Calcium – how and why?

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Calcium is among the most commonly used ions, in a multitude of biological functions, so much so that it is impossible to imagine life without calcium. In this article I have attempted to address the question as to how calcium has achieved this status with a brief mention of the history of calcium research in biology. It appears that during the origin and early evolution of life the Ca^{2+} ion was given a unique opportunity to be used in several biological processes because of its unusual physical and chemical properties.

1. History of calcium research

The recent explosive growth in research related to the cellular roles of calcium has established the importance of this ion throughout the history of a cell. It triggers new life at fertilization, it controls several developmental processes, and once cells have differentiated it functions to control diverse cellular processes such as metabolism, proliferation, secretion, contraction, learning and memory. New roles played by calcium in biological systems are constantly being identified (Berridge *et al* 1999). Indeed, it is difficult to find a physiological process in a cell that is not dependent on calcium. A large number of reviews have dealt with the role of calcium in gene expression and cell physiology, hence I will not discuss these. Instead, I will try to answer the question, as to why calcium is preferred over the other ions in carrying out so many functions? Earlier Ochiai El-Ichiro (1991) had addressed this question, but only regarding a limited number of chemical features of Ca^{2+} ions. However, in this article I will attempt to examine a wider range of chemical and physical features of Ca^{2+} ion that make it unique.

It is possible that acquisition of this special status by calcium was a "frozen accident" in evolution, which the organisms have learnt to live with. However, I will try and present evidence and arguments that favour the possibility that it is the unique chemical and physical properties of Ca^{2+} ion and not a chance event that has made it almost indispensable for the survival of most organisms known today.

The story of calcium began in 1808, when in one of the earliest uses of electrochemistry, Humphry Davy isolated this element from alkaline earth (Trifonov and Trifonov 1982). But it was not until another three-quarters of a century later that Sydney Ringer first demonstrated the biological significance of calcium. In 1883 Ringer first showed that frog hearts need the presence of calcium in the bathing solution in order to continue beating (Campbell 1988). This seminal observation eventually opened up an entire field of study pertaining to the role of calcium in molecular, cellular and even organismal motility. The list of functions for calcium that were identified by the end of 19th century include its role in egg fertilization and development of tissues (bone and calcareous skeleton) (Ringer and Sainsbury 1894), conduction of nerve impulse to muscle, cell adhesion, plant growth (Campbell 1988). The 20th century did not see any slowdown in the process of identifying the cellular functions of calcium, which led to deciphering the importance of calcium in cellular functions necessary for both normal and diseased states of the cell (Mooren and Kinne 1998). Another important discovery regarding the role of calcium was the identification of change in the level of free

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calcium in response to hormone treatments (Hermann 1932). This hinted that the signal that linked hormone treatment to a cellular response might be calcium. More recent work explains how a precise temporal and spatial regulation of Ca^{2+} level, by either an influx from outside or efflux from the stores, play a major role in bringing about the hormone response (Peterson *et al* 1999).

In 1928 Lewis Victor Heilbrunn made an interesting observation that when a cell is torn or broken, it seals itself by a process which is dependent on the presence of calcium ion. He called this process 'surface precipitation reaction' (s.p.r.). He hypothesized that s.p.r. is the basic colloidal reaction of protoplasm and many of the effects produced by chemical or physical agents on protoplasm can be interpreted in terms of it. In an ordinary s.p.r., contents of the cell are exposed to the calcium in the medium that effects the protoplasm in a manner similar to clotting of blood. The same type of reaction can occur if calcium is injected into the cell or if the cell cortex is liquefied, causing a release of calcium into the cell. He proposed that the release of calcium from the cell cortex provokes the s.p.r. and that this reaction in a muscle, was responsible for the shortening of the fibre. Also in a marine egg cell, increase in free calcium results in the formation of the mitotic spindle, leading to further development of the egg. By injecting various cations into frog muscle cells, he demonstrated that it was only calcium that could cause the muscle fibre to contract (Heilbrunn and Wiercinski 1947). Later in 1952, Sandow proposed the term excitation-contraction coupling for this phenomenon. Heilbrunn's views are best summarized by the following statement published in 1937 in his book *An outline of general physiology*: "The sensitivity of protoplasm and its response to stimulation are believed to be due to a sensitivity to free calcium and it is believed that the freeing of calcium and the reaction of this calcium with the protoplasm within the cell is the most basic of all protoplasmic reactions".

