

Mercaptopyruvate Inhibits Tissue-Nonspecific Alkaline Phosphatase and Calcium Pyrophosphate Dihydrate Crystal Dissolution

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ABSTRACT. Objective. The enzymatic activities of tissue-nonspecific alkaline phosphatase (TNAP) including capacity to inhibit calcium pyrophosphate dihydrate (CPPD) crystal dissolution are known to be inhibited by endogenous amino acids, notably cysteine. As cysteine is recognized as a strong TNAP inhibitor, we investigated whether cysteine-related metabolites such as mercaptopyruvate (MPA) could show similar enzyme inhibition effects and, if so, whether these effects might be synergistic with cysteine at approximate physiologic concentrations of the amino acids.

Methods. We studied the inhibitory effects of MPA as well as MPA and cysteine combined in equimolar concentrations on TNAP's phosphatase, inorganic pyrophosphatase, and CPPD crystal dissolution activities. Kinetic parameters V_{max} , K_M , concentration for 50% inhibition (I_{50}), inhibitor constant (K_I), and specific activities calculated from initial velocity, Eadie-Hofstee, Simple, Dixon, and secondary plots were used to assess enzyme inhibition.

Results. MPA significantly inhibited TNAP's phosphatase and pyrophosphatase activities at 10× and 100× physiological concentrations. In the presence of calcium [Ca^{2+}] and [Mg^{2+}] = 1 mM, MPA inhibited uncompetitively TNAP's phosphatase activity and inhibited noncompetitively its pyrophosphatase activity. CPPD crystal dissolution activity was also inhibited. Cysteine and MPA together in equimolar concentrations inhibited TNAP enzyme activities and CPPD crystal dissolution much more effectively than MPA or cysteine alone, reducing CPPD dissolution to 38% of controls at approximate physiologic inhibitor concentrations.

Conclusion. Endogenous amino acids like cysteine and its derivative MPA have the capacity to inhibit TNAP activities at physiologic concentrations. Downregulation of their inhibiting concentration in the cartilage interstitial fluid environment may provide a therapeutic avenue to controlled dissolution of CPPD crystal deposition in tissues. (First Release Nov 1 2009; J Rheumatol 2009;36: 2758–65; doi:10.3899/jrheum.090098)

Key Indexing Terms:

ALKALINE PHOSPHATASE
CALCIUM PYROPHOSPHATE

MERCAPTOPYRUVATE
ARTHRITIS

AMINO ACIDS
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Calcium pyrophosphate dihydrate crystal deposition disease (CPPDD), a common form of arthropathy in the elderly, is characterized by calcium pyrophosphate dihydrate (CPPD) crystal deposition in the articular joints. Symptoms of CPPDD include joint swelling and pain in the knees, wrists, ankles, and other joints, and in some cases result in severe disability. Also referred to as pseudogout, CPPDD can be initially misdiagnosed as gouty arthritis, rheumatoid arthritis, and osteoarthritis because of similar symptoms.

CPPD crystal deposition is restricted to articular connec-

tive tissues, preferentially affecting fibrocartilage, hyaline cartilage, and tissues such as synovium and intervertebral disc, which undergo fibrocartilaginous metaplasia. CPPD crystals are very insoluble¹⁻³. Therapy for CPPDD remains nonspecific, for example, nonsteroidal antiinflammatory drugs (NSAID) such as indomethacin or analgesics. Better understanding of the disease mechanisms related to CPPD crystal formation and dissolution appears to be a prerequisite to the development of novel specific treatment options.

Tissue-nonspecific alkaline phosphatase (TNAP) preferentially hydrolyzes various monophosphate esters at alkaline pH and pyrophosphates at a physiological pH = 7.4⁴⁻⁶. Little is known about the physiological function of TNAP in most tissues except that the bone isoenzyme has long been thought to have a role in normal skeletal mineralization. TNAP is located extracellularly, tethered to the outer cell membrane by a phosphatidylinositol linkage. It is present on the cell membrane of adult articular chondrocytes, particularly in the mid and deep zones of articular cartilage.

