesicles appears (Morisawa, 1999; Morisawa and Cherr, 2002). These aspects suggest that the mode of acrosome reaction is complicated in organisms on the border between invertebrates and vertebrates. Studies on the acrosome reaction in amphioxus have been awaited from both reproductive and phylogenic points of view.

Having obtained naturally spawned spermatozoa, the present study revealed several layers of acrosomal vesicle contents and described an acrosome reaction similar to the mode most often seen in invertebrates. Ionomycin-treatment induced the amphioxus spermatozoa to open the acrosomal vesicle at the apex and to produce an acrosomal process that was at least 2 μm long. The formation of an acrosomal process is usually seen in externally fertilized invertebrate and vertebrate species, hence the mode of acrosome reaction might reflect an ecological adaptation as well as a phylogenic position. On the other hand, the successful induction of acrosomal exocytosis by a calcium ionophore suggests that calcium flow across the plasma membrane is required for the exocytosis of acrosomal vesicle in amphioxus, as has been commonly seen in previously studied animals.

Several aspects of the acrosome reaction in amphioxus remain to be studied, such as the nature of the egg coat material that induces the acrosome reaction, how the acrosomal contents interact with the eggs, and how the core and membrane of the acrosomal process are formed. Further studies of fertilization are thus needed. More detailed observations of the acrosome reaction in the vicinity of eggs, such as those conducted by Colwin and Colwin in *Hydroides* (1961a, b) and in *Saccoglossus* (1963a, b) after the first description of an acrosome reaction in sea urchins by Dan (1952), may be helpful for understanding the reproduction of the genus *Branchiostoma*.

ACKNOWLEDGEMENTS

The authors thank Mr. M. Sekifuji of Misaki Marine Biological

Station for collecting the material used in this study and the staff of the Institute for Ultrastructural Morphology, St. Marianna University, for their technical assistance. This work was supported by Grants-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science to SM (Nos. 08833013 and 13839016).

REFERENCES

Aizenstadt TB, Gabaeva NS, Gulyaev DV (1990) Cytological study of lancelet oogenesis. 2. Oocyte growth and vitellogenesis in *Branchiostoma lanceolatum*. Oogenesis 21: 192–199

Andre J (1963) A propos d'une lecon sur la limule. Annls Fac Sci 26: 27–38

Baccetti B, Burrini A, Dallai R (1972) The spermatozoon of *Branchiostoma lanceolatum* L.. J Morphol 136: 211–226

Cherr GN, Clark Jr. WH (1984) An acrosome reaction in sperm from the white sturgeon, *Acipenser transmontanus*. J Exp Zool 232: 129–139

Colwin LH, Colwin AL (1961a) Changes in the spermatozoon during fertilization in *Hydroides hexagonus* (Annelida). I. Passage of the acrosomal region through the vitelline membrane. J Biophys Biochem Cytol 10: 231–254

Colwin AL, Colwin LH (1961b) Changes in the spermatozoon during fertilization in *Hydroides hexagonus* (Annelida). II. Incorporation with the egg. J Biophys Biochem Cytol 10: 255–274

Colwin AL, Colwin LH (1963a) Role of the gamete membranes in fertilization in *Saccoglossus kowalewskii* (Enteropneusta). I. The acrosomal region and its changes in early stages of fertilization. J Cell Biol 19: 477–500

Colwin LH, Colwin AL (1963b) Role of the gamete membranes in fertilization in *Saccoglossus kowalewskii* (Enteropneusta). II. Zygote formation by gamete membrane fusion. J Cell Biol 19: 501–518

Conklin EG (1932) The embryology of amphioxus. J Morphol 54: 69–151

Conklin EG (1933) The development of isolated and partially separated blastomeres of amphioxus. J Exp Zool 64: 303–375

Dan J (1952) Studies on the acrosome. I. Reaction to egg-water and other stimuli. Biol Bull 103: 54–66

Dan JC (1967) Acrosome reaction and lysins. In "Fertilization Vol 1" Ed by CB Metz, A Monroy, Academic Press, New York, pp 237–293

