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The effect of ions at the surface of calcium oxalate monohydrate crystals on cell-crystal interactions

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Abstract Magnesium is an abundant ion in biologic systems, including renal tubular fluid; however, the precise role of magnesium during the interaction of calcium oxalate crystals with cells has not been previously defined. In addition, the respective roles of calcium and hydrogen ions during the cell-crystal bonding interaction remain poorly defined. Here we report an atomic level three-dimensional study of a single crystal of calcium oxalate monohydrate (COM; whewellite) which was bathed in a solution of magnesium hexahydrate for 1 year. Magnesium was not incorporated into the structure of whewellite to any significant degree. Instead, COM accepted magnesium primarily as an adsorbate in a binding configuration which, as a surface phenomenon, is controlled by localized charge effects. The effect of magnesium and calcium on the efficiency of calcium oxalate crystal binding to renal cells was also investigated. When present in supraphysiologic concentrations (greater than 0.1 M), magnesium progressively inhibited adhesion of pre-formed COM crystals to cultured renal cells. Therefore, even though magnesium does not incorporate into the crystal structure of calcium oxalate, magnesium can exert important surface effects and change the interaction of pre-formed COM with molecules anchored on the cell surface. Similarly, binding was nearly blocked when the exogenous calcium concentration was ≥0.1 M (supraphysiologic range), although in lower concentrations (within the physiologic range) exogenous calcium *promoted* crystal adhesion. Finally, the ambient hydrogen ion concentration also influenced calcium oxalate crystal interactions with renal cells, with maximal binding occurring at a pH of 4. Therefore, hypercalciuria and/or an acidic urine could each promote renal stone formation via increased crystal adhesion to renal cells, a previously under-appreciated potential mechanism.

Keywords Atomic structure · Calcium · Magnesium · pH · Nephrolithiasis · Whewellite

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Introduction

The mechanisms whereby calcium oxalate crystals are retained in the kidney remain poorly understood. Citrate can block crystal adhesion to renal cells [1]; however, the relative contribution of other ionic species, including magnesium, calcium and hydrogen ions, has not been investigated. In the literature there is considerable debate over the interaction that takes place between magnesium and calcium oxalate monohydrate (whewellite: COM), calcium oxalate dihydrate (weddellite: COD) and calcium oxalate trihydrate (COT) [2, 3]. Structural considerations suggest that any interaction should be phase specific [4], even though substitution of magnesium for calcium in the crystal structure of COM, COD and COT should be a thermodynamically unfavorable process because of the different coordination that magnesium imposes, as compared to calcium, with the surrounding anions. Unfortunately, published data merely demonstrate progressive deterioration of calcium oxalate crystal morphology in the presence of magnesium. However, calcium-magnesium exchange should be studied at the atomic level to address these questions critically.

Information available from precipitation studies using solutions rich in magnesium and supersaturated in calcium oxalate suggests that magnesium inhibits the growth and/or nucleation of COM, COD and COT [2, 5, 6, 7, 8]. Under certain conditions, magnesium also appears to favor the precipitation of COD in lieu of COM [8, 9]. However very little, if anything, is known

regarding the effect of magnesium on the processes that control adhesion of COM, COD and/or COT upon a substrate, although several metals and molecular groups are known to influence this process [1, 10]. Furthermore, adhesion of crystals is often crystal-face specific and, as such, highly organized at the atomic level [11, 12].

In humans, calcium oxalate occurs under pathologic conditions, largely as a component of renal stones. But calcium oxalate crystals are also found in other biologic systems. One example are the COM and COD crystals often found within films and crusts that grow over artifacts of historic and artistic interest, in which instance the crystals are associated with magnesium [13, 14]. Often, such crusts are embroiled in a bio-organic matrix [13, 14], organized as cell monolayers whose protein milieu is likely to be a critical mediator of calcium oxalate crystal adhesion. Crystal binding organic molecules on a cell surface, be they glycoproteins [10, 15, 16] and/or anionic phospholipids [17], are expected to induce stereochemical interactions with the atomic interfaces of the crystals.