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However, the presence of TNAP and amount of TNAP in these domains vary greatly, with increased activity recognized in osteoarthritis^{7,8}. Theoretically, in susceptible individuals, inhibitors of TNAP pyrophosphatase activity such as endogenous amino acids could raise pyrophosphate concentrations [PPi], facilitating CPPD crystal formation, and directly inhibiting CPPD crystal dissolution by inhibiting PPi hydrolysis. Studies showed that certain amino acids such as arginine⁹, lysine, histidine^{10,11}, and cysteine¹²⁻¹⁴ inhibited TNAP's phosphatase and pyrophosphatase activity at pH 7.4, 37°C. Of these amino acids, cysteine was found to be the most potent inhibitor¹⁵. However, inhibition of TNAP by cysteine was significant only at high concentrations. As cysteine inhibits TNAP, it was hypothesized that metabolites adjacent to cysteine metabolically^{16,17} may also inhibit TNAP effectively, and possibly may be synergistic with cysteine (Figure 1). We investigated the TNAP inhibitory effects of metabolites close to cysteine, namely glutathione, taurine, cysteamine, cysteine sulfinic acid, and mercaptopyruvate (MPA). As only MPA had TNAP inhibitory activity comparable to cysteine, we chose to assess the effects of MPA and MPA/cysteine together in equimolar concentrations on TNAP phosphatase, pyrophosphatase, and CPPD crystal dissolution activities under the physiologic conditions of pH = 7.4, 37°C, and [Ca⁺⁺] = [Mg⁺⁺] = 1 mM.

MATERIALS AND METHODS

In preliminary experiments, the candidate metabolites glutathione, cysteine, cysteamine, cysteine sulfinic acid, taurine, and MPA were studied for phosphatase activity at approximately 1×, 10×, and 100× physiologic concentrations. As no specific TNAP activity was obtained for metabolites other than MPA and cysteine, further detailed experiments were performed with MPA and cysteine.

Phosphatase (Piase) assay. MPA, an α -keto acid cysteine analog (Sigma Chemical, St. Louis, MO, USA) and cysteine (Sigma) at 0, 20, 200, and 2000 mM were mixed with the substrate p-nitrophenyl phosphate (pNPP, Sigma; at 0, 7.5, 15, 30, 62.5, 125, 250, 500, 1000, and 2000 μ M) in wells of 96-well microtiter plates (Corning CoStar, Lowell, MA, USA). All solutions were made with Tris-HCl (Sigma), pH 7.4. Kidney alkaline phosphatase (ALP; 200 U/l; Calzyme) was added to each well, and the plates were incubated at 37°C for 10 min. The reaction was stopped by the addition of 0.01 M NaOH (Fisher Scientific, Ottawa, ON, Canada). The plates were read at 405 nm on a plate reader (Bio-Tek Instruments, model E1311, Winooski, VT, USA). The results were expressed as nmol of p-nitrophenol generated/unit ALP/min. At least 4 sets of experiments were performed, and each set was done in duplicate. The ions Ca⁺⁺ and Mg⁺⁺ at concentration 1 mM each were also added into the buffer.

Pyrophosphatase (PPiase) assay. Cysteine and/or MPA under the same experimental conditions as those of the phosphatase assay were mixed with a PPI substrate (Na₂P₂O₇; Fisher Scientific) at concentrations corresponding to pNPP. The method for the PPIase assay was similar to that of the Piase assay. Two exceptions to the protocol were the addition of a color reagent of 5% FeSO₄ (Caledon, Georgetown, ON, Canada) and 1% ammonium molybdate (BDH Ltd., Toronto, ON, Canada) in 1 N H₂SO₄ (Van Waters and Rogers Inc., London, ON, Canada) instead of 0.01 M NaOH at the end of the 10 min incubation and the microtiter plates were read at 630 nm instead of 405 nm on the plate reader.

Preparation of CPPD crystals. CPPD (M) crystals were prepared in our laboratory as described¹⁸. The CPPD (M) crystal phase was confirmed by powder x-ray diffraction analysis using a Rigaku Multiflex X-Ray Diffractometer. With polarized light microscopy, the CPPD (M) crystals synthesized had needle shapes and ranged from 1 to 20 μ m in length¹⁸.