2. Outcome of the ability to measure cellular calcium

In spite of insightful views of the early workers, it took until the late 1960s for the fundamental importance of calcium in biology to be widely acknowledged. The reasons behind this delay may have been many, but perhaps one of the most important was the lack of techniques and reagents that could establish definitively a primary role for calcium in cellular functions. A methodological advance that had revolutionary impact on establishing the biological relevance of calcium was the ability to measure cellular calcium levels. The first attempt in this direction was made by Pollack in 1928, when he used alizarin sulphonate to measure free Ca^{2+} level. However, Pollack's method could not be replicated successfully and hence did not gain wide acceptance. A major breakthrough came with the introduction of the Ca^{2+}-sensitive photoprotein aequorin to measure calcium (Ridgeway and Ashley 1967). Other approaches that have also proven useful in measuring calcium include Ca^{2+} sensitive microelectrodes and metallochromic Ca^{2+} indicators (Thomas 1982). But owing to their greater range of sensitivity and ease of usage, calcium sensitive fluorescent indicators such as indo-1 and fura-2 (Tsien 1980; Grynkiewicz *et al* 1985) have been the most widely used tools for calcium measurements (for review see Cobbold and Rink 1987; Hayashi and Miyata 1994). The advances in the field of fluorescence microscopy and imaging further assisted in enhancing the utility of the fluorescent indicators for performing *in situ* measurement of calcium in live cells. More recently the development of a calcium sensitive (GFP-based) fluorescent protein "cameleon", has provided a non-invasive fluorescence-based measurement of calcium (Miyawaki *et al* 1997, 1999). Besides being non-invasive these new generation calcium sensor molecules have also been targeted to various cellular compartments thus enabling a study of the spatial and organellar aspects of calcium homeostasis (Arnaudeau *et al* 2001).

Thanks to these technical advances we have begun to understand the intricate spatio-temporal mechanisms that the cell uses to maintain calcium levels in the cytoplasm and in the various stores, and uses them specifically as the situation warrants. However it is still not clear as to how these calcium stores interact with each other and with the cytoplasm, and how this gamut of interactions generate complex signals that are beyond the reach of a simpleminded cytosolic calcium alteration. Besides the issue of static spatio-temporal signals, is the issue of how the calcium signals are presented in a pulsatile (oscillatory) manner and how the amplitude and frequency of this oscillation act as a signal to regulate gene expression (Dolmetsch *et al* 1997, 1998; Li *et al* 1998).

3. Evolution of calcium as a messenger

Apart from how calcium brings about its effects in the cell, an important question is why, amongst so many other inorganic ions, calcium seems to have a special status. Though there can be no definite answer to this question, I will point out some facts which may throw light on the subject.

A feature that makes calcium such a versatile ion in signalling is the ability of the cell to precisely regulate the cellular concentrations of free and sequestered calcium both in time and space. Calcium stores and calcium specific ion channels are two mechanisms that the cell uses to

regulate its free calcium levels. It is believed that calcium stores in cells could have originated primarily for the purpose of defence against the 'calcium holocaust' (Campbell 1988). When life was originating on earth, calcium was abundant in the igneous rocks present in the earth's hot crust and was unavailable for use by living matter. As the earth cooled various chemical and biological reactions caused the extracellular free calcium levels to rise. The problem was serious since calcium at high concentration causes organellar damage and proteins and nucleic acids to aggregate, and leads to precipitation of the phosphates (necessary for the energy transactions in the cell). Each of these events would cause instant death of the cell. Therefore, it was essential for cells to contain calcium in a manner that it was no longer harmful. This could have been the basis for the selective pressure that led to the evolution of calcium pumps and internal calcium stores, by which cells could maintain their cytosolic free calcium at tolerable levels. Such an arrangement would imply the existence of a huge electrochemical gradient of calcium across the cell membrane. If one was to ignore the contribution of other ions in the cell and consider the external calcium concentration as in table 1, then given that the average transmembrane potential of a cell is – 60 mV, the cytosolic calcium level would be 0·1–0·2 M; nearly 1000-folds higher than what is actually seen in cells (Ochiai 1991).