Given the evidence that magnesium plays a role during calcium oxalate crystal interaction with cell surface molecules in certain biologic systems, we report here the results from a structural study of a single crystal of COM which was bathed in a solution of magnesium hexahydrate for 1 year. These results are then evaluated in light of our ongoing work on the efficiency of calcium oxalate crystal binding to renal cell monolayers by systematically varying the concentrations of magnesium, calcium, and hydrogen ions.

Materials and methods

Single-crystal work

A superior single-crystal of whewellite (0.09×0.018×0.06 mm) with prismatic habit that produced very sharp signals, as indicated by preliminary optical and x-ray diffraction work, was selected as a control. For the powder work a Gandolfi camera was used (114.6 mm; Ni-filtered CuKα radiation; 38 kV, 18 mA, 10^{-2} torr) employing a silicon standard. The single-crystal studies, instead, were initially carried out by precession photography (Zr-filtered MoKα radiation, 40 kV, 20 mA; [010] and [100] projections).[1] The published crystal structure of calcium oxalate monohydrate [18] was used as a frame of reference.

Next, several COM fragments obtained from the control material were introduced into each of the two wells of a depression slide together with 30 µl of a 0.6 M magnesium chloride hexahydrate aqueous solution. After the wells were sealed with a glass cover using silicon grease, the slide was put in a Petri dish that was insulated from the atmosphere by attaching the upper and lower lids to one another with three layers of electrical tape. Finally, the dish was transferred into a glass container and placed in a water bath and heated from 20 to 55°C (at 5°C/h) and maintained for 5 days. The temperature was adjusted in this manner because previous experience indicated that COM is particularly susceptible to any increase in temperature. This promotes structural disorder and expansion [19, 20], thereby potentiating any tendency for magnesium to incorporate into and/or adsorb onto the crystal. Next the water bath was slowly re-equilibrated to 20°C over a 72-h period, and the dish was removed and stored undisturbed for 1 year in order to allow prolonged interaction of magnesium with COM. At the end of this period, the fragments were rapidly dried for analysis in order to prevent deposition effects.

Uptake of magnesium by the COM sample was tested using a Cameca SX-50 electron microprobe in energy dispersive mode (15 keV). A Gandolfi and a precession camera (Zr-filtered MoKα radiation; 40 kV, 20 mA; b and a axis projections) were used for preliminary x-ray diffraction work. All of these observations were then quantified by single-crystal diffractometry (SYNTEX four-circle automated diffractometer; Mo Kα graphite-monochromated radiation, $\lambda = 0.71069$ Å) collecting 2,253 intensity-data using the θ–2θ scan technique. Data reduction and full-matrix least-squares refinement [1,153 independent reflections; $I > 3\,\sigma(I)$, (I = intensity, σ = standard deviation)] were carried out in space group $P2_1/n$[2] using the atomic coordinates of Deganello and Piro [18] from a magnesium-free control, and the computer programs assembled by Sheldrick [21]. Convergence was reached after anisotropic refinement for $R = 0.04$.[3]

Cell-crystal interactions

MDCKI cells (a generous gift of Dr. Carl Verkoelen [22]) and continuous Inner Medullary Collecting Duct (cIMCD) cells (a generous gift of Dr. Jack G. Kleinman [16]) were grown in Dulbecco-Vogt modified Eagle's medium containing 25 mM glucose (DMEM) at 38°C in a CO_2 incubator as described elsewhere [23]. To prepare high-density, quiescent cultures, 1×10^6 cells/35-mm plastic plate (9.62 cm^2) were plated in DMEM containing 10% calf serum and 1.6 µM biotin (Ca 1.8 mM, Mg 0.8 mM, pH 7.4). Two days later, when they were confluent, the medium was aspirated and replaced with a fresh one containing 5% calf serum and 1.6 µM biotin. The monolayer was used for study the next day.