CPPD crystal dissolution assay. MPA (0, 20, 200, and 2000 μ M) was mixed with kidney ALP (200 U/l) and CPPD crystals (100 μ g) in 2 ml microcentrifuge tubes. Ca⁺⁺ and Mg⁺⁺, when present, were each at 1 mM. All solutions were prepared with Tris-HCl, pH 7.4. The mixtures were incubated at 37°C for 2 h with gentle rocking using a test tube rocker (Johns Scientific Thermolyne Speci-Mix; 20 rpm; Ottawa, ON, Canada) to prevent sedimentation of crystals. After incubation, the mixtures were centrifuged at 10,000 rpm in a microcentrifuge (Eppendorf Centrifuge Model 5412). Because [Ca⁺⁺] = 1 mM was present in some experimental solutions, CPPD dissolution was detected by measuring phosphate released from the CPPD crystals.

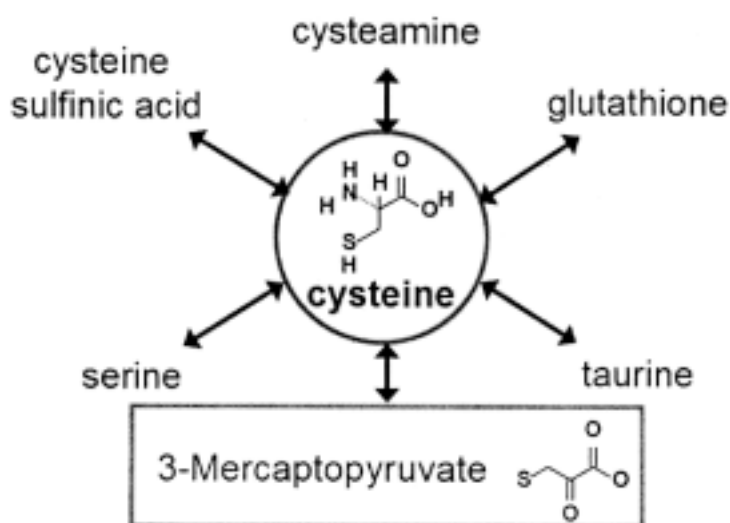


Figure 1. Cysteine and its primary metabolites. Detailed cysteine pathways are available through Kyoto Encyclopedia of Genes and Genomes (cysteine metabolism pathway)¹⁷.

The method for measuring phosphate released from the hydrolysis of pyrophosphate component of CPPD was the same as that outlined in the PPIase assay.

Data analysis. V_{\max} and K_M values were calculated from the y-intercept and the slope of Eadie-Hofstee plots (velocity vs velocity/[substrate]), respectively¹⁹. V_{\max} and K_M were also calculated from initial velocity plots (velocity vs [substrate]) by the computer curve-fitting program KaleidaGraph (Synergy, Windows Version 3.6X)^{20,21}, which uses a nonlinear least-square method from the Levenberg-Marquardt algorithm to fit equations of curves to datapoints²². Confirmation of type of inhibition was obtained from Simple plots ([substrate]/velocity vs [inhibitor])^{23,24}.

Statistical analysis. Statistical analyses were conducted with SPSS (v 12.0.1). Differences in the enzyme kinetic parameters were tested using analysis of variance (ANOVA) followed by post-hoc Dunnett T test. The Dunnett T test compares values between a control group (no analog present) and its test groups (groups where analogs are present). Using a 2-tail test, a p value < 0.05 is regarded as statistically significant.

RESULTS

We analyzed the inhibitory action by MPA on TNAP's phosphatase and pyrophosphatase activities by using initial velocity and Eadie-Hofstee plots. V_{\max} (maximum reaction velocity at substrate concentration when enzyme is saturated) and K_M (substrate concentration at one-half V_{\max}) were derived from initial velocity plots, while Eadie-Hofstee plots were used to determine the type of enzyme inhibition. In the presence of 1 mM Ca^{++} and 1 mM Mg^{++} , MPA is an uncompetitive inhibitor (data not shown). The kinetic parameters V_{\max} and K_M calculated by the KaleidaGraph program from the initial velocity plots (Table 1) both decrease with increasing MPA concentration, consistent with uncompetitive inhibition. MPA inhibited TNAP's Piase activity significantly at 10× and 100× basal concentrations, in the absence or presence of 1 mM Ca^{++} and 1 mM Mg^{++} (Table 1).

