# Minisymposium: Calcium Binding Sites in Proteins

*Convener: R. BRUCE MARTIN; Charlottesville, Va., U.S.A.*

## C1

### Calcium in Biological Systems

R. BRUCE MARTIN

*Chemistry Department, University of Virginia, Charlottesville, Va. 22901, U.S.A.*

In physiological fluids calcium ion takes part in many processes. Among these are muscle contraction, microtubule formation, hormonal responses, exocytosis, fertilization, neurotransmitter release, blood clotting, protein stabilization, intercellular communication, mineralization, and cell fusion, adhesion, and growth. Most of these $Ca^{2+}$ related activities occur by interactions with proteins, which $Ca^{2+}$ may stabilize, activate, and modulate.

In extracellular fluids the free or weakly bound $Ca^{2+}$ concentration is about 1 *mM*. Within many cells the free $Ca^{2+}$ concentration in the cytosol is only 0.1 $\mu M$, $10^{-4}$ times less than in extracellular fluids. Cell membranes contain pumps, Ca-ATPases, that aid in maintaining the extraordinary concentration gradient. However, a substantial amount of $Ca^{2+}$ occurs within cells, some of it bound tightly to proteins. In response to a stimulus the free $Ca^{2+}$ concentration may increase about 10 times. Thus proteins that participate in these responses possess $Ca^{2+}$ dissociation constants in the $\mu M$ range. The cytosolic $Ca^{2+}$ concentration change is achieved rapidly, and free $Ca^{2+}$ serves as a messenger or trigger for other interactions.

$Ca^{2+}$ sites in proteins are composed of negatively charged and neutral oxygen donors; nitrogen donors seem unlikely, and none have been found. Protein oxygen donors derive from carboxylate groups, carbonyl oxygens of the amide backbone, and hydroxy groups of serine and threonine side chains.

$Ca^{2+}$ varies in its coordination number and bond lengths. The frequency of $Ca^{2+}$ coordination numbers decreases in the order $8 > 7 > 6 > 9$. Coordination about $Ca^{2+}$ is basically ionic and spherical. $Ca^{2+}$–O bond distances range from 2.3 to 2.6 Å. In solution, even within a single complex, there may be variability in bond distances and, in many cases, coordination number. To bind $Ca^{2+}$, proteins provide a pocket of appropriate size and shape with two or more negatively charged carboxylate side chains. Specific applications of these general principles appear in other papers in this symposium and in Vol. 17 of *'Metal Ions in Biological Systems'*, H. Sigel, ed.

Except for the charge difference, usually not crucial, tripositive lanthanide ions mimic many $Ca^{2+}$ properties. Energy transfer from a nearby excited aromatic chromophore produces $Tb^{3+}$ luminescence. The spectrum and relative intensity compared to the total luminescence intensity of the circularly polarized luminescence from parvalbumin and troponin-C are nearly identical. Specific $Ca^{2+}$ binding sites in the two kinds of proteins are therefore similar. The excitation spectrum identifies the donor group in the energy transfer process as a phenylalanine side chain in parvalbumin and a tyrosine side chain in troponin-C. The two amine acids comprise homologous pairs in the two proteins.

---

## C2

### $^{25}Mg$, $^{43}Ca$ and $^{113}Cd$ NMR Studies of Calcium Binding Proteins

STURE FORSÉN

*Physical Chemistry 2, Chemical Centre, POB 740, Lund University, S-220 07 Lund, Sweden*

NMR spectroscopy offers many alternative ways of studying the properties of calcium and magnesium binding proteins. One obvious way is to observe the spectra of spin I = ½ nuclei like $^{1}H$, $^{13}C$ and $^{15}N$ under various conditions as regards ion concentration, pH, temperature *etc.* Information pertaining to structure and dynamics of the protein may be gained in this way. As a result of recent developments in methodology and instrumentation NMR of the quadrupolar ions $^{25}Mg$ and $^{43}Ca$ has also developed into a useful tool in the study of calciproteins (*cf.* ref. 1–5). Through the combined use of isotopically enriched $^{25}Mg$ and $^{43}Ca$, FT NMR techniques, high magnetic fields and a solenoid type of probe design, NMR studies of these cations are now feasible at millimolar, or even submillimolar, concentrations. The general type of information that can be obtained from $^{25}Mg$ and $^{43}Ca$ NMR is:

*(i)* association constants in the range $1–10^{4}\ M^{-1}$;

*(ii)* the competition of other cations for the $Ca^{2+}$ and/or $Mg^{2+}$ binding site(s);

*(iii)* the effects of other protein ligands (drugs, *etc.*) on the ion binding;

*(iv)* the apparent pK values of the groups involved in $Ca^{2+}$ and $Mg^{2+}$ binding;

*(v)* dynamic parameters *i.e.* chemical exchange rates and activation parameters or correlation time(s) characterizing the ion binding site(s).