Metabolism of crystals within the joint

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SUMMARY

Monosodium urate (MSU), calcium pyrophosphate dihydrate (CPPD) and basic calcium phosphate (BCP) crystals deposit in joints and surrounding tissues causing acute inflammation and chronic cartilage damage. A number of endogenous substances and physicochemical conditions affect their precipitation, growth and even dissolution, regulating their metabolism and inflammatory activity. We review how MSU and calcium crystals form within the joints and the various factor which regulate their formation.

***Key words**: monosodium urate, pyrophosphate, basic calcium phosphate, crystals, metabolism.*

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INTRODUCTION

Crystal deposition disease occurs when crystals form in joints and surrounding tissues. According to the number and size of the crystals, their deposition may induce acute inflammation and chronic cartilage damage. The most common and well known of these crystals are monosodium urate (MSU) which causes gout, calcium pyrophosphate dihydrate (CPPD) which causes acute (pseudogout) or chronic arthropathy, and basic calcium phosphate (BCP), including carbonate-substituted hydroxyapatite, octacalcium phosphate, and rarely tricalcium phosphate, which may cause chronic joint damage. Furthermore, calcium-containing crystals are thought to play an important part in progression to osteoarthritis (OA) (1). We will review how MSU and calcium crystals form within the joints and the various factors which regulate their formation.

CRYSTAL CHEMISTRY

A crystal structure is defined as the particular repeating arrangement of atoms in 3-dimensional space. Usually, a crystal forms when the solubility limits are exceeded. Solubility depends on a variety of factors, including temperature, pressure, the other species present in the solution, and the perfection and size of the crystals. The first stage of crystal growth is called nucleation. This occurs when the solute molecules dispersed in the solvent start to gather into clusters. These clusters, if stable, constitute the nuclei; otherwise they re-dissolve. Therefore, the clusters need to reach a critical size in order to become stable nuclei. This is dictated by the growth conditions (temperature, supersaturation, etc.). It is at the nucleation stage that the atoms arrange themselves in the defined and periodic pattern that defines the crystal structure.

According to the type of crystal structure (called Bravais lattice), crystals fall into seven different "crystal systems": triclinic, monoclinic, rhombohedral, hexagonal, orthorhombic, tetragonal and cubic. The cubic system is isotropic but the crystals of the other systems are said to be anisotropic, having two or even three refractive indexes, making them birifrangent.

The crystal grows once the nuclei has achieved the critical cluster size. Nucleation and growth continue to occur simultaneously as long as supersaturation persists. Supersaturation is the driving force of crystallization. Depending on the conditions, either nucleation or growth may be the dominant force and as a result, crystals with different sizes and shapes are obtained. However, supersaturation itself is insufficient to cause crystals to form; the crystal embryos must form by collision of molecules of solute in the solution, or sometimes by the addition

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of seed crystals, or other particles, or even particles from the surrounding environment. Any factors which can affect solute concentration, formation of the crystal nuclei and crystal growth around the nuclei will influence crystal formation, and will inhibit or promote crystallization.

MONOSODIUM URATE CRYSTAL METABOLISM

Uric acid is the end product of purine metabolism. It is a weak organic acid that under physiological conditions is mainly found as the urate ion (Figure 1). At a pH below 5.75, as may occur in the urine, the predominant form is non-ionized uric acid. Therefore, in all extracellular fluids in which pH is 7.4 and sodium is the principal cation, 98% of the uric acid is found as monosodium salt.

The vast majority of mammalian species have extremely low serum urate levels (about 1 mg/dL) because uric acid is converted by uricase to allantoin, a highly soluble excretory product. By contrast, in humans in which the homolog uricase gene is structurally modified to an unexpressed state, serum urate concentrations approach the theoretical limit of solubility of urate (a concentration of 6.8 mg/dL). When the serum urate concentration exceeds the solubility of urate, supersaturation of urate in the serum and other extracellular spaces takes place. This state is defined as hyperuricemia, is required, but not solely responsible for the crystallization of MSU crystals and development of gouty arthritis. In fact, hyperuricemia is relatively common, with a prevalence of more than 10% in certain populations, but only a few people with elevated circulating uric acid develop gout (2). These subjects, after a first stage of asymptomatic hyperuricemia, have periodic attacks of acute gout with crystal depositions in joints, followed by asymptomatic periods. Over the years, they develop chronic tophaceous gout with large deposits of crystals which damage the joints (3).

