Biochemical Pharmacology, Vol. 33, No. 12, pp. 1859–1865, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

INTERACTION OF THE HUMAN LEUKOCYTE PROTEINASES ELASTASE AND CATHEPSIN G WITH GOLD, SILVER AND COPPER COMPOUNDS

ANTONIO BAICI*†, ANNAMARIA CAMUS‡ and NAZARIO MARSICH‡

*Department of Rheumatology, University Hospital, CH-8091 Zurich, Switzerland, and ‡Institute of Chemistry, University of Trieste, I-34127 Trieste, Italy

(*Received* 7 *October* 1983; *accepted* 2 *December* 1983)

Abstract—Gold thiomalate and the corresponding silver and copper derivatives were investigated as inhibitors of the human leukocyte proteinases elastase and cathepsin G. The kinetic inhibition mechanism for gold- and silver thiomalate is of the hyperbolic non-competitive type with both enzymes and the inhibitory efficiency of the metals increases in the order Cu < Ag < Au. On the contrary, D-penicillamine derivatives of the three metals do not influence at all the activity of the two proteinases. Although gold thiomalate is the most efficient of the investigated metal compounds (K_i = 33 μM and 25 μM for elastase and cathepsin G, respectively), the hyperbolic nature of the inhibition imposes a serious limit to its practical usefulness since the maximum inhibitory action on both enzymes is about 40%. We suggest that, in order to act as inhibitor, a copper, silver or gold compound must be able to easily transfer the metal to the enzyme.

The human leukocyte proteinases elastase and cathepsin G are thought to be involved in the pathogenesis of lung emphysema [1], clotting disorders and other inflammatory processes [2]. Both enzymes can degrade the two major constituents of articular cartilage, namely collagen and proteoglycans at neutral pH [3] and are possibly involved in the pathophysiology of rheumatoid arthritis [4]. Leukocyte elastase and cathepsin G also degrade the four subclasses of human IgG [5, 6] and IgM [7, 8] and may therefore play a role in the immunopathological processes present in inflammatory diseases.

The beneficial effects of gold therapy in rheumatoid arthritis have been known for a long time and one of the several possible explanations for its action *in vivo* is an interaction with lysosomal hydrolases. Considerable interest has also been centred on the anti-inflammatory activity of copper mainly using animal models [9–11].

Recent work on the role of extracellular proteinases in the processes of tissue destruction in rheumatoid arthritis [12] and tumour invasion [13, 14] led us to the study of the inhibition mechanism of some of these enzymes by agents of established therapeutic significance [15–18].

The medicinally used gold compounds contain Au(I), i.e. gold in its lower oxidation state, which must be stabilized by complexation with sulphur or other soft ligands [19]. We became interested in knowing if the other two transition metals of the group IB, silver and copper, could possibly act similarly to gold.

In the present paper we present a study on the behaviour of the therapeutically used gold(I) thiomalate and of the corresponding silver and copper compounds as inhibitors of the leukocyte proteinases elastase and cathepsin G. A comparison is made with the analogous D-penicillamine derivatives with the twofold purpose of analysing the role of the metal and of the ligand in the inhibition mechanism.

MATERIALS AND METHODS

Enzymes. Human leukocyte elastase (EC 3.4.21.37) and cathepsin G (EC 3.4.21.20) were purified as detailed elsewhere [8]. The active site concentration of elastase was determined with the aza-peptide *N*-acetyl-Ala–Ala–NHN($CH_2CH_2CH_3$) CO-*p*-nitrophenylester [20] in a stopped flow apparatus [16].

Inhibitors. The metal compounds used as inhibitors were prepared from commercial reagents (Merck, Darmstadt, F.R.G.). For the copper derivatives CuI was freshly purified, the preparations were carried out under nitrogen in deaerated solvents and the products stored in inert atmosphere. The reduction of Au(III) to Au(I) was performed in aqueous solution and required three moles of ligand per gram-atom of gold [21]. By convention we give the formula weight of the salts, though they are polymers in solution [22].

Na_2[Au(thiomalate)]·1/2 H_2O: 0.29 g (0.73 mmoles) of $HAuCl_4 \cdot 3H_2O$ dissolved in water (10 ml) were added to a solution of 0.35 g (2.33 mmoles) of thiomalic acid in 10 ml methanol. After 30 min the colourless solution was treated with 1 M NaOH in methanol. The white voluminous precipitate formed was filtered off, thoroughly washed with methanol and dried *in vacuo*. Recrystallization was from water/methanol, yield 78%. Na_2[Ag(thiomalate)]·H_2O: an aqueous solution (10 ml) of 0.38 g (2.24 mmoles) of $AgNO_3$ was added to a solution of 0.35 g (2.33

† Author to whom correspondence should be addressed.

mmoles) of thiomalic acid in 10 ml of methanol. The colloidal, pale yellow solution formed by treatment with 1 M NaOH in methanol coagulated slowly to give a voluminous yellow precipitate, which was filtered, washed with methanol and dried *in vacuo*. Recrystallization was from water/methanol, yield 85%.

