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Gold-containing drugs and the control of proteolytic enzymes

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1 $NaAuCl_4$ and aurothioglucose inhibited trypsin in free solution without the need of a carrier molecule.

2 $NaAuCl_4$, aurothioglucose, aurothiomalate, auranofin and chloro(triethyl phosphine) gold all inhibited the trypsin-like neutral protease on the surface of Ehrlich ascites tumour cell membranes equally well.

3 Crude cathepsin preparations were activated by low concentrations of dithiothreitol and also by aurothioglucose, due to the displacement of an inhibitor.

4 Thiol-activated cathepsins were inhibited by each of the gold derivatives. The gold could be withdrawn from the enzyme by incremental additions of thiols such as reduced glutathione and cysteine with regeneration of enzymic activity.

5 Lineweaver-Burk plots of kinetic data indicated that gold acted as a non-competitive inhibitor of cathepsins.

6 A naturally occurring inhibitor of cathepsins was extracted from cartilage. The mechanism of inhibition was again shown to be a thiol-disulphide exchange, the disulphide being provided by the inhibitor and the thiol being provided by the enzyme.

7 The role of gold in the attempted control of proteolysis in rheumatoid arthritis is briefly discussed in terms of reversible exchange reactions involving gold thiols, disulphides and cartilage inhibitors of proteolytic enzymes.

Introduction

Gold salts have been used for the treatment of rheumatoid arthritis for many years and this is a recent topic of a literature review (Hella, Cassady & Hardin, 1982). Our interest in gold salts was stimulated by an earlier observation that gold inhibited trypsin activity (Griffin & Steven, 1982). The most interesting finding of this study was the requirement for a carrier molecule and the consequent exchange reactions involving gold which could be followed by kinetic analysis. We decided to investigate the mechanisms by which gold, offered in the form of gold salts, might modify three different types of proteolytic enzymes. We employed (a) free trypsin in solution, (b) Ehrlich ascites tumour cells as a convenient source of cell-bound neutral protease having trypsin-like activity to typify proteolytic enzymes located on the cell surface since these systems are known to possess special properties (Steven, Griffin, Itzhaki & Al-Habib, 1980), (c) papain, and (d) a crude preparation of liver cathepsins. Our choice was determined by the fact that trypsin and the cell surface enzymes are both seryl enzymes (having serine as the functional group of their active centres) whilst papain and cathepsin B are thiol enzymes. These studies demonstrated that thio-gold derivatives were capable of numerous exchange reactions which, under defined conditions, caused an initial reactivation of latent cathepsin activity whilst under different conditions (e.g. the presence of added thiols) lead to inhibition of both papain and cathepsins.

Methods

Twice crystallized papain, α-N-benzoyl-DL-arginine-β-naphthylamide HCl (BANA), reduced glutathione, cysteine, dithiothreitol, $NaAuCl_4$ and aurothioglucose were purchased from Sigma, St. Louis, U.S.A. Crystalline bovine trypsin was purch-

ased from Boehringer Mannheim GmbH, Germany. Smith, Kline and French, Welwyn, very kindly provided two gold derivatives for these studies; these were auranofin (SK and F D-39162) with the chemical name of S-(triethylphosphoranediylaurio) -1-thio-β-D-glucopyranose 2,3,4,6-tetroacetate, and chloro(triethyl phosphine) gold (SK and F 36914).

Crude cathepsin preparations were prepared according to the initial steps employed in the preparation of cathepsin B, H and S (Locnikar, Popović, Lah, Kregar, Babnik, Kopitar & Turk, 1981). Fresh bovine liver was homogenized in 5 volumes of distilled water, acidified to pH 4.0 with 2 M acetic acid and allowed to stand at 4°C for 18 h. The homogenate was centrifuged and the resultant supernatant fraction made 20% saturated with respect to ammonium sulphate, employing the addition of 100% saturated ammonium sulphate solution. The protein fraction which remained soluble in 20% ammonium sulphate was collected by centrifugation and exhaustively dialysed; we used this preparation (2.5 mg protein/ml) as our acid protease or cathepsin preparation. Fresh bovine articular cartilage (from three year-old steers) was washed free of blood and synovial fluid with 0.9% w/v NaCl solution (saline) and we then extracted the chopped cartilage fragments in saline at 4°C for 1 h. This extract was collected by centrifugation and used as such in studies on the inhibition of cathepsin by a protein component of the cartilage extracted in saline. It contained 7.5 mg protein/ml.

Protein determination

The protein concentrations in the crude cathepsin and cartilage extracts were determined by the method of Lowry, Rosenbrough, Farr & Randall (1951) employing bovine serum albumin as standard.

