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Presence of Y-Glutamyl Cycle in the Sponge Geodia Cydonium

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In spite of the extremely low concentration of dissolved organic matter (DOM) in the sea water, many marine benthic animals and phytoplankters are able to accumulate and to assimilate dissolved free amino acids (DFAA) to such an extent as to provide a substantial supplemet to the nutritional requirements of the organisms (Stephens, 1972). The phylum Porifera was not included in the uptake experiment so far. Sponges are filter feeders with an impressive flow of water through the organisms. It seems ecologically to be justified to propose that potential energy of the DFAA should be used also in this phylum. Recently we have shown that the transport of amino acids in phytoplankters (Kurelec et al., 1976) and Nereids (unpublished work) is mediated by a cytalytic function of 6 enzymes integrated to form a cycle of chemical events, called y-glutamyl cycle (Meister, 1973). This provides a new form of support to the recent studies in the uptake-experiments. The presence of such a sophisticated transport system would prove the capacibility for the uptake of DFAA. With this idea in mind we looked in the siliceous sponge Geodia cydomium for the presence of the y-glutamyl cycle enzymes.

The enzyme y-glutamyl transpeptidase is firmly bound to a particulate fraction of the sponge homogenate. Purified enzyme (step 2, Tate and Meister, 1974) releases free p-nitroanilin from a y-glutamyl-p-nitroanilide at a rate of 27 nM/mg of protein/min. The same purified enzyme transpeptidates (^{14}C glycine) glutathione with methionine to yield y-glutamyl methionine and cysteinyl-^{14}C-glycine under the conditions described previously (Kurelec et al., 1976).

y-glutamyl cyclotransferase was estimated with a partially purified enzyme as described by Orlowsky and Meister (1970), using y-glutamyl alanine as substrate. The activity of the enzyme was monitored for alanine liberation with the amino acid analyser. Purified enzyme from Geodia revealed an activity of 19 nM/mg/min. 5-oxoprolinase was detected in a purified soluble fraction from the Geodia homogenate using the method of Van der Werf et al. (1971). The enzyme converts 6,9% of the L-(U-^{14}C) pyroglutamic acid radioactivity to amino acids eluting from the Dowex 50 column with ammonia. Further analysis of the eluate using a column chromatograph and a scintillation flow counter showed that the largest part of the radioactivity elutes with the first radoactivity peak from the column. It represents a cyclisation product of the glutamic acid. This peck represents 71% of the eluting radioactivity. It does not appear when boiled enzyme was used. 5,1% of the radioactivity was found in glutamine, 4,1% in proline, 1,7% in glycine and 4,2% in alanine. Assuming that all substances eluting with ammonia were originally glutamic acid, the specific activity of a purified oxoprolinase would be 17,3% nM/mg/min.

Glutathione synthetising enzymes, y-glutamyl cysteine synthetase and glutathione synthetase were not estimated. Glutathione occurs in virtually all cells and since in the absence of evidence for transport of glutathione across the cell membrane (Meister, 1975), we assume that sponge cells also synthetize glutathione.

Thus, the cycle, called y-glutamyl cycle, supposed to function as an amino acid transport system, is apparently present also in the tissue of a sponge Geodia cydonium. If we accept that this is the material basis for the uptake of amino acids representing an adequate physiological function, than the use for that particular nitrogen fraction from DOM will shed new light on the physiology of nutrion in the phylum Porifera.

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DISCUSSION

Questions and comments on papers:

"Dissolved Free Amino Acids in Northern Adriatic Waters" and "Presence of γ - Glutamyl Cycle in the Sponge Geodia Cydonium"

1. What does mean the expression "extreme"? (in the sentence ... most extreme samples) (A. BALLESTER, Spain)

- The world "extreme" refers to the two extreme concentrations of amino acids in the sea waters found in our estimations, i.e. to the extreme low and extreme high concentration shown in our slides.

2. What is the origin of freee amino acids present in sea water? (A. BALLESTER, Spain)

- The origins of the amino acids in sea water are input by rivers (terrestial origin), excretion by plants (after photosynthesis!) and animals and decay of dying organisms (bacterial work). Microorganisms in the water collumn, and especially in the sediments, are quantitatively the most important generator for the balanced amino acid cycling within the ecosystem.

3. Founded concentration of "dissolved free amino acids" is relatively very high, i.e. that one can expect very pronounced interaction between some heavy metals (Fe, Zn, Co, Cu, Pb . . .) and these so called free-amino acids. Obviously the real interaction and distribution of species will be influenced by whole chain of interactions and competition between different metals ions (Mg, Ca, heavy metals) as well as relative ligands concentration (from OH^-, Cl^-, HCO_3^-/CO_3^-, SO_4^- . . . to organic compounds . . .) and will be in relation with corresponding stability constants. (M. BRANICA, Yugoslavia).

4. In the previous discussion and in the presented paper from B. Kurelec et al. was stated "free amino acid". Is it not surprising, even if it is only 1/3 free from total amino acid, as was stated, comparing with very high concentrations of metals, other amino acids or proteins which can react? (L. ŠTILINOVIĆ, Yugoslavia)

- Answer to questions 3 and 4:
  The literature on the free amino acids present in sea water ignores the possibility of complexing with heavy metals (ions, ligands). It is highly probable that the analytical technique for the estimation of amino acids, which includes a treatment of the sample on the strong cationic ion exchanger, has prevented highlighting of the chemical state of amino acids in sea water. Our present knowledge distinguishes only the amino acids that are available to the biochemical mechanisms for their uptake from that part of "primary amines" that is not in the chemical form which enables transport (peptides, proteins). Recent studies using Fluram technique established that half of the "primary amines" could be actively taken up by marine phytoplankton just proving that even 50% / and not only 1/3!/ of the amino acids is in the form that is recognized by the very specific enzymes /regardless whether it is gamma-glutamyl transpeptidase or not!/ involved in their transport.