Na_2[Cu(thiomalate)]·H_2O: a clear colourless solution was obtained by adding 0.43 g (2.27 mmoles) of CuI in 10 ml of acetonitrile to 0.35 g (2.33 mmoles) of thiomalic acid in 10 ml of methanol. The neutralization with 1 M NaOH in methanol afforded a lemon yellow solid, which was filtered, washed with acetonitrile, then with methanol and dried *in vacuo*. Yield 61%. Recrystallization from water/methanol is possible, but the product is extremely sensitive to oxidation. The D-penicillamine derivatives were isolated in good yields (85% for Au, 75% for Ag and 65% for Cu) in protonated form by mixing aqueous solutions of the ligand and of the metal reagent ($HAuCl_4 \cdot 3H_2O$, $AgNO_3$) in the ratios used for the above thiomalato compounds. CuI was added as solid and dissolved promptly giving a yellow solution. After a few minutes the products began to separate as white gelatinous precipitates from the clear solutions. They were filtered off after several hours of reaction, washed repeatedly at first with water and then with acetone and dried *in vacuo*. In neutral or slightly basic medium the Ag derivative of D-penicillamine turns to pale yellow; the copper compound becomes lemon yellow and is extremely air-sensitive. The formulae and analyses of the thiomalates and D-penicillaminates prepared for this study are summarized in Table 1.

Other chemicals. The elastase active site titrant was a generous gift of Prof. J. C. Powers (Atlanta, GA, U.S.A.); Boc–Ala–$OPhNO_2$* and elastin from bovine neck ligament were from Sigma (St Louis, MO, U.S.A.); Suc–Ala–Ala–Pro–Val–NMec was a gift of Dr. T. Payne (Basel, Switzerland); Suc–Ala–Ala–Pro–Phe–$NPhNO_2$ was purchased from Bachem Ltd. (Bubendorf, Switzerland). Commercial gold thiomalate (Tauredon®) was obtained from Byk Gulden (Constance, F.R.G.). D-Penicillamine (Mercaptyl®) was a gift of Knoll Ltd. (Liestal, Switzerland).

Enzyme assays. All inhibition studies were carried out in 50 mM Na^+/K^+ phosphate buffer, pH 7.40, ionic strength 0.13, at 25 ± 1°. The assay methods for elastase with Boc–Ala–$OPhNO_2$, Suc–Ala–Ala–Pro–Val–NMec and elastin were detailed elsewhere [15, 16]. With the fluorogenic substrate Suc–Ala–Ala–Pro–Val–NMec the fluorimetric detection method was used [16]. Cathepsin G activity was assayed with the chymotrypsin substrate Suc–Ala–Ala–Pro–Phe–$NPhNO_2$ by following the release of *p*-nitroaniline at 410 nm [23]. For inhibition studies the thiomalates were dissolved and diluted with the phosphate buffer used for the assay. No differences were found in the inhibitory action when the thiomalates were used alone or dissolved in the presence of excess ligand (1 mM thiomalate), that was added in order to stabilize the compound in solution. In the case of copper thiomalate, solutions were made up in deaerated buffer and measurements were performed in a nitrogen atmosphere. By convention, we will express the concentrations of the three thiomalates as moles/litre based on the formula weights given in Table 1. The D-penicillamine derivatives were prepared in 0.01 M NaOH and diluted with phosphate buffer immediately before use.

Kinetic analysis. Inhibition mechanisms were analysed from initial velocity measurements with the aid of the specific velocity plot, a method which allows the analysis of both linear and hyperbolic enzyme inhibitors [24]. In this plot, the ratio of the substrate concentration vs the Michaelis constant ($\sigma = [S]/K_M$) is used. The K_M values used for elastase with Suc–Ala–Ala–Pro–Val–NMec and Boc–Ala–$OPhNO_2$ as substrates were 8.6×10^{-4} M and 1.8×10^{-4} M, respectively [16]. The K_M value for

* Abbreviations used: Boc–Ala–$OPhNO_2$ *α*, *N*- tert - butyloxycarbonyl - L - alanine - 4 - nitrophenylester. Suc–Ala–Ala–Pro–Val–NMec, succinyl - L - alanyl - L - alanyl - L - prolyl - L - valyl - 4 - methyl - 7 - aminocoumarylamide. Suc–Ala–Ala–Pro–Phe–$NPhNO_2$: succinyl - L - alanyl - L-alanyl - L - prolyl - L - phenylalanyl - 4 - nitroanilide.

