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Volume II

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Preface

The theme of this volume continues that of this open-ended treatise: a timely assessment of the current status of the multifunctional role of Ca^{2+} in cell function. The first volume focuses on calmodulin; this one extends the coverage to the metabolism of Ca^{2+}, other Ca^{2+}-binding proteins, and various Ca^{2+} functions; future volumes will address appropriate topics under active investigation.

The organization of Volume II is divided into three sections. The first three chapters deal with the chemistry and metabolism of Ca^{2+}; the next five describe various Ca^{2+}-binding proteins in addition to calmodulin. The functions of Ca^{2+}, some mediated by calmodulin and some by other proteins, are discussed in the last six chapters. As in the first volume, each chapter reflects the style and interest of the contributors. The length of each chapter varies somewhat, depending on the need and the extent of coverage that was felt necessary.

The field of Ca^{2+} research continues to accelerate noticeably, with a good number of articles focusing on calmodulin. According to a computer search published by a recent Current Contents, the number of articles bearing calmodulin in their titles in 1979 was 213, the term calmodulin having been introduced the year before; by 1980, it quintupled to 1013. The increase appears unlikely to abate in the near future.

One of the aims of this treatise is to keep students and investigators in all disciplines of biological research abreast of the developments in this rapidly expanding field; another is to stimulate new research for a better understanding of the intricate regulatory mechanisms underlying cellular function. I thank all the contributors for their splendid efforts in this endeavor.

This volume is dedicated to my brother, who spared no effort to see a young lad receive a proper education.

Wai Yiu Cheung

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Chapter 1

Calcium Binding to Proteins and Other Large Biological Anion Centers

B. A. LEVINE
R. J. P. WILLIAMS

CALCIUM AND CELL FUNCTION, VOL. II

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I. INTRODUCTION

In a recent article (Levine and Williams, 1981) we described the inorganic chemistry of the calcium ion based upon work with small complex ions. We used the general Eq. (1) to refer to biological activity:

$$\text{Activity} \propto [Ca^{2+}]K_{aq}p(\text{structure factors}) \tag{1}$$

where $[Ca^{2+}]$ is the concentration of the free calcium ion in the compartment under consideration (e.g., in general $>10^{-3}$ M outside cells and $<10^{-7}$ M in cells at rest), K_{aq} is the binding constant of the calcium ion to any free aqueous ligand L to give the complex CaL, p is a partition coefficient which modifies K_{aq} to give the binding in the phase (membrane) or structure where the complex CaL acts. This partition coefficient will also describe the effect of fields, mechanical or electrical, on the stability of CaL in the structure. The product $[Ca^{2+}]K_{aq}p$ therefore describes the binding of calcium to L but does not describe the activity, since activity is related to binding through certain rate constants. The rate constants are a function of the structure and energy of the ground and excited states of CaL. The relevant structures were given in the previous article. In that article data on small calcium complexes were used to describe all four terms in Eq. (1). We also described data for Na^+, K^+, and Mg^{2+}, since the activity of calcium is modulated by the (competitive) activities of these ions (and the proton).

This chapter reviews the conclusions of our previous article before describing the properties of calcium bound to proteins, especially as revealed by our nuclear magnetic resonance (NMR) studies:

1. $[Ca^{2+}]$ can be at any level from about 10^{-3} to 10^{-8} M^{-1} liter in different biological compartments.

2. The binding of calcium to complexes occurs through carboxylate and neutral oxygen donor centers. The binding strength can be varied readily from 10^3 to 10^{12} by varying the number of donor centers and their stereochemical arrangement. Competition from Mg^{2+}, Na^+, K^+, and H^+

can be set at any chosen level by suitable choice of ligand no matter how large K_{aq} is.

3. The partition coefficient p is difficult to describe, but a simple part of it is the effect of an applied potential ψ when p is proportional to $e^{\psi/RT}$; see below.

4. The structure of calcium complexes varies from 6- to 12-coordinate, grouping at about 8. The stereochemistry differs strikingly from that of magnesium in that the *geometry is irregular* both in bond length and bond angle. The calcium ion does not have a fixed geometry and readily forms cross-links.

5. The rates of exchange of ligands, i.e., the ability of the calcium ion to change structure, both in on/off reactions and fluctuational rearrangements of the ligands on the surface of the calcium ion, are fast—much faster than the corresponding rates for the magnesium ion. The energy of "excited" structures is often low.

In many ways, especially related to points (4) and (5), sodium and potassium ions are much more like the calcium ion than the magnesium ion is.

We shall assume that this information from model studies is immediately relevant to the description of calcium activity in biology. This chapter will then be divided into three major sections: a description of calcium-binding proteins, a short description of calcium binding to lipids and saccharides, and a survey of the relationship of these data to calcium activity in biology.

