3: Calcium in Biological Systems

Calcium, like many other "inorganic elements" in biological systems, has during the last decade become the subject of much attention both by scientists and by the general public.[1] The presence and central role of calcium in mammalian bones and other mineralized tissues were recognized soon after its discovery as an element by Davy in 1808. Much later, the insight arrived that Ca^{2+} ions could play an important role in other tissues as well. Experiments of great historical influence were performed by the British physiologist Sidney Ringer a little over a century ago.[2] He was interested in the effects of various cations on frog-heart muscle and somewhat serendipitously discovered that Ca^{2+} ions, everpresent in the tap water distributed in central London, in millimolar concentrations were necessary for muscle contraction and tissue survival.

Today it is widely recognized that Ca^{2+} ions are central to a complex intracellular messenger system that is mediating a wide range of biological processes: muscle contraction, secretion, **glycolysis** and **gluconeogenesis**, ion transport, cell division and growth (for definitions of terms in boldface, see Appendix A in Section IX). The detailed organization of this messenger system is presently the subject of considerable scientific activity, and some details are already known. One of the links in the system is a class of highly homologous Ca^{2+}-binding proteins, to be discussed later on in this chapter, that undergo Ca^{2+}-dependent conformational changes and respond to transitory increases in intracellular Ca^{2+}-ion concentrations. A prerequisite for the proper function of the calcium messenger system in higher organisms is that the **cytosolic** Ca^{2+} concentration in a "resting" cell be kept very low, on the order of 100 to 200 nM. Transitory increases in the Ca^{2+} concentration that may result from hormonal action on a membrane receptor must rapidly be reduced. Several transport proteins, driven either by ATP hydrolysis or by gradients of some other ion like Na^{+}, are involved in this activity.

Ca^{2+} ions are also known to play various roles outside cells. In the plant kingdom Ca^{2+} ions often form links between individual cells and are required for maintaining the rigidity of whole plants; some seaweeds are typical examples. In the blood plasma of mammals, in which the Ca^{2+} concentration exceeds the intracellular by a factor of about 10^4, Ca^{2+} ions are instrumental in joining certain proteins in the blood-clotting system with membrane surfaces of circulating cells. Many extracellular enzymes also contain Ca^{2+} ions, sometimes at the active site but most often at other locations. It is generally believed that Ca^{2+} ions confer on proteins an increased thermal stability, and indeed proteins in heat-tolerant microorganisms often hold many such ions.

Vertebrates require much calcium in their food; in the USA the recommended daily allowance (RDA) for adult humans is 800 mg, and most other countries have comparable recommendations. During gestation in mammals, calcium must be transported across the placenta into the fetus, in particular during those phases of pregnancy when bone formation is most rapid. Interestingly, there appear to be some parallels between intestinal and placental transport that will be discussed further below. The role of calcium in biominerals is a vast subject that we can treat only superficially in this chapter.

To provide a background to the more biologically oriented sections that follow, we begin with a brief recapitulation of some basic facts about calcium. Then we continue with an outline of calcium distribution in biological tissues and organelles, and of the methods that can be used to obtain this information. After this follows a brief section on Ca^{2+} transport, and an account of the mechanism of intracellular Ca^{2+} release as it is presently understood. A discussion of some selected Ca^{2+}-binding proteins of general interest, both intracellular and extracellular, then follows. Before we conclude the chapter, we will summarize some recent observations on Ca^{2+}-binding proteins in prokaryotes.

II. Basic Facts About Calcium: Its Compounds and Reactions

A. Basic Facts

B. Essentials of Ca^{2+} Chemistry

III. Calcium In Living Cells: Methods For Determining Concentrations And Spatial Distributions

A. Measurements of "Free" Calcium Concentrations

1. Ca^{2+}-selective microelectrodes
2. Bioluminescence
3. Complexing Agents with Ca^{2+}-dependent Light Absorption or Fluorescence
4. Complexing Agents with Ca^{2+}-dependent NMR Spectra

B.

Measurements of Total Calcium Concentrations

1. Electron Probe and Electron Energy-loss Techniques
2. Proton-induced X-ray Emisssion (PIXE)
3. Ion Microscopy

C. Summary

Much of our present knowledge about the biological role of Ca^{2+} rests on detailed measurements of the concentration, distribution, and chemical nature of Ca^{2+} and its complexes. Concentrations of uncomplexed, or "free," Ca^{2+} can be measured by Ca^{2+}-selective microelectrodes, bioluminescence and complexing agents with Ca^{2+}-dependent light absorption, fluorescence, or NMR spectra. An outcome of such studies is that the "free" Ca^{2+} concentration in resting **eukaryotic** cells generally is very low, on the order of 100 to 200 nM. Total Ca^{2+} concentrations, uncomplexed and complexed, can be measured by a variety of physical techniques. Some techniques, like atomic absorption, are sensitive but give poor spatial resolution. Others involve the bombardment of the sample with electrons or charged atoms, and can yield spatial resolutions of the order of a few nm; however, there is a trade-off between detectability and resolution

