Minisymposium: Metal Ion Transport and Accumulation in Living Organisms

Convener: W. SCHNEIDER; Zurich, Switzerland

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The Transport and Accumulation of Metal Ions in Living Organisms

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The fundamental chemistry involved in the uptake of metal ions by biological systems is well recognized by now [1]. It is an operational necessity of bioinorganic research to separate problems such as the capture of ions, their transport to the final site, and their function. However, the ultimate goal is certainly the elucidation of complete cycles with respect to the fate of metal ions in all the compartments of a biological system and its environment. We are faced with a vast variety of organisms on one hand and a rather restricted series of metal ions on the other hand.

Copper, calcium, and iron are chosen here to represent chemically widely different metal ions for which characteristic pathways emerged from evolution. In natural water systems the overall concentration level drops in the series Ca $\gg$ Fe $>$ Cu. Actually, biological processes exert a significant perturbation on the fluxes of copper in aquatic eco-systems as outlined in the first paper (P. Baccini and D. Piemontesi). The work reported includes a thorough study of selected peptides and their complex formation. The results are revealing with regard to copper–protein interaction. At this conference, this contribution may well be one of the few which is directed towards the full cycle linking an eco-system via phytoplankton to small molecules.

The capture of calcium ions does not raise problems with respect to abundance or hydrolysis. However, the regulation of local concentrations and fluxes are crucial problems in cells (E. Carafoli). Much work has been devoted to the molecular requirements for selective complex formation of Ca^{2+} (*viz.* minisymposia, Nos. C and H).

The biochemistry of iron is largely governed by the competition of biogenic ligands with hydroxide, *i.e.* hydrolysis, which yields solid oxide-hydroxide phases as the limiting product. There are arguments which support the idea that in intracellular iron transport, a symbiotic effect operates whereby delayed hydrolysis secures the passage of iron from the cell membrane to the storage protein ferritin [2]. The latter has been recognized as an ingenious device to encapsulate ferric hydroxide in cells [3, 4]. The dissolution of ferric hydroxide operates at both ends, *i.e.* in the mobilization of iron from stores, and in the uptake of iron by, *e.g.*, microbes. Spectacular mechanisms of iron acquisition via powerful chelating ligands, such as siderophores and enterobactin, were detected about a decade ago. The third contribution (A. Bindereif, P. E. Thorsness and J. B. Neilands) provides deep insight into the molecular genetics of induction and repression of iron transport pathways in microbial systems. The study pushes the elucidation of mechanisms to a sensational limit. Transferrins are ubiquitously involved in iron transport in vertebrates [5]. The mononuclear binding of ferric ions to the protein provides a safe protection from spontaneous hydrolysis. The two-sited nature of transferrin (P. Aisen) has raised even more severe questions than the structure of the sites and the ligand groups of the chromophore. The work presented here provides a splendid case of *in vitro* and *in vivo* studies which complement each other. It is hoped that in the discussions attention will be given to this point with regard to all the contributions.

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The Role of Peptides in the Copper Transport of Aquatic Ecosystems

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The flux of copper through an aquatic ecosystem is controlled by three types of processes, namely

(a) hydrodynamic mixing and particle transport (physical processes)

(b) abiotic transfers from dissolved to particulate phases and *vice versa* (purely chemical processes)

(c) biological processes (*e.g.* assimilation, excretion, decay)

The study of metal balances in lakes has shown that within the system the phytoplankton are mainly responsible for copper transfer from the dissolved to the particulate phase [1]. In aerobic systems the process of type b) is negligible [2]. In anaerobic systems however copper can be co-precipitated on hydrous ferric oxides formed at the interface of the system [3, 4]. In all three phenomena natural organic ligands play a crucial role. Their copper complexes are not available for phytoplankton. The thermodynamic and physicochemical properties of the natural copper complexing ligands (ligands conc.: $\sim 5 \times 10^{-7}$ mol/mg dissolved organic carbon, copper conc.: $\sim 1 \times 10^{-8}$ *M*) lead to the following structural hypothesis [5]:

(1) a molecule of the fulvic acid type (molecular size: $1-3 \times 10^3$ daltons) is folded to form the most stable copper complex, *e.g.* a CuN_4 or CuN_2O_2 chromophore

(2) any peptide chain has one preferential site for copper at the N-terminal end.

The second hypothesis was investigated with model peptides having different chain lengths (from tri- to heptapeptides) and different side chains [6, 7]. Penta- or higher peptides form more stable complexes of the type $Cu(H_{-3}L)$ (the dominant form together with $Cu(H_{-2}L)$ at pH = 8) than shorter peptides. This effect ($\Delta G = 6$ kJ/mol) is due to the formation of a fifth chelate ring to the axial position (Fig. 1). The side chain in position 5 (R_5) can destabilize the complex because of steric (chirality and volume) and electrostatic (charge of R_5) reasons.

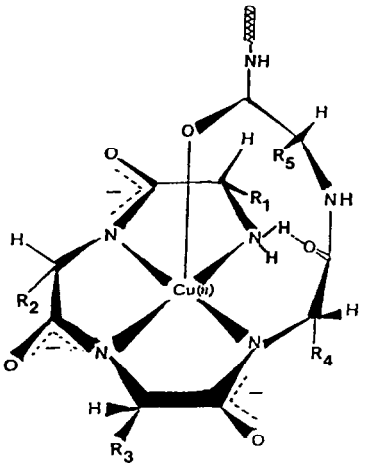

Fig. 1. Structure of the complex $Cu(H_{-3}L)$ for a peptide with more than four amino acids.

It is concluded that certain peptide fragments can compete successfully with inorganic ligands forming copper complexes under natural water conditions at pH > 8. However the model compounds do not reflect the observed characteristics of natural ligands in the pH range from 8 to 9.

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Intracellular Calcium Transport and its Regulation: Calcium Binding Proteins

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In the course of evolution, Ca^{2+} has been chosen as a biological messenger, which regulates the generation of signals at the level of plasma membranes (primary messenger function), and distributes the signals to a large number of targets inside cells (secondary messenger function). Since the plasma membrane signals are generally electrical signals (ionic currents) the primary messenger function involves the modulation by Ca^{2+} of the ionic fluxes (essentially, K^+, Na^+, Ca^{2+} fluxes) between cells and the environment. The secondary messenger function involves complexation of Ca^{2+} with proteins, mostly enzyme proteins, which will then become activated or deactivated and influence various metabolic pathways. The Ca^{2+} that carries out the secondary messenger function may come directly from outside the cell through the plasma membrane. In general, however, it reaches the compartment where a given target is located and eventually the target itself, from storage places inside cells. Storage points may be intracellular membrane bonded organelles, which reversibly sequester Ca^{2+} away from the soluble phase of the cell, or soluble (protein) Ca^{2+} ligands, which act as Ca^{2+} reservoirs that either complex Ca^{2+} or liberate it to the soluble phase of the cell essentially according to the law of mass action. It must be understood, however, that the reversible sequestration of Ca^{2+} into organelles also requires the complexation of Ca^{2+} with specific (protein) ligands, that mediate its transport across the lipid bilayer membrane of the organelles. And it may be added as a corollary that intracellular organelles also contain aqueous spaces which contain Ca^{2+}-sensitive enzymic targets. The reversible sequestration of Ca^{2+} into the intracellular organelles, conventionally seen as a means to modulate Ca^{2+} in the extra-organellar, soluble phase of the cell, is thus essential to the regulation of Ca^{2+}-sensitive targets inside organelles as well.