The inhibitory effects of MPA on TNAP's PPIase activity plotted and analyzed with KaleidaGraph (data not shown) show MPA inhibition of PPIase activity to be noncompetitive in the absence of 1 mM Ca^{++} and Mg^{++} , while in the presence of 1 mM Ca^{++} and Mg^{++} MPA is a noncompetitive or mixed competitive inhibitor of TNAP's PPIase activity. V_{\max} and K_M , generated by KaleidaGraph in Table 2, show a decrease in V_{\max} with increasing MPA concentration, consistent with noncompetitive inhibition, while K_M remains

unchanged or increases at the higher MPA concentrations, consistent with noncompetitive or mixed competitive inhibition.

Previously we have shown that cysteine inhibits TNAP's phosphatase and pyrophosphatase hydrolytic activities¹⁵. Figure 2 shows the results when cysteine and MPA are added together. In the presence of $[Ca^{++}] = [Mg^{++}] = 1$ mM, combining cysteine and MPA suggests uncompetitive inhibition of TNAP's phosphatase activity and noncompetitive inhibition of TNAP's pyrophosphatase activity (Table 3). Combining MPA and cysteine significantly inhibits TNAP's Piase and PPIase activities at approximate physiological concentration of inhibitors compared to cysteine alone, which requires approximately 2× physiologic range (Table 4).

MPA is a much more effective TNAP inhibitor of CPPD crystal dissolution than cysteine (Figure 3). The combination of MPA and cysteine inhibits TNAP CPPD dissolution activity more effectively than cysteine or MPA alone (Figure 3, Table 4), reducing CPPD dissolution to 38% of control levels at approximate physiologic concentrations and totally inhibiting CPPD crystal dissolution at higher inhibitor concentrations studied.

DISCUSSION

CPPD crystals, although quite insoluble, can be dissolved by presence of excess secondary ions such as magnesium³, phosphate excess²⁵, or inhibition of TNAP, which results in local phosphate depletion¹⁵.

TNAP has several roles in biomineralization²⁶⁻²⁹. These include phosphatase activity whereby phosphate is hydrolyzed from organic phosphate; pyrophosphatase activity, whereby pyrophosphate is hydrolyzed thereby decreasing ambient [PPI], a powerful mineralization inhibitor; and CPPD crystal dissolution activity whereby TNAP dissolves CPPD crystals directly³⁰.

Our study extends our previous work that described and characterized the inhibition of TNAP's phosphatase activity and pyrophosphate hydrolase (pyrophosphatase) activity by cysteine at physiological conditions (pH = 7.4 and 1 mM Ca^{++} and 1 mM Mg^{++})¹⁵. The most intriguing observation was the demonstrated inhibition of CPPD crystal dissolution at high cysteine concentrations. Accordingly, we hypothe-

Table 1. Kinetic parameters V_{\max} and K_M of TNAP phosphatase assay: effect of mercaptopyruvate (MPA). At least 4 sets of experiments in duplicates were performed. Values are mean ± standard deviation.

MPA, μ M	$[Ca^{++}] = [Mg^{++}] = 0$ mM		$[Ca^{++}] = [Mg^{++}] = 1$ mM	
	V_{\max}	K_M	V_{\max}	K_M
0	8.7 ± 1	6.1 ± 1	32 ± 10	41 ± 13
20	7.5 ± 1	5.8 ± 1	26 ± 8	33 ± 11
200	2.0 ± 0.1*	9.1 ± 3*	17 ± 1*	19 ± 2*
2000	1.5 ± 0.3*	9.9 ± 5*	10 ± 2*	11 ± 3*

* Calculated value with MPA present is statistically significantly different (p < 0.05) from the value with no MPA.

Table 2. Kinetic parameters V_{max} and K_M of TNAP pyrophosphatase assay: effect of mercaptopyruvate (MPA). At least 4 sets of experiments in duplicates were performed. Values are mean \pm standard deviation.

