Acid release at meiotic maturation of oocytes in the polychaete annelid *Sabellaria alveolata*

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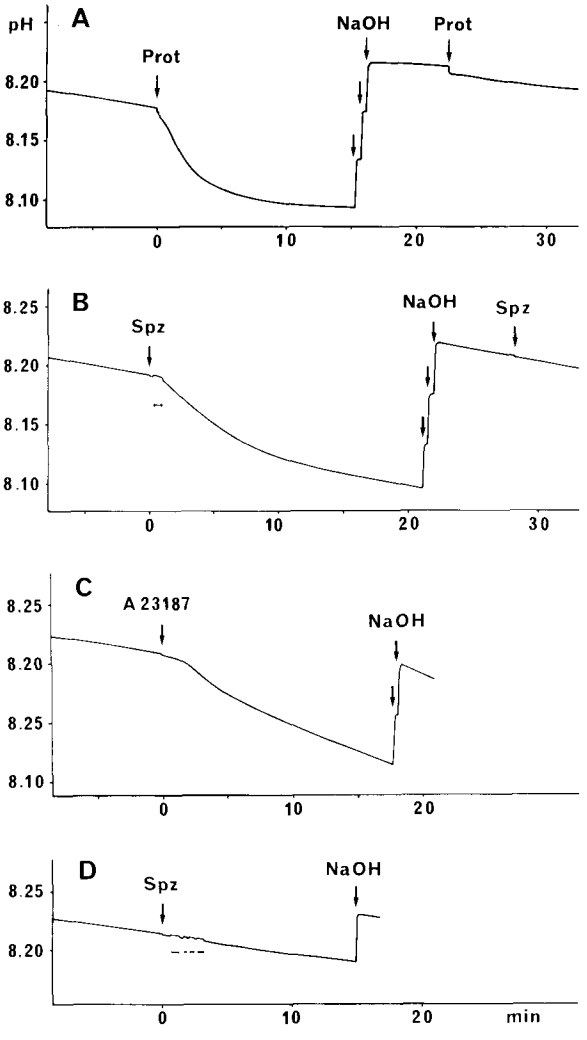
Summary. About 1 pmole of acid per egg is released when prophasic oocytes undergo maturation under the action of sperm, proteases or ionophore A 23187. No similar acid release occurs at fertilization of matured oocytes. These findings are compared with data on *Urechis* and sea urchin.

The release of acid at fertilization of sea urchin eggs has been known for a long time, but its mechanism remained unclear until recently[1]. The discovery that most of this acid does not originate from the content of cortical granules[2] but is an efflux of protons resulting in an increased intracellular pH[3], suggests that this phenomenon plays a key role in the activation process. Nevertheless, acid release has been found, so far, only in sea urchin and the echiurid worm *Urechis*[4]. It has been looked for, at fertilization, at least in 2 species of molluscs, without result. But investigation should, of course, be extended to a wider variety of animals.

Thus, it seemed worthwhile to investigate this question on the eggs of the polychaete annelid *Sabellaria alveolata.* This species is of peculiar interest since fertilization can occur experimentally either when oocytes are in prophase of the 1st meiotic division, or in metaphase I[5]. In the latter case, a maturation process is experimentally triggered by specific proteases isolated from the digestive fluid of adults. This maturation begins by a cortical reaction, similar to that found in sea urchin eggs[6], and leads the unfertilized oocyte from the prophase to the metaphase, where a 2nd arrest occurs until fertilized. This provides a variety of activating mechanisms to which must be added the triggering of maturation and completion of meiosis by the calcium ionophore A 23187[7].

Material and methods. Animals were collected and handled as previously described[8]. Immature oocytes were obtained in the usual way[5], except that the concentration of soybean trypsin inhibitor (SBTI) solution was raised to 1%, dialized before use, to obtain more than 98% of prophasic oocytes. Eggs were suspended in artificial sea water (ASW) buffered at pH 8.2 with 5 mM Hepes-Na (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid). It was observed that buffering stabilizes spontaneous acid release and yields an essentially linear relationship between pH variations and quantities of acid released. Buffering minimizes also the transformation of bicarbonates in gazeous CO_2, by reducing pH decreases. Each experiment was performed with 8 ml egg suspension (1.3×10^5 eggs/ml) in a waterbath at room temperature, the suspension being stirred with a suspended stirring bar. For pH measurements an Ingold 405-M5 combined electrode, a Metrohm E 512 pH-meter and a Sefram recorder were used. Backtitration with 0.1 N NaOH was performed with the microburet of a Metrohm Multi-Dosimat E 415 by 10-µl steps. Protease CT was purified as previously described[5] and the solution used had an esterolytic activity of 0.5 units/ml with N-benzoyl-L-tyrosine ethyl ester as substrate. Spermatozoa were washed free from contaminating proteases by centrifugation through ASW containing 20% Ficoll and 10 µg/ml SBTI and 2 more washes with 5 mM Hepes-ASW. Ionophore A 23187 (gift of E. Lillie) was prepared as a 1-mM stock solution in methanol/DMSO (3/1).

