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Gold(III)-induced oxidation of glycine†

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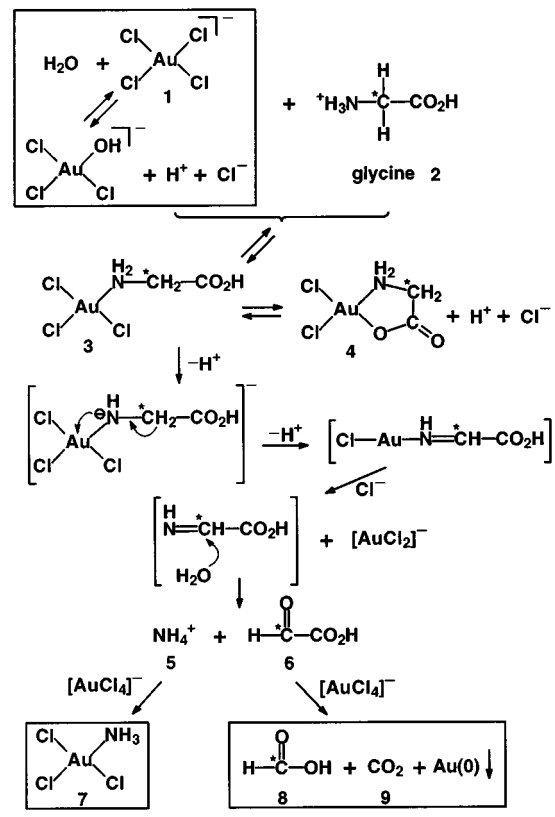
NMR investigations of isotopically-labelled glycine show that Au^{III} induces deamination and subsequent decarboxylation of the amino acid with formation of glyoxylic acid, NH_4^+, formic acid, CO_2 and metallic gold.

Several injectable 1:1 Au(I)–thiolate complexes and an orally-active Au(I)–phosphine complex are used clinically for the treatment of difficult cases of rheumatoid arthritis.[1] Their clinical application is limited, however, owing to severe host toxicity such as kidney damage and blood disorders. The molecular basis for these toxic side-effects of chrysotherapy is poorly understood[2] but recently Gleichmann *et al.*[3] have demonstrated that the oxidation of Au(I) to Au(III) *in vivo* may be responsible for some of the observed toxicity. In inflammatory situations, strong oxidants such as hypochlorite (ClO^-) and hydrogen peroxide (H_2O_2) are potentially available *in vivo* and can oxidise Au(I)–thiolates and Au(I)–phosphines to Au(III).[4] In view of this, and the antitumour activity of some Au(III) complexes,[5] it is important to understand the chemistry of Au(III)–biomolecule interactions. Surprisingly, there are only a few reports of reactions of Au(III) with amino acids, and these have focused on the sulfur-containing amino acids, namely cyst(e)ine and methionine.[6,7,8] Au(III) can cleave the disulfide bond of cystine to give the sulfonic acid[6,7] and can oxidise the sulfur of methionine stereospecifically to the sulfoxide.[8] We report here the first elucidation of the pathway of Au(III)-induced oxidation of the amino acid glycine. The identification of reaction intermediates and final products was made possible by the use of ^{13}C and ^{15}N isotopic labelling and multinuclear NMR spectroscopy.‡ The proposed stepwise oxidation mechanism provides a basis for obtaining further insights into the mechanism of gold drug toxicity.

Reactions of $[Au^{III}Cl_4]^-$ **1** with [^{15}N]glycine [^{15}N] **2** (1:2, starting pH 2.44)§ were monitored by 1D ^1H NMR (data acquired at intervals over a 12 h period) and by 2D [^1H, ^{15}N] HSQC-TOCSY NMR spectroscopy (acquired after 8 h of reaction).[9] On the basis of these results, a mechanism for the reaction of Au(III) with glycine can be proposed (Scheme 1). Initial coordination of Gly to Au(III) *via* the amino group gives $[AuCl_3(Gly\text{-}N)]$ **3** [δ(^1H): CH_2 3.85; δ($^1H/^{15}N$): $^{15}NH_2$ 6.88/2.6], which can undergo chelation to form $[AuCl_2(Gly\text{-}N,O)]$ **4** [δ(^1H): CH_2 3.75; δ($^1H/^{15}N$): $^{15}NH_2$ 6.75/−9.2]. The identification of the O,N-chelate **4** is based on chemical shift arguments compared with analogous Pt(II)–am(m)ine systems.[10] For example, δ(^{15}N) of S,N-chelated Met in [Pt(Hdien-*N*,*N*)(^{15}N-Met-*S*,*N*)] is *ca.* 10 ppm to lower frequency compared to monodentate Met in [Pt(dien)(^{15}N-Met-*N*)].[11] In our case, δ(^{15}N) for $^{15}NH_2$ of coordinated Gly differs by 12 ppm between the monodentate and O,N-chelate Au complexes.

Once formed, the chelate **4** appears to be unreactive. A two-electron transfer from Gly to Au(III) in **3** gives rise to a Au(I)–imine intermediate. Imines with a proton on N are seldom stable and are known to undergo fast hydrolysis to the corresponding aldehyde and NH_4^+.[12] An imine ligand on Au(I) would also be readily displaced by Cl^- and would hydrolyse to give glyoxylic acid **6** [^1H: δ 5.31(s)] with concomitant formation of NH_4^+ **5** [δ 7.09 (d, $^1J_{HN}$ 73 Hz)] and Au(0). The identity of **6** was revealed by further experiments. This species was stable over the pH range 1.45–12.3 and a pH titration gave an associated pK_a of 3.42. ^{13}C–{^1H} DEPT NMR data (CH group, ^{13}C: δ 89.4) suggested assignment to the aldehyde group of glyoxylic acid (lit.,[13] pK_a 3.46 at 298 K, I = 0). NMR spectra of glyoxylic acid recorded at the same pH (^1H: δ 5.36; ^{13}C: δ 173.6 and 89.7) further supported this assignment.

