Modification of the Amino-Acid Efflux During the Osmotic Adjustment of Isolated Axons of *Callinectes sapidus*

Various studies have shown that the regulation of the cell osmotic pressure (intracellular isosmotic regulation) occurring in euryhaline invertebrate tissues when submitted to an osmotic stress is not the result of a passive osmosis phenomenon. It rather implicates an active modification of the concentration of various intracellular osmotic effectors among which amino acids play a prominent part[1-4]. It has been proposed that two different mechanisms could be implicated in the regulation of the concentration of the amino acids. The first mechanism involves modifications of the permability of the cellular membrane to the amino acids[5-7] and the other one implicates modifications of the metabolism of these organic osmotic effectors[5,8,9]. We therefore undertook, together with a study of the volume modifications, a study of the efflux of labelled alanine on axons isolated from the walking legs of the euryhaline crustacea *Callinectes sapidus*, when submitted to a hyper or a hypoosmotic stress.

The stresses are achieved following a method previously described[5]. The volume modifications are followed on an isolated axon, with a microscope provided with a camera attachment. The volume changes are calculated from the modifications in the axon diameter measured on enlargments of the pictures taken during the experiments. For these calculations, the length of the axon is assumed to be constant. The efflux of alanine is studied by dropping the axons, preloaded by soaking them for 90 min in an isotonic saline containing alanine-U-^{14}C (20 m*M*, 5 μC/ml), in scintillation counting vials containing 2.5 ml of a non-radioactive saline. The axons are washed for 5 min in each vial. At the end of the experiment, the axons are blotted on filter paper, weighed and then digested in 2 ml of soluene (Packard Instrument Co.) before being counted for residual radioactivity. 15 ml of Tt 21[10] are added to the vials containing the washing saline. The vials are then counted in a Beckman scintillation counter set at 2% error. The radioactivity appearing in the washing saline is due for 75.5% to labelled alanine as shown by chromatographic analysis.

When submitted to a hypoosmotic stress, the axon shows a rapid volume readjustment (Figure 1). Within the first 5 min following the application of the stress, the axon swells of about 30%. A few hours later, the volume is completely regulated. However, more than 50% of this readjustment takes place during the first hour. In these conditions, there is an important increase in the alanine efflux immediately after the application of the stress. The outflux is then progressively slowed down and returned to values slightly higher than the control (Figure 2). This is best shown when considering the half renewal times calculated from the slope of the residual activity curve correspond-

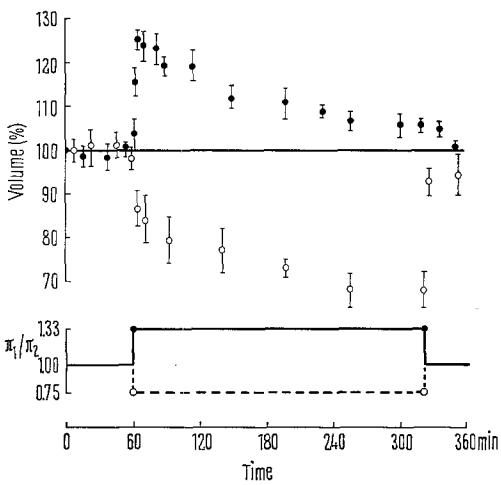

Fig. 1. Modification of the volume of isolated axons of *Callinectes sapidus* during a hyperosmotic (○) or a hypoosmotic (●) stress. The given data are mean values for 5 experiments. The hypoosmotic stress is achieved by dropping the axons of a sea water crab from a saline corresponding to the blood composition of a crab adapted to sea water (osmotic pressure π_1 = 1100 mOsm/l) to a saline corresponding to the blood composition of a crab adapted to twice diluted sea water (osmotic pressure π_2 = 825 mOsm/l). The hyperosmotic stress is achieved by dropping the axons of a crab adapted to twice diluted sea water from a saline corresponding to the blood composition of a crab adapted to twice diluted sea water (osmotic pressure π_1 = 825 mOsm/l) to a saline corresponding to the blood composition of a sea water adapted crab (osmotic pressure π_2 = 1100 mOsm/l). The volume measured during the control period is considered as 100%.

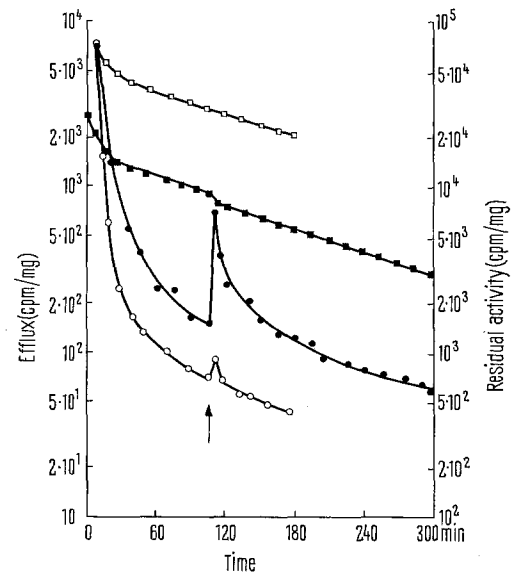

Fig. 2. Modification of the efflux (○, ●) and of the residual activity (□, ■) of alanine-^{14}C in isolated axons of *Callinectes sapidus* submitted to a hypoosmotic (●) or a hyperosmotic (○, □) stress. The stress is applied at the time marqued by the arrow and is achieved in the way described in the legend of Figure 1.

