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Magnesium chemistry and biochemistry

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Introduction

Magnesium is abundant within all cells and essential for life, but its general roles in cellular function are poorly understood for a variety of reasons. Below we will review some of the chemical properties of Mg^{2+} that make it virtually unique among biologically relevant cations. The remaining reviews in this special issue of *Biometals* will cover individual aspects of Mg^{2+} functions in cells.

Magnesium in the natural environment

Origin

Magnesium apparently derives its name from Magnesia, a district in the Volos area of Thessaly in northeastern Greece. It was used to refer to any of several minerals ranging from magnesite (magnesium carbonate or 'magnesia alba') to magnetite (manganese dioxide or 'magnesia nigra') and also magnesia stone (talc or soapstone), a magnesium silicate. However, there are also two ancient towns in Asia Minor named Magnesia from the Greek word *magnes* used to refer to the magnetic iron ore discovered in this area. Magnesia could have been derived from *magnes* but used for another mineral in a different place, just as magnesia in Greece referred to several minerals.

Magnesium metal was first prepared by Sir Humphrey Davy around 1810 by making the amalgam of magnesium with mercury and then distilling off the mercury electrolytically (Treneer 1963). Davy named the new metal *magnium* and the word *magnesium* was initially used for manganese, derived from the mineral 'magnesia nigra'. The pure metal is highly reactive and all magnesium in the biosphere is either the free cation Mg^{2+} in aqueous solutions or some salt or mineral form. There are several common mineral forms of magnesium in the environment. Dolomite [$MgCa(CO_3)_2$] derives its name from the Dolomite range in the Italian Alps. The story of the recognition of dolomite and various controversies that have ranged and still range around this mineral makes interesting reading for the historian of science (Hacking 1999; McKenzie 1991). Epsomite ($MgSO_4 \cdot 7H_2O$) derives its name from the Epsom district in England, originally south of London but now incorporated into greater London. Epsom water originally came from a spring that arose on the common of Epsom village. Epsomite proved to be a major constituent of the waters. Application proved efficacious in healing of external ulcers, and the water became a destination for the sick. Until the first decade of the 18th century, Epsom was a well-known spa with many visitors partaking of the waters internally, where it acts as a purgative (Sakula 1984). Other minerals containing large amounts of magnesium are olivine (Mg_2SiO_4), magnesium calcite ($MgSO_4$) and chrysolite [asbestos, $Mg_3Si_2O_5(OH)_4$], as well as garnet and spinel, which also contain aluminum with the magnesium.

Occurrence in the biosphere

While mineral deposits containing magnesium tie up much of the Earth's magnesium, by far the most abundant source of magnesium as Mg^{2+} available to biological systems is the hydrosphere (oceans, rivers, etc.). In the ocean, the concentration of Mg^{2+} averages about 55 mM. Because many Mg^{2+} salts are highly soluble in water, Mg^{2+} is readily available to cells, unlike some transition metals or even calcium that, in general, precipitate from aqueous solutions at much lower concentrations than the corresponding Mg^{2+} salts.

Magnesium and cells

Mg^{2+} is the 8th most abundant element on earth, the fourth most abundant element in vertebrates and the most abundant divalent cation within cells. It's content in the whole body of most animals is about 0.4 g kg. From 60–65% of total Mg^{2+} resides in bone, about 35% in tissue compartments and only about 1–2% in extracellular fluid including the plasma (Marier 1990; Meyer & Zentek 1990). The amount of Mg^{2+} in bone varies between different animals, decreases with age, and is not completely bioavailable under conditions of Mg^{2+} deprivation. Mg^{2+} levels in the blood do not vary greatly under most conditions, but subtle differences are being realized in various diseases such as diabetes (de Valk 1999; Kao *et al.* 1999). In addition, as reviewed elsewhere in this issue by Romani and Maguire, hormonal regulation of Mg^{2+} flux and content of a variety of cells is now recognized although the physiological relevance is not known in many cases.

