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Tommaso D'Anna

Copper as limiting factor of respiratory rate in the unfertilized egg of Ciona intestinalis (Ascidian)

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Embriologia chimica. — *Copper as limiting factor of respiratory rate in the unfertilized egg of* Ciona intestinalis *(Ascidian)* (*). Nota di TOMMASO D'ANNA, presentata (**) dal Corrisp. G. REVERBERI.

RIASSUNTO. — L'uovo di *Ciona intestinalis* a seguito della fecondazione subisce una diminuzione del contenuto di rame totale. Tale risultato, messo in relazione con gli effetti del DECA (chelante ad alta affinità per il rame) sia sulla respirazione che sull'attività della succinodeidrogenasi nelle uova vergini, porta a ritenere che il rame sia uno dei fattori limitanti della respirazione. La rimozione del rame operata dal DECA o dalla fecondazione potrebbe essere responsabile in parte dell'aumento respiratorio dell'uovo alla fecondazione.

INTRODUCTION

The interest of many Authors has for some time been devoted to the problem of the mechanism or factors which control the respiratory rate of unfertilized eggs.

Regarding the Ascidian egg, it has been observed that the increase in oxygen consumption which occurs in the egg following fertilization is related to an increase of cytochrome oxidase and succinic dehydrogenase activity [1, 3], and to a decrease of the ATP content [2].

Unfertilized eggs treated with sodium diethyldithiocarbamate (DECA) reveal a marked increase in oxygen consumption [3, 4]; moreover the DECA increases succinic dehydrogenase activity in homogenate of unfertilized eggs [3]. DECA specifically chelates copper and its action on the respiration of unfertilized eggs could be related to a removal of copper from some enzymes to which it could be bound in a dissociable form.

The hypothesis that copper concentration could control the activity of enzymes involved in oxidative metabolism of unfertilized Ascidian eggs was put forward [3, 4]. In order to support such hypothesis the copper content was measured both in unfertilized and in fertilized eggs of *Ciona intestinalis*.

MATERIAL AND METHODS

The eggs were removed from the oviduct of a number of animals and, after washing with artificial sea water [5], suspended in a defined volume and divided into two batches one of which was fertilized.

Unfertilized and fertilized eggs (2 blastomeres) were collected with a clinical centrifuge at 3,000 rpm and homogenized by hand in a tight all-glass homogenizer after the addition of two volumes of all-glass distilled water.

(*) Lavoro eseguito presso l'Istituto di Zoologia dell'Università di Palermo.
(**) Nella seduta del 12 gennaio 1974.

The copper content was measured on portions of the homogenate by a modification of the Eden and Green method [6]; such a modification allowed the detection up to 0.2 μg of copper in a sample.

To 300 μl of homogenate, in pyrex tubes 125 × 15 mm, 300 μl 96% H_2SO_4 and 300 μl 60% $HClO_4$ were added; the tubes were then suspended in a fume chamber over a melted mixture of KNO_3 and $NaNO_2$ (10 : 8.5 W/W) in an electric plate for 12 hours and further oxidation carried out with a weak gas flame by continuously shaking the tubes. Finally 300 μl 65 % HNO_3 were added to the tubes after cooling and oxidation was completed with the gas flame as above.

The oxidation product was then diluted with 1 ml distilled water and neutralized cold with 1 ml 28 Bé NH_4OH.

Copper in the samples was revealed by a yellow color which appeared after addition of 200 μl 0.5 % DECA and 0.5 ml 4 % sodium pyrophosphate. The color was absorbed by 300 μl isoamylalcohol which was then centrifuged at 9,000 rpm with a OLE DICH microcentrifuge to separate the suspended water. The optical density was read at 440 nm in a ZEISS PMQ II Spectrophotometer by using microcells. Copper content was calculated with reference to known samples of copper sulphate (containing from 0.4 μg to 0.8 μg of copper), which were run simultaneously with homogenate samples.