β-Naphthylamidase assay

Both cathepsin B and trypsin cleave α-N-benzoyl-DL-arginine β-naphthylamide HCl (BANA) with the formation of β-naphthylamine which may be recorded by fluorimetric assay (MacDonald, Ellis & Reilly, 1966). Trypsin activity or trypsin-like activity was measured in 3 ml Tris-HCl buffer 0.1 M pH 8.0 at 37°C for 1 h and cathepsin or papain activities were measured in 3 ml acetate buffer 0.1 M, pH 6.0 at 37°C for 1 h. In each case we added 40 μl BANA solution (32 mg BANA/ml dimethylsulphoxide) as substrate, each reaction being carried out in screw-capped plastic bijou tubes. The fluorescence was recorded on an Aminco-Bowman spectrofluorimeter in cuvettes with a 1 cm light path, employing an excitation wavelength of 335 nm and an emission wavelength of 410 nm.

The inhibition studies were carried out with a fixed quantity of enzyme e.g. 2 μg of trypsin per tube plus incremental additions of the potential inhibitor. After 10 min preincubation, the buffer and substrate were added and the reactions begun at 37°C. The activation experiments contained latent enzyme plus incremental additions of dithiothreitol or, partially activated cathepsin plus 166 μM aurothioglucose followed by incremental additions of glutathione or cysteine.

Expression of results

The inhibition experiments present data for the percentage inhibition induced by incremental addition of an inhibitor to a fixed quantity of enzyme, the control value being taken as 100%. The re-activation experiments present data expressed as a percentage of the original control activity whilst the activation of the latent enzyme is represented as net production of β-naphthylamine fluorescence since the original latent enzyme had no activity prior to adding activator, dithiothreitol in this case. Lineweaver-Burk plots were obtained by plotting ($^1/_V$) against ($^1/_{[S]}$), where V is expressed in nmol of product formed per min at 37°C by 100 μl cathepsin preparation and $[S]$ is the

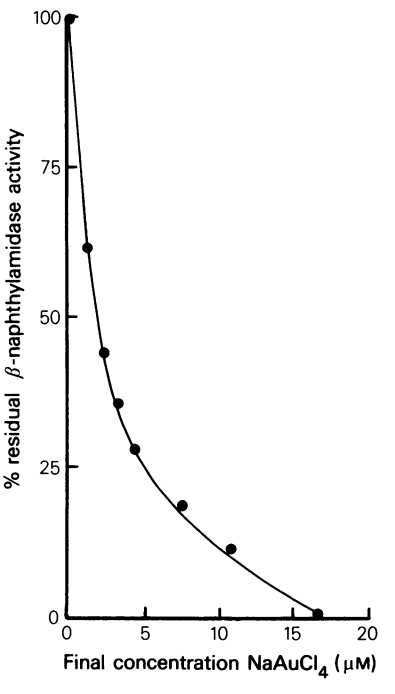


Figure 1 Inhibition of trypsin β-naphthylamidase activity by incremental additions of $NaAuCl_4$. The control, 2 μg trypsin in 0.1 M Tris/HCl buffer pH 8.0 for 1 h at 37°C, had activity equal to 100 on the vertical axis. The degree of inhibition is expressed as a percentage of the control trypsin activity.

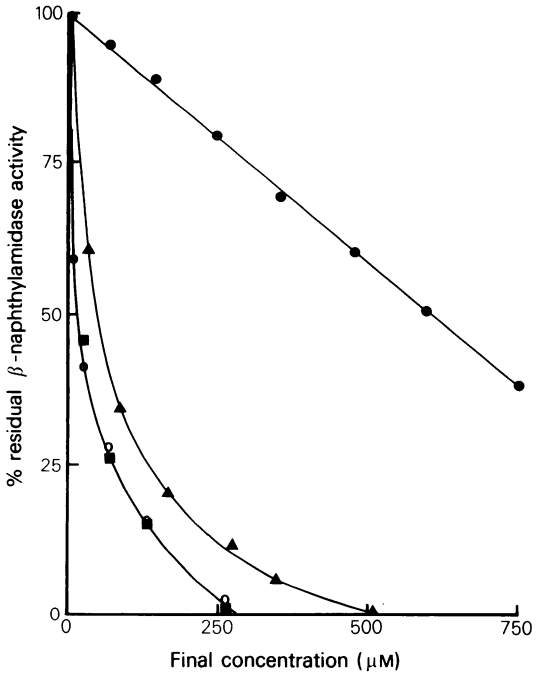


Figure 2 Inhibition of Ehrlich ascites tumour cell surface β-naphthylamidase by incremental additions of gold salts. Each tube contains 2×10^8 cells and the control β-naphthylamidase activity is represented by 100 on the vertical axis. Auranofin (▲); $NaAuCl_4$ (○) chloro(triethyl phosphine) gold (■) and aurothioglucose (●).

substrate concentration in mM. Each plot represents three experimental runs within a ± 5% degree of variation.