The cell would have been forced to evolve mechanisms to control these pumps, which it could have then used for triggering cell activation. Thus the presence of calcium pumps and varied calcium stores in the cell could be a feature that contributed to the use of this ion as a messenger (Campbell 1988). Since prokaryotes appear to make much less use of calcium, it could be argued that calcium signalling mechanisms probably evolved after the divergence prokaryotes and eukaryotes.

But since it is hard to tell if the lesser prevalence of calcium signalling in prokaryotes is due to a selective loss or due to a delayed acquisition of this ability, it is likely that a 'calcium holocaust' affected the observed prevalence of the Ca^{2+} ion in biology.

The above hypothesis appears to be a plausible explanation for the abundance of calcium in biology, but it falls short of answering why calcium is preferred to other cations such as H^+, Mg^{2+}, Zn^{2+}, Na^+, and K^+, which are also abundant in biological systems. In most living cells Ca^{2+} and Na^+ ions are pumped out of the cell and thus maintained at low concentrations inside (10^{-7} M and 10^{-4} M respectively) relative to the outside environment, while K^+ and Mg^{2+} are pumped into the cell and are hence present in much higher concentrations in the cytoplasm (10^{-1} M and 10^{-3} M respectively). Moreover, there are many proteins and substrates inside the cells that would form a complex with either Ca^{2+} or Mg^{2+} with a binding constant of 10^{-3}–10^{-4} M. Thus under physiological conditions Mg^{2+} is bound to them while Ca^{2+} is not. However, many cells have proteins with binding constant of as much as 10^{-6} M for calcium. Alteration in the activity of the Ca^{2+} pump can cause a rapid increase in the intracellular calcium (up to a 100-fold), increasing the cytosolic calcium con-

Table 1. Abundance of various elements.

	Abundance (per cent)[a]			Total cellular conc. (mM)[b]			
				Intracellular		Extracellular	
Element	Ocean	Plants	Human body[b]	Squid axon	Human erythrocyte	Squid axon	Human erythrocyte
Hydrogen	10·7	16	10				
Oxygen	85·7	49	65				
Carbon	$2{\cdot}8 \times 10^{3}$	21	18				
Nitrogen	$6{\cdot}7 \times 10^{-5}$	3	3				
Calcium	0·0411	0·10	1·50	5×10^{-4}	0·1	10	2·5
Magnesium	0·129	0·04	0·05	7	2·5	55	1·5
Sodium	1·08	0·01	0·15	10	11	440	152
Potassium	0·0392	0·10	0·20	300	92	22	5
Iron	$< 10^{-7}$	0·005	0·006				
Zinc	$< 10^{-7}$	0·02	0·003				
Silicon	$2{\cdot}9 \times 10^{-4}$	0·10	0·002				
Copper	$< 10^{-7}$	0·0006	0·0002				
Chloride	1·94	0·01	–	40–150	560	4	110
Barium	3×10^{-7}	–	$3{\cdot}0 \times 10^{-6}$				
Strontium	8×10^{-5}	–	$4{\cdot}6 \times 10^{-5}$				

[a]*Encyclopaedia Brittanica*; [b]Kaim and Schwederski 1996.

centration to 10^{-5} M. This can trigger binding of calcium to these proteins, leading to a specified cellular activity. Since the intracellular level of Mg^{2+} ion is already high, a rapid and large (> 10-fold) increase is not possible for this ion, limiting its use as a trigger for rapid cell activation. The cells have indeed made use of the rapidity of calcium-mediated signal in variety of cellular signalling pathways. This unique feature of calcium could be one of the causes for Ca^{2+} ion attaining the distinction of most widely used 'second messenger' ion.

Ba^{2+} ion is one of the few ions that in some cases have been found to be able to substitute the requirement of Ca^{2+} ion, for example in regulating enzyme activity (Price 1975; Goodwin and Anthony 1996). But Ba^{2+} ions are present at such low concentrations in the cell that it cannot substitute the second messenger functions that is displayed by the Ca^{2+} ions. Thus, even though cells do make use of Ba^{2+} ions, their usage is very limited as compared to Ca^{2+} ions.