IV. The Transport and Regulation of Ca^{2+} Ions in Higher Organisms

A. Ca^{2+} Uptake and Secretion

B. Intracellular Ca^{2+} Transport

1. The Ca^{2+}-ATPases
2. The Na^{+}/Ca^{2+} Exchanger of the Plasma Membrane
3. Mitochondrial Ca^{2+} Transport: Influx
4. Mitochondrial Ca^{2+} Transport: Efflux
5. Ca^{2+} Efflux from Non-mitochondrial Stores
6. Other Voltage-gated or Receptor-activated Ca^{2+} Channels

C. Inositol Trisphosphate and the Ca^{2+} Messenger System

D. Summary

The fluxes of Ca^{2+} ions and their regulation in higher organisms, as well as in microorganisms, depend on several transport proteins in addition to vesicular and gated processes. An important class of transport proteins are the Ca^{2+}-ATPases, which are particularly abundant in muscle cells. These proteins translocate Ca^{2+} ions against large activity (or concentration) gradients through the expenditure of ATP. Transport of Ca^{2+} ions against activity gradients across membranes may also be accomplished by coupled transport of other ions, like Na^{+}, with a gradient in the opposite direction.

As a result of some external stimulus—the action of a hormone, for example—the "free" Ca^{2+}-ion concentrations in the cytoplasm of many cell types may transiently increase several orders of magnitude. This increase largely results from the release of Ca^{2+} from intracellular stores (ER, SR) in response to the initial formation of a new type of messenger, 1,4,5-IP_3. The activity of Ca^{2+}-transport proteins eventually restores the Ca^{2+} concentration levels to resting levels. This sequence of events forms the basis for Ca^{2+}'s role in the regulation of a wide variety of cellular activities (see Section V).

V. Molecular Aspects of Ca^{2+}-regulated Intracellular Processes

A. Calmodulin

B. Troponin C

C. Parvalbumin and Calbindins D_{9K} and D_{28K}

D. Sarcoplasmic Calcium-Binding Protein from *Nereis diversicolor*

E. Membrane Cytoskeleton and Phospholipid Binding Proteins

F. Ca^{2+}-dependent Proteases

G.

Protein Kinase C

H. Summary

Many different biological processes in eukaryotic cells are regulated by intracellular Ca^{2+} concentration levels. Examples of such processes are muscle contraction, transport processes, cell division and growth, enzyme activities, and metabolic processes. A link in this regulatory chain is a number of intracellular Ca^{2+} receptors with Ca^{2+} affinities such that their binding sites are largely unoccupied at resting Ca^{2+} concentration levels, but are occupied at Ca^{2+} levels reached as a result of some external stimulus. This class of Ca^{2+} receptors is often called the "calmodulin superfamily" and includes the well-known members troponin C (regulating muscle contraction in striated muscle) and calmodulin (playing an important role in the regulation of many cellular processes). Amino-acid sequence determinations as well as x-ray and 2D ^1H NMR studies have revealed a strong homology between the regulatory Ca^{2+}-binding proteins. The Ca^{2+}-binding sites are located in a loop flanked by two helices, and the Ca^{2+} ions are ligated with approximately octahedral or pentagonal bipyramidal symmetry. The ligands are six or seven oxygen atoms that are furnished by side-chain carboxylate or hydroxyl groups, backbone carbonyls, and water molecules. Pairs of these Ca^{2+} sites, rather than individual sites, appear to be the functional unit, and a common consequence of their arrangement is cooperative Ca^{2+} binding. Ca^{2+} binding to the intracellular receptor proteins is accompanied by structural changes that expose hydrophobic patches on their surfaces, thereby enabling them to bind to their target proteins.

VI. Extracellular Ca^{2+}-binding Proteins

A. Ca^{2+}-binding in Some Extracellular Enzymes

B. Summary

In higher organisms, the Ca^{2+} concentration in extracellular fluids generally is considerably higher than the intracellular concentrations. In mammalian body fluids, the Ca^{2+} concentration is typically on the order of a few mM. The extracellular concentration levels are highly regulated and undergo only minor variations. A consequence of these high levels of Ca^{2+} in extracellular fluids is that the binding constant need be only 10^3 to 10^4 M^{-1} in order for a protein site to be highly occupied by Ca^{2+}. Several extracellular enzymes and enzyme activators have one or more Ca^{2+} ions as integral parts of their structures. Some Ca^{2+} ions are bound at, or near, the active cleft and may take part in the enzymatic reactions (e.g., phospholipase A_2, α-amylase). In other molecules, for example, serine proteases like trypsin and chymotrypsin, the Ca^{2+} ion is not essential for enzymatic activity, and may play more of a structural role. Ca^{2+} ions are involved in the cascade of enzymatic events that results in blood clotting in mammals. Several of the proteins in this system contain two new amino acids, γ-carboxyglutamic acid (Gla) and β-hydroxyaspartic acid (Hya), which are strongly suspected to be involved as ligands in Ca^{2+} binding. In the presence of Ca^{2+} ions, prothrombin and other Gla-containing proteins will bind to cell membranes containing acidic phospholipids, in particular, the platelet membrane. It appears likely that Ca^{2+} ions form a link between the protein and the membrane surface.