Results

Sodium auric chloride ($NaAuCl_4$) inhibits trypsin in free solution (Figure 1) and also the trypsin-like neutral protease on the surface of Ehrlich ascites tumour cells (Figure 2). The inhibition of this tumour cell surface neutral protease by gold transferred from the thio-gold drugs, chloro(triethyl phosphine) gold, auranofin and aurothioglucose is also presented in Figure 2.

The activation of our cathepsin preparation by incremental additions of dithiothreitol is summarised in Table 1.

At low concentrations of added dithiothreitol e.g. 4.3 μM, the partially activated cathepsin exhibited a biphasic plot of β-naphthylamidase activity in the presence of incremental additions of the thio-gold drug, auranofin (Figure 3). In this plot, low concentrations of auranofin caused an activation of latent cathepsin, whilst at higher concentrations of auranofin the inhibitory effect of gold became dominant.

Table 1 Activation of latent cathepsin at pH 6.0 by incremental addition of dithiothreitol.

Dithiothreitol (μM)	*Fluorescence*
0	0
2.7	70
5.5	160
8.5	260
11	300
13	350
17	410
20	450
23	485
27	518
30	590
33	615

Cathepsin activity is recorded as the fluorescence of β-naphthylamine, formed as the product of BANA cleavage after 1 h at 37°C.

At higher concentrations of added dithiothreitol, e.g. 33 μM, the highly active cathepsin preparation exhibited simple inhibition kinetics in the presence of incremental additions of thio-gold drugs, leupeptin and dimethyl disulphide (Figures 4,5).

The inhibition of cathepsin by gold was demonstrated to be reversed by addition of a competitive binding agent for gold, e.g. an excess of a thiol in the

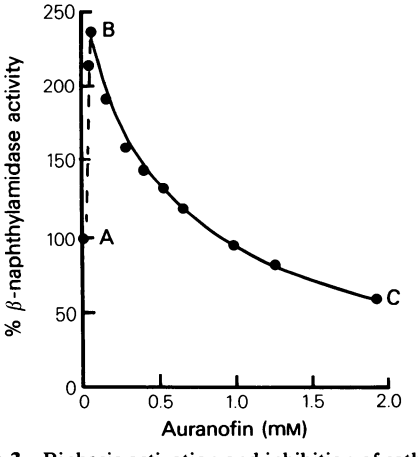


Figure 3 Biphasic activation and inhibition of cathepsin by incremental additions of auranofin. Each tube contained 100 μl cathepsin partially activated by addition of 4.3 μM dithiothreitol, with initial β-naphthylamidase activity equivalent to 100%, point A on vertical scale. Incremental additions of auranofin led to activation of cathepsin (line AB) followed by subsequent inhibition, curve BC.

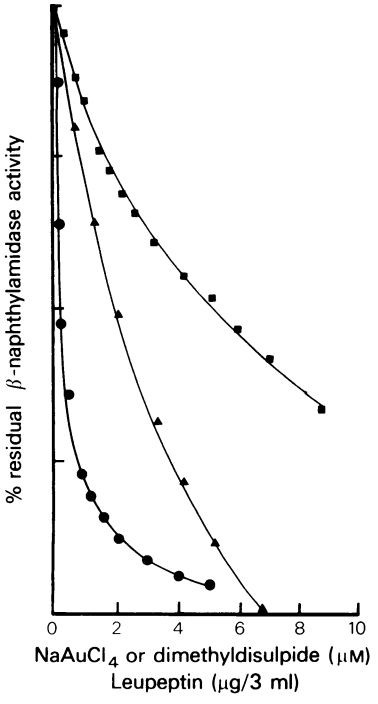


Figure 4 Inhibition of dithiothreitol-activated cathepsins by incremental additions of leupeptin, dimethyl disulphide and $NaAuCl_4$ followed by β-naphthylamidase assay. Each tube contained 100 μl cathepsins (0.25 mg protein) with dithiothreitol pre-incubation to give final concentration of 33 μM dithiothreitol. Incremental additions of inhibitors were made and after 10 min pre-incubation, the residue cathepsin activity was determined with BANA at pH 6.0, 37°C for 1 h. The control activity is represented by 100 on the vertical axis. Leupeptin (●); $NaAuCl_4$ (▲) and dimethyldisulphide (■).