What is perhaps most intriguing about Mg^{2+} in the tissues of animals is that compartmentation and the degree of exchange with plasma Mg^{2+} differ markedly. In heart, kidney and adipocytes total intracellular Mg^{2+} can exchange with plasma Mg^{2+} within 3–4 h (Elliot & Rizack 1974; Page & Polimeni 1972; Polimeni & Page 1973; Rogers 1965; Rogers & Mahan 1959); in contrast, only about 10% of brain Mg^{2+} and 25% of skeletal muscle Mg^{2+} can exchange, with equilibrium occurring only after 16 hours or more (Rogers 1965; Rogers & Mahan 1959). Even more striking, no more than 5–7% of intracellular Mg^{2+} in lymphocytes can exchange with extracellular Mg^{2+} even after 40 h incubation (Grubbs *et al.* 1985); In contrast, in the same lymphocytes, 100% of intracellular Ca^{2+} can be exchanged within 3–4 h. The inability to exchange Mg^{2+} is surprising since cytosolic ATP binds up to 50% of total cellular Mg^{2+} in virtually all cells. These observations clearly indicate that different tissues and cell types handle Mg^{2+} very differently and that Mg^{2+} and Ca^{2+} are not handled in the same way.

Chemical properties of magnesium

Insight into the interesting biological roles and properties of Mg^{2+} may be gleaned from consideration of the basic chemical properties of Mg^{2+}, properties that make it unique amongst biological cations. The size of a Mg^{2+} cation and some derivative properties are compared to those of K^+, Na^+ and Ca^{2+} in Table 1. The ionic radius of Mg^{2+} is substantially smaller and it's hydrated radius substantially larger than that of the other three cations. Indeed, the ionic radius of Mg^{2+} is among the smallest of all cations while it's hydrated radius is the largest of all cations. Since volume is a third power of the radius, this difference is far more obvious when comparing the ratio of the hydrated volume to the ionic volume of each cation. The hydrated Mg^{2+} cation is approximately 400× larger than it ionic, dehydrated form. In contrast, there is only a 25× change for Na^+ and Ca^{2+} and only a 4× change for K^+. Coupled with the very slow exchange rate of solvent waters around the hydrated Mg^{2+}, these properties impose interesting dilemmas when considering the binding sites of these cations on proteins and other biological molecules. For example, since a transport protein is generally thought to transport a largely and probably completely dehydrated cation, a Mg^{2+} transport protein must first interact with and recognize the hydrated cation yet be able to remove the hydration shell and deliver the bare ion into the transport pathway through the membrane. While transporters for other cations face the same issue, the challenge for a Mg^{2+} transporter is far greater than for any other cation transport system because of the volume change and relative strength of interaction with the waters of hydration. This kind of problem is not merely theoretical since Mg^{2+} transporters are generally quite unusual members of the transport family (see review articles by Maguire, by Shaul, and by Romani & Maguire in this issue).

The coordination number and solvent exchange rate for Mg^{2+} also likely play a role in determining its biochemical functions. Mg^{2+} is invariably hexacoordinate as is Na^+. Ca^{2+} in contrast is relatively promiscuous and can adopt 6, 7, or 8 coordinate bonding arrangements. Indeed, even 9-coordinate arrangements are known for Ca^{2+}. Moreover, the coordination sphere, an octahedron for the hexacoordinate cations, is highly flexible with Ca^{2+} but much more constrained with Mg^{2+}. With Ca^{2+}, the distances between the cation and the oxygen atom of proteins and small molecules, as determined by crystal structure studies, vary widely, between 2.2 and 2.7 Å. For Mg^{2+} this variation is only 2.05 to 2.25 Å. The much greater flexibility in structures formed in part with Ca^{2+} allows a much greater angular deviation from the 90 ° angle expected in an octahedral configuration. Deviations from the expected 90 ° bond angle are as much

Table 1. Properties of the ions of common biological cations.