Radioactive [^{14}C]-labeled COM crystals were prepared from supersaturated solutions by Y. Nakagawa (University of Chicago) as reported previously [24]. [^{14}C] oxalic acid (30–60 mCi/mmol, ICN Biomedicals, Irvine, CA) was added to a sodium oxalate solution in order to achieve a specific activity of 10^5 cpm/ml. Sufficient calcium chloride was then added to form a supersaturated solution. The COM crystals that precipitated were 1–2 µm in size as measured along the prism direction and had a specific activity of 300–450 cpm/µg. Crystal size, shape and cell parameters were assessed by light, scanning electron microscopy and x-ray powder diffraction as previously reported [25].

To quantify adhesion of [^{14}C]COM crystals to cellular apical membrane proteins, the culture medium was aspirated and replaced with 5 ml of Tris-buffered saline (TBS; 10 mM Tris, 155 mM NaCl, 5.4 mM KCl, pH 7.4, 38°C) containing specific concentrations of magnesium chloride (0–0.6 M) or calcium chloride (0–0.5 M). To assess the independent effect of pH, magnesium and calcium were omitted from the TBS and the pH was adjusted to values between 1 and 9. [^{14}C]COM crystals were then added to the buffer to achieve a final concentration of 200 µg/ml (41.6 µg/cm^2 of cells). The culture dishes were gently agitated for 5 s to allow crystals to settle to the surface of the cell monolayer under the force of gravity. After 2 min the buffer was aspirated and the cells were washed three times with TBS (5 ml). The cells were then liquefied in 0.5 ml of 6 N NaOH for 5 min, transferred into a scintillation vial to which 5 ml of Ecoscint (National Diagnostics, E. Palmetto, FL) was added, and the amount of cell-associated radioactivity counted.

[1] The [010] and [100] projections are down the b and a axis of the COM crystal lattice, respectively.

[2] The space group identifies the set of all symmetry operations of a crystal pattern in three dimensions: P identifies the primitive lattice 2_1 a screw axis, and n a glide plane normal to the latter.

[3] R is the reliability factor. This is a measure of the agreement between a set of observed data and a set of calculated values for the same data; a value of 0.04 is considered very good.

Table 1 Values of crystallographic unit cell of COM before and after exposure to magnesium hexahydrate*

Control	After exposure
a = 9.976 (3) Å	a = 9.976 (4) Å
b = 14.588(4)	b = 14.588 (4)
c = 6.291(3)	c = 6.291 (3)
β = 107.03(4)°	β = 107.03 (4)°
S.G. P 2_1/n	S.G. P 2_1/n

* Data obtained using silicon (ASTM) as the internal calibration standard

To assess viability of cells exposed to the non-physiological buffers for 2 min, cells were rinsed with PBS and processed with the Live-Dead stain as per the manufacturers instructions (Molecular Probes, Eugene, OR). The percentage of cells staining live or dead was counted and averaged from ten low power fields per condition under an immunofluorescence microscope.

Supplies

Reagents were purchased from Sigma Chemical, St. Louis, MO, USA, unless otherwise indicated.

Statistics

Data for the crystal-cell interactions were compared by Student's *t* test; *P* values less than 0.05 were accepted as significant. Values presented are means ± SEM. When no measure of variance appears on a graph, it is because the variance is smaller than the symbol used for the mean.