Table 1. Analytical data for the metal compounds*

Formula	Analysis calculated/found (%) C	H	Metal	Na	N
Na_2[Au(tm)]·½H_2O†	12.03	1.01	49.40	11.52	—
	12.1	1.1	49.4	11.2	—
Na_2[Ag(tm)]·H_2O	15.05	1.58	33.82	14.42	—
	14.8	1.5	33.4	13.6	—
Na_2[(Cu(tm)]·H_2O	17.49	1.83	23.13	16.75	—
	17.9	1.9	23.5	16.9	—
H[Au(Dpen)]·½H_2O‡	16.95	3.12	55.68	—	3.96
	17.1	3.14	56.2	—	3.8
H[Ag(Dpen)]·½H_2O	22.66	4.18	40.69	—	5.28
	22.8	4.31	40.5	—	4.9
H[Cu(Dpen)]·H_2O	26.13	5.27	27.65	—	6.10
	26.6	5.23	26.9	—	6.1

* By convention we give here the minimal formulae, though the compounds are polymers in solution [22].

† tm = $^-OOCCH_2CHS^-COO^-$ (thiomalate).

‡ Dpen = $(CH_3)_2CS^-CH(NH_2)COO^-$ (D-penicillaminate).

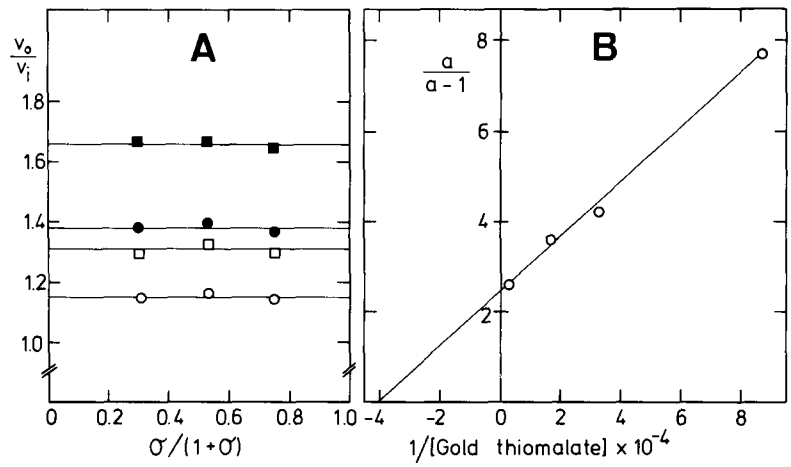


Fig. 1. Inhibition of human leukocyte cathepsin G by gold thiomalate. The substrate was Suc–Ala–Ala–Pro–Phe–$NPhNO_2$; $\sigma = [S]/K_M$ was calculated using for K_M the value 1.9 mM. A = specific velocity plot [24] with gold thiomalate at the following concentrations (μM): 11.0 (○), 30.1 (□), 58.8 (●), 331.0 (■). B = replot of the intercepts in A vs the reciprocal of the inhibitor concentration. In this particular case the intercepts (a) of the curves in the specific velocity plot, extrapolated for $\sigma/(1+\sigma) = 0$ or $\sigma/(1+\sigma) = 1$, coincide and the ratio $a/(a-1)$ is equal to $v_o/(v_o - v_i)$ [24].

cathepsin G with Suc–Ala–Ala–Pro–Phe–$NPhNO_2$ as substrate was calculated with the method of Eisenthal and Cornish-Bowden [25] and was 1.9×10^{-3} M.

Apparatus. Stopped flow measurements were carried out with an apparatus described elsewhere [26]. Fluorescence was monitored with an Aminco SPF-500 recording spectrofluorimeter operating in the ratio mode. For spectrophotometric measurements an Uvikon 810 apparatus (Kontron Ltd., Zurich, Switzerland) was used.

RESULTS

Inhibition of cathepsin G by gold, silver and copper thiomalates. Figure 1A shows the specific velocity plot for inhibition of cathepsin G by gold thiomalate. The plot consists of a family of parallel lines and the replot (Fig. 1B) is a straight line with ordinate intercept equal to 2.40. From the intercepts of the replot a β value of 0.58 and $K_i = K_i' = 2.5 \times 10^{-5}$ M can be calculated (see also Scheme 1 for nomenclature). An inhibition profile of cathepsin G by gold thiomalate is given in Fig. 2 in order to show that the fractional inhibition is independent on the substrate concentration and that the curve is hyperbolic. The solid line in Fig. 2 was obtained with the experimental values of K_i and β and the specific velocity equation [24], which, in this particular case, reduces to $v_o/v_i = \{1+[I]/K_i\}/\{1 + \beta[I]/K_i'\}$.