MPA, μM	[Ca ⁺⁺] = [Mg ⁺⁺] = 0 mM		[Ca ⁺⁺] = [Mg ⁺⁺] = 1 mM	
	V_{max}	K_M	V_{max}	K_M
0	20 \pm 2	35 \pm 12	56 \pm 3	126 \pm 29
20	20 \pm 4	31 \pm 10	52 \pm 3	133 \pm 16
200	15 \pm 2.2	54 \pm 39	39 \pm 4*	137 \pm 23
2000	12 \pm 2.3*	50 \pm 19	27 \pm 5*	181 \pm 73

* Calculated value with MPA present is statistically significantly different ($p < 0.05$) from the value with no MPA.

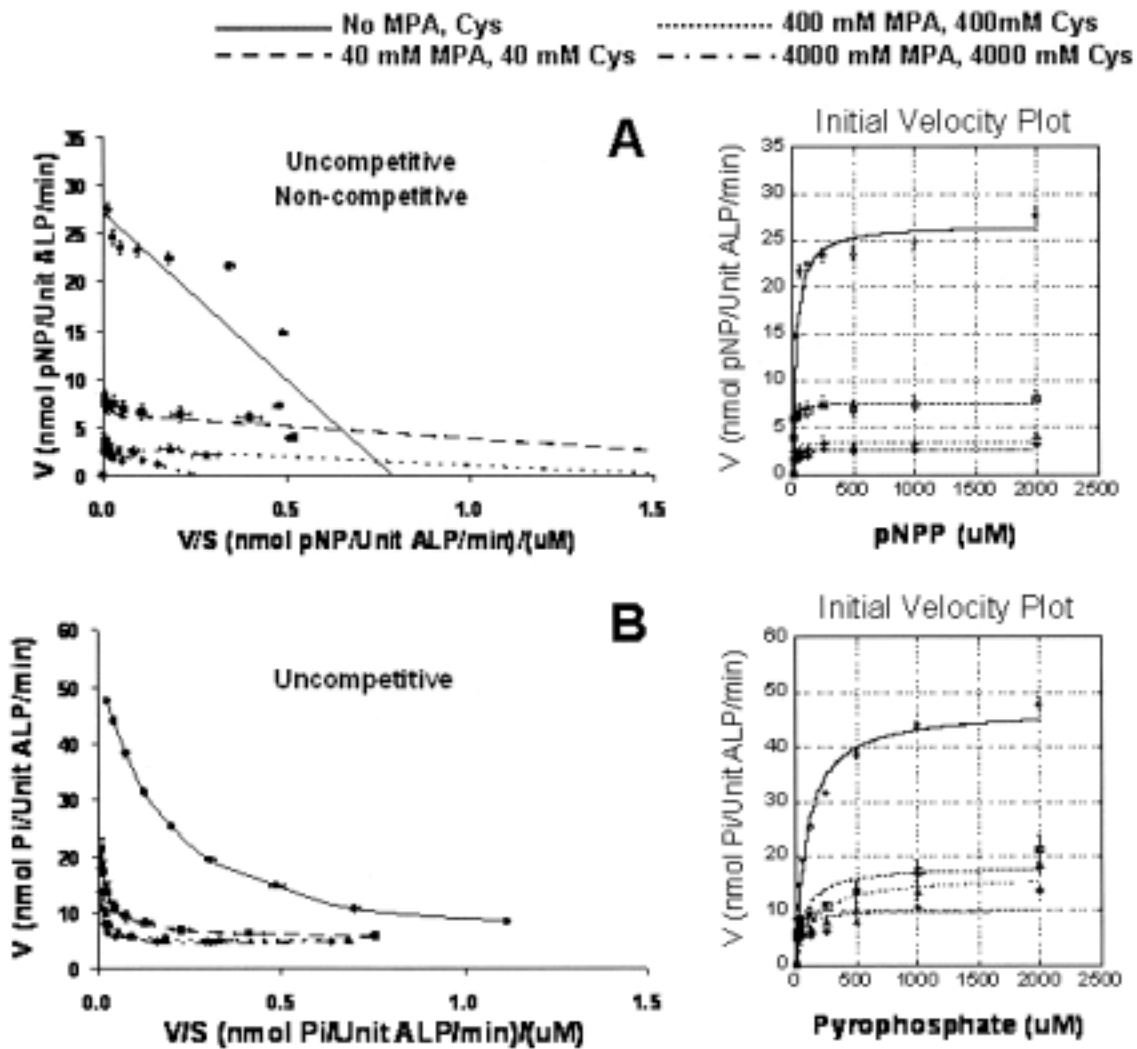


Figure 2. Eadie-Hofstee and initial velocity plots showing the effect of MPA + cysteine on TNAP phosphatase and pyrophosphatase activity. A. Phosphatase activity, with p-nitrophenol phosphate as substrate. B. Pyrophosphatase activity, with pyrophosphate as substrate. All incubations were done in the presence of [Ca⁺⁺] = [Mg⁺⁺] = 1 mM.

sized that TNAP might also be inhibited by one or more of several primary metabolites of cysteine, all of which share some structural similarity with this amino acid. Of the metabolites tested, including cysteine, taurine, cysteine

sulfenic acid, glutathione, and MPA, only MPA significantly inhibited activities of TNAP.

In physiologic conditions more closely resembling the ionic milieu, 1 mM [Ca⁺⁺] = [Mg⁺⁺] = 1 mM, MPA uncom-

Table 3. Kinetic parameters V_{\max} and K_M of TNAP pyrophosphatase and pyrophosphatase assay: effect of mercaptopyruvate (MPA) and cysteine combined. At least 4 sets of experiments in duplicates were performed. Values are mean \pm standard deviation.

MPA, μM Cysteine, μM	Phosphatase [Ca ⁺⁺] = [Mg ⁺⁺] = 1 mM		Pyrophosphatase [Ca ⁺⁺] = [Mg ⁺⁺] = 1 mM	
