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FREE AMINO ACIDS AND ISOSMOTIC INTRACELLULAR REGULATION IN THE SHRIMP CRANGON CRANGON

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The concentrations of non-protein nitrogenous material and individual free amino acids in muscle tissue of the euryhaline shrimp Crangon crangon are measured. The cellular ninhydrin-positive component closely follows blood osmolarity over a range of ambient salinities and temperatures. The three most abundant amino acids, glycine, proline and alanine, are primarily involved in adjusting intracellular osmotic concentrations to the salinity-induced variation in blood. The cellular adjustment to changed salinity is rapid; parallel observations on blood concentrations suggest that the cellular regulation involves an exchange of nitrogenous material through the cell membranes.

Introduction

The presence of small organic molecules as significant intracellular osmotic effectors has been recognized since the turn of the century (1). In the last decade the role played by amino acids has become recognized particularly by Florkin and co-workers (2). Since the contents of tissue cells invariably appear isosmotic to blood or haemolymph, the processes by which the amino acids appear to adjust the intracellular osmotic concentrations to those in the blood are described by the term "isosmotic intracellular regulation" (3). In regards to the mechanism involved Schoffeniels (4) postulates that the intracellular free amino acids (FAA) are regulated by

blood cation levels, which differentially affect the various enzyme systems involved in amino acid metabolism. Experiments with isolated nerves of chinese crabs, Eriocheir sinensis (5) indicate that in the cells, the concentrations of the non-essential amino acids (i.e. those which the animal can synthesize) are regulated by direct synthesis and degradation, whereas the essential amino acids and proline, are at least in part, regulated by modification of the permeability of the cell membranes, and extrusion from the cells.

We studied the contribution of amino acids to the regulation of cellular osmotic pressure in the euryhaline shrimp Crangon crangon. To gain further insight into the regulation processes involved, the levels of ninhydrin-positive substances (NPS) and FAA were determined over a wide range of salinities and temperatures, in both muscle and blood. These studies were performed in both fully acclimated animals and animals undergoing salinity adaptation (following a change of sea water concentration).

Materials and Methods

Specimens of adult Crangon crangon (L.) collected from the Dutch Wadden Sea were acclimated to various salinities between 10 and 40 $^{o}/oo$ and to temperatures of 5, 15 and 21 ^{o}C for at least 5 days in glass aquaria provided with a bottom filter of sand and shell grit (6). The NPS concentration was determined in deproteinized samples (methanol-acetone) of haemolymph and homogenized abdominal musculature following the method Moore & Stein (7). Solutions of DL-leucine were used as standard. Amino acid analysis were performed with a Bio-Cal BC-200 automatic amino acid analyzer using a two column system (8).

Results and Discussion

a. Muscle concentrations

The muscle NPS concentrations obtained are shown in Fig. 1, which

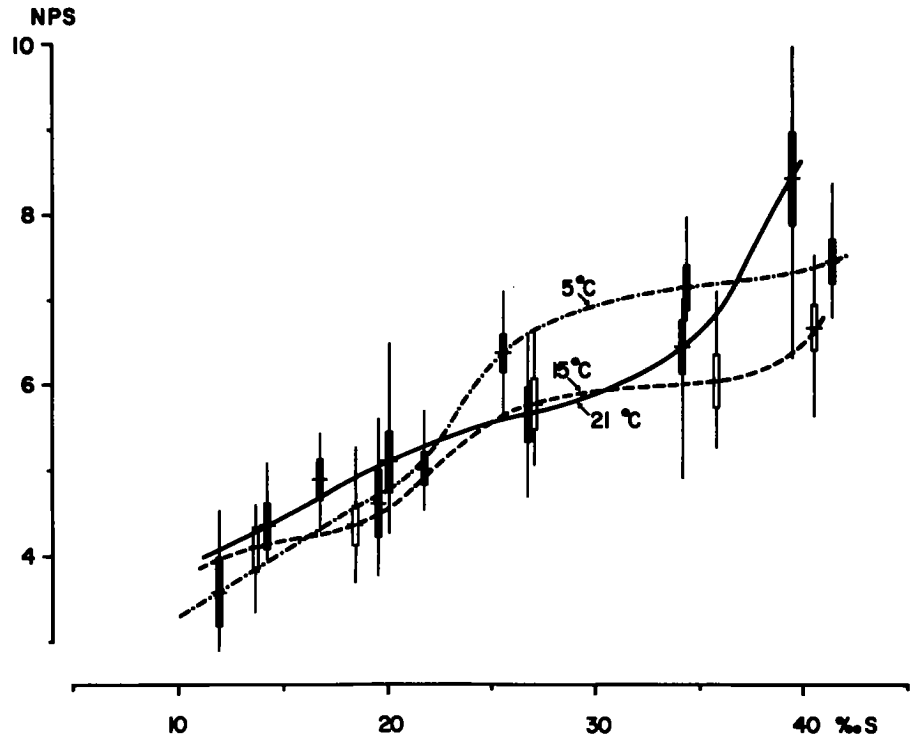

Fig. 1. Variation in concentration of ninhydrin-positive substances, NPS in muscle tissue (in mg N/g fresh weight) as a function of acclimation salinity at temperatures of 5, 15 and 21°C.