Results

The electron microprobe results indicated that magnesium was present only to a minor degree (about four times the standard deviation of the background at the peak position). If magnesium had entered the lattice of COM, the values of its unit cell would have been changed. Instead, x-ray powder data taken after exposure to magnesium chloride hexahydrate for a full year (Table 1) indicate that the values of the unit cell parameters remained unchanged (within the value of the standard deviation). The precession work confirmed this result. Sets of [010] and [100] projections of the COM crystal lattice, compared with those from the magnesium-free control, failed to identify changes in the intensity distribution of the reflections. In addition, there was no evidence of magnesium-induced order-disorder phenomena in these projections, either in the form of diffuseness and/or streaking of those reflections. Most importantly, a detailed study of the electron density maps generated by the single crystal work failed to identify any calcium-magnesium exchange in the calcium sites, or any other residue in the crystal structure which could be ascribed to magnesium. By difference Fourier analysis the maximum peak height measured for any residue was only of the order 0.7 $Å^3$, which is barely in line with that expected for a hydrogen atom, and far from what is expected for magnesium (≈25 $Å^3$). Table 2 reports the values of the atomic coordinates for the control and magnesium-exposed sample. These agree remarkably well.

It is expected that magnesium cannot exchange for calcium in COM, primarily due to the different coordinating number of oxygens that the two cations require (six for magnesium versus eight for calcium). Therefore, even prolonged exposure of COM to magnesium is unlikely to produce significant dissolution of the crystal with formation of magnesium oxalate. This is an important point to consider since magnesium oxalate

Table 2 Values of the atomic parameters of whewellite before and after exposure to magnesium hexahydrate. Note the lack of changes before and after magnesium exposure

	Whewellite[a]			Whewellite after exposure to magnesium[b]		
	x/a	y/b	z/c	x/a	y/b	z/c
Ca(1)	0.0546(3)	0.1243(2)	0.0870(3)	0.0542(1)	0.1240(1)	0.0882(2)
Ca(2)	0.4357(3)	0.1236(2)	0.4389(3)	0.4353(1)	0.1235(1)	0.4399(2)
Ox(1)	0.1322(3)	0.2826(2)	0.1566(3)	01312(5)	0.2820(4)	0.1595(7)
Ox(2)	0.1395(2)	0.4660(2)	0.1329(3)	0.1400(5)	0.4661(4)	0.1319(8)
Ox(3)	0.3555(3)	0.2819(3)	0.3751(3)	0.3545(5)	0.2811(4)	0.3774(8)
Ox(4)	0.3614(2)	0.4658(2)	0.3541(3)	0.3616(5)	0.4662(4)	0.3539(8)
Ox(5)	0.0690(3)	0.1418(2)	0.2924(3)	0.0699(5)	0.1431(4)	0.2920(8)
Ox(6)	0.1975(3)	0.1226(2)	0.5271(3)	0.1973(5)	0.1222(4)	0.5260(7)
Ox(7)	0.2958(3)	0.1229(3)	0.0519(2)	0.2955(5)	0.1224(4)	0.0528(7)
Ox(8)	0.4263(4)	0.1066(3)	0.1809(3)	0.4262(5)	0.1065(4)	0.1807(8)
C(1)	0.2452(3)	0.3201(3)	0.2620(3)	0.2449(7)	0.3194(6)	0.2621(9)
C(2)	0.2493(3)	0.4270(4)	0.2481(3)	0.2494(7)	0.4286(6)	0.2483(9)
C(3)	0.1812(3)	0.1264(4)	0.3377(3)	0.1803(7)	0.1266(6)	0.3366(9)
C(4)	0.3130(3)	0.1175(5)	0.1375(3)	0.3127(7)	0.1172(6)	0.1359(10)
OW(1)	0.6023(2)	0.1542(2)	0.2090(4)	0.6010(6)	0.1540(5)	0.2088(9)
OW(2)	0.1093(3)	0.1172(2)	0.2940(3)	0.1089(7)	0.1125(5)	0.3016(10)

[a] Synthetic specimen. Values of atomic coordinates are as they were reported to Cambridge Data Bank in 1981. Some uncorrected proof errors were in fact detected in the original paper [18]

[b] Natural sample: Field Museum (Chicago). MoKα graphite-monochromated radiation; θ–2θ scan technique; total of 2,253 intensity data; 1,153 intensities with $\sigma > 3\sigma(I)$. R = 0.04

has much higher solubility than calcium oxalate. To exclude further the possibility of significant magnesium oxalate formation, an x-ray powder film was taken of a very few, extremely small grains of material that had deposited on the inner side of the slide after prolonged incubation of COM with magnesium hexahydrate. Only magnesium hexahydrate, and *not* magnesium oxalate, was detected.