Ion	Ionic radius (Å)[a]	Hydrated radius (Å)	Ratio of radii	Ionic volume (Å^3)	Hydrated volume (Å^3)	Ratio of volumes	Coordination number	Water exchange rate[b] (sec^{-1})	Transport number[c]
Na^+	0.95	2.75	2.9	3.6	88.3	24.5	6	8×10^8	7–13
K^+	1.38	2.32	1.7	11.0	52.5	4.8	6–8	10^9	4– 6
Ca^{2+}	0.99	2.95	3.0	4.1	108	26.3	6–8	3×10^8	8–12
Mg^{2+}	0.65	4.76	7.3	1.2	453	394	6	10^5	12–14

[a]Ionic and hydrated radii are taken from ref. (Diebler *et al.* 1969; Eigen 1963).
[b]Solvent exchange rates are taken from ref. (Diebler *et al.* 1969).
[c]The transport number estimates the average number of solvent molecules associated with an ion sufficiently tightly that they migrate through the solution as the cation diffuses. It is a measure of the electrostatic ordering around the cation. The larger the transport number the larger the macromolecular complex that is present and must migrate through the solvent. Values are taken from (Cowan 1995).

as 40 ° with Ca^{2+} and less than half that with Mg^{2+} (Einspahr & Bugg 1984).

This comparatively rigid structure of Mg^{2+} complexes would appear to reflect in significant part the very different roles of Ca^{2+} and Mg^{2+} in basic biochemical processes. Ca^{2+} is generally a signaling molecule that must bind to a variety of proteins and modulate a conformational change. This implies that the geometry of the Ca^{2+} binding site must exhibit some flexibility to accommodate Ca^{2+} bound to at least 2 states of the protein in question (Ashby & Tepikin 2001; Bootman *et al.* 2001; Brown & MacLeod 2001). In contrast, the most common physiological role of Mg^{2+} is to bind ATP or another nucleotide triphosphate in the catalytic pocket of an enzyme. When bound to ATP in the active site of an enzyme, not all of the normal six coordination positions for ligands are filled by interaction with either the protein or ATP. One or more waters remain coordinated with the Mg^{2+}. The purpose of Mg^{2+} binding to the phosphoryl moieties of ATP in many cases appears to be activation of the phosphate ester toward hydrolysis. For those (non-ATP-dependent) enzymes that bind magnesium as an essential cofactor, the magnesium ion may serve to hold a water molecule in a specific position, either to help form a particular structure or to place a water molecule in correct position so that it can participate directly in the enzymatic mechanism. The relatively slow exchange rate of water in the hydration shell of Mg^{2+} may also play a role here making it somewhat harder to lose a water molecule and therefore somewhat easier to allow formation of a structure containing a water in a particular geometry. This ability of Mg^{2+} to position a water for participation in the catalytic mechanism of an enzyme is an example of outer sphere complexation, a reaction mechanism exhibited by few metals. Most metals in enzymes or chemical reactions react via direct or inner sphere complexation where the metal participates directly in catalysis (see review by Cowan, this issue). Thus the biochemistry of Mg^{2+} is multifaceted and unusual.

Related to inner *versus* outer sphere complexation and of great importance in the biochemistry of Mg^{2+} is its Lewis acidity. Lewis acidity reflects the influence of a cation on the (partial or complete) ionization of a bound water molecule, that is, the ability of Mg^{2+} to polarize a functional group such as a carbonyl in amides or esters or its ability to stabilize the charge of an anion. Mg^{2+}'s high charge density makes it suitable to act as a Lewis acid in enzyme catalysis and is typically employed biologically in hydrolysis of phosphate esters and phosphoryl transfers. Thus, the common role assigned to Mg^{2+}, chelation of ATP as MgATP in a multitude of enzymes, is actually a functional part of the enzyme catalysis rather than a simple binding and neutralization of charge (see review by Cowan in this issue).

Other properties of Mg^{2+} may need to be kept in mind when considering its functions and those of associated proteins or other macromolecules. For example, the transport number of Mg^{2+} is significantly higher than that for the other common biological cations. The transport number reflects the number of solvent molecules associated with the cation and diffusing through the solvent with the cation. Since the solvent exchange rate for Mg^{2+} is over 3 orders of magnitude less than that for other common cations (Table 1), this implies that a given Mg^{2+} ion in solution is effectively much larger than other cations. This would, again, have ef-

fects on the nature of Mg^{2+} binding sites on proteins, but also would have a significant but largely unstudied effect on ordering of solvent and molecules within cells.