The specific velocity plot for inhibition of cathepsin G by silver thiomalate (not shown) consisted of a family of parallel lines analogous to that in Fig. 1A and the replot gave a straight line with ordinate intercept equal to 3.38 (Fig. 3). For this inhibitor β was 0.70 and $K_i = K_i' = 1.2 \times 10^{-4}$ M.

Serious technical problems arose with copper thiomalate. This compound revealed a poor stability in aqueous media even if the solutions were thoroughly deaerated and treated in a nitrogen atmosphere (a violet colour was indicative of the decomposition of copper thiomalate). Repeated opening of the vials for transferring inhibitor solutions to the spectrophotometer cells caused inevitable air infiltrations and made long series of measurements virtually impossible. For these reasons the detailed kinetic mechanism of inhibition could not be studied. Therefore, the inhibition of cathepsin G by this compound was tested at few inhibitor concentrations and had a maximum value of about 25% with 1 mM copper thiomalate.

Inhibition of elastase by gold, silver and copper thiomalates. The inhibition patterns of elastase by silver thiomalate, analysed with the specific velocity plot, were identical to those shown in Fig. 1. The

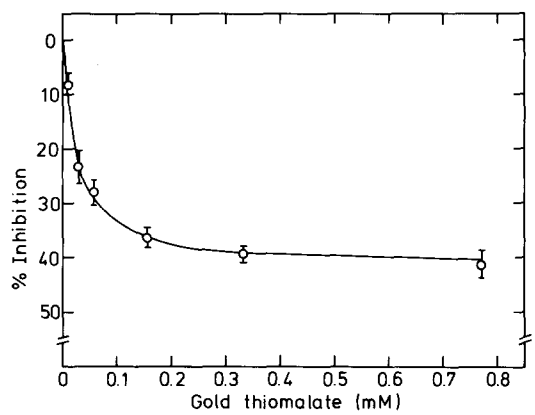


Fig. 2. Inhibition profile of human leukocyte cathepsin G by gold thiomalate. The substrate was Suc–Ala–Ala–Pro–Phe–$NPhNO_2$, which was tested at three concentrations (0.84, 2.17 and 5.60 mM). No differences in the percent inhibition were found with these substrate concentrations over the whole inhibitor concentration range. The experimental points represent the mean ± S.D. of six separate experiments (each point determined twice with the three above substrate concentrations). The solid line is a calculated curve obtained with the specific velocity equation [24] and $\beta = 0.58$, $K_i = 2.5 \times 10^{-5}$ M.

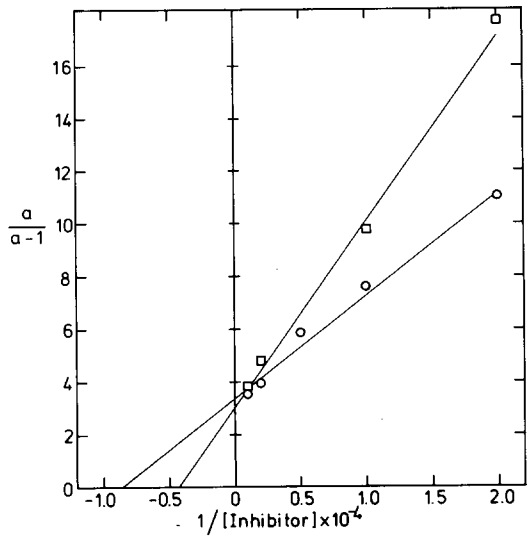


Fig. 3. Replots of the specific velocity plot intercepts for the inhibition of human leukocyte elastase (□) and cathepsin G (○) by silver thiomalate. For other details see Fig 1.

replot is shown in Fig. 3 together with the corresponding plot for inhibition of cathepsin G by silver thiomalate. Results are summarized in Table 2.

The same problems encountered in the studies with copper thiomalate and cathepsin G were also found in the case of elastase. With 1 mM copper thiomalate the elastase activity was reduced by about 30%.