Previously we characterized the interaction of COM and COD crystals with renal epithelial cells [10, 11, 26]. These studies strongly suggested that the stereochemical organization of the atomic interfaces that form between cellular surfaces and COM or COD crystals must be essentially alike, even in different cell types. This interaction, in fact, is primarily dictated by the requirements for bonding which are imposed by the calcium cations and the oxygen atoms from the water molecules that are present both in COM and COD [26]. Consequently we used these cells to elucidate the potential role, if any, of magnesium, calcium and hydrogen ions in the process of adhesion between COM and cellular structures. Indeed, as the magnesium concentration was increased from 0 to 100 mM, adhesion of COM crystals to a confluent monolayer of renal cells (MDCKI line) increased by 33%, as shown in Fig. 1. However, when the magnesium concentration was further increased (from 100 to 600 mM), COM crystal adhesion fell in a nearly linear fashion, reaching a low value of 13% control at concentrations ≥500 mM (Fig. 1).

The prevailing calcium concentration also had profound effects on COM crystal adhesion to renal cells. Crystal adhesion was low when no calcium was present, and rose to maximal values at a concentration of 50 mM (a 3.5-fold increase compared with zero calcium, Fig. 2). At a calcium concentration of ≥100 mM, COM crystal adhesion again returned to low values. The biphasic nature of calcium on crystal adhesion paralleled that of magnesium (Fig. 1), but the increase in binding at low concentration was much more dramatic.

Crystal adhesion to cells was also pH-dependent, since binding was quite low at pH 1 (Fig. 3). Although crystals did not visibly dissolve at this pH, partial crystal dissolution cannot be absolutely excluded since the solubility of COM crystals increases markedly below pH 3. However, since cell-associated radioactivity was low (and not high) at these acidic pH values when crystal dissolution was possible, cellular uptake and/or adhesion of free [^{14}C] oxalate does not appear to be a confounding factor in these experiments. As the pH was increased from 1, crystal binding also increased, reaching a maximum at pH 4, and then decreased again as the pH was raised to 9. Of note, crystal adhesion to cells at pH 7 was only 44% of what it was at pH 4 (Fig. 3).

A nearly identical pattern of crystal adhesion to confluent monolayers of cIMCD cells was observed when magnesium, calcium and pH were varied over the same ranges (data not shown).

To determine the effect of the non-physiologic buffers on cell viability, Live-Dead staining was used as de-

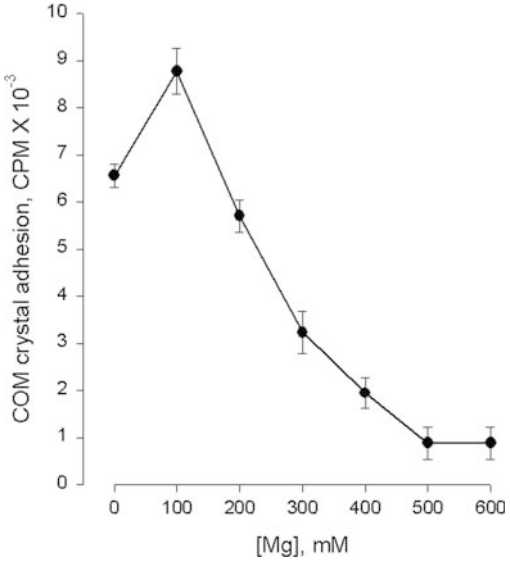

Fig. 1 Effect of magnesium on COM crystal adhesion to cells. Cultures of MDCKI cells were prepared as described in Materials and methods. The affinity of cells for crystals was assessed by replacing the medium with TBS with a set magnesium concentration (0–600 mM) and measuring the amount of exogenous [^{14}C]COM crystals that bound to cells during a 2-min period. Crystal adhesion fell progressively as the magnesium concentration was increased from 100 mM, reaching low baseline levels at concentrations ≥0.5 M. Each point is the mean ± SE of six samples