4. Favourable chemical nature of calcium

A further possible answer to the question regarding the ubiquitous role of calcium lies in the chemistry of this element. This involves its molecular structure, valence state, binding strength, ionization potential and kinetic parameters in biological reactions. In order to have a wide-range of biological effects, a metal ion should be able to interact with the biological ligands such as proteins, lipids and carbohydrates. For such interactions it is necessary that the metal ion should be able to bind the commonly available reaction centres in these biomolecules, for which it ought to be able to display the requisite coordinate chemistry. Unlike Mg^{2+} and Zn^{2+}, which bind with greater affinity to nitrogen ligands, Ca^{2+} ion has the maximum affinity for carboxylate oxygen. Acidic amino acids like aspartic acid, and glutamic acid that contain the carboxylate oxygen occur more frequently in proteins as opposed to the nitrogen containing ones like asparagine, histidine and glutamine, making Ca^{2+} more widely used than the other two cations (Ochiai 1991). Humans have an excess of 0·82% of (Glu + Asp) over (Asn + His + Glu) amino acids. The important thing is that this translates into an excess of 77860 (Glu + Asp) as compared to (His + Gln + Asn). If this excess happens to be in proteins, specifically involved in cell signalling and not spread over all classes of proteins, then this difference can have significant effect on making Ca^{2+} an ion preferred over Mg^{2+} for the purpose of signalling. Another relevant parameter for a good second messenger is the rapidity of its binding kinetics. As a measure of such a parameter, the rates of water exchange of metal aquo complexes can be utilized. Such a rate (in units of s^{-1} at 25°C) has been found to be 10^8 for Ca^{2+}, 2×10^7 for Zn^{2+} and Mn^{2+}, 7×10^5 for Mg^{2+}. Thus, Ca^{2+} binds to and dissociates from a protein 100-fold or so faster than Mg^{2+} (Ochiai 1991).

The Ca^{2+} ion typically exhibits high (6–8) coordination numbers and often irregular (i.e. protein induced) coordination geometry due to its favourable ionic radius (100–120 pm) (table 2) and its electronic structure (Swain and Amma 1989; Carugo *et al* 1993).

The Cd^{2+} (95 pm) and Pb^{2+} (119 pm) ions are similar to Ca^{2+} but are biologically harmful due to their strong coordination with thiolates (Cys-). Mn^{2+} (83 pm) and the heavier homologue Sr^{2+} (118 pm) are less toxic calcium substitutes, the possible biological importance of Sr^{2+} perhaps being obscured by the much more abundant Ca^{2+}. Unlike the irregular coordination chemistry of Ca^{2+}, which allows it to be accommodated into a larger number of proteins, Mg^{2+} exhibits a strong preference for an octa-

Table 2. Geometric-parameter for metal-carbonyl interactions in protein (Chakrabarti 1990).

Protein	Metal	Residue	M–O distance (pm)	M–O=C angle (deg)	Angle (deg)	
Calmodulin	Ca	T26	246	164·1	187·0	74·5
		T62	217	170·9	179·8	80·2
		Y99	206	170·2	188·1	85·3
		Q135	238	147·6	188·6	58·3
Cytochrome P450cam	Ca	E84	269	157·1	170·0	67·6
		G93	275	148·8	148·8	88·5
		E94	281	113·6	228·9	39·3
		Y96	264	142·5	208·5	64·5
Bacteriochlorophyll A	Mg	L234	204	155·1	204·6	86·4
Trp repressor	Na	E60	274	117·1	241·6	74·3
Thermolysin complex	Zn	O	200	119·7	120·9	74·0
		O1	210	121·0	124·2	65·2
		O1	285	94·9	97·0	47·0