Although the different phases of the disease, as well as the mechanisms of urate crystal inflammatory response, have been extensively described over recent years, the triggering cause of MSU crystal precipitation in asyntomatic hyperuricemia has still not been identified. What can be inferred from the literature is that crystal formation and deposition is a multifactorial process.

It has been shown that a number of endogenous substances and physicochemical conditions affect crystal precipitation, growth and even dissolution, regulating not only MSU crystal metabolism but also its inflammatory activity.

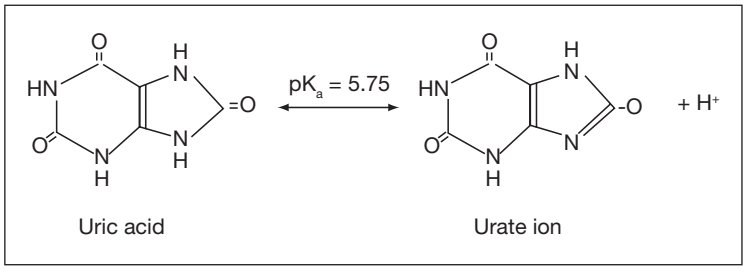

Figure 1 - Dissociation of uric acid.

Factors which influence formation of MSU crystals

Various factors may influence the formation of MSU crystals affecting solubility, nucleation and crystal growth (Figure 2).

As mentioned above, a supersaturation of urate is needed for crystals to form. All conditions which reduce urate solubility will obviously favor crystal precipitation. Temperature, pH value, and pressure, are well known factors which affect the concentration of a solute in a solution. While pressure has hardly any effect on the solubility of urate, it has been observed that the solubility of urate decreases with a reduction in temperature (4, 5) enhancing nucleation of MSU crystals. It has been suggested that reduced solubility of urate at lower temperatures accounts for the predilection of gout in the peripheral joints, such as the foot and the ankle, which are exposed to sustained cooler temperatures.

Urate solubility is affected by a lowering of the pH value, which increases the tendency of crystals to nucleate. It is known that a low pH also increases the amount of ionized calcium present in serum by reducing calcium

binding to macromolecules, phosphates, etc. (6). Calcium ion dramatically enhances urate nucleation and growth (7). It has been suggested that the initial nucleus may be a calcium urate, and that calcium ion readily replaces sodium ion in the salt crystal lattice. This means that any factor which lowers the pH value greatly increases the probability of urate crystallization by both a direct and, through the increase of the calcium ion activity, an indirect mechanism. Serum acidosis increases significantly with strenuous exercise, insufficient respiration and alcohol consumption (8) and these events have been reported to often trigger gout attacks. In fact, a high lactic acid content has been found in acute synovial effusions of gout (9). It has been suggested that a local reduction in pH can be caused by phagocytosis of existing crystals and local trauma (7), or occur in intra-articular, avascular areas where glycolysis is the primary source of energy, resulting in the production of large quantities of lactic acid (10).

A study using flow cytometry to detect small amounts of crystals produced in a supersaturated solution of uric acid showed that physiological endogenous concentration of lactic acid (less than 1.0 mg/L) did not affect the formation rate, but that 2.0 mg/mL of lactic acid enhanced the formation of a mixture of MSU and uric acid crystals (11).

Examining the effect of g-globulin on urate crystal formation, the same authors showed that g-globulin accelerates the formation of MSU crystals. The rate of acceleration depends on both rate time and dose.

The possibility that components of synovial fluid could influence the formation of urate crystals was suggested in 1975 by Wilcox, who observed that synovial fluid from a gouty patient accelerated nucleation of crystals (7).