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ding to the intracellular compartment of the axon (Figure 2). The period during the hypoosmotic stress is 125 min compared with 160 min as the control.

During the hyperosmotic stress, on the contrary, there is an immediate shrinkage of the axon and no volume regulation can be observed even after 6 h of incubation in the hypertonic saline (Figure 1). In this case, no significant modification of the alanine efflux can be recorded (Figure 2).

It appears therefore that an increase in alanine efflux is associated with the rapid volume readjustment occurring after the application of a hypoosmotic stress. A same modification of efflux with various other organic molecules such as urea or propylene-glycol. On the other hand, a similar change in the efflux of 2-amino-isobutyric acid has been observed on bundles of muscle fibers isolated from *Callinectes* and submitted to a hypoosmotic stress[11]. Moreover, an increase in the permeability to potassium has been recently demonstrated on duck erythrocytes during the volume readjustment following the application of a non-hemolytic hypoosmotic stress[12]. An increased release of amino acids has also been observed on the isolated mussel heart under hypoosmotic stress conditions[13]. Although modifications of the extracellular space can partly account for the changes in efflux, it appears thus that an increase in the permeability of the cellular membrane is implicated in this phenomenon.

The increase in the alanine efflux we observe is not modified when the axons are submitted to the hypoosmotic stress in salines where NaCl has been replaced by LiCl or choline chloride or in calcium free salines; although in this last condition, there is a general increase in the alanine efflux. The increase in efflux is however completely suppressed when the axons are dropped in a saline, the ionic composition of which is identical to the ionic composition of the hypotonic saline but which is kept isosmotic to the control saline by addition of sucrose. It is therefore concluded that the increase in efflux is induced by the swelling of the tissue produced by the modification of the osmotic pressure 'per se'.

Thus, in hypoosmotic media, *Callinectes* axons, after an initial phase of swelling, revert towards their original volume at least partly by virtue of a loss of cellular osmotic effectors. This loss is a consequence of a temporary increase in efflux which requires changes in membrane characteristics. These changes in efflux seem to be induced by the modification of the osmotic pressure 'per se' and not by the alteration, in the incubating saline, of the concentration of a specific ionic species.

That such a mechanism of isosmotic intracellular regulation can be at play during the adaptation in vivo of a euryhaline species to diluted media is still unknown. This possibility is now under investigation[14,15].

Résumé. Au cours du choc hypoosmotique, le volume d'un axone isolé d'une patte de crabe euryhalin *Callinectes sapidus* est rapidement régularisé. En même temps, on observe une augmentation du flux sortant de l'alanine marquée. Cette augmentation paraît être due à une modification de la membrane axonale, produite par la variation de la pression osmotique dans le milieu d'incubation.

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Chemical Promotion of Pistillate Flower Formation in *Cucurbita*

Flower sex expression in the cucumber proceeds through a nodal sequence in the appearance of flower types[1]. The early nodes formed produce only staminate flowers, followed by a monoecious phase and finally successive nodes of pistillate flowers. This pattern generally occurs even though all floral buds pass through a common bisexual phase during development[2,3].

Flower sex expression can be modified by environmental[4,5] and chemical[5–7] factors. The staminate phase is favored by gibberellins[5,6] and the pistillate phase by auxins[7], growth retardants[8], and some unsaturated hydrocarbons[9,10]. Recently, much interest has been directed to the role of ethylene[11–13]. This report describes the marked promotion of pistillate flower formation with 1,1,5,5-tetramethyl-3-dimethylaminodithiobiuret (MATB) on nodes that normally would produce only staminate flowers and the reversal of this effect with gibberellin A_3(GA_3).

Materials and methods. Cucumber (*Cucumis sativus* L. cv. National Pickling) were cultured in a greenhouse at a 14-h photoperiod and minimum day and night temperatures of 25 and 21 °C, respectively. Foliar sprays of MATB (0, 1.4 and 7.0×10^{-4}, 1.4 and $2.8 \times 10^{-3} M$) were applied when the first true leaf was approximately 5 cm in diameter. The interaction of MATB ($7.0 \times 10^{-4} M$) and GA_3 ($1.4 \times 10^{-3} M$) at saturating doses was also established.

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