In a previous paper [16] we analysed the inhibition of elastase by gold thiomalate using the ester substrate Boc–Ala–$OPhNO_2$. It was shown that the inhibition mechanism was complicated by an apparent interaction occurring between substrate and inhibitor. In order to test whether this interaction was possibly due to impurities in the commercial gold thiomalate, we repeated the measurements with a sample synthesised by us. When using Boc–Ala–$OPhNO_2$ as the substrate we could confirm the previous results: at relatively low substrate and inhibitor concentrations the inhibition had a hyperbolic mixed type character. Increasing the substrate and inhibitor concentrations caused a non-linear increase of the inhibitory activity.

The inhibition profile of elastase by gold thiomalate using the tetrapeptide substrate Suc–Ala–Ala–Pro–Val–NMec is shown in Fig. 4. The curve was sigmoid and the percentage of inhibition was the same when using a commercial sample of gold thiomalate or our own compound. The specific velocity plot, as well as the double reciprocal plot of Lineweaver–Burk, were not applicable in this case and consisted of concave curves. Therefore, a quantitative steady-state analysis can obviously not be performed. The K_i value was calculated from the experiments with Boc–Ala–$OPhNO_2$ as substrate at low concentrations [16] and Table 2 summarises the results. Using insoluble elastin as substrate for elastase the inhibition profile had the usual hyperbolic shape (Fig. 4) and the maximal inhibition was about 40%, in agreement with the β value of 0.6 calculated from steady-state analysis [16].

In order to analyse in more detail the complex kinetic behaviour of the elastase inhibition by gold thiomalate with synthetic substrates we performed the stopped-flow experiments shown in Fig. 5. Figure 5A shows two reaction traces in the absence (b) and in the presence (a) of gold thiomalate. In Figure 5B the reaction shown in Aa is analysed in more detail using different observation times. The amplitude of the pre-steady-state phase determined by extrapolating back to zero time the linear part of the curve with observation time 16 s, was $\Delta A = 0.42$. Since $\Delta\varepsilon$ at 360 nm is 6,700/M/cm [16] this corresponds to a product concentration of 63 μM, or about 5.5 times the enzyme active site concentration. Therefore, the amplitude of the pre-steady-state phase does not have a stoichiometric relationship with the enzyme concentration and does not describe a definite (e.g. 1 : 1 or 1 : 2) occupancy of binding sites. The slope of the steady-state curve (dashed line in Fig. 5B) corresponded to 80% inhibition.

Studies with D-*penicillamine derivatives.* The D-penicillaminates of gold (I), silver (I) and copper(I) were tested as potential inhibitors of both leukocyte elastase and cathepsin G up to a concentration of 2 mM for the gold derivative and 1 mM for the other two. Elastase was assayed with Boc–Ala–$OPhNO_2$, Suc–Ala–Ala–Pro–Val–NMec and elastin, whereas cathepsin G was tested with Suc–Ala–Ala–Pro–Phe–$NPhNO_2$. Experiments were performed both in the absence and in the presence of D-penicillamine (1–2 mM). In no case the activity of the two enzymes was influenced.

Table 2. Inhibition of human leukocyte elastase and cathepsin G by gold, silver and copper thiomalates*

	Au-thiomalate		Ag-thiomalate		Cu-thiomalate	
	K_i (M)	β	K_i (M)	β	K_i (M)	β
Elastase†	3.3×10^{-5}	0.60	2.3×10^{-4}	0.67	$> 10^{-4}$	0.75
Cathepsin G‡	2.5×10^{-5}	0.58	1.2×10^{-4}	0.70	$> 10^{-4}$	0.70

* The values given were obtained in 50 mM phosphate buffer, pH 7.40 and 25°. The meaning of K_i and β is explained in Scheme 1.

† K_i and β for elastase and Au-thiomalate were measured with Boc–Ala–$OPhNO_2$ as substrate, whereas the other parameters refer to measurements with Suc–Ala–Ala–Pro–Val–NMec.

‡ The substrate was Suc–Ala–Ala–Pro–Phe–$NPhNO_2$.

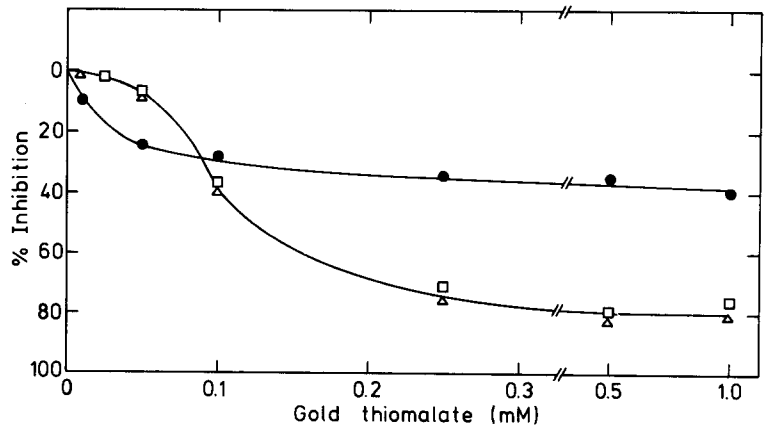
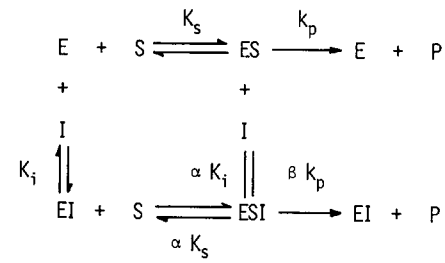