McGill and Dieppe, with their fundamental studies on *in vivo* and *in vitro* crystal growth, showed that serum, synovial fluid, high molecular weight fraction of serum, insoluble collagen and gamma globulin not only promote urate crystal nucleation, but also result in smaller and more uniform urate crystals (12, 13). These crystals exhibited "bow-like" morphology consistent with a central nidus of nucleation with radiating crystal growth, like urate crystals formed in the articular cartilage. Urate

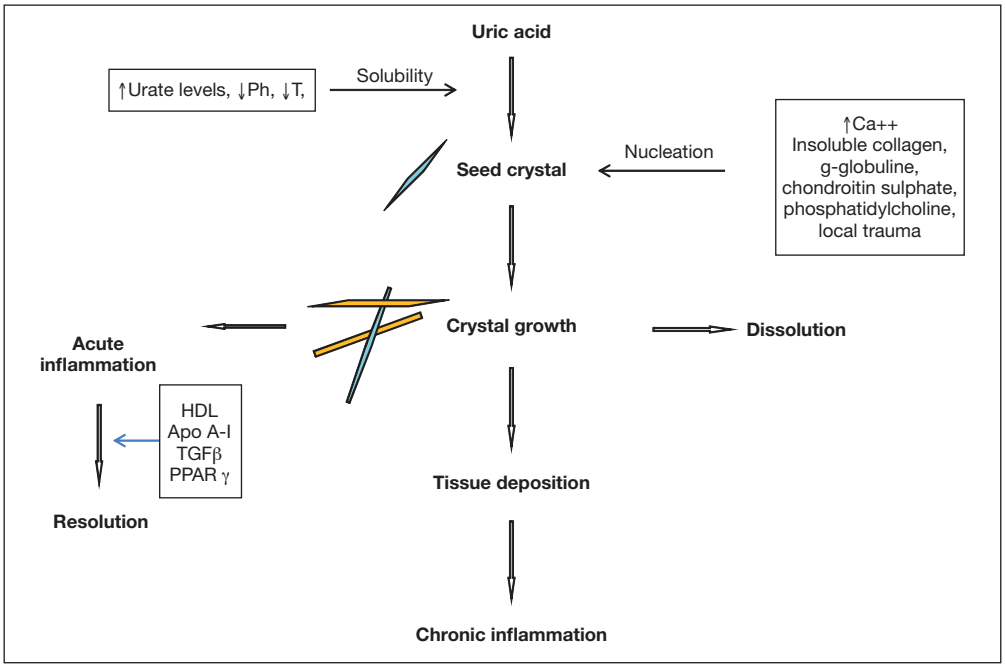

Figure 2 - Metabolism of MSU crystal formation.

crystals formed *in vivo* are most commonly seen in synovial fluid where they are found as single crystals. It is likely that these crystals have been shed from the site of their formation in the synovium or cartilage, and hence the morphology of urate crystals in synovial fluid may not be representative of the forming crystal (13).

An important study carried out in 1986 by Burt and Dutt, on the growth kinetics of MSU crystals, demonstrated that some components of cartilage and synovial fluid, such as hyaluronic acid, proteoglycan monomer and aggregate, and phosphatidylserine, had no significant effect on the growth rate constant of MSU. On the contrary, chondroitin sulphate and phosphatidylcholine increased the growth rate constant, possibly by promoting further nucleation in the growth medium (14).

IgG antibodies isolated from synovial fluid of patients suffering from gout have been shown to accelerate the appearance of new MSU crystals from a supersaturated solution of the salt *in vitro*. The same effect was not observed for IgG antibodies isolated from the joint fluids of patients with other joint diseases, such as pseudogout, rheumatoid arthritis or osteoarthritis, suggesting the presence of crystal-specific antibodies (15). These antibodies would bear an imprint of the crystal surface and consequently behave as a nucleating matrix in a new crystallization event.

Another well-recognized clinical feature of gout is the tendency for an acute attack to be precipitated by physical trauma, such as stubbing the toe or following physical activity. Enhanced MSU crystal nucleation has been reported *in vitro* following repeated snapping of urate supersaturated solutions (7). Mechanical shock caused nucleation at much lower supersaturations than those required for spontaneous nucleation.

There are contrasting data on the effect of albumin on urate crystal growth. It has been reported that albumin significantly inhibited MSU crystallization (14), increasing urate solubility. The proposed mechanism is the adsorption of the albumin molecules on the crystal surface with subsequent poisoning of the active growth sites (4).