Approaches for the study of magnesium

Perhaps the central issue in understanding the roles of Mg^{2+} in biological systems is the lack of suitable techniques and probes for determining its concentration under various circumstances. Certainly atomic absorption spectrometry is accurate and sensitive in determination of the total mass of Mg^{2+} in a sample. However, the total amount of a substance is not, in general, a very useful parameter. With many substances such as glucose or potassium, the total concentration approximates the 'free' or unbound concentration. It is this latter concentration that is thermodynamically relevant in determining the rate and extent of reactions. Unfortunately with Mg^{2+}, techniques for measuring the free concentration of Mg^{2+} in biological systems are inadequate or nonexistent. The relative numbers of free and bound cations in a typical cell are illustrated in Table 2.

Isotopes of magnesium

Of all biologically relevant elements, Mg is unique in that it exists as three different isotopes in measurable natural abundance, ^{24}Mg (78.7%), ^{25}Mg (10.1%) and ^{26}Mg (11.2%). With other elements, the dominant isotope is $\geq$99% natural abundance. To date there are no known biological reactions that favor or are significantly influenced by one or the other of the Mg isotopes, but determination of individual isotopes is feasible using induction coupled plasma mass spectrometry. Thus, for example, it would be feasible to measure membrane flux of Mg^{2+} by determining changes in isotopic ratios in cells exposed to only one of the three isotopes. For example, if cells were grown in Mg^{2+} of natural abundance and then suspended in a medium containing only ^{24}Mg, measurement of the appearance of ^{25}Mg and/or ^{26}Mg in the medium with time gives the rate of efflux. This method however is relatively expensive although the stable isotopes are available.

^{25}Mg can be measured by NMR, but its value is limited (Drakenberg *et al.* 1997; London 1991; Malmendal *et al.* 1998; Tsai *et al.* 1987). While the sensitivity is reasonable, about 0.3% that of ^{1}H, it has a spin quantum number of 5/2. Its nucleus is therefore quadrupolar resulting in a high relaxation rate which in turn results in very broad spectral peaks. While this broadening can be used in some circumstances for equilibrium studies in isolated systems, it gives virtually no structural information.

The only really useful radioactive isotope of Mg is ^{28}Mg with high energy β and γ emission and a half-life of 21.3 h. Other radioactive isotopes have half-lives of a few minutes or seconds. Unfortunately, while $^{28}Mg^{2+}$ was readily available at reasonable cost from 1960–1990 from Brookhaven National Laboratory in New York, policy decisions in the 1980s by the United States government emphasized extensive cost recovery on government services so that the estimated price currently for $^{28}Mg^{2+}$ is about $30,000 per mCi. It is therefore not used today to any significant extent. Previous studies of transport using $^{28}Mg^{2+}$ in both microorganisms and to a lesser extent eukaryotic cells have however established a set of baseline properties that will be useful in studies of new systems or when using substitute isotopes such as $^{63}Ni^{2+}$ in place of $^{28}Mg^{2+}$. Transport work is reviewed in this issue by Romani and Maguire in mammalian systems, Shaul in plants and Maguire in prokaryotes.

Absorption and fluorescent spectrophotometry of magnesium

A number of dyes, usually fluorescent, have been developed for Ca^{2+} and with somewhat less success for K^+ and Na^+. These dyes rely on measurement of the ratio of emission at two different wavelengths as a measure of free concentration of Ca^{2+} or other ion based on a separate determination of the affinity of the cation for the dye. The early fluorescent dyes for Ca^{2+} such as Quin-2 were not useful in many circumstances and cell types because of their relatively poor affinity for Ca^{2+}. Newer dyes like Fura-2 however work quite well in virtually all cells types. Many variants on the basic dyes can be used in special circumstances.

In contrast to Ca^{2+}, currently commercially available dyes for Mg^{2+} such as Mag-Fura are only about the equivalent of Quin-2 with Ca^{2+}, that is, their usefulness is restricted to a few cell types. In the case of dyes like Mag-Fura however, the problem is not their relatively poor affinity for Mg^{2+}, but rather their retention of significant affinity for Ca^{2+}. Indeed, the available Mg^{2+} dyes all have significantly better affinities for Ca^{2+} than for Mg^{2+}, although they bind Ca^{2+} with less affinity than dyes like Fura-2. They

Table 2. Cellular concentrations of the common biological cations.