Fig. 4. Inhibition profile of human leukocyte elastase by gold thiomalate. (●) = insoluble elastin as substrate. The open symbols represent inhibition using the tetrapeptide substrate Suc–Ala–Ala–Pro–Val–NMec: (△) = gold thiomalate synthesized in our laboratory; (□) = commercial gold thiomalate.

DISCUSSION

Mechanistic considerations. The inhibition mechanism for elastase by silver thiomalate and for cathepsin G by silver and gold thiomalates is of the hyperbolic, non-competitive type. The mechanism for elastase inhibition by gold thiomalate can be classified as hyperbolic mixed type [16]. These mechanisms can be adequately described with the general modifier mechanism shown in Scheme 1. For both elastase and cathepsin G the efficiency of the inhibition by metal thiomalates increases in the order Cu < Ag < Au. This result correlates well with the increasing ionic radius in the lower oxidation state



Scheme 1. The general modifier mechanism. E = enzyme, S = substrate, I = inhibitor, P = product. $K_i = [E][I]/[EI]$, $K_s = [E][S]/[ES]$, $\alpha K_i = K_i' = [ES][I]/[ESI]$, k_p = catalytic constant.

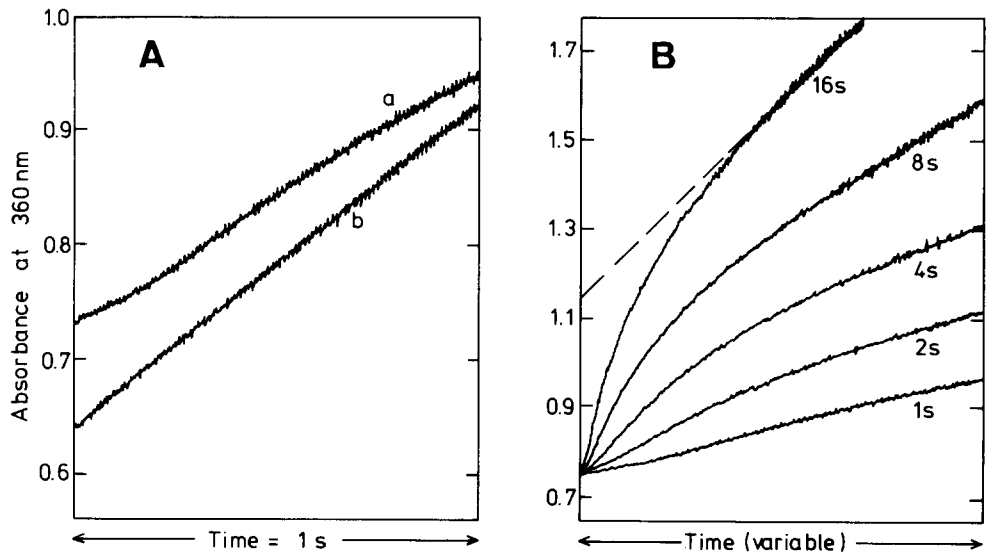


Fig. 5. Stopped-flow kinetic measurements with human leukocyte elastase. Conditions: temperature 25°, wavelength 360 nm, light path 1.0 cm, dead time of the instrument 0.9 ms [26]. The substrate was Suc–Ala–Ala–Pro–Val–NMec and the final concentrations were always: substrate = 0.90 mM, enzyme active sites = 11.4 μM, gold thiomalate 2.4 mM. In A (trace b) the enzyme was rapidly reacted against the substrate solution. All other experiments (Aa and the five traces in B) were obtained by rapidly mixing enzyme with a preincubated solution of substrate and inhibitor. The variable observation times in B are shown in the figure. The starting points of the various curves are determined by the absorption of the substrate at 360 nM and the starting point at higher absorbance in the experiments with gold thiomalate is due to the absorption of gold thiomalate.

of the three transition metals and with the ability to form coordination complexes with several ligands.

