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THE INTERACTION OF AUROTHIOMALATE AND CYSTEINE

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Abstract—Aurothiomalate and cysteine react under appropriate conditions to produce an insoluble complex of aurocysteine. Although alkylation of the SH group of cysteine completely blocks the reaction, the organomercurials have no effect. The results are compared with, and found to be compatible with, the interaction between aurothiomalate and the cysteinyl residue of human serum albumin.

Sodium aurothiomalate is a drug used frequently in the therapy of rheumatoid arthritis. However, its pharmacology is little understood. Previous workers have shown that aurothiomalate in serum is bound mainly to albumin [1–3], the interaction involving the free thiol group of mercaptalbumin [4, 5]. However, the effects of thiol-blocking agents on the interaction between aurothiomalate and the cysteinyl residue of albumin are anomalous [5]. In an attempt to resolve these anomalies and to gain a greater understanding of the pharmacology of aurothiomalate, the present study has investigated the interaction of aurothiomalate and cysteine as a simple model for the more complex system involved in the interaction of aurothiomalate and the cysteinyl residue of albumin.

MATERIALS AND METHODS

Materials. Sodium [^{195}Au]aurothiomalate, L-[U-^{14}C]cysteine hydrochloride and *p*-chloro [^{203}Hg]mercuribenzoic acid were purchased from Radiochemical Centre, Amersham, Bucks., U.K.; 5,5′-dithiobis(2-nitrobenzoic acid) and thiomalic acid were obtained from B.D.H. Ltd., Poole, Dorset, U.K. and sodium aurothiomalate from May & Baker Ltd., Dagenham, Essex, U.K. L-cysteine, *N*-ethylmaleimide, *p*-chloromercuribenzoic acid and *p*-chloromercuriphenyl sulphonate were purchased from Sigma London Chemical Co. Ltd., Surbiton, Surrey, U.K.

Methods. Sodium aurothiomalate, sometimes spiked with sodium [^{195}Au]aurothiomalate, and L-cysteine, sometimes spiked with L-[U-^{14}C]cysteine hydrochloride, were allowed to react at room temperature in a total vol of 1.0 ml aqueous solution, and at various concentrations up to 2.0×10^{-2} M. In all experiments, degassed solutions were used and air was displaced from the reaction vessels by bubbling with oxygen-free nitrogen. After incubation for varying periods of time, any precipitate formed was spun off and assayed for gamma-emission from ^{195}Au or beta-emission from ^{14}C. The supernatant was assayed for thiols as well as radioactivity.

Thiol assay. Thiols in the supernatants were assayed by a method modified from that of Ellman [6]. Ten μl test solution was added to 2.5 ml 5.5′-dithiobis(2-nitrobenzoic acid) (5×10^{-4} M) in Tris–HCl (10^{-2} M) buffer pH 8.0 at 4°C. The increase in absorbance at 412 nm was continuously monitored until either a plateau was reached or 60 min, which ever was the shorter. The rate of reaction of Ellman's reagent with cysteine was considerably faster than that with thiomalate. At 4°C the reaction with cysteine was essentially complete by 5 sec, whereas the reaction with thiomalate did not plateau until about 20 min, with a half-time of 3 min. By differentiating between fast-reacting SH groups (cysteine) and slow-reacting SH groups (thiomalate), the concentrations of these two thiols could be easily assayed in mixtures of the two. The validity of the method was tested by measuring the rates of reaction at 4°C of various mixtures of cysteine and thiomalate (Fig. 1). The proportions calculated from these results agreed well with the actual composition (Fig. 2).