Results and discussion. As shown in record A, 100% protease-induced maturation is accompanied by an important acid release which lasts 9 min. A 2nd addition of protease after completion of maturation has no effect, except for a small pH drop due to imperfect adjustment of the protease solution pH. If one assumes that the spontaneous acid release before addition of protease corresponds to a metabolic acid production unaffected during maturation, and that the small immediate pH drop is due to the acidity of the protease solution, it can be calculated, by deduction, that maturation is accompanied by a specific acid release of about 1.3×10^{-12} M of H^+ per egg during the 1st 9 min. Record B shows that a similar acid liberation occurs at fertilization of prophasic oocytes (99% of fertilization), but



Acid release during maturation and fertilization of eggs from the same batch. *A* initiation of maturation by addition of 100 µl of protease CT (Prot); *B* fertilization of prophasic oocytes with 100 µl of a protease free sperm suspension (Spz) containing 3.4×10^8 spz/ml; *C* parthenogenetic activation of prophasic oocytes with 20 µl of ionophore A 23187 (1 mM); *D* fertilization of metaphasic oocytes by addition of 100 µl of sperm suspension (Spz). Backtitration with 0.1 N NaOH is indicated, each arrow corresponding to an addition of 10 µl. Horizontal bars indicate when stirring was stopped to improve fertilization.

is completed only in 14 min, which can be attributed to a poorer synchrony. Similar calculations yield a specific acid release of 1.2×10^{-12}M H^+ per egg. This result is in agreement with that of the previous experiment, since the 1st effect of fertilization is to lead eggs to the metaphase I stage with a timing similar to maturation. In experiment C only 70% of eggs matured upon addition of ionophore, probably because of insufficient mixing of this highly hydrophobe drug. The graph was corrected as if 100% activation had been obtained, for direct comparison with the precedent records. It shows that an acid release occurs which stabilizes itself in about 12 min. Calculations give a specific acid release of 9×10^{-13} M H^+ per activated egg, which is only slightly less than the values obtained in other experiments where 100% activation was induced. The kinetic is slow by comparison with records A and B and shows a noticeable lag. This is characteristic of threshold activation since it is not observed with more intensely activated eggs, as already noticed in sea urchin eggs[9]. A special feature of activation by ionophore is the persistence of an increased acid release after completion of maturation, which corresponds to almost twice that of unstimulated eggs. This may correspond to an ionophore-mediated exchange of intracellular protons for extracellular divalent cations[10] or to an indirect effect of permeability changes in the cytoplasmic membrane or intracellular organites membranes. Experiment D shows an attempt to detect an acid extrusion at fertilization of metaphasic oocytes, previously matured under the action of protease. Fertilization ratio is always low because of the high density of stirred egg suspension necessary for acid release studies. So in experiment D, only 27% of eggs were fertilized and, as there is no significant change in acid release, the curve cannot be corrected to simulate 100% fertilization. It can thus be concluded that fertilization of metaphasic oocytes is not accompanied by an acid release, or at least not of the same order of magnitude as that observed during maturation.

So the acid release in *Sabellaria* eggs shows striking similarities with that of *Urechis* and sea urchin eggs. The kinetic of acid release is roughly similar and the total amount of acid liberated is comparable, especially if one considers the ratio of acid released (in pmoles) per egg volume (in $\mu m^3 \times 10^6$) which is here 9.7 with protease, 9.3 with sperm and 7.0 with ionophore while they are 5.2 for *Urechis*, 6.7 for *Strongylocentrotus purpuratus* and 7.2 for *Dendraster excentricus*[4]. Special features are that fertilization can occur in *Sabellaria* without noticable acid release, while maturation is always accompanied by it. However it must be noticed that *Urechis* eggs are fertilized as prophasic oocytes, so that acid release occurs in this species during the same cytological processes as maturation in *Sabellaria*. There is at present no indication on the biochemical basis of acid release in *Sabellaria*. At first sight, the temporal correlation which exists between acid extrusion and cortical reaction could suggest that acid might originate from the content of cortical granules, but recent discoveries on sea urchin eggs seem to argue against such an interpretation.

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Etude du développement des membres postérieurs de *Pleurodeles waltlii* Michah. (Amphibien, Urodèle) après la résection de l'épiderme du bourgeon

Study of the hind-limb development in the newt *Pleurodeles waltlii* Michah. (Amphibiae, Urodela) after epidermis removal of the bud

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Summary. Epidermis removal of the hind-limb bud of the newt *Pleurodeles waltlii* throughout early stages of development is followed by the overlaying of stripped mesodermal bud by the epidermis of the dorsal and ventral fins. This removal never gives any deficiency of the proximo-distal sequence of the limb.

Chez les amniotes, et chez le poulet en particulier, l'épiderme apical est différencié en une crête apicale épidermique. Selon l'hypothèse de Saunders[1], reprise par la majorité des auteurs, cette crête apicale épidermique est en interaction avec le mésoderme sous-jacent au cours de l'établissement de la séquence proximo-distale du membre. Chez le pleurodèle, par contre, l'épiderme des bourgeons du membre postérieur ne présente, au cours de leur morphogenèse, aucune différenciation histologique, ultrastructurale ni histoenzymologique particulière[2,3] et nous avons suggéré que si l'épiderme du bourgeon de membre postérieur joue un rôle inducteur tout l'épiderme est concerné et non seulement, comme chez les amniotes, celui de l'extrémité apicale; cependant la réalité et le rôle morphogénétique d'éventuelles interactions doivent être démontrés. Nous avons analysé, dans un premier temps, les effets de la résection de l'épiderme du bourgeon aux stades précoces de son développement. Les résultats obtenus font l'objet de cette note.

Matériel et techniques. Nous avons effectué des résections de l'épiderme du bourgeon de membre postérieur à partir du stade I jusqu'au stade V[3]. Les larves ont été anesthésiées à l'aide de MS 222 (Sandoz) et opérées dans la solution physiologique stérile de Steinberg[4]; les résections ont été réalisées chriurgicalement et tout l'épiderme du bourgeon a été enlevé ainsi qu'une portion importante de l'épiderme du flanc. Un certain nombre de larves ont été fixées immédiatement après l'opération; les autres ont été élevées individuellement dans des cristallisoirs. Certaines d'entre elles ont été sacrifiées en cours de développement et fixées