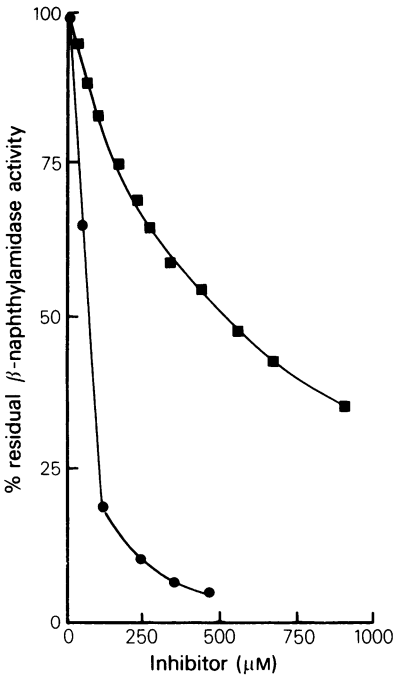


Figure 5 Inhibition of dithiothreitol-activated cathepsins by incremental additions of aurothioglucose and auranofin followed by β-naphthylamidase assay. Conditions as for Figure 4. Aurothioglucose (●); auranofin (■).

form of glutathione or cysteine (Figures 6,7). In these two figures the data represent a family of plots obtained with an initial dithiothreitol activation (defined as × μM in the legends and ranging from 3.3 to 33.3 μM). The partially activated cathepsin was then reacted with a fixed concentration of aurothioglucose, 166 μM, and subsequently re-activated by incremental additions of reduced glutathione (Figure 6) or cysteine (Figure 7).

Lineweaver-Burk plots obtained with 100 μl cathepsin in the presence of 4.3 μM dithiothreitol alone and with dithiothreitol in the presence of 100 μM aurothioglucose, are presented in Figure 8 to determine the nature of gold inhibition of cathepsin.

The saline extract of bovine cartilage possessed a protein which inhibited cathepsin (Figure 9); this inhibition could be reversed by addition of reduced glutathione (data not shown, but similar to Figure 6b).

Discussion

We deliberately used a crude cathepsin preparation in this study of gold exchange and the effect of gold on cathepsin activity. In the pathology of rheumatoid disease, crude enzyme systems are involved in producing articular cartilage damage rather than the highly purified enzyme as we often imply by *in vitro* studies. For similar reasons we made no attempt to purify an inhibitor of cathepsins present in cartilage.

We have previously demonstrated that the gold of negatively charged sodium aurothiomalate had no inhibitory action on free trypsin unless a suitable carrier was added to the test system (Griffin & Steven, 1982). The carrier molecule exchanged the gold from the drug and then transferred the gold to the active centre of trypsin, causing inhibition of β-naphthylamidase activity. Sodium auric chloride has no negative charge and can readily transfer gold to trypsin (Figure 1) with consequent inhibition of β-naphthylamidase activity. Similar results were ob-