The dry weight of samples of the homogenate was determined with microweighing bottles (1 ml total volume) and copper content expressed as ng Cu/mg dry weight.

RESULTS

Table I summarizes the data obtained from a series of experiments carried out on eggs of the same batch. Preparation of extracts and measurement of copper content were made contemporaneously both in unfertilized and in fertilized eggs. Each value is the average of two or three samples of the same homogenate.

TABLE I.

Copper content in the eggs of Ciona intestinalis ng/mg *dry weight*

Exper. n.	Unfertilized	Fertilized	% Variation
1	14.92	12.81	— 8.10
2	14.06	9.66	—31.29
3	13.96	12.50	—10.45
4	15.22	13.04	—14.32
5	15.44	9.60	—37.17
6	14.94	12.37	—17.20
7	13.02	10.35	—20.50
8	13.88	10.68	—23.04
Average	14.43	11.50	—20.26
Standard error . .	±0.29	±0.51	±3.54

Even if there are variations from one experiment to another the decrease of copper content is evident following fertilization in the egg. Copper content of unfertilized egg gives an average value of 14.43 ng per mg of dry weight and 11.5 ng in the fertilized egg with a 20.26 % decrease.

Discussion

The decrease of copper content in the egg of *Ciona intestinalis* upon fertilization is very significant if related to the results obtained in a previous work [3]. The data of that paper are summarized in Table II and compare the effect of DECA both on respiration and on succinic dehydrogenase activity of unfertilized and fertilized eggs.

TABLE II.

Respiration and succinic dehydrogenase activity in the eggs of Ciona intestinalis

	Respiration nl O_2/egg/hr.			Succinic dehydrogenase nl O_2/μg prot./hr.		
	Controls	3.5 mM DECA	% Δ	Controls	0.2 mM DECA	% Δ
Unfertilized . .	0.38	0.54	42	6.53	7.92	20.3
Fertilized . . .	0.62	0.70	13	7.62	8.01	5.2
% Δ	63.00	29.50	—	16.70	1.13	—

In can be seen that unfertilized eggs treated with DECA largely increase their respiratory rate (42 %) as related to the increase which usually occurs following fertilization (63 %). Eggs treated with DECA at fertilization do not increase their respiration like the controls but to a lesser extent (30 %), owing to the fact that their breathing had been previously stimulated by DECA.

Similar results were obtained when DECA was tested on succinic dehydrogenase activity. In fact DECA stimulates enzyme activity of unfertilized eggs by 20 % and only by 5 % that of fertilized eggs.

On the basis of those data the following conclusions were drawn: DECA operates both on respiration and on succinic dehydrogenase with a mechanism similar to fertilization. Removal of copper either by DECA treatment or following fertilization might cause increase of respiratory rate of the eggs.

It is difficult to explain how copper could control respiration of the unfertilized Ascidian egg: one might suppose that copper binds to enzymes of oxidative metabolism and modifies their normal activity.

A number of data support such a hypothesis; in fact copper inhibits enzymes of glycolisis and of the pentose phosphate cycle [7], mitochondrial respiration and microsomal ATPase activity [8]. Large amounts of copper

bind to protein binding sites in the membrane of bovine heart mitochondria, and cause alteration of the normal mitochondrial activities owing to a strong inhibition of oxidative enzymes [9]. Chelating agents having a strong affinity to copper inhibit such a binding and restore the normal activities even in mitochondria which had been previously treated with copper [10].

Moreover copper inhibits a number of dehydrogenases binding to free —SH groups and forming dissociable complexes. Copper removal restores the normal activity of those enzymes [11].

As a conclusion, the decrease of the copper content detected in the fertilized eggs of *Ciona intestinalis* could be attributed to a flux of copper ions out of the egg following fertilization; such a factor could contribute to the removal of the block of respiratory activity of the unfertilized egg. Therefore copper could be considered one of the limiting factors of respiration in unfertilized eggs of *Ciona intestinalis* by a control on succinic dehydrogenase activity and perhaps on other enzymes of oxidative metabolism.

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