Aurothiomalate was found to interfere with the assay of thiols by Ellman's method* so that the plateau absorbance at 412 nm could not be used as a measure of thiol concentration. However no interference was found when the initial rate of reaction was

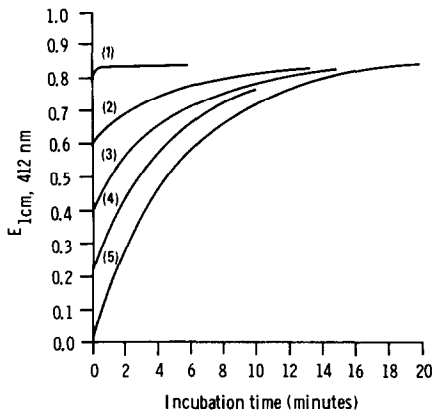

Fig. 1. Assay for thiomalate and cysteine. Trace from a continuous recording of the change in absorbance at 412 nm when Ellman's reagent was reacted with 15 μl of a mixture of cysteine and thiomalate at 4°. Cysteine (nmole): Thiomalate (nmole) = 150:0 (1), 112.5:37.5 (2), 75:75 (3), 37.5:112.5 (4) and 0:150 (5) respectively.

* C. J. Danpure. Details to be published separately.

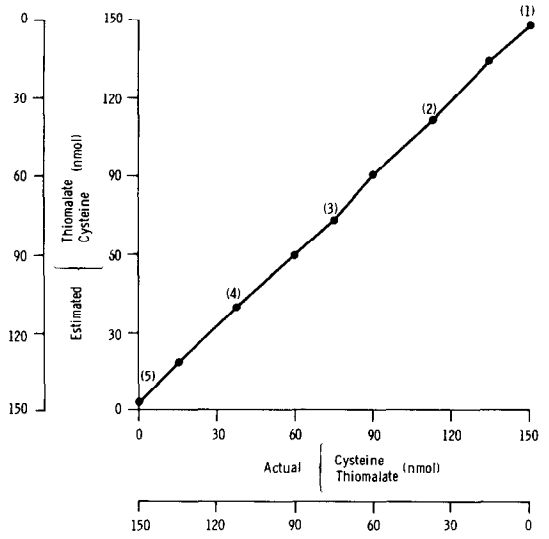

Fig. 2. Assay for thiomalate and cysteine. The concentrations of thiomalate and cysteine were measured in mixtures of the two by comparing the absorbance at 412 nm after 5 sec (cysteine) and the plateau (total SH) from Fig. 1. For details see Fig. 1.

used as a measure of the thiomalate concentration. Therefore in the experiments where aurothiomalate was present during the thiol assay (i.e. when added in excess) the intial rate assay was used. Appropriate plateau and initial rate standard curves for thiomalate and cysteine were made.

RESULTS

Aurothiomalate reacted with 10^{-2} M cysteine in aqueous solution at room temperature to form a precipitate over a period of several hours. The amount of precipitate increased with increasing concentration of aurothiomalate and became maximal when aurothiomalate reached an equimolar concentration with cysteine. The precipitate decreased on the addition of excess aurothiomalate. When a vast excess was added, no precipitate was formed. On the basis of ^{195}Au distribution, all the gold was precipitated until excess aurothiomalate was added, when all the additional gold remained in the supernatant (Fig. 3). Concomitantly with the precipitation of ^{195}Au there was a decrease in supernatant fast-reacting SH groups (cysteine) and an increase in slow-reacting SH groups (thiomalate) (Fig. 3). Addition of a molar excess of aurothiomalate caused no further change in the supernatant SH content. The loss of cysteine from the supernatant, on addition of aurothiomalate, was confirmed by the appearance of ^{14}C in the precipitate when the cysteine was previously spiked with L-[U-^{14}C]cysteine hydrochloride. Although complete precipitation of the reaction product took several hours, the initial reaction was quick in so far as no cysteine could be detected 10 sec after the admixture of equimolar concentrations of cysteine and aurothiomalate.

Addition of increasing concentrations of cysteine to 10^{-2} M aurothiomalate produced a precipitate only when the aurothiomalate was in 2-fold excess or less. Thiomalate was the only thiol present in the reaction mixture or supernatant, after the precipitate was removed, and increased in proportion to the amount of cysteine added (Fig. 4). When cysteine was added in excess no further increase in thiomalate was found. The excess cysteine was totally confined to the supernatant.