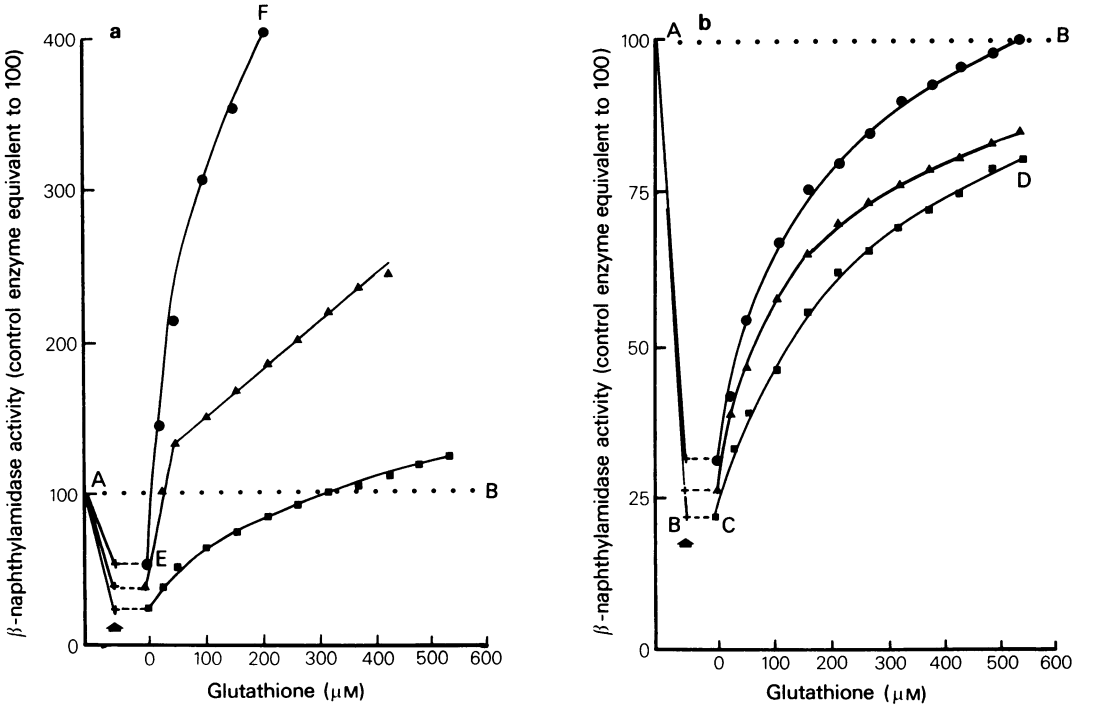


Figure 6 Gold inhibition of cathepsin followed by re-activation by incremental additions of reduced glutathione followed by β-naphthylamidase assay. Each tube contained 100 μl cathepsin (0.25 mg protein) with × μM dithiothreitol followed by aurothioglucose to 166 μM (at arrow). Incremental additions of reduced glutathione were made to each tube and after 10 min pre-incubation the β-naphthylamidase activity was determined at 37°C for 1 h at pH 6.0. The β-naphthylamidase activity of control tubes which lacked both gold and glutathione is represented by 100 (dotted line AB); the data for enzymic activities in the test systems are presented as a percentage value of this control (see text). The values of × μM dithiothreitol in each series of tubes of (a) were 3.3 μM (●); 6.6 μM (▲); 13.2 μM (■) and of (b) were 16.6 μM (●); 20.0 μM (▲) and 33.3 μM (■).

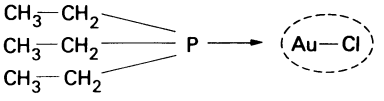
tained with aurothioglucose as the donor of gold (data not shown here) but this reagent was required in higher concentrations than sodium auric chloride. The inhibition of trypsin by Ag^+ has been described by Martinek, Savin & Berezin (1971). The Ag^+ was shown to bind to the histidine in the active site of trypsin and it is likely that gold also binds to this histidine, resulting in inhibition of β-naphthylamidase activity (Figure 1).

Ehrlich ascites tumour cells possess a cell surface neutral protease similar to trypsin (Short, Steven, Griffin & Itzhaki, 1981). This enzyme exhibited trypsin-like specificity and played a crucial role in the activation of procollagenase exported by these cells (Steven, Griffin, Itzhaki & Al-Habib, 1980). This trypsin-like enzyme is also responsible for the tumour cell induced target cell cytolysis (Di Stefano, Beck, Lane & Zucker, 1982; Steven, Hulley, Griffin & Itzhaki, 1982). Gold is readily transferred from thio-gold drugs and the simple inorganic gold salt ($NaAuCl_4$) to the tumour cell surface trypsin-like enzyme with consequent inhibition of both β-naphthylamidase activity (Figure 2) and tumour cell induced target cell cytolysis (Steven, Hulley, Griffin & Itzhaki, 1982). Both trypsin in free solution and the trypsin-like enzyme on the surface of tumour cells are inhibited by gold. In the case of $NaAuCl_4$, the gold is transferred directly to the enzyme whereas, with auranofin, aurothioglucose and chloro(triethylphosphine) gold, the gold is transferred by an exchange from the substituted thiol group of the drug molecule. The structures of these gold-containing drugs is diagrammatically presented in Scheme 1, to indicate the thio-gold bonding and to emphasize that the exchange of gold will generate a free thiol (SH). The generation of a thiol is of importance when considering the action of thio-gold drugs on cathepsin B and papain since these enzymes are activated by thiols and inhibited by heavy metals. Cathepsin B and papain are described as 'thiol enzymes' and their activity depends upon having a reactive thiol group located in their active centres (Barrett, 1977).

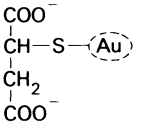
The thiol enzymes (e.g. papain and cathepsin B) require thiol activation to attain expression of maxi-

Structure of thio-gold drugs

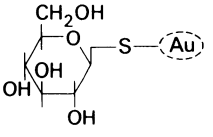
Chloro(triethylphosphine) gold (SK and F 36914)



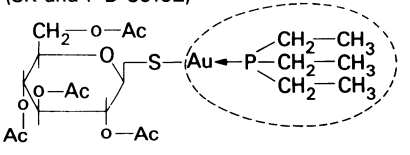
Aurothiomalate



Aurothioglucose



Auranofin (SK and F D 39162)



Note Ac ≡ (CH_3—CO) or acetyl residue. The exchangeable gold moiety is enclosed in a dotted cage.