	V_{\max}	K_M	V_{\max}	K_M
0	27 \pm 1.7	27 \pm 1.6	48 \pm 1.4	89 \pm 17
40	7.5 \pm 1.8*	5.7 \pm 1.9*	18 \pm 5.1*	71 \pm 18
400	3.3 \pm 0.9*	5.2 \pm 0.7*	16 \pm 6.9*	139 \pm 118
4000	2.6 \pm 0.4*	15 \pm 2.9*	11 \pm 3.7*	85 \pm 88

* Calculated value with MPA present is statistically significantly different ($p < 0.05$) from the value with no MPA.

Table 4. Effect of inhibitors on TNAP enzyme activity. When cysteine and mercaptopyruvate (MPA) were combined, each inhibitor at concentration shown, except MPA alone (0, 20, 200, 2000 μM). At least 4 sets of experiments in duplicates were performed. Values are mean \pm standard deviation.

Enzyme	Substrate	Inhibitor Concentration, μM	Cysteine (%)	Inhibitors	
				MPA Nmol Pi/Unit ALP/Min (%)	Cysteine + MPA (%)
TNAP (Piase)	pNPP 2000 μM	0	29 \pm 1.9 (100)	32 \pm 10 (100)	28 \pm 1.7 (100)
		40	28 \pm 2.8 (98)	26 \pm 8 (81)	8 \pm 1.6* (29)
		400	11 \pm 1.3* (37)	17 \pm 1.0* (53)	4 \pm 0.8* (14)
		4000	4 \pm 1.0* (12)	10 \pm 2.0* (31)	3 \pm 0.2* (12)
TNAP (PPiase)	Pyrophosphate 2000 μM	0	27 \pm 1.9 (100)	56 \pm 3.0 (100)	55 \pm 16.4 (100)
		40	30 \pm 2.0 (110)	53 \pm 3.0 (93)	21 \pm 4.5* (39)
		400	18 \pm 1.3* (66)	39 \pm 5.0* (70)	19 \pm 4.7* (34)
		4000	2 \pm 1.1* (6)	27 \pm 5.0* (54)	14 \pm 3.7* (25)
TNAP (CPPD crystal dissolution)	CPPD 345 μM (100 μg)	0	0.46 \pm 0.07 (100)	0.26 \pm 0.11 (100)	0.26 \pm 0.02 (100)
		40	0.49 \pm 0.09 (107)	0.25 \pm 0.01 (96)	0.23 \pm 0.01 (88)
		400	0.43 \pm 0.04 (93)	0.24 \pm 0.01 (92)	0.10 \pm 0.007* (38)
		4000	0.15 \pm 0.02* (33)	0.07 \pm 0.01* (27)	0.00 \pm 0.05* (0)