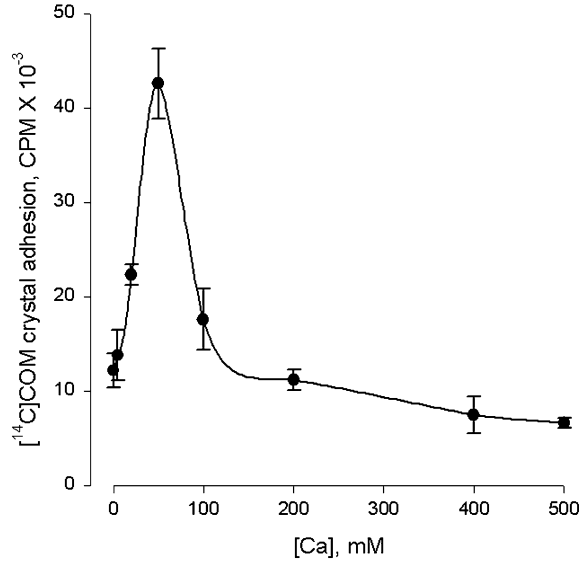

Fig. 2 Effect of calcium on COM crystal adhesion to cells. Cultures of MDCKI cells were prepared as described in Materials and methods. The affinity of cells for crystals was assessed by replacing the medium with TBS with a set calcium concentration (0–500 mM) and measuring the amount of exogenous [^{14}C]COM crystals that bound to cells during a 2-min period. Crystal adhesion increased as the calcium concentration was increased from 0 to 50 mM, and then fell progressively as the calcium concentration was further increased, reaching low baseline levels at concentrations ≥500 mM. Each point is the mean ± SE of six samples

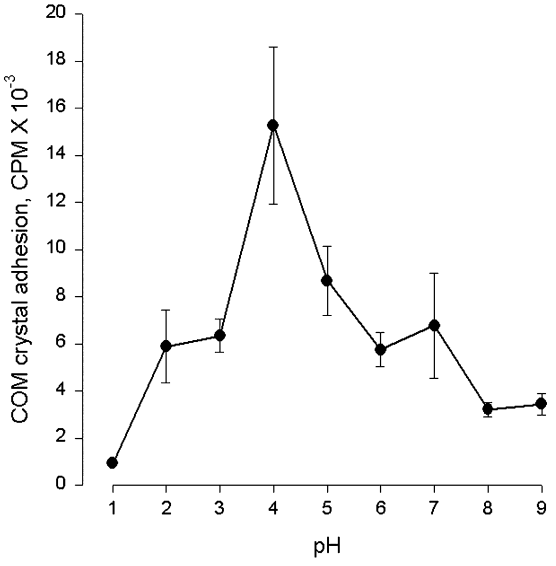

Fig. 3 Effect of pH on COM crystal adhesion to MDCKI cells. Cultures were prepared as described in Materials and methods. The affinity of cells for crystals was assessed by replacing the medium with TBS with a set pH (1–9) and measuring the amount of exogenous [^{14}C]COM crystals that bound to cells during a 2-min period. Crystal adhesion was low at pH 1, increased to a maximum at pH 4, and then decreased as the pH was increased to 8. Each point is the mean ± SE of six samples