Scheme 1

mal enzymic activity (see Table 1 for the thiol activation of cathepsin). The reason for thiol activation is that these enzymes readily form intermolecular disulphide bridges with inhibitor molecules to form latent enzymes or even exist naturally as the proenzyme in a form of intramolecular disulphide bonded molecules which may be activated subsequently by a rearrangement of disulphide bonds (Brocklehurst & Kierstan, 1973). In the absence of added thiols, papain exhibits a biphasic activation and subsequent inhibition in the presence of incremental additions of sodium aurothiomalate (Griffin & Steven, 1982). Thus, low concentrations of aurothiomalate actually stimulate papain activity (also cathepsin activity, data not presented here) by the loss of gold from the drug with consequent generation of a thiol (thiol succinate in this case) which consequently exchanges with the latent papain to produce active papain. Even at low concentrations of added thiol (e.g. 4.3 μM final concentration of dithiothreitol) the incremental addition of auranofin to cathepsin resulted in a biphasic plot of activation of latent cathepsin followed by a subsequent inhibition phase at higher concentration of auranofin (Figure 3). This situation can only be demonstrated when the cathepsin or papain preparation still contains some latent enzyme, i.e. when

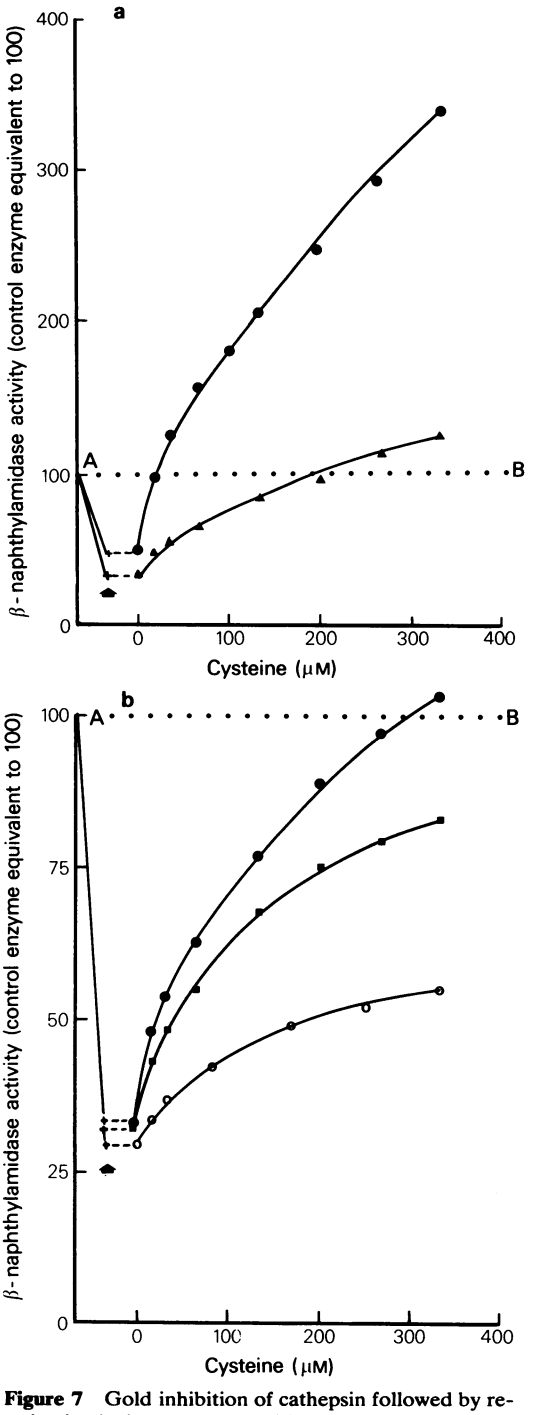


Figure 7 Gold inhibition of cathepsin followed by reactivation by incremental additions of cysteine followed by β-naphthylamidase assay. Conditions as for Figure 6. The values of × μM dithiothreitol in each series of tubes of (a) were 3.3 μM (●); 8.3 μM (▲) and of (b) were 13.3 μM (●); 16.6 μM (■) and 26.6 μM (○).

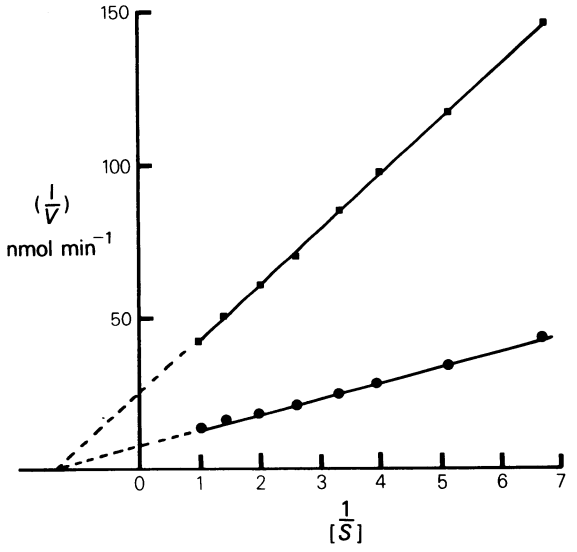


Figure 8 Lineweaver-Burk plots of cathepsin in the presence and absence of aurothioglucose. Each tube contained 100 μl cathepsin in the presence of 4.3 μM dithiothreitol. The vertical axis represents $(\frac{1}{V})$ where V represents nmol min^{-1} and the horizontal axis represents $^1/_{[S]}$ where $[S]$ is mmol BANA. The cathepsin alone is represented by (●); the cathepsin plus 100 μM aurothioglucose is represented by (■). This plot is typical of non-competitive inhibition.