In contrast, it was found that human serum albumin accelerates (by up to ten times) the nucleation of MSU crystals at a pH above 7.5, but only to a much lesser extent (1.2 times) at pH 7.0. Protein denaturation, as well as blocking exposed carboxylate groups on the protein, substantially reduced the nucleating effect (16).

Potassium and cupric ion slightly increase urate solubility (4). Hyaluronic acid, proteoglycan monomer and aggregate all caused very small increases in MSU solubility (14).

Dissolution/disappearance of MSU crystals

As described above, an increase in urate serum levels and, therefore, a decrease in solubility, is the first step in urate crystal formation. It is reasonable to expect that any condition leading to a decrease in hyperuricemia will promote crystal dissolution or inhibit formation of other crystals and, consequently, will trigger acute attacks of gout. This is achieved primarily by using urate-lowering drugs. MSU crystals disappear from the SF after serum uric acid is reduced to normal levels. The time required for the crystals to disappear depends on the duration of gout (17). Sonographic signs of deposition of MSU crystals on the surface of hyaline cartilage disappeared completely once serum urate levels were stable at 6 mg/dL for seven months or more (18) with urate lowering drug therapy.

However, if not treated, acute gout attacks are self-limiting. Both physical and humoral factors are involved in this process. Physical factors interfere with the initial crystal-cell interaction after binding or adsorption to urate crystals and specifically inhibit a range of cellular responses. These are represented by IgG (19), apoB, LDL (20), HDL (21, 22).

Humoral factors are released during the inflammatory process induced by crystals and do not have a direct effect on crystal metabolism but rather act through inhibition of specific signaling pathways which activate inflammatory genes (23-25). These will be discussed by others in this issue of Reumatismo.

CALCIUM CRYSTAL METABOLISM

Unlike MSU crystals, which form in supersaturated solutions, the formation of calcium crystals almost certainly occurs within cartilage and other connective tissues, and they are presumably released into the synovial space by a process of shedding (26). The precise mechanisms through which calcium crystals form are not known but alterations in the concentration of inorganic pyrophosphate (PPi) seem to play a crucial role in their production. PPi is produced in one or more steps in a wide variety of biochemical pathways that lead to the synthesis of most of the major cell constituents (26).

Several families of enzymes in chondrocytes are capable of converting ATP and other nucleoside triphosphates (NTP) to PPi (Figure 3). To date, at least two such enzymes with nucleoside triphosphate pyrophosphohydrolase (NTPPPH) activity have been identified: the cartilage intermediate layer protein (CILP) and the nucleotide pyrophosphatase/phosphodiesterase (NPP)1, an ecto-enzyme also called plasma cell membrane glycoprotein-1 (PC-1) (27, 28).

A substantial portion of ATP used by chondrocytes to generate extracellular PPi is provided by the mitochondria.

PPi is then hydrolyzed in orthophosphate (Pi) by inorganic pyrophosphatases (Figure 3), such as tissue non-specific alkaline phosphatase (TNAP) and glucose-6-phosphatases, which are mainly located in the cytosol. Pyrophosphatases are specific for PPi and require Mg2+ for activity, pH of 7-8, and are strongly inhibited by other divalent metal cations, such as Ca2+ and Fe2+.

At enzymatic and molecular levels, NPP1 and TNAP have antagonistic effects (29) on mineral formation due to their opposing activities: production of PPi by NPP1 or its hydrolysis by TNAP.

Physiological levels of extracellular PPi are maintained by articular chondrocytes and resident cells in many other tissues in order to prevent pathological calcification.

Extracellular PPi levels are normally held in check by an equilibrium in PPi generation by NPP/NTPPPH activity relative to PPi degradation by pyrophosphatases, by the balancing effects of cytokines and growth factors, and by transport of PPi from inside the cell involving the multiple-pass transmembrane protein ANK.

These mechanisms become dysrgulated in aging, and osteoarthritic (OA) cartilage and extracellular PPi excess accumulates, mediated in large part by upregulation of NPP1 and ANK expression. Such an accumulation leads to deposits of pathological CPPD crystals (30, 31).