scribed in Materials and methods. Exposure of MDCKI cells to pH 1 for 2 min resulted in nearly 100% cell death, whereas pH 9 was associated with 31% death. Very high concentrations of calcium (0.5 M) and magnesium (0.6 M) also resulted in a significant but smaller percentage of cell death (10.9 and 17.1%, respectively). Therefore, adhesion data at these extremes of pH, magnesium and cacium could be influenced by cellular events associated with cell death or damage, and not necessarily be the result of ionic changes at the cell or crystal surface. However, exposure of cells for 2 min to each of the other buffer extremes (e.g., pH 2 or 8, magnesium 0 or 0.5 M, and calcium 0 or 0.4 M) did *not* cause significant cell death, as assessed using this assay. Therefore, the bulk of the points in Figs. 1, 2 and 3 represent crystal adhesion to *living* cells.

Discussion

The results of the structural refinement indicate that magnesium is not incorporated into the structure of COM to any significant degree. Therefore, preformed calcium oxalate monohydrate accepts magnesium primarily as an adsorbate in a binding configuration which, as a surface phenomenon, is primarily controlled by localized charge effects, in agreement with mobility data presented by Curreri [27, 28]. Our current results verify this earlier electron microprobe data and clarify why the COM crystal lattice is insensitive to prolonged magnesium exposure (Table 1). All available data, together with theoretical considerations regarding the COM crystal structure, suggest that magnesium adsorption onto COM can only reach the trace element range ($<$3–5,000 p.p.m.), even after exposures as long as 1 year. Detailed electron microprobe and atomic force microscopic work would be needed to quantify this amount precisely.

For other calcium-containing crystals, the interaction with magnesium can differ. For example, in calcite (calcium carbonate) magnesium at very low concentrations ($<$0.3–0.4% in weight) also behaves as an adsorbate with a limited exchange capacity for calcium [29, 30, 31]. However, in calcite calcium is coordinated by six rather than eight oxygens. Therefore, at equilibrium, up to eight mole percent of magnesium can incorporate into calcite when the crystal is grown exposed to supersaturated magnesium solutions for prolonged periods of time [30]. Under non-equilibrium conditions magnesium incorporation can become much higher ($>$20 moles percent; i.e., [29]).

Our current study also demonstrates that the ionic milieu importantly influences the adhesion of preformed COM crystals to structures on the surface of renal cells. As the magnesium concentration was increased from 100 to 500 mM, crystal binding to cells also fell progressively. However, the urinary concentration of magnesium is typically in the range of 2–5 mmol/L, and changes within this narrow range do not appear to have quantitatively important effects (Fig. 1). In addition, inhibition of crystal binding to cells at magnesium concentrations above 100 mM is not likely to have physiologic significance in the human urinary tract. However, the change in crystal binding to cells in relation to the ambient calcium concentration is of considerable pathophysiological interest. Crystal adhesion increased nearly 3-fold as the calcium concentration increased across a physiologically achievable range (0–0 mM; Fig. 2). Therefore, hypercalciuria could promote nephrolithiasis by increasing crystal adhesion to cells, a previously unappreciated potential mechanism. Finally, as the pH increased from 4 to 7, COM crystal binding to cells decreased by 56% (Fig. 2), suggesting that a pH value of 4–5 is optimal for COM crystal binding to cellular proteins. This observed pH-dependence of crystal adhesion confirms that observed by Verkoelen and colleagues [32]. Therefore, a urinary pH at the lower limit observed in humans, i.e., pH 5 (or even slightly less) may predispose to kidney stone formation by increasing the likelihood of calcium oxalate crystal adhesion to cells and subsequent retention in the kidney.