The interaction between 10^{-2} M cysteine and 10^{-2} M aurothiomalate was pH-dependent (Fig. 5). As the pH was increased from 5.0 to 9.1 the rate of formation of the precipitate was decreased, so that even when the reaction was left for 24 hr at pH 9.1 only 70 per cent of the ^{195}Au was precipitated. However no cysteine was detectable in the supernatant even at pH 9.1.

Preincubation of 10^{-2} M cysteine with 10^{-2} M *N*-ethylmaleimide completely prevented the formation of a precipitate on addition of 10^{-2} M aurothiomalate (Table 1). On the other hand neither 10^{-2} M *p*-chloromercuribenzoate or 10^{-2} M *p*-chloromercuriphenyl sulphonate inhibited the precipitation of ^{195}Au (Table 1). In fact the insoluble organomercury–cysteine complexes at pH 7.0 could be redissolved by aurothiomalate and replaced by ^{195}Au-containing

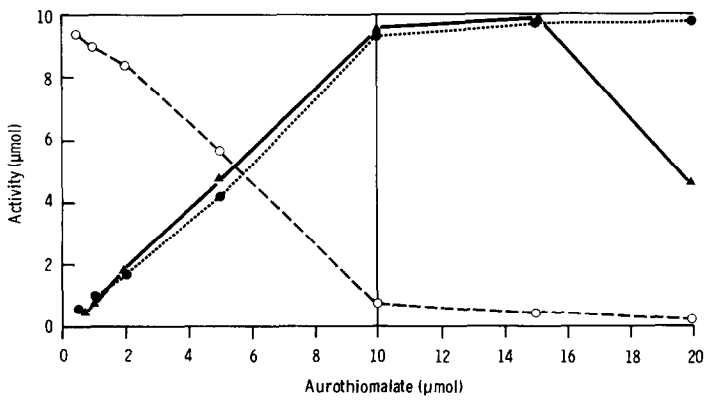

Fig. 3. Reaction between aurothiomalate and cysteine. Varying amounts of ^{195}Au-labelled aurothiomalate were reacted with 10 μmole cysteine in 1 ml total vol. ▲——▲ μmole ^{195}Au precipitated. ○--○ μmole cysteine (fast-reacting SH) in supernatant. ●----● μmole thiomalate (slow-reacting SH) in supernatant.

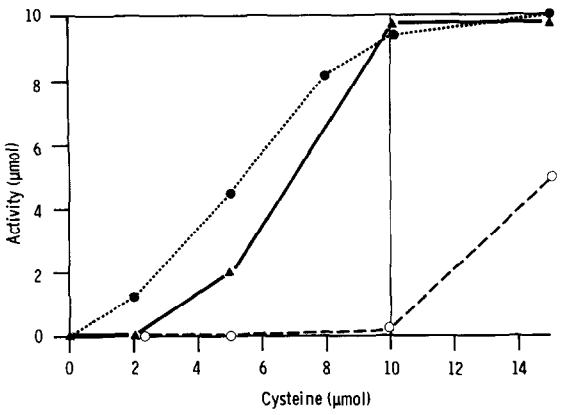

Figure 4. Reaction between aurothiomalate and cysteine. Varying amounts of cysteine were reacted with 10 μmole ^{195}Au-labelled aurothiomalate. ▲——▲ μmole ^{195}Au precipitated. O—O μmole cysteine in supernatant. ●——● μmole thiomalate in supernatant.