BCP crystal deposition may be stimulated according to extracellular availability of substrate PPi, the activity of TNAP, the availability of substrate ATP, the activity of adenosine triphosphatase (ATPases), and other factors, such as substantial local Mg2+ concentrations. It has been shown that, at low concentrations, PPi prevents the nucleation and crystal growth of hydroxyapatite (HA) and other basic calcium phosphate crystals (32). On the other hand, the excessive PPi generation provides a source for increased extracellular Pi generation via PPi hydrolysis, and may promote BCP crystal deposition (33). In this

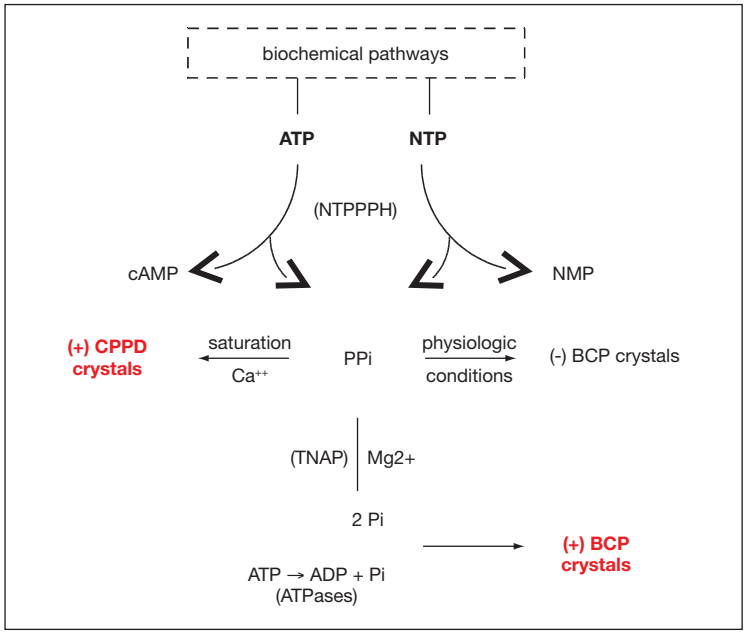

Figure 3 - Metabolism of CPPD and BCP crystal formation.

way both types of crystal formation may be jointly promoted in cartilage, an event that commonly occurs clinically in OA.
Factors which stimulate PPi elaboration include transforming growth factor-β (TGF-β) (34), retinoic acid (35), thyroid hormone, and ascorbate (36). TGF-β stimulates NPP1 expression and NPP1 subcellular movement to the plasma membrane. This drives the elevation of extracellular PPi. Interleukin (IL)-1β, suppresses both NPP1 expression and extracellular PPi in chondrocytes, and blocks the effects of TGF-β on PPi (37). The insulin-like growth factor-1 (IGF-1) normally suppresses extracellular PPi in chondrocytes, thus increasing cAMP production (38).

The role of cartilage matrix vesicles in cartilage mineralization

Recent observations have shown that cartilage matrix vesicles (MVs) are the initial site of mineral formation (39). Cartilage MVs are extracellular cell-derived organelles whose production is modulated by hypertrophic chondrocytes (40). MVs from OA cartilage contain enzymes necessary for Ca2+ uptakes (annexins) (41), as well as for Pi homeostasis, TNAP, 5' adenosine monophosphatase (5'AMPase), ATPases (42), and NPP1, (43) and are able to specifically generate either CPPD and BCP crystals. Preformed crystals are then released from lumen of MVs into the extracellular matrix, so that crystals continue to grow.
Although the factors regulating the quantity and type of crystal produced by these vesicles are still unknown, the state and composition of vesicle membranes is of fundamental importance in determining mineralization capacity (44). Derfus *et al.* (45) demonstrated that the state of the surrounding matrix may influence the ability of the vesicle to mineralize *in situ*. Substrate availability, particularly the availability of PPi-generating ATP, also influences the type of crystal generated by MVs.