Ion	[Total] (mM)	[Free] (mM)	Total atoms per Cell[a]	Free atoms per cell
Na^+	12	8	3.6×10^6	2.4×10^6
K^+	140	120	4.2×10^7	3.6×10^7
Ca^{2+}	3	0.0001	9×10^5	30
Mg^{2+}	30	0.3	7.5×10^6	90,000

[a]Calculated for a 1 μM diameter cell; this approximates the volume of an *E. coli* cell. For a larger cell such as a lymphocyte, multiply these numbers by the ratio of the cell radius cubed ($[r_1/r_2]^3$). Thus for a lymphocyte of diameter 20 μM, multiply the above values by ($[10/0.5]^3$) = 8000.

are useful for measurement of Mg^{2+} only within cells where the resting concentration of Ca^{2+} is less than 1 μM and therefore does not appreciably interfere with Mg^{2+} binding to the dye.

What is needed clearly is a highly fluorescent dye with an affinity for Mg^{2+} of 1 mM or less and a Ca^{2+} affinity of >500 μM. Recent work by Otten *et al.* (2001) may have finally solved this problem. These workers made use of the observation that fluorinated quinoline antibiotics are known to chelate Mg^{2+} (Kawai *et al.* 1996; Lecomte *et al.* 1994) and have made a number of derivatives of 4-oxo-4*H*-quinolizine-3-carboxylic acids that are quite selective for Mg^{2+} over Ca^{2+}. Indeed some of the dyes show no change in fluorescence even at 100 mM Ca^{2+}! The various dyes exhibited affinities for Mg^{2+} in the range of 0.5–5 mM, precisely the concentration range needed. The various dyes are rationable fluorescent indicators with emission maxima between 450–550 nm. Since the dyes are carboxylic acids, appropriate esters could be synthesized as for the Ca^{2+} dyes for loading into cells. These dyes show great promise.

Microelectrodes

Ion electrodes for Mg^{2+} are only now becoming useful. Electrodes for Mg^{2+} have suffered from a myriad of problems including relative insensitivity, interference from monovalent cations and pH sensitivity. However, the latest ionophores used to formulate the electrode tip have properties sufficiently selective for Mg^{2+} that microelectrodes can routinely be made and used to measure intracellular free Mg^{2+} with some sensitivity. These microelectrodes and their properties are discussed further by Günzel in this issue.

Magnesium chelation

Chelation of metal cations is one of the most common forms of error in biological experiments, primarily because of a lack of understanding of chelator selectivity for different cations. A simple fact of bioinorganic chemistry at present is that virtually no existing chelator is highly selective for a *single* divalent cation. There are a few agents that have far better affinities for Cu^{2+} or sometimes Cu^{2+} and Fe^{2+} than all other divalent cations. There is certainly no such thing as a Mg^{2+}-specific or even a Mg^{2+}-selective chelator (or for that matter a Ca^{2+}-specific or -selective chelator). A few chelators bind all *except* one of the common biologically important transition metal divalent cations with high affinity, thus potentially leaving only one cation present at a possibly biologically active concentration. Examples of these properties are shown in Table 3.

To illustrate the problem, consider the common chelators EDTA and EGTA (Table 3). The common wisdom or assumption about EGTA is that it is a Ca^{2+}-specific or at least Ca^{2+}-selective chelator, usually with the further assumption that it has a very high affinity for Ca^{2+}. There are several problems that arise from these assumptions. First, inspection of the values listed in Table 3 shows that EGTA has virtually the same binding constant for Ca^{2+} as EDTA. It has slightly worse binding constants for transition metal cations than does EDTA, but even so, transition metal cations bind to EGTA or EDTA with affinities orders of magnitude greater than their affinities for Ca^{2+}. Consequently, addition of EDTA or EGTA to a solution will reduce the concentration of *all* transition metal cations in that solution to levels that are biologically irrelevant. Indeed, a 10× excess of EGTA over the expected Ca^{2+} concentration would also chelate as much as 20% of the Mg^{2+} present in the solu-

Table 3. Metal-ligand stability constants.

