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THE ENERGETIC EFFORT OF MUSSELS' ADAPTATION TO LOW SALINITY MEDIA

ION NEACŞU[1]*, VALER CRĂCIUN[2], ADRIAN TELEMBICI[3], PINCU ROTINBERG[2], DANIELA GHERGHEL[2]

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Abstract: The transfer of mussels from marine water with a salinity of 13 g/L to low salinity water induces a reduction in Na^+-K^+-ATP-ase's activity, and in the energetic level corresponding to the ionic pump's activity, with a restoring tendency at 6 and 4 g/L salinity, and irreversible at 2 g/L. The results suggest certain osmoregulation possibilities of mussels, involving an Na^+-K^+-ATP-ase possessing suitable functional characteristics.

INTRODUCTION

As generally known, one the main factors limiting the geographical preading of aquatic animals is the osmotic one. Thus, the low and variable salinity of the Black Sea prevents several Mediterranean and oceanic organisms' penetration in the Pontic basin.

Animals' adaptation to low salinity conditions assumes the existence of certain mechanisms, capable of eliminating the water which penetrates osmotically through epithelia in the internal medium and, from here, in the intracellular one, and, concomitantly, of reintroducing into the cells the salts tending to be lost in the external – more diluted – medium.

The general evolution of aquatic animals is manifested from the osmoconforming to the osmoregulating ones although, actually, different speciesmay be situated, somewhere between these two limits, which means that they are neither typically osmoconforming nor perfectly osmoregulating ones, evidencing only certain osmoregulating possibilities.

SCOPE OF THE INVESTIGATIONS

Previous researches of ours (Neacşu et al., 1969; Telembici et al., 1969; Crăciun et al., 1989) showed that the Black Sea mussels are generally osmoconforming-animals, yet they show, too, certain osmoregulating possibilities, more obvious between some limits of water's salinity values. The present study deals with the implications of the Na^+-K^+ pump in mussels' osmoregulation process and the corresponding energetic consumption.

This mechanism is represented by the activity of a Na^+-K^+-depending ATP-ase which, through ATP hydrolysis, releases the energy necessary for 3 Na^+ expulsion from the cells and for the reintroduction, into the cells, of 2 K^+, which actually requires a considerable energetic consumption (Skou, 1969).

MATERIAL AND METHODS

The experiments were developed on mussels (*Mytilus galloprovincialis*, Lmk.) transferred from the Black Sea waters, the Agigea area, wih 13 g‰ salinity, to salinity values of 6; 4 and 2 g‰, obtained through marine water's dilution with sweet water.

Determination of ATP-ase activity at 6 g‰ salinity, was made 1; 3 and 7 days after the transfer, and at 4 and 2 g‰ salinity after 1 and 2 days, as the animals did not survive longer than this. To this end, entire mussels (the fleshy part) have been utilized as, in the case of bi-valve mollusca, no organ specialized in the transport of ions had been described, the whole bodily surface being involved in this phenomenon.

The Na^+-K^+-ATP-ase's activity has been determined by dosing of the anorganic phosphate (Pi) resulted from ATP hydrolysis (Artenie and Tănase, 1981). Incubation of the enzymatic preparation occurred at a temperature of 19^0 C, for 30 minutes, the results obtained representing the mg Pi released through the enzymatic activity, and the corresponding energy (kcal) – calculated for 100 g living mussel tissue.

Data's statistic processing was based on Student test.

RESULTS AND DISCUSSIONS

In the first day after salinity's reduction, a considerable diminution of ATP-ase's activity is recorded with all salinity values taken into study (Table 1).

În the following days, at salinity values of 6 and 4 g‰ a tendency of enzyme's activity returning to the initial value of normal marine water is observable. Instead, with salinity values of 2 g‰, enzyme's activity is continuously decreasing, animals' death occurring two days after the transfer.

Table 1. ATP-ase activity and the corresponding energetic consumption with mussels transferred into low salinity water (All values are statistically significant)

Salinity (g‰)	Days	ATP-ase activity (mg Pi/100 g) (X±ES)	n	Energetic consumption (kcal/100 g)
13 (control)	0	168.5±2.3	5	0.040
6	1	97.8±2.1	5	0.023
	3	126.2±1.8	5	0.030
	7	139.4±2.5	5	0.033
4	1	109.7±2.1	5	0.026
	2	129.6±3.1	5	0.031
2	1	118.1±2.6	5	0.028
	2	97.9±3.3	5	0.023

Such results may be interpreted *versus* certain properties of Na^+-K^+-ATP-ase, as well as *versus* the hydroelectrolytic modifications suffered by mussels after their transfer to low salinity water. Consequently, it is known that this enzyme is strongly stimulated by the increase of Na^+ intracellular concentration, as well as by the increase of K^+ extracellular concentration (Skou, 1965; Harris, 1967; Alberts et al., 1989).