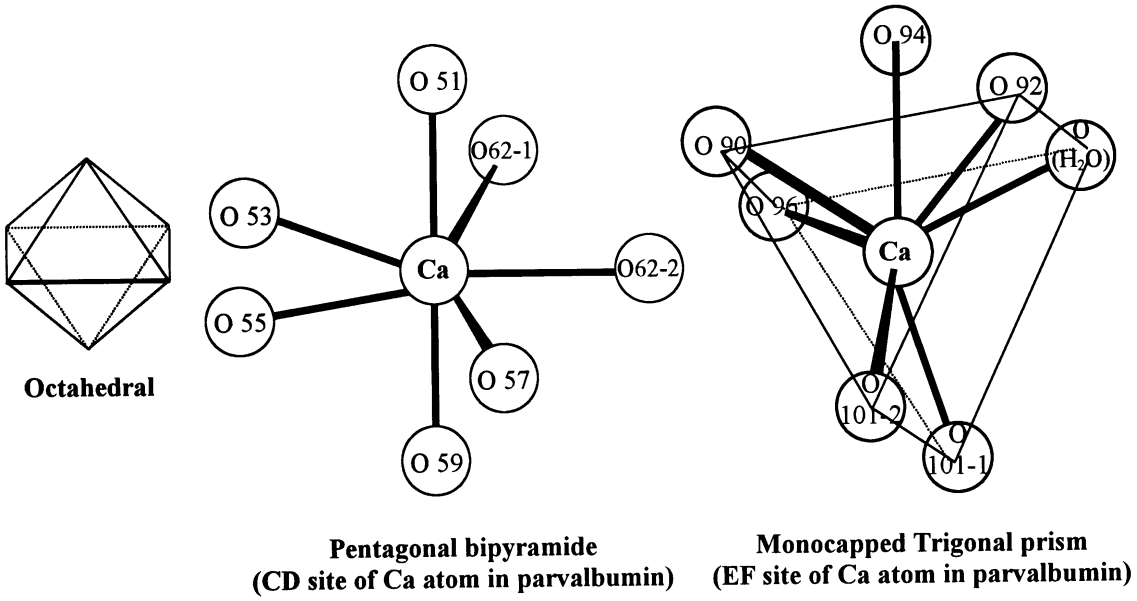

Figure 1. The coordination polyhedron of the Ca(II) ion (Swain and Amma 1989).

hedral configuration (Carugo *et al* 1993). The rigid stereochemistry of Mg^{2+} restricts accommodating any external influences. This results in Mg^{2+} being present in a limited set of proteins that have the requisite geometry to accommodate this ion. With a coordination number of 7, unlike Mg^{2+} ion, Ca^{2+} ion can achieve either pentagonal bipyramid (CD site in parvalbumin) or a trigonal prismatic arrangement with a capped rectangular face (EF site of parvalbumin) (figure 1).

An irregular geometry and ability to display high coordination numbers also puts calcium at an advantage in acting as a cross-linking agent in biology. Unlike the –S–S– and sugar-peptide bridges which are (almost) irreversible cross-links, the cross-links mediated by calcium ion are reversible and thus responsive to a change of conditions. Biological systems have made extensive use of this cross-linking ability of calcium, as the vast preponderance of calcium based structural elements in biology shows (table 3). Calcium is indispensable for the maintenance of the cytoskeletal architecture of all cells and for the process of muscle contraction. Moreover, since the cell has elaborate mechanisms to pump out calcium, various extracellular enzymes have made use of calcium as a cofactor.

Another feature that is crucial for the feasibility of a molecular interaction is the thermodynamics (free energy change) of the reaction. For any aqueous reaction, the important factors affecting the thermodynamics of the reaction are the size, charge and the hydration status of the ion. Unlike simple chemical anions, biological ligands are complex with regards to their stereochemistry. Thus, steric factors inherent in the 'radius ratio effect'***** dominate the order of cation selectivity such that when interacting with a biological ligand Ca^{2+} is preferred over other abundant divalent cations such as Mg^{2+}. Besides the steric factor, the hydration status of the metal ion also effects its binding to the ligand. Dietrich *et al* (1973) showed that macrocyclic ligands (which closely resemble the biological ligands) could successfully distinguish mono- from divalent cations and not on the basis of size alone. Due to this when sodium and calcium ions, cations of similar size, compete for ligands, there are circumstances in which one or the other cation is bound more strongly. This is achieved by the means of differences in the number of oxygen atoms available in the ligand for binding, thickness of the ligand and the hydration status of the ion.

