The dissolution of monosodium urate monohydrate crystals: formulation of a biocompatible buffer solution with potential use in the treatment of gouty arthropathies

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Abstract

The dissolving abilities (DAs) of several aqueous media for microcrystalline monosodium urate monohydrate (MSU, NaC₅N₃O₇H₂·H₂O) have been investigated using UV spectrophotometry for quantitative analytical determinations and X-ray diffraction, scanning electron microscopy and polarized light optical microscopy to assess structural aspects. High DAs were found for a buffer labeled TMT which contains tris(hydroxymethyl)aminomethane (TRIS), tris(hydroxymethyl)aminomethane hydrochloride (TRIS-HCl), D-mannitol (MAN) and taurine (TAU) and gave DA₃₀=1298(5) mg/L for synthetic MSU after 30 min incubation at 37°C and pH 7.4, most of the dissolution taking place within the first 5-10 min. Semiempirical molecular modelling techniques (ZINDO/1) show a favorable energy balance for the formation of a TRISurate–TRIS adduct which might explain the high DA values. Buffers containing linear or dendrimeric polylamines gave DA values which suggest that complex formation toward sodium cations is less important. An ex vivo MSU sample was found to have a significantly lower DA value (DA₀₃₀=1124(5) mg/L in TMT) as well as a lower crystallinity than its synthetic counterpart, possibly related to the presence of a non-crystalline impurity such as endogenous proteins. Cytotoxicity tests based on the MTT assay were used to check the bio-compatibility of the TMT buffer and showed only moderate cell mortality after 24 h contact with the buffer solution.

Introduction

Biomineralizations are an important issue in arthropathies. One group of disorders, represented by chondrocalcinosis and pseudogout, are associated to calcium pyrophosphate dihydrate (CPPD, Ca₇P₂O₁₀·2H₂O) and hydroxyapatite (HAP, Ca₁₀(Po₄)₆(OH)₂), which deposit in the cartilage and soft tissue around the joint, e.g. in the synovial membrane. CPPD forms slowly and is found in many elderly individuals (~40% at age 80 and older). The crystals, usually small (∼5 µm) and of compact shape, contribute to various degenerative processes. In some instances, e.g. due to trauma, the crystals may also cause inflammation (pseudogout), usually through lysis of neutrophil polymorphonuclear (PMN) leukocytes which phagocytize freely suspended crystals in the synovial cavity.

Beyond symptomatic treatment of the inflammation, e.g. by oral medication with non-steroidal anti-inflammatory drugs (NSAIDs), a method for the removal of the crystals themselves has recently been proposed, based on joint lavage with Ca-complexing buffer solutions, that seems interesting to prevent pseudogout attacks and mitigate some of the degenerative processes due to crystal deposits.

A quite different situation is found in gout, which develops in the presence of monosodium urate monohydrate crystals (MSU, 2,6,8-trioxopurine·(H) sodium salt monohydrate, NaC₅N₃O₇H₂·H₂O, Figure 1).

In contrast to the phosphates above, MSU is not an ionic, but a van der Waals solid, i.e. the bonds between the single ions or molecules are much weaker and the crystal easily undergoes plastic deformation. The soft MSU crystals can therefore hardly be responsible for a mechanical degeneration of the joint, but they may still provoke inflammation (gout) in a similar way as described above. Another peculiarity of MSU is that its formation is clearly related to high levels of urate in the blood serum (uricemia) but, although necessary, this condition is not sufficient to provoke gout, either because crystallization in the synovial liquid may be inhibited or because the crystals are somehow blocked to develop their pathogenic actions. Much ongoing research is concerned with these facts and it seems that surface covering of crystal faces may play a role, but the therapy of gout is so far restricted to the symptomatic treatment of the inflammation itself and to the prevention of urate crystal formation through the control of uricemia, either reducing the urate production from purine (e.g. using xanthine oxidase inhibitors) or increasing the rate of uric acid elimination (e.g. using uricosuric drugs).