Indeed, lowering of water's salinity causes the increase of Na^+ intracellular concentration, although it might be expected that water's osmotic penetration into the cells should dilute the intracellular Na^+. However, as already evidenced elsewhere

(Crăciun et al., 1999), Na^+ enters the cells concomitantly with water's osmotic penetration, according to the so-called "*solvent drag*" effect, the result of which is that water's osmotic flow through the membrane causes, too, an increase in the flow of salts dissolved in water, which results in the stimulation of Na^+-K^+-ATP-ase's activity.

On the other hand, with the decrease of water's salinity, a certain dilution of K^+ extracellular concentration, alongwith an increase in the K^+ eflux – may be noticed, with a possible inhibition of Na^+-K^+-ATP-ase activity.

The question therefore arises whether it is exactly this latter effect to be observed with mussels in the first day following reduction of water's salinity?

The investigations performed on the Na^+-K^+-ATP-ase's activity of certain fish species (Maetz, 1974) have shown that, during their transfer from marine to sweet water and, reversely, from sweet to marine water, synthesis of a new type of ATP-ase occurs – situated at the opposite pole of the epithelial cells, and showing modified affinities towards Na^+ and K^+, the concentration of which had been changed by changing water's salinity.

Based on this, one may assume that, in the case of mussels, too, there might exist a similar adaptative process – which, for the time being, had not been evidenced experimentally.

Thus, after mussels' transfer to low salinity water, synthesis of a new ATP-ase might be developed, the activity of the old enzyme being gradually reduced. It is only after several days that the activity of a new ATP-ase will be observable, alongwith the tendency of the Na^+ and K^+ active transport's return towards the initial values – which had been observed by the authors at salinity values of 6 and 4 g‰. Acceleration of the Na^+ active transport outside the cell induces to this ion the behaviour of an "impermeable" one (Macknight and Leaf, 1977), thus counterbalancing the excess of intracellular osmotic pressure and reducing the osmotic flow of water.

Besides the energetic effort necessary for the sythesis of a new type of Na^+-K^+-ATP-ase, the increase – in time – of the Na^+-K^+ pump's activity also requires a certain energetic consumption. Thus, if, for the expulsion of 3 Na^+ off the cells and re-introduction of 2 K^+ in the cells, one ATP molecule is hydrolyzed (Skou, 1965; Thomas, 1972; Alberts et al., 1989), one may calculate the amount of energy consumed by the ionic pump, if assuming that ATP hydrolysis in the living cell releases 7.3 kcal/mol (Alberts et al., 1989).

The results obtained (kcal), ascribed to 100 g living tissue, represent the energetic consumption over the 30 min duration of enzyme's incubation, coresponding to the calculation of ATP-ase's activity. The values do not appear as exaggerated (Rüegg, 1971), if considering that mussels are poikilothermal organisms, so it might be possible that only part of the energy consumed should be useful energy, the largest part of it being possibly dissipated in the enviroment. Yet, for the elucidation of such aspects, other studies are still necessary.

CONCLUSIONS

– Mussels' transfer from marine water with normal salinity of 13 g‰ to low salinity water, causes a significant reduction in Na^+-K^+-ase's activity and in the energetic level corresponding to the ionic pump's activity.

– At 6 and 4 g‰ values of salinity, a certain tendency of these parameters's returning towards normal values is observable the second day after the transfer, while, at values of 2 g‰, animals' death is recorded.

– The results obtained suggest the involvment – in mussels' osmoregulation process – of an ATP-ase possessing new characteristics, different from that active in conditions of normal salinity, and active at the opposite pole of the cells, at salinity of 6 and 4 g‰.

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1. Faculty of Biology, "Al. I. Cuza" University, Bd. Carol I, 20A, 6600 Iaşi, Romania
E-mail: ineacsu@uaic.ro
2. Institute of Biological Research, Bd. Carol I, 20A, 6600 Iaşi, Romania
3. IRCM Constanţa, Bd. Mamaia 300, 8700 Constanţa, Romania
*Corresponding author: Ion Neacşu