With regard to the mechanism of inhibition at the molecular level it is very likely that the metal must be soft-liganded in order to manifest enzyme inhibition properties and that an exchange of the metal between ligand and enzyme is a prerequisite for the inhibitory action. The metal ion, once transferred from thiomalate, probably interacts in some way with a reactive side chain of the enzymes (e.g. histidine or methionine) as suggested for trypsin and gold thiomalate by Griffin and Steven [27]. Interestingly, gold thiomalate alone was not capable of inhibiting trypsin. Only in the presence of a suitable thiol carrier gold was dissociated from thiomalate and became bound to the enzyme [27]. This could also be the case for gold, silver- and copper-D-penicillaminates, which failed to inhibit the two leukocyte serine proteinases. In the case of elastase and cathepsin G, enzyme inhibition could be achieved with gold thiomalate alone so that an exchange reaction possibly takes place directly between the enzyme and the thiolate.

The complexity of the inhibition mechanism of leukocyte elastase by gold thiomalate when using synthetic substrates deserves a particular comment since this topic has been the subject of several studies and the discussion did never embrace the mechanistic aspect, that was rather neglected as an unwanted complication. With the elastase-specific tetrapeptide substrate Suc–Ala–Ala–Pro–Val–NMec the inhibition profile of leukocyte elastase by gold thiomalate (percent inhibition vs inhibitor concentration) and the specific velocity plot were sigmoid and concave, respectively. This suggests that the general modifier mechanism shown in Scheme 1 is no longer valid and that the inhibition pathway must contain at least one species accounting for squared concentration terms in the velocity equation. Some mechanisms can be proposed which account for the observed curvatures. In particular, for linear inhibitors, Reiner analysed the case of substrate depletion by *SI* formation, the case in which the true inhibitor is the *SI* complex and a combination of the two mechanisms [28]. None of these systems can be directly applied to our problem because the inhibition is further complicated by its hyperbolic character. However, the curvatures observed in the plots are consistent with the idea of an *SI* species or, in general, with a species carrying a modified structure of the inhibitor due to the presence of substrate. Both situations of apparent substrate depletion at high inhibitor concentration and inhibitor depletion at high substrate levels, as demonstrated by our experiments, imply the introduction of squared terms in the velocity equation. This causes the plots to be curved and makes them useless without the use of very complex computer calculations, which are outside the scopes of this paper. Using the insoluble (and more 'natural') substrate elastin the inhibition profile was hyperbolic and the maximal inhibition was about 40%. The lag phase, with a characteristic sigmoid shape, preceding the steady-state in the gold thiomalate inhibited reaction of leukocyte elastase could be interpreted as: (1) The displacement of gold from thiomalate to the enzyme; (2) a slow rearrangement of a ternary complex between enzyme, substrate and inhibitor; (3) the formation of a *SI* species competing with *S* for the enzyme active site; (4) a combined effect of 1, 2 and/or 3. We feel quite likely that a *SI* species is formed after binding of S and I to elastase giving rise to an *ESI** ternary complex, different from the *ESI* complex shown in Scheme 1. This hypothesis that the substrate-depleting species (*ESI**) is formed 'on the enzyme' is not only supported by our steady-state and stopped-flow experiments, but also by the lack of similar effects during cathepsin G inhibition by gold thiomalate. Independently of the origin of this complex kinetic behaviour, it occurs at quite high gold thiomalate concentrations, it is a characteristic of elastase assayed with synthetic substrates and remains therefore of pure mechanistic interest.

Perhaps these effects find an explanation in the very rich coordination chemistry of gold (I), which in turn originates a potentially very complicated biochemistry. For example, in aqueous solution at neutral pH gold thiomalate is a polymer (apparently a hexamer with a ring structure) in which each gold atom is linearly coordinated by two sulphur atoms [22]. Its structure is, however, influenced by ionic strength, pH [29] and probably by its concentration, with formation of oligomers, clusters, bis-thiolate and mixed-thiolate species. Furthermore, gold thiomalate undergoes exchange reactions in which the thiomalate is readily released [29, 30]. This complex pattern of gold thiomalate chemistry in aqueous solution may thus be the origin of the complicated inhibition behaviour observed with elastase.

Biological relevance. From the results exposed above it appears that the function of gold thiomalate as possible inhibitor of the human leukocyte proteinases elastase and cathepsin G is of secondary interest. In fact, despite K_i values of the order of magnitude of 10^{-5} M, the maximal inhibition that can be achieved under saturating conditions is about 40% for both enzymes. Any inhibitory effect greater than 40% observed for elastase is likely due to an interaction between gold and synthetic substrates. Silver and copper derivatives, that mimic the action of the corresponding gold compounds and have been the subject of some *in vivo* studies are even less efficient than gold as enzyme inhibitors.