While the systemic treatment of uricemia is efficient to prevent urate crystal formation, the removal of the crystals, once they have formed, remains a problem. The systemic approach is in principle possible because urate would spontaneously diffuse from the synovial fluid (concentrated compartment) to the blood plasma (diluted compartment) until both compartments have the same concentration, insufficient to reach the solubility product of MSU crystal formation. However, this diffusional process takes place through a quite limited membrane area defined by the blood capillaries at the inner wall of the synovial membrane and it would take 3 months to 3 years to dissolve MSU crystal deposits in this way.

In search for an alternative, the present study is aimed at finding a suitable buffer solution for the direct dissolution of the MSU deposits through joint lavage. Beside the dissolving ability of the different chemical agents which can be added to the buffer and may specifically sequester either sodium (dendrimers etc.) or the urate anion (hydrogen-bridge formers), particular attention has been
Materials and Methods

Materials

Crystals of sodium hydrogen urate monohydrate (2,6,8-trioxopurinate(4H) sodium salt monohydrate, MSU, NaC₅N₄O₃H₂O, MM=208.11 g/mol, Figure 1) were analytical grade, a.g., from Sigma, Milan. Tris(hydroxymethyl)aminomethane (1,3-dihydroxy-2-hydroxymethyl-2-aminopropane, TRIS, C₃H₇NO₃), TRIS-HCl, D-mannitol (D-2,3,4,5,6-pentahydroxy-hexan-1-ol, MAN, C₁₀H₁₂O₅) and taurine (2-amino-ethansulfonic acid, TAU, C₁₂H₁₄O₄S) were from Carlo Erba, Milan. Spermine (1,12-diamino-4,9-diazadodecan, SPE, C₁₂H₂₆N₄) was from Fluka, Basel. Polyamidoamine (N,N,N’,N’-(3-one-4-aza-6-amino-hexyl)-1,2-diaminoethane, PAMAM, C₃₀H₅₈N₁₄O₂) tetrazolium salt (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide, MTT, C₁₈H₁₄N₅SBr) and Dulbecco’s modified Eagle’s medium (DMEM) were from Sigma, Milan. NaCl, KCl, KH₂PO₄, NaHPO₄·12H₂O (PBS buffer) were a.g. from Carlo Erba, Milan. Na₂HPO₄·12H₂O (PBS buffer) were a.g. from Carlo Erba, Milan. All reagents have been used as purchased without further purification. In addition to the synthetic reagents, MSU was also used in form of ex vivo crystals obtained from tophaceous subcutaneous deposits and on ex vivo crystal suspensions in synovial fluid aspirated from an acute inflamed knee.

Dissolving ability

Synthetic crystals (12 mg, stored at room temperature, 22(1)°C, in ambient air atmosphere) were incubated in 5 mL of the dissolving solutions at pH 7.4 (1) (pH-Meter E603, Metrohm), osmolality 290(10) mOsm (Osmostat, DIC) and 37°C for different incubation times (1-60 min), under stirring (numbers in parentheses give the estimated standard deviations and are referred to the last digit). The dissolving solution was, in most cases, either TM, which consists of a TRIS buffer (TRIS-HCl 5.72 g/L, TRIS 1.66 g/L) containing MAN (36 g/L), or TMT, which stands for TM plus TAU (3 g/L).

Further dissolution tests were performed using the following alternative formulations: deionized water with diluted NaOH to reach pH 7.4 (5 mOsm), aqueous NaCl (8 g/L), TRIS buffer alone (80 mOsm), PAMAM (2.38 g/L) with HCl to reach pH 7.4 (33 mOsm), SPE (2.38 g/L) and HCl to reach pH 7.4 (34 mOsm), SPE (2.38 g/L) with MAN (50 g/L) and HCl to reach pH 7.4 (309 mOsm), PBS (NaCl 8 g/L, KCl 0.2 g/L, Na₂HPO₄·12H₂O 2.87 g/L, KH₃PO₄ 0.2 g/L, pH 7.4, 300 mOsm), PBS without NaCl (27 mOsm), PBS with SPE (2.38 g/L, 334 mOsm), PBS without NaCl and with SPE (2.38 g/L, 334 mOsm). After incubation, the mixtures were filtered using syringes equipped with a surfactant-free cellulose acetate membrane filter (Schleicher & Schuell, disk diameter 30 mm, mean pore diameter 0.22 μm). The determination of DA values for MSU was carried out, in the clear filtrates, via UV spectrophotometry (Perkin Elmer-Model EZ-201 spectrophotometer) at λ=291.0 nm.