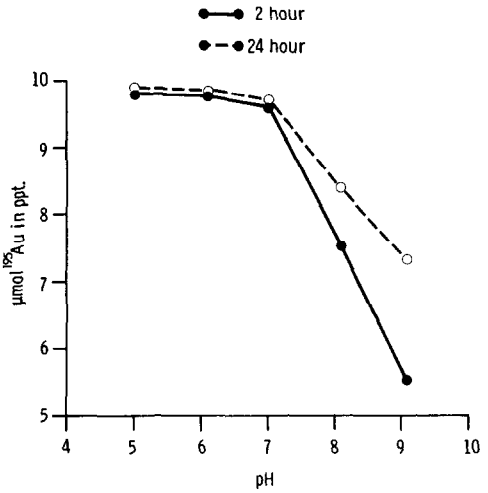

Fig. 5. Effect of pH on ^{195}Au precipitation. 10 μmole ^{195}Au-labelled aurothiomalate were reacted with 10 μmole cysteine in 0.1 M Tris (pH adjusted with HCl). The mixture was left for 2 hr (●——●) or 24 hr (O----O) before the precipitate was spun off.

precipitate. When 10^{-2} M cysteine and 10^{-2} M *p*-chloromercuribenzoate were reacted in 10^{-1} M NaOH and then 10^{-2} M aurothiomalate added before neutralisation, precipitation of the cysteine–organomercury complex on neutralisation was completely prevented.

Experiments using *p*-chloro [^{203}Hg]mercuribenzoate showed that when ^{195}Au was precipitated in the presence of cysteine and *p*-chloromercuribenzoate, the ^{203}Hg remained in the supernatant. In none of the experiments using thiol-blocking agents were any thiols detectable in the supernatants or otherwise.

DISCUSSION

The preceding observations on the interaction of cysteine and aurothiomalate, in the absence of thiol-blocking agents, are compatible with the reaction shown in Fig. 6, in which there is an exchange of gold from thiomalate to cysteine to produce an insoluble complex of aurocysteine. Although the overall reaction is slow, especially at high pH, as shown by the delay in ^{195}Au precipitation, the initial reaction is fast. On the basis of this and the pH data, an intermediate step may be postulated involving a molecule such as $CH_2(COOH)CH(COOH)S \cdot Au^- \cdot S \cdot CH_2 \cdot CH(COOH)NH_2$. Formation of the intermediate might be expected to be promoted by increasing pH, whereas formation of aurocysteine from the intermediate would be inhibited. Delayed precipitation caused by high pH may be taken as an indication of the relative higher solubility of the unstable intermediate compared to aurocysteine. Alternatively, as it is known that aurothiomalate polymerises in aqueous solution,* the precipitate could be due to the formation of insoluble polymeric aurocysteine from the otherwise soluble monomer.

When the interaction of aurothiomalate and mercaptalbumin was studied under identical conditions to the present experiments, the release of thiomalate could not be detected.† This is in agreement with the

* A. Isab, P. J. Sadler, C. J. Danpure, D. A. Fyfe and P. A. Charlwood. Submitted for publication.

† C. J. Danpure. Unpublished observations.

Table 1. Effect of SH-blocking agents on the interaction between aurothiomalate and cysteine

SH-blocking agent	Concentration	Gold precipitated (μmole)	
NEM	10^{-2} M	0	0
	5×10^{-3} M	2.46	2.61
PCMPS	10^{-2} M	9.75	9.69
PCMB	10^{-2} M	9.12	9.08*
		6.58†	
Control		9.81	9.39

The SH-blocking agents *N*-ethylmaleimide (NEM), *p*-chloromecuriphenyl sulphonate (PCMPS) and *p*-chloromercuribenzoate (PCMB) were incubated for 10 min at room temperature with 10^{-2} M cysteine. The mixture was then incubated for 2 hr with 10^{-2} M aurothiomalate. The precipitate formed was spun off and its content of ^{195}Au determined. The total reaction volume was 1 ml (i.e. 10 μmole cysteine and 10 μmole aurothiomalate).

* PCMB, not being water-soluble at this concentration, was dissolved in 10^{-1} M NaOH. Aurothiomalate was added to the PCMB–cysteine reaction mixture before neutralisation. The total reaction mixture was then neutralised with 10^{-1} M HCl.

† PCMB in 10^{-1} M NaOH was reacted with cysteine and then precipitated by neutralisation. Aurothiomalate was then added.

