Tamm-Horsfall Glycoprotein - Inhibitor or Promoter of Calcium Oxalate Monohydrate Crystallization Processes?

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Summary. The processes of calcium oxalate monohydrate (COM) crystal nucleation, growth and aggregation (agglomeration) generally have been studied using a wide variety of assay systems/conditions. This paper reviews the apparently conflicting data on the effects of Tamm-Horsfall glycoprotein (THP) on COM crystallization processes in vitro, with the main emphasis on crystal aggregation. According to its well-known physico-chemical properties, THP has a dual role in modifying crystal aggregation: at high pH and low ionic strength (IS), THP is a powerful crystal aggregation inhibitor. Upon lowering pH and raising IS, THP viscosity increases, leading to reduced crystal aggregation inhibition. In the presence of additional calcium ions, some THPs even become strong promoters of crystal aggregation. This phenomenon seems to be more pronounced in THPs isolated from recurrent calcium stone formers whose proteins exhibit an abnormally high tendency of polymerization. Recent studies suggest an inherited molecular abnormality of THP among some severe recurrent calcium stone formers.

Key words: nephrolithiasis, calcium oxalate, inhibitors and promoters, Tamm-Horsfall glycoprotein

Introduction

When addressing crystallization within the urinary tract, two major aspects have to be considered (14): a thermodynamic one including high urinary supersaturation during which crystal nucleation occurs, and a kinetic one comprising rates of nucleation, growth and aggregation (agglomeration) of crystals. When reviewing studies on urinary compounds that modify calcium oxalate crystallization kinetics, a somewhat confusing picture emerges. In many studies on "crystallization", it is often not clear whether authors refer to nucleation, growth or aggregation of crystals, and a wide variety of assay systems/conditions is being applied for measuring these processes. Furthermore, the term "inhibitor" has been used for compounds that act as chelators of calcium or oxalate ions as well as for molecules binding to the surface of preformed calcium oxalate crystals (17). Whereas chelators reduce free ion activity and, therefore, supersaturation, "real" inhibitors do not influence supersaturation, since they bind to crystal surfaces and block growing sites ("crystal poisoning") at very low (usually micromolar) concentrations (1). Additionally, some molecules may act as promoters, probably by providing preformed surfaces...
for heterogeneous nucleation, epitaxial growth and aggregation of crystals (1). Since urinary compounds can have such varying effects on different crystallization processes, the term "modifier" should be used (23), and it should always be stated whether a specific molecule inhibits or promotes nucleation, growth or aggregation of crystals.

Tamm-Horsfall glycoprotein

The pathophysiology of Tamm-Horsfall glycoprotein (THP) - also called uromucoid - has been reviewed extensively (12, 15, 18). THP is a glycoprotein produced by the kidneys; its carbohydrate content amounts to 30%. In its monomeric form, THP has a molecular weight (MW) of about 80 kD (15). The exact function of THP remains enigmatic (12, 15, 18), but recent studies suggest that THP might be a specific ligand for cytokines in the kidney (15). Because THP is present in various amounts in renal stones (7), it has been proposed to play a role in renal stone formation. In several studies no difference was found between normals and calcium oxalate stone formers in terms of daily urinary excretion of THP, averaging 40-50 mg (2, 7, 21, 26); in patients with distal renal tubular acidosis, however, THP excretion seems to be significantly lower than in controls (28).

The results of available studies on THP effects on calcium oxalate crystal nucleation, growth and aggregation seem very contradicting. Kitamura and Pak reported 15% inhibition of calcium oxalate crystal nucleation by 50 mg/l of normal human THP at pH 6.5, ionic strength (IS) 0.15 and 1 mM CaCl₂ (13). Lowering pH to 6.0, however, produced 25% promotion of nucleation (29). In the presence of very high calcium (6.76 mM) and oxalate (1.03 mM) concentrations at pH 5.7 in a synthetic urine, THP at only 1 mg/l promoted nucleation by 183% (4). The addition of 35 mg human THP to 1 liter of extremely concentrated urine (evaporated to 1250 mOsmole/kg) at pH 5.3 promoted the nucleation of amorphous calcium oxalate crystals by 250% (19).