* Calculated value with inhibitor present is statistically significantly different ($p < 0.05$) from the value with no inhibitor. TNAP: tissue-nonspecific alkaline phosphatase; CPPD: calcium pyrophosphate dihydrate; pNPP: p-nitrophenyl phosphate; ALP: alkaline phosphatase.

petitively inhibited TNAP's Piase activity and noncompetitively inhibited its PPiase activity.

TNAP is a metalloenzyme that requires the presence of Mg^{++} and Zn^{++} at its active site^{31,32}. Insufficient magnesium further decreases TNAP activities at all concentrations of cysteine and MPA (Tables 1, 2, and 3). Conversely, excessive magnesium will also lead to the inhibition of TNAP, as Mg^{++} will occupy Zn^{++} binding sites. Indeed, CPPD arthropathy is often associated with metabolic diseases such as hypomagnesemia, hypercalcemia, hyperparathyroidism, and hypothyroidism^{33,34} in which there are abnormalities in serum, in calcium or magnesium concentrations.

The type of inhibition by MPA of TNAP's PPiase activity was noncompetitive because the change in K_M was constant for PPiase activity in the presence of 1 \times and 10 \times basal concentrations of MPA compared to PPiase activity in the absence of the inhibitor (Table 2). These results suggest the presence of 2 different allosteric sites on 2 TNAP subunits (one allosteric site per subunit), one that binds MPA (low substrate affinity, high K_M) and another that binds substrate

or endproduct (high substrate affinity, low K_M), which can modulate the TNAP's PPiase activity. The exponential rise in K_M with increasing substrate concentration in each curve is indicative of the allosteric effect of either the increase in substrate concentration or endproduct accumulation. The linked PPiase subunit suggestion is further supported by the mixed competitive cysteine inhibition of TNAP PPiase activity, which also indicates the presence of more than one modulatory site on TNAP.

Our results indicate that MPA inhibited TNAP's Piase and PPiase activities at inhibitor concentrations of 20 μM and above. Cysteine accumulation could also lead to an increase in MPA levels since it is one of the primary metabolites in the cysteine cycle that is involved in various antioxidant and detoxifying functions³⁵. In addition, the daily serum amino acids concentration could vary considerably with dietary conditions. Elevated amino acids have been found in the synovial fluid of patients with various arthritic conditions, including CPPD, further suggesting the presence of higher levels of cysteine metabolites such as MPA^{36,37}. In