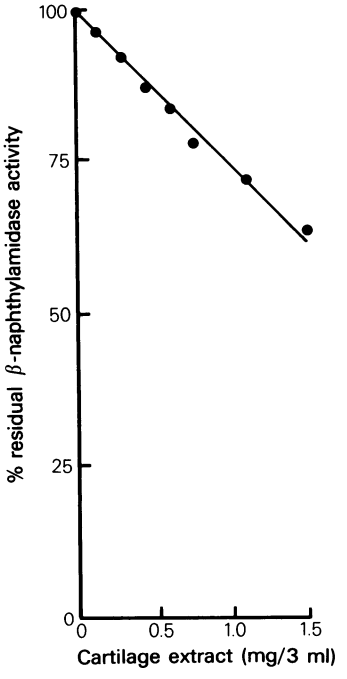
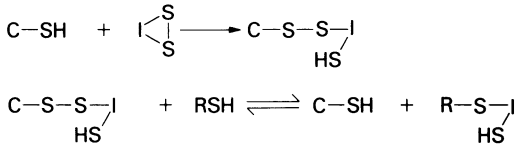


Figure 9 Inhibition of cathepsin by incremental additions of cartilage extract followed by β-naphthylamidase assay. Each tube contained 100 μl cathepsin 0.25 mg protein/tube pre-incubated with dithiothreitol to give a final concentration of 33 μM dithiothreitol. Incremental additions of cartilage extract were made to each tube and after 10 min pre-incubation the residual cathepsin activity was assayed as in Figure 4.

insufficient thiol has been added to produce maximal activation. From an appreciation of the evidence presented in Figure 3, it is clear that the initial concentration of thiol in the test system controls the type of kinetic plot which will be obtained on adding incremental additions of thio-gold drugs to cathepsin and papain (see Figures 6 and 7, in which the dithiothreitol concentration was much higher). It would therefore be of little value to present these types of kinetic plots by I_{50} values, since in Figure 3 we get both activation *and* inhibition by an added thio-gold drug.

The latent cathepsins in our crude liver cathepsin preparation were readily activated by thiols (Table 1). The mechanism of this activation is presented in Scheme 2, in which the cathepsin (C – SH) and inhibitor (I<S-S) complex to form an intermolecular disulphide-linked latent enzyme. The latter may be re-activated by added thiols (RSH) with increased



Scheme 2

β-naphthylamidase activity as a consequence of re-activation.

In order to demonstrate the transfer of gold from thio-gold drugs to cathepsin and the consequent inhibition of the cathepsin, it is necessary to employ sufficiently high thiol concentrations to activate the cathepsin (see Table 1 and Figure 3). The plots in Figures 4 and 5 were carried out with a final concentration of 33 μM dithiothreitol and incremental additions of thio-gold drugs. The plots in Figures 4 and 5 show that the most efficient gold inhibitor of cathepsins was $NaAuCl_4$ which had comparable activity to leupeptin and dimethyl disulphide as inhibitors of cathepsin B (Locknikar *et al.*, 1981; Mort, Leduc & Recklies, 1981; Barrett, 1974). Both auranofin and aurothioglucose were capable of exchanging gold to inhibit cathepsin with consequent inhibition of β-naphthylamidase activity (Figure 5).

If the gold binds to the reactive thiol in cathepsin leading to inhibition of enzyme activity or, alternatively, if the gold binds to a second site, modifying the active centre, then this inhibition should be reversed by a subsequent addition of excess thiol to compete

for the bound gold. This reversal of gold inhibition of cathepsin is shown in the plots of Figure 6(a) and (b), employing reduced glutathione as a competitive binding agent for gold.

Figure 6(a) and (b) consists of a family of plots obtained with a fixed concentration of enzyme, inhibitor and variable dithiothreitol concentration as initial activator of the cathepsin. It is best understood by analysing the plot in Figure 6(b) for the highest concentration of dithiothreitol (33 μM), which caused the maximal activation of cathepsin for the plots in Figure 6. The point A in Figure 6(b) represents the cathepsin activity in the presence of dithiothreitol but the absence of gold and absence of glutathione. This is the activity of the control enzyme tubes. All the other tubes contained a fixed quantity of aurothioglucose (166 μM) and this resulted in a 78% inhibition (line AC). At this stage incremental additions of reduced glutathione were added to successive tubes, resulting in a partial recovery of cathepsin activity to 80% of the original activity along the curve CD. The data show that the increasing thiol concentration in the test system resulted in the removal of inhibitory gold from the cathepsin with consequent reactivation of β-naphthylamidase activity. The line AC may be expressed as follows:

$$C - SH + \text{Au-glucose} \rightarrow C - S - Au + \text{thioglucose}$$

and the curve CD may be expressed as

$$C - S - Au + GSH \rightarrow C - SH + GS - Au$$

where GSH represents reduced glutathione and CSH represents the active cathepsin.