Fukumoto M (1990) The acrosome reaction in *Ciona intestinalis* (Ascidia, Tunicata). Dev Growth Differ 32: 51–55

Holland ND, Holland LZ (1989a) The fine structure of the testis of a lancelet (= amphioxus), *Branchiostoma floridae* (Phylum Chordata: Subphylum Cephalochordata = Acrania). Acta Zoologica (Stockholm) 70: 211–219

Holland ND, Holland LZ (1989b) Fine structural study of the cortical reaction and formation of the egg coats in a lancelet (= amphioxus), *Branchiostoma floridae* (Phylum Chordata: Subphylum Cephalochordata = Acrania). Biol Bull 176: 111–122

Holland ND, Holland LZ (1991) The fine structure of the growth stage oocytes of a lancelet (= amphioxus), *Branchiostoma lanceolatum*. Invert Reprod Dev 19: 107–122

Holland LZ, Holland ND (1992) Early development in the lancelet (= amphioxus) *Branchiostoma floridae* from sperm entry through pronuclear fusion: Presence of vegetal pole plasm and lack of conspicuous ooplasmic segregation. Biol Bull 182: 77–96

Holland LZ, Gorsky G, Fenaux R (1988) Fertilization in *Oikopleura dioica* (Tunicata: Appendicularia): Acrosome reaction, cortical reaction and sperm-egg fusion. Zoomorphology 108: 229–243

Jaana H, Yamamoto TS (1981) The ultrastructure of spermatozoa with a note on the formation of the acrosomal filament in the lamprey, *Lampetra japonica*. Jpn J Ichthyol 28: 135–147

Jamieson BGM (1984) Spermatozoal ultrastructure in *Branchiostoma moretonensis* Kelly, a comparison with *B. lanceolatum* (Cephalochordata) and with other deuterostomes. Zool Scr 13: 223–229

Jamieson BGM (1991) Fish evolution and systematics: Evidence from spermatozoa. Cambridge University Press, Cambridge

Lin J, Chen X, Wang D (1987) Studies on ultrastructure in spermatogenesis of amphioxus (*Branchiostoma belcheri* Gray). Oceanologica Limnologia Sinica 18: 432–436

Mizuta T, Kubokawa K (2004) Non-synchronous spawning behavior in laboratory reared amphioxus *Branchiostoma belcheri* Gray. J Exp Marine Biol Ecol 309: 239–251

Morisawa S (1999) Acrosome reaction in spermatozoa of the hagfish *Eptatretus burgeri* (Agnatha). Dev Growth Differ 41: 109–112

Morisawa S, Cherr GN (2002) Acrosome reaction in spermatozoa from hagfish (Agnatha) *Eptatretus burgeri* and *Eptatretus stouti*: Acrosomal exocytosis and identification of filamentous actin. Dev Growth Differ 44: 337–344

Niijima L, Dan J (1965) The acrosome reaction in *Mytilus edulis*. II. Stages in the reaction, observed in supernumerary and calcium-treated spermatozoa. J Cell Biol 25: 249–259

Okamura F, Nishiyama H (1978) The passage of spermatozoa through the vitelline membrane in the domestic fowl, *Gallus gallus*. Cell Tissue Res 188: 497–508

Takashima R, Takashima Y (1963) Electron microscope studies on the fine structure of *Nereis* spermatozoa before and after the acrosomal reaction. Tokushima J Exptl Med 10: 117–123

Yan S (1999) Contribution of late professor T. C. Tung to the experimental embryology of amphioxus. Dev Growth Differ 41: 503–522

Yanagimachi R, Noda YD (1970) Ultrastructural changes in the hamster sperm head during fertilization. J Ultrastruct Res 31: 465–485

Yoshizaki N, Katagiri C (1982) Acrosome reaction in sperm of the toad, *Bufo bufo japonicus*. Gamete Res 6: 343–352

(Received June 21, 2004 / Accepted August 31, 2004)