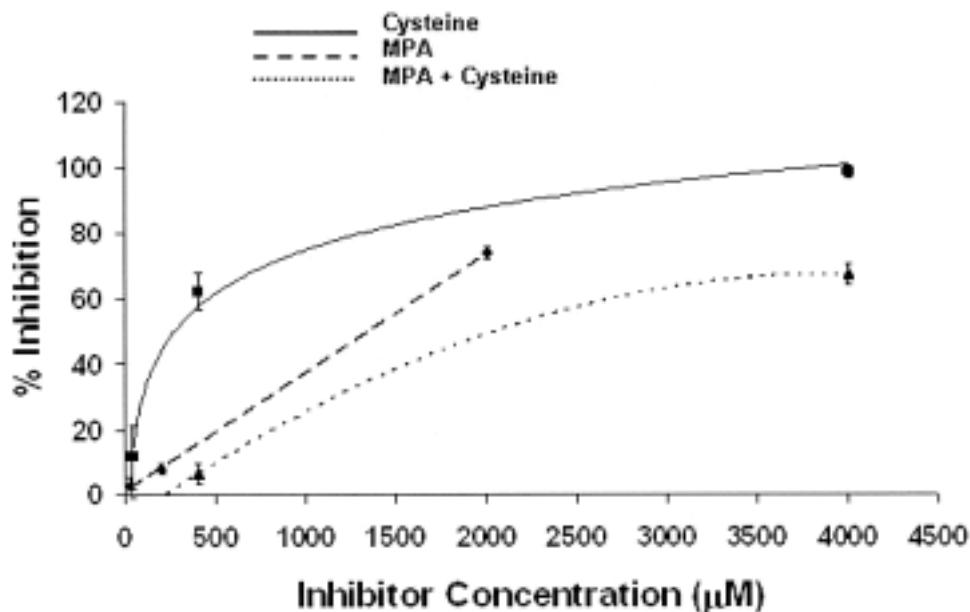


Figure 3. Inhibition of alkaline phosphatase CPPD crystal dissolution by cysteine, MPA, cysteine + MPA. Percentage inhibition was determined by measuring phosphate liberated from CPPD.

the elderly, the MPA concentration could also increase due to the presence of excess cysteine, as a result of the significant decrease in the conversion of cysteine to glutathione³⁸.

It is of immense interest that cysteine and MPA demonstrated more TNAP inhibition than either cysteine or MPA alone. This is evident for phosphatase and PPIase activity at inhibitor concentrations of 20 to 40 μM and for CPPD crystal dissolution at inhibitor concentrations of 200 to 400 μM . Regarding possible clinical implications, consideration must be given to the relative concentrations of enzyme substrates and inhibitors compared to the known or inferred concentration of these substances within cartilage extracellular fluid. The enzyme and ionic concentrations in these experiments correspond to the human physiologic range. Specifically, the experimental concentration for organic phosphate approximates physiologic concentrations, whereas that of pyrophosphate is about 100 times physiologic extracellular [PPI]. The implications from these considerations are that under physiologic conditions and in the presence of endogenous inhibitors, at the experimental concentrations, PPIase inhibition would tend to be greater at 10 μM PPI than at 2000 μM PPI used in these experiments. That TNAP dissolved CPPD crystals in detectable amounts, and that crystal dissolution could be inhibited by cysteine and MPA, indicates that biologic conditions of CPPD crystal dissolution are being modeled by these experiments.

Although early studies suggested that serum cysteine = 40 to 50 μM ³⁹, more recent investigations indicate that serum cysteine = 220 to 265 μM with a range for individuals of $\pm 20\%$ ⁴⁰⁻⁴⁴. Serum concentrations for MPA are unknown but can be inferred to be around 100 μM or less

compared to metabolites such as cysteine 250 μM and pyruvate 30 to 266 μM .

The experiments indicate that conditions that increase [cysteine] and [MPA] preferentially inhibit TNAP and provide conditions favorable both for CPPD crystal formation and for inhibition of CPPD crystal dissolution. MPA in particular accumulates when the enzymes 3-mercaptopyruvate sulfotransferase (which catalyzes both pyruvate to 3-mercaptopyruvate and lactate to 3-mercaptolactate) and lactate dehydrogenase (which converts lactate to pyruvate and 3-mercaptolactate to 3-mercaptopyruvate) are active. These conditions favorable to TNAP inhibition are present in local tissue hypoxia¹⁶. This suggests that CPPD crystal formation may be associated with a local chronic hypoxic state within the involved articular tissues. Our experiments suggest that the modulation of TNAP activity *in vivo* by sequestration of inhibitory amino acids or other small molecular-weight inhibitors could be a therapeutic option for CPPD crystal deposition disease. TNAP activity is also central to tissue culture as well as to cartilage cell culture and chondrocyte cell and matrix vesicle models of mineral formation, each of which could be used to further confirm the role of TNAP inhibition to prevent CPPD formation or accelerating crystal dissolution in these *in vivo* systems^{26,45-47}.

Elucidating the mechanisms in which altered enzymatic activities of TNAP might contribute to the pathogenesis of CPPD crystal deposition disease is key to the development of novel specific therapeutic strategies. While other factors, such as protein coating, may affect CPPD crystal dissolution, our study indicates that sequestration or removal of TNAP inhibitors such as cysteine and MPA in articular cartilage may lead both to decreased CPPD crystal formation

(by decreased hydrolysis of pyrophosphates available for crystal formation) and to increased CPPD crystal dissolution. Consequently, the regulation of TNAP activities by endogenous amino acids might be an important target for future developments in CPPDD therapy.

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