As was pointed out earlier, the controlling factor in all these kinetic plots is the initial concentration of dithiothreitol employed to activate the cathepsin. As the concentration of dithiothreitol is decreased (Figure 6a,b) so the activity of the cathepsin decreases, and consequently the effect of gold inhibition decreases in numerical terms, i.e. point C moves closer to point A in Figure 6a,b, i.e. C is at 78% inhibition for 33 μM dithiothreitol and 50% inhibition for 3.3 μM dithiothreitol in the presence of 166 μM aurothioglucose. This results in a family of kinetic plots for decreasing concentrations of dithiothreitol (Figure 6). We know that at 3.3 μM dithiothreitol, only a part of the potential activity of the cathepsin has been activated (Table 1). It is therefore not surprising that gold inhibition of cathepsin in 3.3 μM dithiothreitol can be reversed by addition of glutathione to produce more active enzyme than the activity of cathepsin control (point A). All that is happening is that the bound gold is captured by glutathione and the excess glutathione then activates latent cathepsin which had not been previously activated by dithiothreitol at 3.3 μM (curve EF, Figure 6a).

The plots in Figure 7(a) and (b) show a similar family of inhibition and re-activation curves for cathepsin inhibited by aurothioglucose and reactivated with the thiol of cysteine. Similar results were obtained when papain was used in place of our cathepsin preparation (results not shown, to preserve space).

The plot of $(1/v)$ against $(1/[S])$ for cathepsin in the presence of 4.3 μM dithiothreitol and 100 μM aurothioglucose exhibits a typical non-competitive irreversible inhibition (Figure 8). That is to say, it is not reversed by excess substrate (BANA) but it is reversed by excess thiol such as glutathione or cysteine (Figures 6,7).

In view of the fact that the drugs used in this study may be prescribed in rheumatoid arthritis to alleviate cartilage damage caused by proteolytic enzymes, it is important to consider the action of these enzymes on the protein constituents of cartilage. It is well known that normal cartilage contains an inhibitor of trypsin (Rifkin & Crowe, 1977) and we were not surprised to find that normal bovine cartilage contains an inhibitor of cathepsins (Figure 9). The inhibition of cathepsin by this cartilage extract was reversed by incremental additions of reduced glutathione (data not shown but similar to Figure 6). The evidence of Figure 9 suggests that normal cartilage is protected against attack by cathepsins. The evidence suggests that cartilage which is degraded in an inflammatory disease process either (a) lacks inhibitors for free enzymes such as trypsin and cathepsin, or (b) that the damage to cartilage is carried out by membrane-bound enzymes on the surface of cells which are known to be protected from the approach of protein inhibitors (see Steven *et al.*, 1982); the latter are inhibited by thio-gold drugs.

It is of interest that the inhibition of trypsin-like enzymes and cathepsins by gold, can be modified by thiol exchange reactions (see also Griffin & Steven, 1982). Both types of gold-inhibited enzymes are re-activated by thiols, the thiol extracting the gold from the active centre (or from a thiol controlling the active centre in the case of cathepsin). By contrast, thiols inhibit trypsin-like enzymes (Steven & Podrazký, 1978; Short *et al.*, 1981) whilst thiols activate cathepsins (see Table 1). The treatment of rheumatoid patients with a sequence of gold drugs and the thiol reagent, penicillamine, therefore makes little scientific sense in terms of controlling cartilage damage by free cathepsins (Mowat & Huskisson, 1975; Hella *et al.*, 1982). It should be noted that the gold concentrations employed in this study were reacted with partially pure or purified enzyme systems; it is suggested that *in vivo* much higher concentrations of gold would be used to achieve comparable effects, due to the gold exchanging with the mass of non-enzymic protein present in the inflamed joint.

The usual dosage is 30–50 mg metallic gold per week, the whole course of treatment involving 2 g of metallic gold. The actual concentration of gold in the typical inflamed joint is not known to the authors.

We conclude that there can only be proteolysis by cathepsin-like enzymes when the thiol concentration is relatively high, resulting in excess enzyme activity in relation to the naturally occurring disulphide-containing inhibitors of cathepsins present in cartilage. The control of excessive cathepsin activity may be achieved by increasing the concentration of disulphides or by increasing the concentration of heavy metal ions such as gold.

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