九州大学学術情報リポジトリ
Kyushu University Institutional Repository

CHANGES IN MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE ACTIVITY OF FISH EGGS AT FERTILIZATION

OHTSUKA, EIJI
Biological Laboratory, College of General Education, Kyushu University

https://doi.org/10.15017/7344043

出版情報：Sieboldia : acta biologica . 4 (2), pp.95-98, 1969-03-30. 九州大学教養部生物学教室
バージョン：
権利関係：

KYUSHU UNIVERSITY

CHANGES IN MITOCHONDRIAL ADENOSINE TRIPHOSPHATASE ACTIVITY OF FISH EGGS AT FERTILIZATION

Eiji OHTSUKA

Biological Laboratory, College of General Education, Kyushu University, Ropponmatsu, Fukuoka, Japan

Most of the work on the fertilization response of fish eggs has been carried out in the morphological alterations of the cortical layer where numerous alveoli are observed in the unfertilized state. These studies have accumulated evidences that the cortical changes following sperm entry consist of a series of events ending in the breakdown of the cortical alveoli followed by chorion separation (cf. Yamamoto, 1961), which events are generally common to those in sea urchin eggs (Sugiyama, 1956). However, less is known about the biochemical reactions underlying the cortical changes. The present study was undertaken to obtain some information concerning the chemical aspect of the fertilization wave which is an invisible change preceding the alveolar breakdown (Yamamoto, 1944, 1961). Special attention was paid to the adenosine triphosphatase (ATPase) activity of the mitochondria, based on the possibility that the activation of ATPase is involved in the fertilization wave of the fish egg (Ohtsuka, 1964). Now, as it is very difficult to secure the fertilized eggs for assay immediately after the establishment of the fertilization wave, a comparison of the enzymatic activity of mitochondria was obliged to be done between unfertilized eggs and fertilized ones in which the cortical changes have been accomplished.

Material and Methods

Mature unfertilized eggs of the fresh water fish, *Oryzias latipes*, were obtained from isolated gonads in Ringer's solution described by Yamamoto (1944). Each batch of eggs was divided into two to four groups, one of which remained unfertilized while the rest was inseminated and collected at different intervals of time after the beginning of alveolar breakdown near the animal pole of the egg. Eggs were washed twice with distilled water before they were subjected to the experimental procedures.

For the measurement of acid soluble inorganic phosphate, the eggs were homogenized in an adequate volume of 10% trichloroacetic acid,

and, after centrifugation, the supernatant solution was employed for determinations according to the method of Lohmann and Jendrássik (1926). In those experiments in which the activity of mitochondrial fractions was to be tested, mitochondria were isolated by slight modifications of the method of Schneider and Hogeboom (1950) and taken up for use in cold isotonic (0.25 M) sucrose.

The assay of ATPase was done as follows. The reaction mixture usually contained 2 mM of $MgCl_2$, 3mM of ATP (Na-salt), 50 mM of Tris-HCl buffer of pH 7.2, and the mitochondrial suspension. The mixture was made isotonic by the addition of sucrose and the final volume was 3.0 ml. In some experiments, 2,4-dinitrophenol (DNP) at a final concentration of 0.2 mM was applied to the reaction mixture of the same total volume. Incubation was performed at 35° C for 15 minutes with occasional stirring. The reaction was terminated by adding 1.0 ml of 10 % trichloroacetic acid, and an aliquate (3.0 ml) of the deproteinized supernatant was assayed for the inorganic phosphate released by the method of Lohmann and Jendrássik (1926).

Results and Discussion

A study was first made of the content of inorganic phosphate in acid soluble fractions of both unfertilized and fertilized eggs. Figure 1 illustrates results of these experiments. As it can be seen, the level of acid

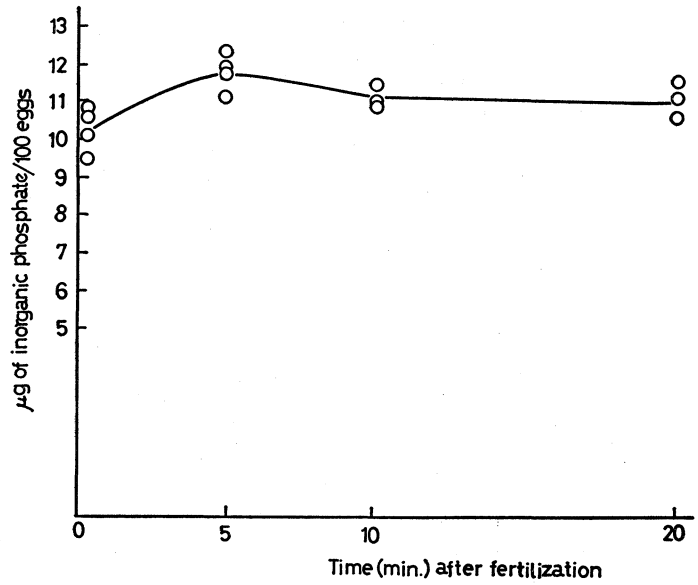

Fig. 1. Changes in the content of acid soluble inorganic phosphate of *Oryzias* eggs at fertilization.

soluble inorganic phosphate increased immediately after fertilization, followed by a slight decrease until 20 minute time point. This temporary increase in phosphate content, however, does not supply a conclusive evidence that fertilization elicits the potential ATP-dephosphorating activity of the egg, because the acid soluble fraction may include inorganic phosphate derived from various kinds of phosphate compounds.