In addition to the pathogenic implications, these data shed insight into the bonding interactions that take place between COM crystals and molecules on the cell surface. As the magnesium concentration increased from 0 to 100 mM, adhesion of COM crystals to a confluent monolayer (MDCKI line) increased by 33%. This trend, however, reversed itself when the magnesium concentration increased further from 100 to 500 mM. The variation in observed crystal adhesion must be related to the influence magnesium exerts on the interface that

links COM to the apical surface of the cells. In principle, magnesium could exert this action either at the COM surface, the cell surface, or both. The latter possibility, however, does not appear likely since the carboxyl groups that lay on the cell surface tend to bind magnesium at far lower concentrations (<100 mM [33]) than were necessary to inhibit crystal adhesion. Therefore, most likely, magnesium influences cell-COM interactions once it is adsorbed onto the crystal surface of COM. Here, in fact, magnesium can bind with available oxygen anions. However, once the sites in the crystal structure of COM available to magnesium are fully occupied, any additional increase in magnesium results in local repulsion, as has been described for similar interactions in several polymers and proteins [34]. In our system, such a threshold is reached at a magnesium concentration of $\approx$100 mM.

A similar behavior is observed for calcium. When present in the optimum concentration ($\cong$50 mM), calcium markedly promoted crystal adhesion to cellular proteins. At higher concentrations calcium, instead, blocked adhesion of COM to cells. In addition, calcium was a far more efficient promotor of crystal binding to the same cells than magnesium (Figs. 1 and 2).

These observations can be rationalized if one considers the overall process of adhesion and incorporation of ions into the COM structure. Excess calcium is, in fact, readily incorporated into the structure of COM (within calcium sites and voids) and, like magnesium, can also adsorb upon the surface of COM. Magnesium, however, achieves a six-fold coordination with the oxygen anions, rather than the eight-fold coordination required by the structures of both COM and COD. Consequently, and contrary to calcium, magnesium cannot substitute for calcium in the structure of COM but is only able to adsorb on the crystal surface, as was formally demonstrated by the structural refinement reported in Table 1. It is expected that the calcium concentration threshold for optimal adhesion and ionic incorporation occurs when the calcium-binding sites are optimally occupied at both the cellular and crystal level (including calcium sites, voids and surface adsorbed calcium). This occurs under conditions of high calcium density per atomic volume at which point any further increase in the local calcium concentration eventually fosters repulsion. Computational models of ionic interaction [35] indicate that ligand-ligand repulsion effects are considerably higher around calcium than magnesium in the hydrated complexes that both ions form as part of their interaction with proteins. This repulsion differential is in part due to the relative coordination number of the oxygen anions around calcium (eight) versus magnesium (six), which also results in longer distances between calcium and oxygen relative to magnesium and oxygen. This differential demand for atomic space between the two ions is consistent with the lower threshold value for a fall in crystal adhesion to cells observed for calcium (50 mM, Fig. 2) as compared with magnesium (100 mM, Fig. 1).

Finally, in order for calcium and magnesium to form hydrated complexes with cell surface proteins over the physiologic pH range (pH 4–8, Fig. 3), hydrogen ions must be readily available, and our results confirm that hydrogen ions promote crystal binding to cells (Fig. 3). This pH-dependency of crystal binding is *unlikely* to be mediated by cell surface sulfates or carboxyl groups, since both are protonated in the 4–8 pH range [33].

In conclusion, our study demonstrates that variations in the ambient calcium and hydrogen ion concentration, within the biologically relevant range, have important effects on calcium oxalate crystal adhesion to cells. Therefore, hypercalciuria and/or an acid urine could favor renal stone formation via unexpected pathways (i.e., crystal binding to cells rather than crystal growth). In addition, although magnesium does not incorporate into the crystal structure of COM, the cation does exert important effects on the crystal surface, and influences the interaction of preformed calcium oxalate crystals with cellular proteins, as does the prevailing concentration of calcium and hydrogen ions. Although variation of urinary magnesium within the biologically relevant range is not likely to alter crystal binding, increased understanding of the mechanisms whereby magnesium alters the atomic interaction of COM and COD crystals with minerals and proteins could shed important insights into the mechanisms whereby calcium oxalate deposits in human kidneys resulting in nephrolithiasis.

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