5. Biological relevance of calcium-lipid binding

Another important biological outcome of binding of calcium to biological molecules is its effect on orientation,

*****In a simple chemical reaction it is only the decrease in the free energy associated with the interaction of the participating ions that determines the stability of the product. But, in complex reactions (such as in biological reactions), free energy change associated with the stereochemistry (packing state) of the product molecules also needs to be taken into account. This free energy change, due to the packing state of the product molecules, depends significantly not only on the charge but also on ratio of the size of the interacting ions. This dependence on the steric features of the ions is known as the 'radius ratio effect'.

Table 3. Calcium based biominerals (Kaim and Schwederski 1996).

Chemical composition	Mineral form	Occurrence and function
$CaCO_3$	Calcite, aragonite, vaterite amorphous	Exoskeletons (eggs, shells, corals), spicules, gravity sensor
$Ca_{10}(OH)_2(PO4)_6$ $Ca_{10}F_2(PO_4)_6$	Hydroxyapatite Fluoroapetite	Endoskeletons (vertebrate bones and teeth)
$CaC_2O_4(nH_2O)$ (n = 1, 2)	Whewellite, weddelite	Calcium storage and passive defense of plants
$CaSO_4{\cdot}2H_2O$	Gypsum	Gravity sensors

fluidity and fusion of the various cellular membranes. Biological membranes are composed of long-chain alkyl carboxylates and phospholipids [like phosphatidylserine (PS), phosphatidylcholine (PC) etc.]. Both the alkyl carboxylates and PS bind to calcium. The binding constant of calcium to the head groups of PS is in the millimolar range, which causes the calcium in the blood plasma to bind calcium while intracellular calcium (10^{-7} M) cannot do so. This makes the head groups of PS pointing outwards to be more stable. (Perhaps the membranes of the primitive cell had the PS residues pointing inwards, hence after the sudden increase in the extracellular calcium cells needed to employ special enzymes called 'flippases', that help maintaining the PS residue point inwards.) The carboxylate anions cannot bind to univalent cellular cations like Na^+ and K^+, but can bind highly charged ions like Mg^{2+} and Ca^{2+}. After binding to these anions Mg^{2+} stays partially hydrated and cannot co-ordinate large numbers of the anions, thus stabilizing the membrane. The larger Ca^{2+} ion on the other hand binds to several such anions after the loss of its water of hydration, leading to reduced membrane fluidity at that site. Besides the carboxylate ions, calcium can effectively bind to strong acid anions and the di-ester phosphates, while magnesium does so with a very low binding constant (ca. 10^3 M^{-1}). Thus in the event of an increase in calcium (10^{-6} M), either due to intake from outside or release from the stores, calcium can co-ordinate the rapid coming together of these ions from two or more membranes causing the membranes to collapse into a single structure. Compared to the rate of membrane fusion in the presence of K^+ ($4{\cdot}6 \times 10^{-3}$ min^{-1} mM^{-1}), Mg^{2+} cause a 3·6 fold, while Ca^{2+} cause a 12-fold increase (Maeda and Ohnishi 1974). Fusion of intracellular membranes would cause the release of the contents of the fusing vesicles (as in the case of neurotransmitter release). Such a mode of vesicle fusion would thus be more rapid, as compared to receptor mediated vesicle fusion, which requires either fresh synthesis or relocalization of the existing receptor protein molecules. Besides, unlike the receptor mediated fusion, recovery of the membranes would not require any additional signal that turn off the expression of receptors, as the diffusion of calcium away from the site, and its subsequent removal from the cell would immediately prevent any further membrane fusion.

6. Conclusion

It appears that various chemical and biological constraints imposed on cells during the process of evolution presented cells with a vast reservoir of both extra and intracellular calcium. The inherent chemistry of this ion further presented them with a unique opportunity to use calcium as intracellular messenger. The opportunity thus presented was evidently not overlooked during evolution. Thus, the widespread use of calcium in biological systems as we see today, is clearly not a freak evolutionary accident.

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