X-ray crystallography

X-ray powder diffraction patterns were collected for the different samples of MSU in order to check their crystallographic identity, phase purity and grain size before and after solution treatment. An automated Philips PW1710 powder diffractometer with Bragg-Brentano geometry was used in combination with CuKα (λ=1.54060 Å) radiation and a graphite monochromator. Optical slit and scan parameters were: in plane divergence 1°, receiving slit 0.2 mm, step size/time 0.04°/2s. Peak widths in terms of FWHM were obtained from a single peak curve fitting procedure and used to estimate the apparent grain size s following the Scherrer equation and allowing for a correction term which accounts for the instrumental contribution to the peak width.15

Crystal morphology

A scanning electron microscope (SEM, Philips XL30, acceleration voltage 20 kV) equipped with an energy dispersive fluorescent X-ray detector (EDAX DX4) for elemental analysis (Z≥6) was used to assess morphology and composition of ex vivo MSU samples and their synthetic counterparts before and after solution treatment. The samples were mounted on Al-holders and metallized in a graphite evaporator (Balzers CED 020, 50 Pa), which allows for EDAX analysis of all possible elements heavier than carbon. For polarized light optical microscopy observations of the crystals and their dissolution behavior in the buffer, a polarizing microscope (Leica DMLSP) permitting 360° azimuthal sample rotation was used. A suspension of MSU crystals was placed in a closed cuvette at temperatures from 20 to 50°C, and pictures were taken at time intervals with a CCD camera system (JVC-TK-C1380) directly coupled to the microscope photo tube exit.

Toxicity test in vitro

Measurements of cellular vitality have been performed on chondrocytes and synovial cells. The cells were obtained from three patients with hip osteoarthritis undergoing surgery for total hip prostheses. Chondrocyte and synovio-cyte cultures were obtained using the method described in Cini et al.5 Cells were then incubated with varying concentrations of TRIS-HCl/TRIS and D-mannitol (TM) as well as the typical (see above) concentration of TRIS-HCl/TRIS, D-mannitol and taurine (TMT) for 24 hours at 37°C. The control culture was obtained by incubating cells with Dulbecco’s modified Eagle’s medium without foetal calf serum for 24 hours. Cytotoxicity was determined two days after exposure to TM and...
TMT using the MTT assay, which measures the number of metabolically active cells by a colorimetric technique. In the case of TRIS-HCl+TRIS+MAN+TAU (5.72+1.66+36.00+3.00 g/L) toxicity experiments were performed also at 0.5, 2.0, 4.0, 12.0 h.

Structure simulations and adduct formation energies

For uric acid, the urate anion in its four possible configurations with respect to deprotonation (H1, H3, H7, H9), the TRIS ligand and the TRIS-urate and TRIS-uric acid systems, calculations were made applying molecular orbital semi-empirical AM1 and ZINDO/1 methods as well as molecular mechanics MM using a MM2-type force field in order to identify the most stable structures and to get an estimate of the corresponding adduct formation energies, normally in the gaseous state and in some cases also in presence of solvent (water) as simulated via a continuum field (in MM calculations). Calculations have been performed using the HyperChem 5.1 (for AM1 and ZINDO/1) and MacroModel 6.0 (for MM) program systems, graphic representations have been obtained using ORTEP-3.

Results

Dissolving ability values

Most dissolution experiments were conducted using synthetic MSU crystals. The highest DA values were obtained using the TRIS+MAN, TM, mixture which gave, after 30 min incubation, $DA_{30}=1303 (5) \text{ mg/L}$ (Table 1 and Figure 2).

Table 1. Dissolving ability (DA) values (mg/L) for synthetic MSU crystals (12 mg) obtained by using TRIS+MAN, TM, and TRIS+MAN+TAU, TMT, aqueous buffer solutions (5 mL, pH 7.4, 290 mOsm), at different incubation times $t$ (min). Estimated standard deviations are 5 mg/L as calculated from averaging over multiple determinations.