illustrates an overall positive correlation with ambient salinity. Parallel determinations of the muscle water content have shown that the percentage muscle water (W) is related to salinity in °/oo (S) by the equation W = -0.108S + 80.04 (N = 24, corr. coeff. = 0.81) at 5 °C. This shows that Crangon possesses a relatively efficient water regulation (or isosmotic intracellular regulation) and that the measured NPS variation obtained is clearly in excess of salinity-dependent changes in tissue hydration.

It is remarkable that this regulatory pattern of cellular NPS is strictly analogous to the regulation of total osmotic pressure in shrimp haemolymph (6). Corresponding features include the stronger regulation at

intermediate salinities, where the temperature effect is inverse (decreasing NPS content with increase in temperature), and greater salinity dependence at more extreme salinities, where the temperature correlation is positive. This relationship is further illustrated in Fig. 2 where the freezing point of haemolymph is related to that due to intracellular NPS (the latter being

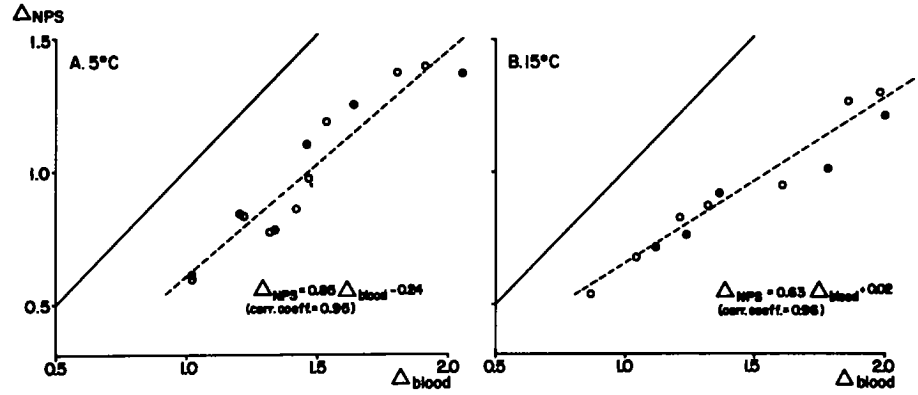

Fig. 2. Relation between freezing point depression attributable to muscle ninhydrin-positive substances (Δ_{NPS}), and that in haemolymph (Δ_{blood}) at 5 and 15 °C. (Open and solid circles refer to separate experimental series).

calculated on the basis of the water content of muscle, and the molal freezing point depression of water (1.858 °C) assuming the presence of one ninhydrin-positive nitrogen atom per molecule of NPS). It will be seen that NPS accounts for the greater part of cellular osmolarity. The straight line relationships obtained illustrate how closely cellular NPS follows blood osmotic concentrations. Assuming that the cellular content is isosmotic with blood, the lower gradient obtained for 15 °C indicate that at this temperature, increases in cellular osmolarity cannot solely be ascribed to NPS increase.

From analysis of the individual free amino acids by the chromatographic technique some twenty amino acids were identified. Comparison with NPS data reveals that the free amino acids constitute some 70 to 80% of muscle ninhydrin-positivity and illustrate the same salinity correlation (Fig. 3).

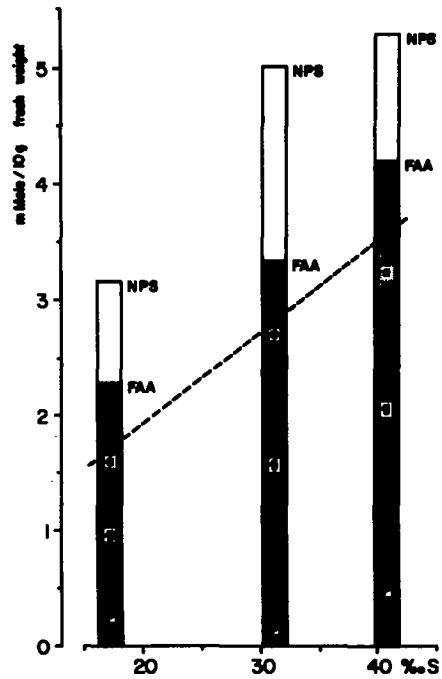

Fig. 3. Histograms representing the concentrations of alanine (a), proline (p), glycine (g), total free amino acids (FAA) and ninhydrin-positive substances (NPS), in muscle tissue of shrimps acclimated to three salinities at 5 °C.