Studies were then conducted with isolated fresh mitochondria to investigate whether any change takes place in the activity of the mitochondrial ATPase of newly fertilized eggs. The activity was determined in terms of inorganic phosphate set free in the assay system with and without the activator present. The activator used was magnesium or calcium, but calcium ion was found to be quite ineffective in stimulating the ATPase at the concentration (2 mM) applied; the assay was therefore carried out in the presence of added magnesium ions. The results obtained were collected in table 1. The mitochondria of the newly fertilized eggs

Table 1. ATPase activity in mitochondria of *Oryzias* eggs. The reaction mixture contained $MgCl_2$ (2 mM), ATP (Na-salt, 3 mM), Tris-HCl buffer, pH 7.2 (50 mM) and the mitochondrial suspension. The total volume was 3.0 ml. The activity is given as μg inorganic phosphate released per 400 eggs in 15 minutes at 35°C.

Exp. No.	Unfertilized		5 mins. after fertilization	
	No activator	Mg	No activator	Mg
1	2.30	3.85	2.47	5.72
2	1.46	1.95	1.90	4.33
3	2.53	4.18	2.44	5.10
4	1.15	1.86	1.35	3.97
5	1.78	2.70	2.62	5.83

possessed higher activity of magnesium-activated ATPase than those of unfertilized eggs. Although the variability in the values between experimental cases was observed, thus it seems most likely that the response of the fish egg to fertilization comprises a significant increase in mitochondrial ATPase activity, probably at an early step of fertilization. However, there is at present no indication of the character of the cortical change in which this enzymatic reaction is involved. Similar results have been reported in sea urchin eggs by Monroy (1957), who estimated the ATPase activity in isolated mitochondria and demonstrated that it rises during the first few minutes following fertilization. According to him, the mitochondria of sea urchin eggs undergo physiological or structural rearrangements upon fertilization.

On the other hand, it has been known that fresh rat liver mitochondria have low ATPase activity, but a great increase in activity is induced by

treatment with DNP (Potter and Recknagel, 1951). This was confirmed for fish eggs in the next experiments in which freshly prepared mitochondria from unfertilized eggs were assayed under the presence of DNP. The concentration of DNP was 0.2 mM, a concentration at which it causes the optimal activation of apyrase activity in the total homogenate of developing fish embryos (Maruyama and Ishida, 1955). As is seen in table 2, the ATPase activity was enhanced by the addition of DNP. It

Table 2. Effect of DNP on mitochondrial ATPase activity of unfertilized *Oryzias* eggs. The reaction mixture contained DNP at a final concentration of 0.2 mM. Other conditions were the same as those in table 1.

Exp. No.	No DNP	DNP
1	1.95	4.63
2	1.30	4.82
3	2.17	5.57

gives an activity comparable to that obtained from the mitochondria of newly fertilized eggs. Thus the results of the present study indicate a conversion of the mitochondrial ATPase occurring in the egg at fertilization, which produces an increase in enzyme activity.

Summary

1. An acid soluble inorganic phosphate of the eggs of *Oryzias latipes* increases in the early process which follows fertilization, and later indicates a slight decrease until 20 minutes.

2. The ATPase of mitochondria from unfertilized eggs is sensitive to magnesium. The increase in activity occurs upon fertilization.

3. The ATPase activity of unfertilized egg mitochondria becomes high in the presence of added DNP.

The author is indebted to Professor I. Kawakami of Kyushu University for his constant encouragement during this investigation.

References

Lohmann, K., and L. Jendrássik, Biochem. Z., **178**, 419 (1926).
Maruyama, K., and J. Ishida, Annot. Zool. Jap., **28**, 131 (1955).
Monroy, A., J. Cell. Comp. Physiol., **50**, 73 (1957).
Ohtsuka, E., Embryologia, **8**, 101 (1964).
Potter, V. R., and R. O. Recknagel, Phosphorous metabolism, **1**, 377, Baltimore (1951).
Schneider, W. C., and G. H. Hogeboom, J. Biol. Chem., **183**, 123 (1950).
Sugiyama, M., Exptl. Cell Res., **10**, 364 (1956).
Yamamoto, T., Annot. Zool. Jap., **22**, 126 (1944).
———, Internat. Rev. Cytol., **12**, 361 (1961).