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*These values depend on the total amount of MSU crystals used in the experiments. 50% means that 6 mg of the total of 12 mg MSU per 5 mL have been dissolved. See text for explanation of the choice of 12 mg for total amount of MSU in the DA measurements.

Addition of TAU to the buffer (TRIS+MAN+TAU, TMT) has little influence on the dissolving ability and shows $DA_{30}=1298 (5) \text{ mg/L}$. It has to be noted that crystal dissolution happens mostly during the first 5-10 min incubation time. In fact, DA values after 5, 10, 30, 60 min incubation at 37°C are 1217(5), 1241(5), 1298(5), 1298(5) mg/L, respectively (Figure 2A).

The DA values measured for pure water are somewhat smaller than those for TM and TMT, but still relatively high with $DA_{30}=1146(5) \text{ mg/L}$ at pH 7.4. The datum is in agreement with earlier findings and shows that water is, in principle, an effective dissolving agent. Its use is, however, precluded for in vivo applications because of its poor buffer capacity and the low osmolality. In the case of PBS both the buffering ability and the osmolality (300 mOsm) would be suitable for in vivo treatments but the dissolving ability was found to be very poor, $DA_{30}=85(5) \text{ mg/L}$, in agreement with Wang et al.

The reason for the decreased effectiveness is the common ion (Na+) effect, PBS being rich in sodium chloride (8 g/L). In the case NaCl is removed from PBS (27 mOsm), $DA_{30}$ increases up to 592(5) mg/L (it has to be recalled that sodium ions are still present due to the presence of Na2HPO4·12H2O).

Additional tests were performed to check the alkali ion and urate ion complexing power of linear amines or dendrimers. In the case the system contains SPE (pH 7.4, 34 mOsm), the dissolving efficiency is again high, $DA_{30}=1359(5) \text{ mg/L}$. The addition of MAN to adjust the osmolality of the buffer to 309 mOsm gives a small increase in effectiveness,

Figure 2. Dissolving ability values (mg/L) for synthetic MSU crystals (12 mg) of TRIS+MAN+TAU, TMT, buffer solution (5 mL, pH 7.4, osmolality 290 mOsm): A) as a function of time at 37°C; B) as a function of temperature in the range 27- 47°C, after 30 min incubation time.

Figure 2. Dissolving ability values (mg/L) for synthetic MSU crystals (12 mg) of TRIS+MAN+TAU, TMT, buffer solution (5 mL, pH 7.4, osmolality 290 mOsm): A) as a function of time at 37°C; B) as a function of temperature in the range 27- 47°C, after 30 min incubation time.
DA₃₀=1383(5) mg/L. A less efficient behavior, with DA₃₀=1141(5) mg/L, was found for the systems containing the dendrimeric molecule PAMAM (and HCl to reach pH 7.4). In view of these results it seems that H-donor/acceptor properties from the molecules in the dissolving media might explain the increase of solubility with respect to pure water. Some answers to this question will come from molecular modeling analysis (see below). It has to be noted that an ex vivo tophus taken from a gouty patient and consisting mainly of MSU, gave a concentration of 1124(5) mg/L in TMT buffer when 12 mg of solid were incubated in 5 mL at 37°C for 30 min. This is considerably less than for synthetic MSU and might be important for the understanding of the crystallization mechanism of MSU (see discussion).

Finally the influence of temperature on dissolution of synthetic MSU was studied. On changing the temperature in the range 27-47°C, the concentration of MSU in TMT (using 12 mg of crystals, 5 mL of buffer, and incubation times of 10 min) increases from 948(5) to 1644(5) mg/L (Figure 2B). An approximate heat of dissolution of 4.9(3) kcal/mol (in TMT) could be computed from these data. A similar value (5.5 kcal/mol) can be calculated from data given by Allen et al.8 for the solution of monosodium urate in water. These values may be compared with the heats of dissolution for uric acid dihydrate where a much larger difference (4.0 kcal/mol in TRIS buffer and 15.4 kcal/mol in water) was found and attributed to the exothermic acid-base reaction H₂U+TRIS → HU−+TRISH that occurs in the dissolution of uric acid.25

