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.

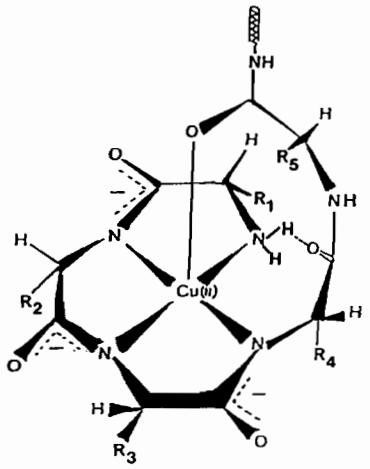

Fig. 1. Structure of the complex $Cu(H_{-3}L)$ for a peptide with more than four amino acids.

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.

1 P. Baccini et al., *Schweiz. Z. Hydrol.*, *41*(2), 202–227 (1979).
2 P. Baccini et al., *Schweiz. Z. Hydrol.*, *44*(1), 99–116 (1982).
3 D. P. H. Laxen and E. R. Sholkovitz, *Env. Techn. Letters*, *2*, 561–568 (1981).
4 P. Baccini and Th. Joller, *Schweiz. Z. Hydrol.*, *43*(1), 176–199 (1981).
5 P. Baccini and U. Suter, *Schweiz. Z. Hydrol.*, *41*(2), 291–314 (1979).
6 D. Perret, Ph.D. Thesis, Univ. of Neuchâtel (1980).
7 D. Piemontesi, Ph.D. Thesis, Univ. of Neuchâtel (1982).

J3

Intracellular Calcium Transport and its Regulation: Calcium Binding Proteins

ERNESTO CARAFOLI

Laboratory of Biochemistry, Swiss Federal Institute of Technology (ETH), 8092 Zürich, Switzerland

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.

The evolutionary choice of Ca^{2+} as a biological messenger has in all likelyhood been dictated by its chemical properties, which greatly favor it over the other cations available in the original environment (K^+, Na^+, Mg^{2+}, NH_4^+). The divalent ion Ca^{2+}, which has a radius of slightly less than 1 Å, is ideally qualified to fit irregular binding cavities of the type normally encountered in folded proteins. A comparison with the other divalent cation originally available, Mg^{2+}, (ionic radius 0.65 Å) offers a plausible explanation for this. The smaller polarising power of Ca^{2+} imposes no stringent geometric demands on the cavity designed to accept it, whereas the greater polarizing power of Mg^{2+} does. The flexibility in accepting binding cavities is evidently a decisive advantage for an ion designed to act as a messenger. This is so because the messenger function requires that the ion be kept at very low activity levels inside cells, where the targets of the messenger function are found. This evidently requires easy binding of the ion to complex X_1 (protein) ligands of the type found inside cells. The messenger function also requires that the activity of the messenger ion inside cells be rapidly and precisely modulated around the low background level, and this in turn demands that the interaction of the ion with the complex ligands be easily reversible. Soluble and membrane-bound ligands are both convenient, the latter having the additional advantage of modulating the activity of Ca^{2+} into cells by mediating its reversible transport across membrane phases into different aqueous compartments.

The importance of the messenger function of Ca^{2+} is underscored by the existence of a large number of intracellular specific binding proteins, and by the fact that a variety (up to 7) of systems exist for its transport across cellular membranes. The redundancy of the transport systems is a particularly striking reminder of the absolute necessity of controlling the levels of free Ca^{2+} in all compartments of the cell. The fact that completely different transport modes often coexist in the same membrane is evidently dictated by the necessity of controlling Ca^{2+} with precision and flexibility. This is so because the various transport systems have characteristics (K_m, V_{max}, specificity, inhibition, regulation) that qualify them ideally for intervening at specified moments of the physiological life of cells.

So far, the following Ca^{2+} transport modes have been described in different membranes: 1) 2 specific pumping ATPases, 2) 2 Ca^{2+}/Na^+ exchange systems, 3) at least one Ca^{2+}/H^+ exchange system, 4) one electrophoretic, charge uncompensated transport system. Some of these transport systems have been solubilized, purified, reconstituted, and are now amenable to molecular studies. Others can at the moment only be studied functionally, in situ. Of particular interest is the finding that calmodulin, perhaps the most interesting of all intracellular Ca^{2+} binding proteins, and undoubtedly the most important carrier of the Ca^{2+} message to (enzyme) targets, stimulates some of the systems for the transport of Ca^{2+} across membranes. A comprehensive picture would thus have Ca^{2+} not only regulating scores of important enzymic activities within cells, but even its own balance between cells and the environment, and among the various compartments of the cell.

1 E. Carafoli and M. Crompton, 'The Regulation of Intracellular Calcium', *Current Topics in Membranes and Transport, 10*, 151 (1978).
2 F. L. Siegel, E. Carafoli, R. H. Kretsinger, D. H. Mac Lennan and R. H. Wassermann (eds.), *'Calcium-Binding Proteins: Structure and Function', Developments in Biochemistry*, Vol. 14, Elsevier, Amsterdam, 1980.

J4

Deletion Mapping of the Aerobactin Gene Complex of Plasmid ColV

ALBRECHT BINDEREIF, PETER E. THORSNESS and J. B. NEILANDS*

Department of Biochemistry, University of California, Berkeley, Calif. 94720, U.S.A.

With the possible exception of certain strains of lactic acid bacteria, iron is known to be an essential element for all species of microbes and for all higher organisms. Within the cell, iron protein catalysts participate in some of the reactions most fundamental to life, including aerobic and anaerobic energy metabolism, nitrogen fixation, photosynthesis, and generation of the deoxyribotides required for synthesis of DNA. Iron is abundant in the environment but may be insoluble or in some other way unavailable. Mechanisms for assimilation of iron have been evolved by all species but, because of the plasticity and diversity of microbial metabolism, those in unicellular organisms are the most readily studied.

The particular system of microbial iron acquisition investigated in this laboratory is labelled *high-affinity* and consists of low molecular weight, virtually Fe(III) specific ligands, termed siderophores, and the matching membrane receptors for the complexed Fe(III) ion. This system, which is coordinately expressed under iron starvation, has been detected in virtually all aerobic and facultative anaerobic microorganisms carefully examined for its presence [1].

In general, siderophores can be relegated to one of two chemical classes, *viz.*, hydroxamic acids and catechols. The number of siderophores characterized to date, some by crystallography and/or high resolution nuclear magnetic resonance spectroscopy, must now number several score. The complete system, comprised of siderophore and specific receptor, is