Factors which influence calcium crystal deposits

Matrix components modulate pathological crystal growth and inhibition. For example, HA and CPPD deposits are preferentially located in degenerating articular cartilage matrix (39). Alterations in matrix proteoglycan (PG) content and aggregation affect inhibition of BCP crystal growth. PG monomers are less effective inhibitors than PG aggregates in solution and reduction in cultured chondrocyte PG synthesis and/or aggregation associated with increased monolayer calcium crystal deposition (46). CPPD crystals are frequently observed in the matrix that has severe degenerative changes in collagen fibers or collagenolysis (47). This suggests that degenerated collagen fibers may promote crystallization by nucleation, as demonstrated *in vitro* (48).
Therefore, crystals seem to be able to grow and increase in number under a variety of conditions that are associated with the degradation of collagen fibers, the presence of proteoglycans and their subsequent degradation in focal areas in which crystallization occurs.
Derfus et al. demonstrated that OA matrix contains at least two populations of vesicles with different enzyme profiles capable of precipitating different mineral types, heavy vesicle fraction (HVF) and light vesicle fraction (LVF) (39). He postulated that CPPD may form on MV surfaces if NTPPPH remains an ectoenzyme, with PPi generating activity directed toward the matrix. While BCP may form within MV where calcium and phosphate may be concentrated in a site protected from other inhibitors of mineralization.
Another factor which represents a turning point in differentiating between physiological and pathological mineralization is the Pi/PPi ratio. This is a determinant parameter leading to HA or CPPD formation (49). It has been demonstrated by an *in vitro* model of MVs isolated from chick embryo growth plate cartilage that an initial Pi/PPi ratio higher than 140 leads to HA deposition, mimicking conditions occurring during arthritic crystal deposition. When Pi/PPi ratio is lower than 70, it inhibits the MV-induced seeding of HA, which corresponds to the conditions in which mineralization is inhibited. An initial Pi/PPi ratio below 2.8 leads to deposits of pathological

CPPD, while an initial Pi/PPi ratio higher than 28.4 inhibits CPPD formation.

A rise in PPi concentration could result from either enhanced production or decreased removal of PPi from the joint. In hypophosphatasia, for example, in which alkaline phosphatase is deficient, body fluid concentrations of PPi are raised, presumably because of defective enzymatic removal. This condition is associated with chondrocalcinosis (50).

Ca2+/Pi ratio and the Ca2+xPi product are critical factors affecting the kinetics of the biomineralization process. The rise in Ca2+, along with PPi, are both necessary for the formation of crystals. It is possible that, under abnormal conditions, bound calcium might be released during degradation of proteoglycans and this could contribute to the formation of calcium crystals. Enhanced Ca2+ concentrations occur in some pathological conditions, such as hyperparathyroidism, which is also known to be associated with CPPD deposition.

Hypercalcemia may promote CPPD crystal deposition by mechanisms beyond cartilage matrix supersaturation with ionized calcium, such as calcium functioning as a cofactor in NPP1 catalytic activity, as well as chondrocyte-activating effects mediated by the calcium-sensing receptor. In addition, normal articular chondrocytes express parathyroid hormone (PTH) and PTH-related protein receptors, and functional responses of chondrocytes to PTH can promote proliferation, altered matrix synthesis, and mineralization (51).

Other metabolic disorders such as hypophosphatasia, hypomagnesemic conditions (including the Gitelman's variant of Bartter's syndrome), and hemochromatosis, are linked to an increase in joint fluid PPi levels and to secondary CPPD crystal deposition disease. Mg2+ is the cofactor of TNAP. Decreased alkaline phosphatase activity due to hypomagnesemia could theoretically result in high extracellular levels of PPi and, therefore, in an increase in the ionic product PPixCa2+ which predisposes to CPPD crystal deposition (52).

Hypophosphatasia is due to deficient activity of the ectoenzyme TNAP, the major physiological antagonist of the NPP1-mediated elevation of extracellular PPi (50).

Factors which affect the solubility of calcium crystals also indirectly affect the shedding of crystals from cartilage. The level of ionized calcium appears to be the most important regulator of CPPD crystal dissolution while the PPi concentration has relatively little effect (53). When Ca2+ levels decrease (below 5 mg/100 mL), a rapid increase in CPPD solubility occurs. Crystals become smaller and are shed into the joint space where they can cause an acute inflammatory episode of pseudogout.

In conclusion, bioregulation of PPi levels in cartilage is clearly an important factor in maintaining tissue integrity and function. The homeostatic mechanisms involved in maintenance of physiological PPi levels are complex and involve several factors: aging, growth factors, chemokines, transporters, enzymes, and enzyme substrates. A understanding of the pathophysiology of crystal formation will contribute to the prevention and treatment of pathological crystal deposition disease.

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