Crystal structure and morphology

X-ray diffraction patterns (Supplementary Figure 1) match with PDF Card #31-1890 showing that all MSU samples are essentially phase pure materials having the triclinic structure reported in Mandel et al.26,27

The apparent grain size ε estimated from the peak width is 520(120) Å for the original synthetic MSU crystals prior and their residues after incubation in TMT (30 min), and 380(80) Å for MSU extracted from ex vivo gouty tophi.
Under the polarizing microscope, a MSU suspension of tophaceous deposits in synovial fluid showed crystals of about 5×15 µm² (Figure 3). At low temperature, these crystals did not dissolve appreciably during incubation periods up to about 30 min. Once the temperature was raised above 30°C, dissolution became rapid and, without changing their acicular habit, many crystals disappeared within 5-10 min (Figure 3).

As can be seen from the scanning electron micrograph on Supplementary Figure 2, an apparent single crystal of ~6×15 µm² is actually made up of many small laths, evidently the product of decomposition under electron beam bombardment. The thickness of these laths (~1000 Å) comes near to the grain size estimated from diffraction peak broadening, i.e. the coherent domain size within which the crystal structure has no defects, and suggests that, in the real crystal, such submicroscopic domains are packed together forming a mosaic-like aggregate (Figure 4).

Toxicity of dissolving media

The results of cytotoxicity tests on human synoviocytes and chondrocytes at increasing overall concentrations (24 h incubation time) of TRIS (pH 7.4, 280-300 mOsM) are reported in Table 2 and indicate that the concentration of TRIS that corresponds to the highest DA values was also the less cytotoxic for cultured chondrocytes and fibroblast-like synoviocytes, with a percentage of metabolically active cells of 57(5) and 67(12), respectively. On increasing the concentration of TRIS·HCl/TRIS beyond 5.72/1.66 g/L, the toxicity of the buffer solution increases significantly, and for concentrations of 11.44/3.32 g/L approximately one third only of the cells remain metabolically active. Toxicity measurements performed at 0.5, 2.0, 4.0, 12.0 h for TMT buffer reveals that at ca 1 h incubation time (time requested for possible joint treatments) the toxicity for synoviocytes and chondrocytes is acceptably low.

Molecular modeling and complex formation energies

In an attempt to understand the dissolving abilities of the different solutions, the structure of urate, selected dissolving agents and possible adducts were studied using molecular modeling techniques. The structure of the hydrogen urate anion (HU⁻) was optimized both at the AM1 and the ZINDO/1 level. The latter level produced the better result as the simulated molecule was planar and computed distances and angles are in good agreement with experimental results from X-ray structure determination of MSU in the solid state (distances deviate less than 0.05 Å excluding the N-H deviations which average 0.09 Å).27 Geometrical parameters are also in good agreement with the data obtained via density functional methods and reported in Chandra et al. It is also noteworthy that the most stable optimized HU⁻ anion, as computed at the gas phase, is deprotonated at H⁺ in agreement with the X-ray results. The standard formation enthalpies for the MSU anions are: HU⁻-H₁, -3117.86; HU⁻-H³, -3138.77; HU⁻-H⁷, -3112.32; HU⁻-H⁹, -3132.77 kcal/mol.

The structure of TRIS is flexible and several minima can be found on the conformational surface. The structure found in the solid state is close to one minimum energy structure (TRIS-A, Supplementary Table 1, Figure 5) found in the gas phase via ZINDO/1,²⁵,²⁶ but it is less stable than another one (TRIS-B, Figure 5) that has two strong intramolecular hydrogen bonds which lack in the conformation corresponding to the solid state structure. The computed formation energy (ZINDO/1) for a TRIS-HU⁻-TRIS aggregate is -175.94 kcal/mol, without any solvation effect. This is a quite large energy which lies clearly beyond the difference between the energies of the various conformations of TRIS alone and has its origin in the cooperative effect of several

![Figure 5. Selected structures and aggregates as obtained from semi-empirical molecular orbital methods at ZINDO/1 level or from molecular mechanics methods at MM2 level.](image-url)
Discussion

Dissolving abilities and their origin

The amount of MSU (both synthetic and ex vivo) used in the experiments for the determination of the dissolving ability was 12 mg/mL, which is about two times the solubility at 37°C and was chosen for two main reasons. First, to have enough sample to keep the relative weighing error below ca. 0.8%. Second, to be in conditions far from complete dissolution of the crystals and through this to reduce the effect of possible grain size inhomogeneities within the sample. When using at least 12 mg of crystalline sample, a good reproducibility was achieved as expressed by the low value of the relative standard deviation (0.4%) obtained from averaging.