VII. Calcium in Mineralized Tissues

Summary

Calcium is, along with iron, silicon, and the alkaline earth metals, an important constituent of mineralized biological tissues. Some Ca^{2+}-based biominerals, like bone or mother-of-pearl, can be regarded as complex composites with microscopic crystallites embedded in a protein matrix. The formation of calcified biominerals is a highly regulated process, and human bone, for instance, is constantly being dissolved and rebuilt. When the rates of these two counteracting processes are not in balance, the result may be decalcification, or *osteoporosis*, which seriously reduces the strength of the bone.

VIII. Ca^{2+}-binding Proteins in Microorganisms: The Search for a Prokaryotic Calmodulin

Summary

The role of Ca^{2+} ions in the regulation of biological activities of procaryotic organisms is still largely unsettled. Over the last decade, however, evidence has gradually accumulated that calcium ions are involved in diverse bacterial activities, such as chemotaxis and substrate transport, sporulation, initiation of DNA replication, phospholipid synthesis, and protein phosphorylation.[168] An important landmark is the recent demonstration that the intracellular Ca^{2+} concentration in *E. coli* is tightly regulated to about 100 nM, a level similar to that typical of resting eukaryotic cells.[169] Furthermore, increasing numbers of calcium-binding proteins, some of which also have putative EF-hand Ca^{2+} sites characteristic of the calmodulin superfamily of intracellular regulatory proteins, have been isolated in bacteria.[168]

IX. Appendixes

A. Definition of Biochemical Terms

Antiport	A transport protein that carries two ions or molecules in opposite directions across a membrane.
Basal lateral membrane	The membrane in intestinal epithelial cells that is located on the base of the cells, opposite the microvilli that face the intestinal lumen.
Cytosol	The unstructured portion of the interior of a cell—the cell nucleus excluded—in which the organelles are bathed.
Electrogenic	A biological process driven by electric field gradients.
Endocytosis	The process by which eukaryotic cells take up solutes and/or particles by enclosure in a portion of the plasma membrane to (temporarily) form cytoplasmic vesicles.
Endoplasmic reticulum (ER)	Sheets of folded membranes, within the cytoplasm of eukaryotic cells, that are the sites for protein synthesis and transport.
Epithelial cells	Cells that form the surface layer of most, if not all, body cavities (blood vessels, intestine, urinary bladder, mouth, etc.).
Erythrocytes	Red-blood corpuscles.
Eukaryotic cells	Cells with a well-definied nucleus.
Exocytosis	The process by which eukaryotic cells release packets of molecules (e.g., neurotransmitters) to the environment by fusing vesicles formed in the cytoplasm with the plasma membrane.
Gluconeogenesis	Metabolic synthesis of glucose.
Glycolysis	Metabolic degradation of glucose.
Hydropathy	A measure of the relative hydrophobic or hydrophilic character of an amino acid or amino-acid side chain.
Lamina propria mucosae	The layer of connective tissue underlying the epithelium of a mucous membrane.
Mitrochondrion	A double-membrane organelle in eukaryotic cells that is the center for aerobic oxidation processes leading to the formation of energy-rich ATP.
Organelle	A structurally distinct region of the cell that contains specific enzymes or other proteins that perform particular biological functions.
Osteoporosis	Brittle-bond disease.
Phorbol esters	Polycyclic organic molecules that act as analogues to diacylglycerol and therefore are strong activators of protein kinase C.
Prokaryotic cells	Cells lacking a well-defined nucleus.
Sarcoplasmic reticulum	The ER of muscle cells.
Trophoblasts	The cells between the maternal and fetal circulation systems.

Tryptic digest	Fragmentation of proteins as a result of treatment with the proteolytic enzyme trypsin.
Uniporter	A transport protein that carries a particular ion or molecule in one direction across a membrane.

B. One-Letter Code for Amino-Acid Residues

A—alanine, C—cysteine, D—aspartate, E—glutamate, F—phenylalanine, G—glycine, H—histidine, I—isoleucine, K—Iysine, L—Ieucine, M—methionine, N—asparagine, P—proline, Q—glutamine, R—arginine, S—serine, T—threonine, V—valine, W—tryptophan, Y—tyrosine.

C. The Activity of a Transport Protein

This is usually described in terms of the classical Michaelis-Menten scheme:

$$V(=transport\ rate) = V_{max} \cdot \frac{[S]}{[S] + K_m}, \tag{3.1}$$

where [S] is the concentration of the solute to be transported and K_m = $(k_{-1}+k_2)/k_1$ is the Michaelis constant (dimension "concentration") for the reaction

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} R. \tag{3.2}$$

Approximated as the reciprocal ratio between on- and off-rate constants relevant to the solute-protein complex, $1/K_m = k_1/k_{-1}$ may be taken as a lower limit of the affinity of the protein for the solute.

X. References

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