Ligand	Mg^{2+}	Ca^{2+}	Cu^{2+}	Fe^{2+}	Mn^{2+}	Zn^{2+}	H^{+}
Ethylenedinitrilotetra(3-propanoic acid)	1.8	?	14.9	6.2	4.7	9.3	9.7
1,3-Phenylenedinitrilotetraacetic acid	2.0	2.4	8.3	?	3.1	5.2	5.8
N-(2-Pyridylmethyl)iminodiacetic acid	4.0	4.9	14.1	9.0	7.0	10.6	8.2
EDDA (Ethylenediiminodiacetic acid)	4.0	?	16.2	8.6	7.0	11.1	9.6
Ethylenediiminodi-2-propanoic acid	2.8	1.7	14.8	8.5	6.1	10.1	9.6
EDDM (Ethylenediiminodipropanedioic acid)	4.9	5.5	15.9	?	8.5	11.1	9.7
DL-1-Methylethylenedinitrilotetraacetic acid N,N′-diamide	5.1	6.9	15.3	10.0	9.8	9.5	7.6
DL-1-Ethylethylenedinitrilotetraacetic acid N,N′-diamide	4.9	6.7	15.0	9.7	9.4	11.2	7.6
(2-Hydroxytrimethylene)dinitrilotetraacetic acid	5.3	6.7	17.2	11.9	9.1	13.7	9.5
1,4,10,13-Tetraoxa-7,16-diazacyclooctadecane-N,N′-diacetic acid	?	7.7	15.0	7.9	8.7	8.0	8.0
MIDA (N-methyliminodiacetic acid)	3.5	3.8	11.0	6.7	5.4	7.7	9.6
N-Propyliminodiacetic acid	?	3.4	11.3	6.6	?	8.0	10.0
DL-2-(2-Methylthioethyl)nitriloacetic acid	1.5	1.4	9.2	4.7	3.2	5.5	10.7
EDTA (Ethylenedinitrilotetraacetic acid)	8.8	10.7	18.8	14.3	13.9	16.5	9.5
EGTA (Ethylene bis(oxyethylenenitrilo) tetraacetic acid)	5.3	10.9	17.7	11.8	12.2	12.6	9.3
TAPEN (Ethylenedinitrilotetrakis(trimethyleneamine))	?	?	19.6	7.9	6.1	12.3	10.7
DPA (Iminobis(methylene-2-pyridine) (Di-2–picolylamine))	?	?	13.9	6.1	3.5	7.6	7.2
2-(2-Pyridylmethylenehydrazino)pyridine	?	?	11.0	17.2	2	6.2	5.8
2-Chloro-1,10-phenanthroline	?	?	5.1	11.6	?	3.3	4.2
TLA (Nitrilotris[methylene(6-methyl-2-pyridine)])	?	?	10.4	4.5	2.6	5.5	6.7
(Ethylenedithio)diacetic acid	?	1.7	11.2	2.7	1.0	2.7	4.0

From R.M. Smith and A.E. Martell, NIST Standard Reference Database 46, NIST Critically Selected Stability Constants of Metal Complexes: Version 6.0 (Smith and Martell 2001).

tion. The chelation of essentially all cations in solution may or may not have any effect on one's experiment since, since for the most part the contaminant levels of transition metal cations in most laboratory buffers are probably 1 μM or less and their further reduction would have little effect except on systems with nanomolar affinity. Nonetheless, it is possible that one can be fooled into thinking a result is Ca^{2+}-dependent when in fact the result derives from the lack of another cation. In general, addition of a chelator shows only that a system is metal dependent but does not show which metal the system responds to biologically. 'Add-back' experiments, where a chelator is added and then a *single* cation is added back at a concentration slightly in excess of the concentration of chelator, are much more likely to give a biologically meaningful answer as to a system's metal dependence. Even 'add-back' experiments can give incorrect answers however, especially if only a single cation is tested.