It is not yet possible to gather definitive clues about the complex mechanisms of action of transition metal compounds in the treatment of inflammatory diseases. Occasionally, the failure of silver and copper derivatives to produce the wanted effects, could be partly explained with a reduced availability of these metals, with respect to gold, for *in vivo* receptors.

Acknowledgements—This work has been partly supported by the Swiss National Fund, Grant 3.596-0.79 and by the Fondo Incentivazione Programmi Scientifici of the University of Trieste.

REFERENCES

1. J. E. Gadek, G. W. Hunninghake, G. A. Fells, R. L. Zimmerman, B. A. Keogh and R. G. Crystal, *Bull. Eur. Physiopath. Resp.* (Suppl.) **16**, 27 (1980).

2. M. Jochum, H. Fritz, K-H. Duswald and E. Hiller, in *Selected Topics in Clinical Enzymology* (Eds. D. Goldberg and M. Werner), p. 85. Walter De Gruyter, Berlin (1983).
3. P. M. Starkey, A. J. Barrett and M. C. Burleigh, *Biochim. biophys. Acta* **483**, 386 (1977).
4. A. J. Barrett, *Agents Actions* **8**, 11 (1978)
5. A. Baici, M. Knöpfel, K. Fehr, F. Skvaril and A. Böni, *Scand. J. Immun.* **12**, 41 (1980).
6. A. Baici, M. Knöpfel and K. Fehr, *Scand. J. Immun.* **16**, 487 (1982).
7. A. Baici, M. Knöpfel, K. Fehr and A. Böni, *Immun. Lett.* **2**, 47 (1980).
8. A. Baici, M. Knöpfel and K. Fehr, *Molec. Immun.* **19**, 719 (1982).
9. D. H. Brown, W. E. Smith, J. W. Teape and A. J. Lewis, *J. Med. Chem.* **23**, 729 (1980).
10. A. J. Lewis, *Agents Actions* **8**, 244 (1978).
11. M. W. Whitehouse and W. R. Walker, *Agents Actions* **8**, 85 (1978).
12. A. Baici, P. Salgam, G. Cohen, K. Fehr and A. Böni, *Rheumatol. Int.* **2**, 11 (1982).
13. M. Graf, A. Baici and P. Sträuli, *Lab. Invest.* **45**, 587 (1981).
14. A. Baici, M. Gyger-Marazzi and P. Sträuli, *Invasion Metastasis*, **4**, 13 (1984).
15. A. Baici, P. Salgam, K. Fehr and A. Böni, *Biochem. Pharmac.* **29**, 1723 (1980).
16. A. Baici, P. Salgam, K. Fehr and A. Böni, *Biochem. Pharmac.* **30**, 703 (1981).
17. A. Baici, P. Salgam, K. Fehr and A. Böni, *Z. Rheumatol.* **40**, 44 (1981).
18. A. Baici and M. Gyger-Marazzi, *Eur. J. Biochem.* **129**, 33 (1982).
19. C. F. Shaw, III, *Inorganic Perspectives in Biology and Medicine* **2**, 287 (1979).
20. J. C. Powers and B. F. Gupton, *Meth. Enzym.* **46**, 208 (1977).
21. D. H. Brown, M. Paton and W. E. Smith, *Inorg. Chim. Acta* **66**, L51 (1982).
22. A. A. Isab and P. J. Sadler, *J. Chem. Soc. (Dalton)*, 1657 (1981).
23. E. G. Delmar, C. Largman, J. W. Brodrick and M. C. Geokas, *Analyt. Biochem.* **99**, 316 (1979).
24. A. Baici, *Eur. J. Biochem.* **119**, 9 (1981).
25. R. Eisenthal and A. Cornish-Bowden, *Biochem. J.* **139**, 715 (1974).
26. M. Hadorn, V. A. John, F. K. Meier and H. Dutler, *Eur. J. Biochem.* **54**, 65 (1975).
27. M. M. Griffin and F. S. Steven, *Br. J. Pharmac.* **75**, 333 (1982).
28. J. M. Reiner, *Behavior of Enzyme Systems*, Chap. 8. Van Nostrand-Reinhold, New York (1969).
29. A. A. Isab and P. J. Sadler, *J. Chem. Soc. (Dalton)*, 135 (1981).
30. P. J. Sadler, *J. Rheumatol.* (Suppl. 8) **9**, 71 (1982).