It is furthermore evident that the variation in FAA is in turn caused by variation in the three most abundant amino acids, namely, alanine, proline and glycine, although individually they lack a linear correlation with salinity.

b. Haemolymph concentrations

The haemolymph NPS concentrations are extremely low (equivalent to 0.05 to 0.09 mg $N.ml^{-1}$) and essentially independent of salinity. These values may be calculated (as above) to represent freezing point depression values of less than 0.01 °C.

The major amino acid constituents of the haemolymph are glycine, proline and alanine, but their fractional contribution is considerably lower (approximately 42%, compared with 80% in muscles). Whereas the non-essential amino acids constitute approximately 83% of muscle FAA, they form only some 50% in haemolymph, due to the greater proportions of essential amino acids (particularly of lysine) in the latter tissue. Although non-essential, the concentration of proline is greater in haemolymph than muscle. These data seem to agree with Gilles and Schoffeniels' (5) observations on isolated nerves of Eriocheir, that the cellular concentrations of essential amino acid and of proline are regulated in part by modification in permeability of the cell membrane.

c. Accommodation rate

The rate of adaptation of cellular concentrations to ambient salinity was studied by sudden intercharge of hypo- and hyperosmotic media (respectively 17 and 33 $^{o}/_{oo}$S) to which shrimps had been acclimated at 5 ^{o}C.

During adaptation to hypo-osmotic medium cellular NPS accommodates rapidly having a "half-time" value of about 3 hours. During this process no significant changes in haemolymph NPS are observed (9). In the opposite transfer (to hyper-osmotic medium) the muscle content decreases rapidly, tending to show an "undershoot". It is, however, significant that under these conditions the haemolymph values increased and persisted for 3-5 days. The increase in haemolymph amino acid supports the concept that extrusion from the cells is involved in the salinity-dependent regulation of cellular levels.

The rapid responses of muscle NPS to hypo- and hyperosmotic conditions in Crangon is interesting when compared to Lang and Gainer's (10) experiments on volume regulation of muscle fibres of the blue crab, Callinectes sapidus. Here transfer to hypertonic salines showed only the expected volume change (decrease) without a secondary tendency to revert to original dimensions as was observed in hypotonic media. Vincent-Marique and Gilles (11) interpret

these data as evidencing a rapid mechanism of volume regulation during hypo-osmotic stress, and a slower one under hyperosmotic conditions. In view of the already evidenced exchange of nitrogenous material between blood and tissues it may, however, be pointed out that Lang and Gainer's hyperosmotic experiment does not represent true in vivo conditions, because of the absence of blood, which in life will serve as a link with amino acids and their precursors in other tissues ("liver", the digestive system).

References

1. L. FREDERICQ. Bull. Classe Sciences 1, 428 (1901).
2. M.N. CAMIEN, H. SARLET, G. DUCHATEAU and M. FLORKIN. J. Biol. Chem. 193, 881 (1951).
3. C. JEUNIAUX, S. BRICTEUX-GRÉGOIRE and M. FLORKIN. Cahiers Biol. Marine 2, 373 (1961).
4. E. SCHOFFENIELS. Archs int. Physiol. Biochim. 68, 696 (1960).
5. R. GILLES and E. SCHOFFENIELS. Comp. Biochem. Physiol. 31, 927 (1969).
6. R.E. WEBER and D.H. SPAARGAREN. Neth. J. Sea Res. 5, 108 (1970).
7. S. MOORE and W.H. STEIN. J. Biol. Chem. 176, 367 (1948).
8. D.H. SPACKMAN, W.H. STEIN and S. MOORE. Analyt. Chem. 30, 1190 (1958).
9. R.E. WEBER and W.J.A. VAN MARREWIJK. Neth. J. Sea Res. 5 (4), in press.
10. M.A. LANG and H. GAINER. J. gen. Physiol. 53, 323 (1969).
11. C. VINCENT-MARIQUE and R. GILLES. Life Sciences 9, 501 (1970), and Comp. Biochem. Physiol. 35, 479 (1970).