Most authors agree that THP is a weak inhibitor of calcium oxalate crystal growth in vitro (13, 28, 29). At pH 6.5 and IS 0.15, 50 mg/l of THP inhibited by 38% in the presence of calcium and oxalate at 0.44 mM each (13). At pH 6.0, IS 0.15 and calcium and oxalate concentrations of 1 mM and 0.2 mM, respectively, 50 mg/l of THP inhibited by 3% (29), whereas 8 mg/l did not affect crystal growth (28).

Measuring calcium oxalate crystal aggregation (agglomeration) in vitro, Scurr and Robertson found 22% inhibition by 48 mg/l of isolated THP at pH 6.0, IS 0.15, 2 mM calcium and 0.4 mM oxalate (22). Upon addition of oxalate to metastably supersaturated ultrafiltered urine from healthy humans in the presence of isolated THP (50 mg/l), predominantly small COM crystals, but not aggregated calcium oxalate dihydrate "envelopes" are formed (20). Using the newly developed spectrophotometric method at pH 7.2, IS 0.1 and only equilibrium concentrations of calcium and oxalate (saturated conditions), we found 90% aggregation inhibition by normal human THP at 40 mg/l (8). In a subsequent study, however, lowering pH to 5.7 and increasing IS to 0.21 at otherwise unchanged conditions revealed 35% promotion of aggregation by 40 mg/l of THP isolated from calcium renal stones (9). More recently, studying the effects of 5 mM calcium at pH 5.7 and 200 mM NaCl (11), we were able to demonstrate that 4 out of 5 THPs from severely recurrent stone formers promoted COM crystal aggregation (inhibition between -68% and +7%), whereas 6 THPs from healthy controls had either no effect or inhibited aggregation (inhibition between -3% and +50%).

These apparently paradoxical influences on the various crystallization processes may be explained by the well-known physicochemical properties of THP: increasing the concentrations of the protein itself (16), divalent cations like calcium and magnesium (3, 24), sodium (16, 24) and hydrogen ions (16) all increase polymerization of THP molecules, leading to reversible gel formation (increased viscosity) (6). Scanning electron microscopy studies revealed that THP molecules formed elongated fibers about 20 nm thick and up to 1000 nm long in solutions containing NaCl (100 mmol/l) or CaCl₂ (1 mmol/l), when NaCl and CaCl₂ were simultaneously present, however, thick bundles of fibers could be seen, like those found in hyaline casts from human urine (27). It is obvious from all available studies that reduced THP inhibition or even promotion of calcium oxalate crystallization processes always occurred at lower pH and higher IS.

Studying the effects of increasing IS from 0.01 to 0.21 at pH 5.7 on individually purified THPs (40 mg/l) from normal men and male patients with severe recurrent idiopathic calcium oxalate stone disease (>20 stones), we found that COM crystal aggregation inhibition was reduced more markedly in stone former THPs (10). This seems to be the consequence of a molecular abnormality of stone former THP, which exhibits an increased tendency to polymerize at lower pH and higher IS, as evidenced from viscosity measurements and molecular weight determinations (10). Studies on THPs of the family members of one severe stone former indicate that this apparent abnormality, the molecular basis of which remains unknown, might be inherited (10).

Most authors agree that the mechanism by which urinary macromolecules inhibit crystal growth and aggregation in vitro, is their binding to the crystals, which induces a more negative surface charge (Zeta potential) on the crystal surface (5, 22). In comparison with glycosaminoglycans and RNA, however, THP produces a less negative Zeta potential on COM crystals (22). Furthermore, two studies have demonstrated that upon raising THP concentrations within physiologic urinary limits, e.g. above 10⁻⁷ M, Zeta potential does not become more negative, e.g. the potential curve flattens off at about -20 mV (8, 22). Our own studies did not reveal Zeta potential differences between THPs from healthy men and severely recurrent stone formers, nor was COM crystal aggregation inhibition by physiologic THP concentrations correlated to Zeta potential values (10). We found, however, a negative linear correlation between crystal aggregation inhibition and intrinsic viscosity of THP at pH 5.7 and 200 mM NaCl, e.g. THPs with lower viscosities allowed for more crystal aggregation.