A. Use of Nuclear Magnetic Resonance Spectroscopy

Elsewhere we have described the use of NMR spectroscopy in the study of proteins (Campbell *et al.*, 1975; Levine *et al.*, 1979). Here we give an outline of the method, since many of the observations described below depend directly on an understanding of the procedure. The proton NMR spectrum of troponin C is shown in Fig. 1. Different regions of the spectrum have been assigned to particular types of amino acids, and for some resonances the assignments are to particular amino acids in the sequence (Levine *et al.*, 1977a). The assignment of peaks in such detail allows us to follow the effect on the protein of changes in solution conditions such as those involving pH, $[Ca^{2+}]$, salt concentration, and temperature. Now we can interpret the changes in position of the resonances in terms of changes in structure (Levine *et al.*, 1977b). This is possible because the energy of a transition, an NMR absorption peak, depends upon the chemical groups that are nearest-neighbors to the atom which has absorbed the energy. It is especially helpful to an understanding of solution structure at this stage

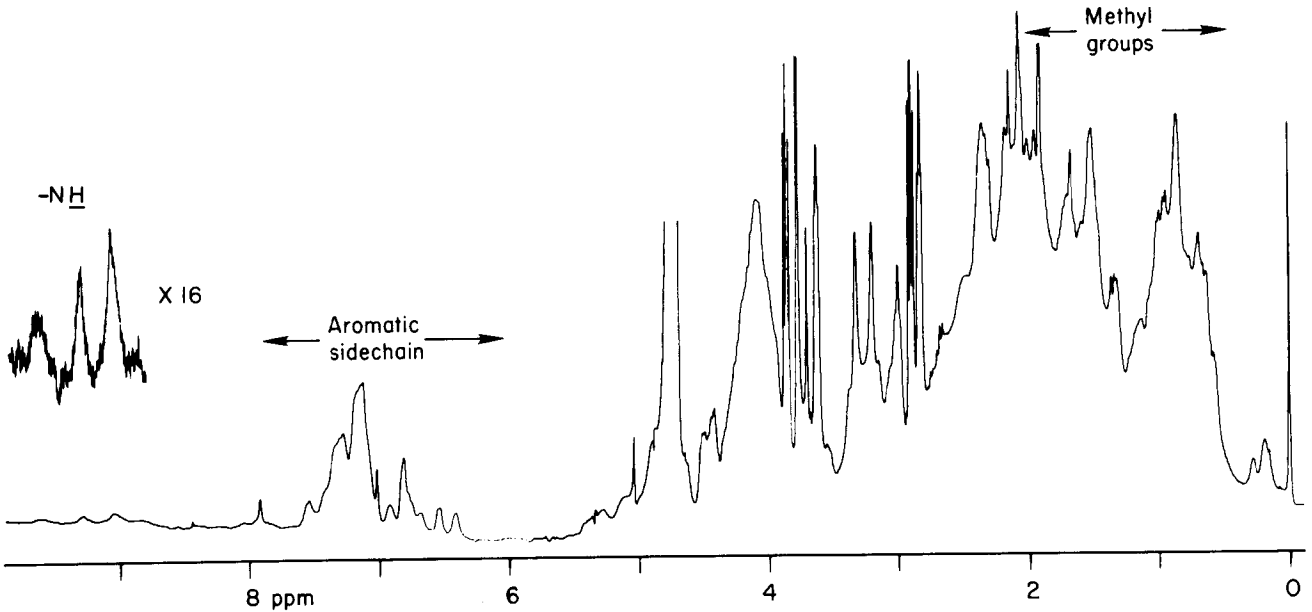

Fig. 1. Proton magnetic resonance spectrum of native (Ca_4P) troponin C in D_2O solution at neutral pH. Resonances in the spectral range 6.5–8 ppm derive from groups of aromatic side chains; in the range 0–2 ppm are methyl group resonances. For assignments see Levine *et al.*, 1977a.

if a crystal structure is available, even though the two structures may not be too similar. For example, the NMR resonance energies of groups in phospholipase A_2 are entirely consistent with the fold found in the crystal structure (Aguiar *et al.*, 1979). Various techniques are available for augmenting the structural evidence from direct absorption NMR spectroscopy, for example, by studies in the presence of (lanthanum) shift probes (Levine *et al.*, 1979). Lanthanide ions usually replace calcium ions fairly exactly.

Apart from evidence from line positions we can use the line width or relaxation properties. Especially valuable are nuclear Overhauser effects (NOEs) (Noggle and Schirmer, 1971) which are seen as changes in line intensity on irradiation of another line belonging to a nearest-neighbor amino acid. These NOE data give distances in molecules directly. Line widths can also be affected by relaxation probes, e.g., Gd^{3+} or Mn^{2+}, cations which readily replace calcium and give structural information (Campbell and Dobson, 1979; Levine *et al.*, 1979).

Considerable information about molecular tumbling and internal segmental or side-chain motion is also available from the NMR spectra. Again without going into detail, differences in relaxation times of different lines often seen in line widths can be used to assess (1) surface residue motion (such as that of lysines), (2) restricted motions (e.g., flipping of aromatic rings, valines, and leucines), and (3) motion of the main chain based on studies of α-CH or NH protons (Williams, 1978; Levine *et al.*, 1979). A major finding is that many calcium proteins have mobile interiors.

In this chapter we shall rarely refer to the primary NMR data, since we prefer to illustrate the major conclusions of our work, but a detailed appreciation does require reference to the original NMR studies.

B. The Distinction between Proteins and Small Molecules as Ligands

It is important to observe that proteins, as ligands, have specific features. Because of their size, their fold energy may equal or exceed that of the binding energy of the metal to the protein. It follows that the way in which the metal binds, its energy and stereochemistry, and the way in which the protein folds are mutually dependent (Williams, 1977; Levine and Williams, 1981). Furthermore the mobility of the protein is constrained by the metal. One way of seeing this is to consider a ligand such as EGTA, with four carboxylates on a highly mobile chain, in comparison to four glutamates in a protein. When EGTA binds to a metal, the binding has a stereochemistry and energy dictated by the metal ion and the en-