The data from this work show good dissolving properties of solutions based on TRIS buffers (TM and TMT) for both synthetic and ex vivo MSU crystals. The high dissolving ability comes from several factors. First, the buffering effect of the media that excludes the precipitation of uric acid (pKₐ=5.5) [20] once the HU⁻ anions pass into solution from the crystal surface. Second, the absence of any common ion effect. The PBS buffer, that contains a common ion with respect to MSU ([Na⁺] = 137 mmol/L), is much less effective. Third, the molecules of TRIS (in both their neutral and protonated forms) help solvation of HU⁻ anions via hydrogen bond formation. It has to be recalled that the solubility of MSU in TMT is significantly higher than in pure water, the increase in terms of DAₚ being about 13%.

On the other hand, the complex formation ability of the molecules contained in the buffering media toward sodium cations seems to be of less importance as shown by the small or null increase of the DA values in solutions containing SPE and PAMAM when compared to the corresponding values for TMT and pure water, and considering that the expected chelating effects from polyamines are larger than those from TRIS or TRISH⁻ molecules, at least in the gas phase or in media with low dielectric constants.

TRIS-HU⁻ adduct formation seems therefore more important to explain the high DA values for TM and TMT media. This is confirmed by the results from the molecular modeling analysis which shows that the TRIS and TRISH⁻ molecules have a high affinity for the HU⁻ anions owing to the formation of many strong hydrogen bonds. The stabilizing contribution has an upper limit of ca. -22 kcal/mol for each H-bond linkage in the TRIS-HU⁻-TRIS system. This value is reasonable when compared to the computed (gas phase, density functional theory) [21] formation energy of ca. -27 kcal/mol for the 6-thiopurine…6-thiopurinate pair having one N-H…N and two C-H…S hydrogen bond type interactions and for the guanine…cytosine pair in DNA [22,23]. The adduct formation energies should be much lower once solvent effects are taken into account especially for aqueous systems. The preliminary treatment of solvent (water) effects was carried out at the MM level to model the selected aggregates. Comparing the TRIS-HU⁻-TRIS system calculated without and with solvent effects, it appears that the overall scheme is maintained but, in the presence of bulk water, the two TRIS molecules are located at larger distances from HU⁻. It has to be noted that TRISH⁻ cations (that are massively present in the buffer at pH 7.4) should reasonably give stronger interactions with HU⁻ anions than neutral TRIS molecules do, because of more favorable electrostatic contributions.

Therapeutic applications

For the incubation time necessary to attain maximum DA values (~30 min, Figure 2), TRIS buffer has no appreciable toxicity on human chondrocytes (Table 2), possibly due to an oxygen radical scavenger activity stemming from the MAN and TAU co-adjuvants as can be predicted on the basis of previously reported data [24]. Furthermore, the experiments in this work were conducted with a well-defined volume of dissolving agents and the relative dissolution data were obtained at conditions close to equilibrium (as regards the dissolution process). However one can hypothesize that, in vivo, the dissolving agent could be administered in a continuous flow regime, i.e. joint lavage, that could be more effective owing to two main effects: i) the mechanical removal of the crystalline MSU aggregates; ii) an improvement of the dissolution kinetics if the crystals are exposed to a continuously renewed fresh buffer solution (Figure 2B). Under these conditions, the exposure of the synovial cavity to the buffer solution could be considerably shortened (a few minutes would be sufficient) and toxic interferences would be further reduced.