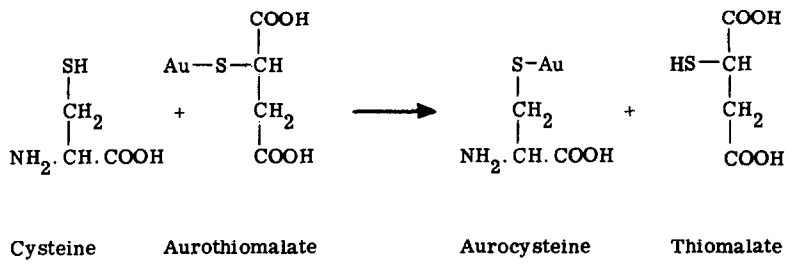

Fig. 6. Suggested reaction between aurothiomalate and cysteine.

findings of Gerber [4]. Therefore it is possible that the postulated unstable intermediate in the reaction between aurothiomalate and cysteine could be stable in the environment of the cysteinyl residue of albumin.

The inhibitory effect of *N*-ethylmaleimide is in accord with the reaction in Fig. 6. If the reaction involves the —S^- moiety of cysteine, then alkylation would be expected to block this ionisation and therefore the interaction. On the other hand the lack of inhibition by the organomercurials is somewhat difficult to interpret. In fact the gold from aurothiomalate seems able to displace the mercurial which is already bound to the thiol of cysteine. The effect of thiol-blocking agents on the reaction between aurothiomalate and cysteine is especially interesting in relation to the effect of the same agents on the interaction between aurothiomalate and the cysteinyl residue of human serum albumin. In the case of the latter, alkylating agents such as *N*-ethylmaleimide, iodoacetate and iodoacetamide inhibit the interaction, whereas *p*-chloromercuribenzoate does not [5]. In addition evidence suggests that both the organomercurial and aurothiomalate are bound to the same cysteinyl residue simultaneously.* This postulated organomercurial–aurothiomalate–cysteine complex (Fig. 7) may also have been formed in the present studies, but presumably its stability is somewhat less than the equivalent complex with the cysteinyl residue of albumin, resulting in the precipitation of aurocysteine with the organomercurial–thiomalate complex remaining in solution.

* C. J. Danpure. Unpublished observations.

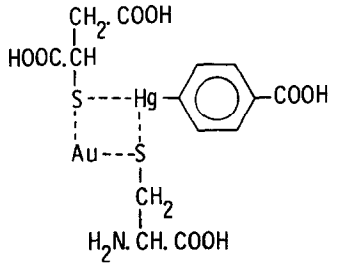

Fig. 7. Suggested complex of aurothiomalate, cysteine and *p*-chloromercuribenzoate.

The present studies have several interesting implications with respect to the pharmacology of the antiarthritic drug aurothiomalate. Firstly, they provide an explanation for some of the anomalous results found by Danpure [5] on the interaction between aurothiomalate and albumin in human serum. Secondly, they indicate that, although bound strongly to albumin in the plasma [5], the majority of intracellular non-protein-bound gold would be expected to exist as complexes of aurocysteine or perhaps gamma-glutamyl-aurocysteinyl-glycine, due to the relatively high intracellular concentrations of cysteine and glutathione.

Gold, in the form of aurothiomalate, has been shown to inhibit many enzymes [7–11]. In at least some cases the inhibition probably involves interaction of the drug with a susceptable enzyme SH group [10, 11]. Cysteine may be very important in the translocation of gold from one intracellular site to another. Whether only the gold moiety of aurocysteine binds to the intracellular protein SH groups or whether the whole molecule forms complexes, similar to those between aurothiomalate and albumin, remains to be discovered. In addition, it would be fruitful to investigate the form in which gold is excreted in patients undergoing chrysotherapy for rheumatoid arthritis. From the foregoing results it might be expected that complexes of aurocysteine or *S*-auroglutathione might be the predominant gold metabolites in the urine.

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