In contrast to the use of chelators in cellular studies, chelators are quite useful for determining the concentration of Mg^{2+} or other cations in defined solutions as would be the case in most enzymatic assays. A convenient shareware program for calculating metal ion concentrations in the presence of various chelators can be found at http://www.med.umich.edu/biochem/enzresources/software.htm. Again however, one must be careful not to mistake a result as arising from addition of a cation when chelators remove essentially all other metals from a solution.

Surrogates for magnesium

Because only minimal spectroscopic or other technical methods are available for Mg^{2+}, substitute cations have been employed in some circumstances. The most common surrogate has been Mn^{2+} which can replace Mg^{2+} in the majority of enzymes where MgATP is used as substrate. For some enzymes, MnATP is as active or sometimes even more active than MgATP. Mn^{2+} has similar ligand preferences, exhibits both inner and outer sphere complexes, and is usually hexacoordinate. Mn^{2+} also is paramagnetic and has been used as an EPR probe successfully as has VO^{2+} (Buy *et al.* 1996; Houseman *et al.* 1994; Zimmermann

et al. 1999, 2000). Nonetheless, Mn^{2+} is significantly larger than Mg^{2+}, and this can lead to some differences in biochemical interactions. By the same logic, it becomes reasonable that Ca^{2+} is usually a poor substitute for Mg^{2+} since it is much larger than either Mg^{2+} or Mn^{2+} and would not be expected to fit very well into binding sites designed by evolution for Mg^{2+}.

Interestingly, despite the relatively close chemistry and size of Mg^{2+} and Mn^{2+}, Mn^{2+} does not appear to be transported by the known Mg^{2+} transport systems, although Mn^{2+} is a relatively weak inhibitor of some Mg^{2+} transport systems. In contrast to Mn^{2+}, Ni^{2+} appears to be a good substrate for many Mg^{2+} transport systems, presumably because its ionic size is similar to that of Mg^{2+} and it can adopt an octahedral geometry in its hexacoordinate state. Similarly Co^{2+} can also be a substrate for some Mg^{2+} transport systems and inhibits others (Grubbs *et al.* 1989; Hmiel *et al.* 1989; Smith *et al.* 1995; Snavely *et al.* 1989; Townsend *et al.* 1995).

Mn^{2+}, Ni^{2+}, and Co^{2+} are used as surrogates for ionic Mg^{2+}. In contrast, inorganic complexes of Co^{3+}, and sometimes Ru^{2+} and Ru^{3+} can be used as substitutes for a hydrated Mg^{2+} cation. The most common substitute is cobalt(III) hexaammine $[Co(III)(NH_3)_6]$. In such compounds, the ammine moiety is covalently bound to the cation. Some such compounds are quite stable in aqueous solution under biologically relevant conditions. Cobalt(III) hexaammine has a half-life at pH 7 of about 6 months (Basolo & Pearson 1967). Substitutions of one or more ammines can also be made, even the addition of carbon chains or other organic moieties. Cobalt(III) hexaammine is almost exactly the size of a hydrated Mg^{2+} and thus would be expected to bind to any site where the size of the Mg^{2+} cation rather than its charge was the dominant determinant. For example, the initial binding site for Mg^{2+} on the bacterial CorA Mg^{2+} transport protein appears to interact with the completely hydrated Mg^{2+} ion since cobalt(III) hexaammine is a potent inhibitor with an affinity for the transporter even better than Mg^{2+} itself (Kucharski *et al.* 2000). Where charge might be important, Ru(II) and Ru(III) hexaammines can be used in addition to cobalt(III) hexaammine. Ni(II) hexaammine is larger than the cobalt or ruthenium complexes and does not appear to bind well to this transporter.

Conclusions

Although much has been learned about Mg^{2+} and its associated biochemistry, the lack of sufficient tools, largely derived from the unique chemistry of the Mg^{2+} ion, has hampered progress. Nonetheless, the advent of newer ionophores for microelectrodes, the possibility of using quinolizine carboxylic acid derivatives as Mg^{2+} indicator dyes and the use of selective surrogate cations promises to rapid progress in the future. The remaining reviews in this special issue elaborate on this promise.

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