However, cytotoxicity might not be the only aspect to be considered for a therapeutic application of the buffer solution. The aetiologic role of MSU crystals in the development of gout is well established [1,13] but the exact mechanism of the inflammatory response is still a matter of debate. Independently from the factors that trigger crystal induced arthritis, the idea to consider the crystal itself the target of the therapeutic strategy is, in our opinion, quite intriguing. To discuss this concept, few data are available from literature. Bennet et al. [26] observed that injecting a joint affected by chondrocalcinosis with potent calcium chelating agents such as disodium EDTA in order to dissolve CPPD aggregates, a pseudogout attack had been triggered. This paradox was interpreted with the occurrence of a phenomenon of crystal shedding of CPPD deposits from the tissues into joint cavities. The released crystals would be small and more phlogistic than the bigger ones and those anchored to the tissue, in agreement with the finding that the crystal dimensions have much importance on phagocytosis of MSU and CPPD crystals by polymorphonuclear leukocytes [27]. On the other hand, Bennet et al. [26] cite that no phlogistic effects are observed when a PBS buffer solution alone is injected. Resuming the above, a therapy based on the direct dissolution of MSU crystals appears possible, but the risk of inflammation and other side effects should be carefully assessed before its clinical application.

Dissolution mechanism and microstructure of monosodium urate monohydrate crystals

Optical microscopy (Figure 3) and the analysis of X-ray diffraction peak widths (Supplementary Figure 1) show that the dissolution of MSU rapidly reduces the crystal size while the grain size ε remains constant during the process. This means that, during incubation, grains near to the exterior of the crystals (Figure 4) are first dissolved before the underlying grains are attacked, i.e. the dissolution process is not likely to create more crystals than initially present. Regarding microstructure and morphology, the ex vivo extracted MSU material (Supplementary Figure 2B) is similar to its synthetic counterpart, but its grain size ε is smaller. While the absolute value of the grain size estimate may be in error by 20-25%, the difference is significant and potentially important for the mechanism of crystal growth and dissolution. The reason for the smaller grain size of the ex vivo material remains at present unclear, although an alkalization compositional variance can be excluded from EDAX evidence. Similar differences have also been reported in earlier studies, e.g. for the average size of the entire crystal which is greater for synthetic than for ex vivo MSU material [28,29] and for the decomposition under the electron beam which leaves porous residues with different texture at the submicron-length scale [30]. It should be remembered, at this point, that the present grain size estimates are based on a non-destructive method.
(X-ray diffraction) and therefore more reliable for a description of the actual microstructure of MSU crystals.

Finally, it has to be recalled that the DA values of TM or TMT for MSU crystals from ex vivo samples from a gouty patient is ca 10% smaller than the corresponding values for synthetic samples. This might be related to the presence of endogenous proteins or other impurities, linked to the ex vivo crystals. The EDAX and XRD analyses on the ex vivo sample were in agreement with a high purity of MSU as Na+ salt, without detectable amounts of other cations (Ca2+, Mg2+, K+) or crystalline impurities. Endogenous proteins linked to MSU crystals are therefore more likely to account for the reduced dissolving ability, either simply because they add up to the total mass of the sample or because they act in some way as a protective coating. Endogenous proteins have been the subject of extensive discussions regarding the mechanism in which MSU provokes the development of gout,12,13,15 and recently, such materials have also gained much interest for new theories about the nucleation and growth of crystals where protein-induced clusters are thought to play a central rôle.

Conclusions

The present study demonstrates that monosodium urate crystals can effectively be dissolved at physiological pH (7.4) and temperature (37°C) using an appropriate biocompatible buffer solution which shows the way to new therapeutic alternatives in the treatment of gout. Test results for dissolving media of different composition and energy balance parameters obtained from experiment and molecular modeling point out that the dissolving efficiency is based on (a) the buffer action which prevents formation of the more insoluble uric acid, (b) the avoidance of a common ion (Na) effect, and (c) the presence of a molecule (here the buffer itself, TRIS) able to form hydrogen bridge adducts with urate. The addition of dendrimers (PAMAM) to sequester proteins has been the subject of extensive modeling that point out that the dissolving efficiency, which remains similar as that of pure water. It has to be noticed that the comparative analysis that has been performed on different buffers (TM, PAMAM, PBS, PBS+sPE, etc.) showed that TMT was the most versatile and efficient buffer as regards dissolving ability and other important parameters, like osmolarity and pH (see Dissolving Ability paragraph, under Materials and Methods and under Results sections). These facts are of general interest for work with biomineralizations involving urate.

References

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