Both the Au(III)– and Au(I)–imine intermediates are too unstable to be observed by NMR during the reactions. The NH_4^+ produced reacts with $[AuCl_4]^-$ to give Au(III)-ammine adducts such as $[AuCl_3(NH_3)]$ **7**. This species was identified by reaction of **1** with $^{15}NH_4Cl$ (1:2, pH 2.98). It gave rise to a major HSQC cross-peak ($^1H/^{15}N$: δ 5.93/−12.9), which exhibited $^1H/^2H$ isotope shift effects[14–16] and chemical shifts very similar to those of **7**. Further substitution of Cl^- ligands by NH_3 is known to be difficult to achieve, and formation of $[Au(NH_3)_4]^{3+}$ requires the use of a large excess (super-saturated solutions) of NH_4^+ together with NH_3 gas.[17] Oxidative decarboxylation of glyoxylic acid *via* further reaction with Au(III) gives rise to formic acid **8**, carbon dioxide **9** and Au(0). This was confirmed by two separate reactions of **1** with glyoxylic acid to give formic acid **8** (^1H: δ 8.20, pH 1.52) and with 2-[^{13}C]Gly **2** to give [^{13}C]**8** (^1H: δ 8.20, $^1J_{CH}$ 218 Hz, ^{13}C: δ 166.4). Therefore formic acid is formed from the aldehyde



Scheme 1

† Reactions of $[Au^{III}Cl_4]^-$ **1** with [^{15}N]glycine as monitored by 1D ^1H NMR and 2D [^1H, ^{15}N] HSQC-TOCSY NMR can be viewed in electronic form, see: http://www.rsc.org/suppdata/cc/1999/1359/.

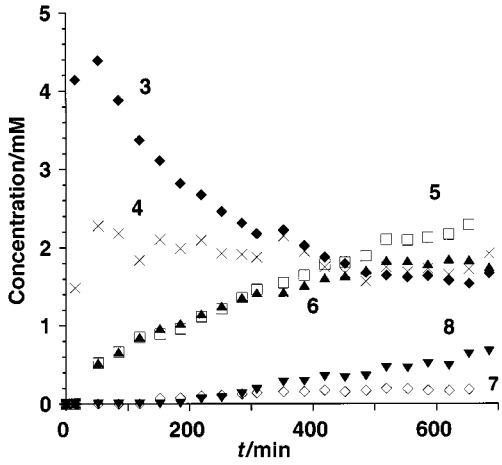


Fig. 1 Concentration *vs.* time profiles for species observed during the reaction of $[AuCl_4]^-$ **1** (15 mM) with [^{15}N]glycine ([^{15}N]**2**) (30 mM) at pH 2.87, 298 K. Concentrations were obtained from peak integrals using TSP as a standard. The profile for free Gly (*ca*. 70% unreacted after 10 h) is not shown.

group of glyoxylic acid. Formic acid can be further oxidised by Au(III) to give Au(0) and carbon dioxide,[18] but this step was not observed in the current study using 2-[^{13}C]Gly, probably due to the slow rate of the reaction at low pH.[18] The variations in concentration of the species observed by NMR during the reaction of **1** with [^{15}N]**2** with time are shown in Fig 1.

Reactions of **1** with [^{15}N]**2** at higher molar ratios gave rise to similar NMR spectra but higher product yields. For example, in the presence of a 10-fold excess of **1**, all [^{15}N]**2** reacted within 14 h. The ^{1}H NMR spectrum of a solution containing **1** and [^{15}N]**2** in a 1 : 2 molar ratio, pH 7.14, recorded after 14 h showed peaks for the CH_2 signal of [^{15}N]**2** at δ 3.56, together with singlets at δ 5.07 (assigned to the CH of glyoxylate) and at δ 8.46 (assigned to formate). The course of the reaction between **1** and **2** at physiological pH§ therefore appears to be similar to that at low pH. However, rapid proton exchange at pH 7[19] made it impossible to carry out a detailed ^{1}H,^{15}N NMR study.

The mechanism in Scheme 1 predicts that the products from the reaction§ of DL-alanine with $[AuCl_4]^-$ should be NH_4^+, pyruvic acid and acetylaldehyde. This was verified by ^{1}H and ^{13}C NMR spectroscopy, although the reaction was slower than that of Gly.

A parallel mechanism has recently been proposed for Fe(III)-assisted oxidative cleavage of a C–N bond.[20] Fe(III)-bound bis(2-pyridylmethyl)aminoacetate (BPG) is transformed into Fe(III)–bis(2-pyridylmethyl)amine *via* an Fe(III)–imine intermediate which undergoes hydrolysis with release of glyoxylic acid. Photodecomposition of complexes such as $[Co^{III}(Gly_3)]$ and $[Co^{III}(Ala_3)]$ have been reported to give rise to dipeptide amides, together with CO_2 and aldehydes.[21] Photoredox transformation of an Fe(III) complex with *N*-(phosphonomethyl)glycine has also been reported, and when irradiated with UV light the Gly derivative decomposes to give formaldehyde, ammonia and a new species containing phosphorus.[22] The reactions with Au(III) studied here were not activated by light.¶

Gold(III)-induced oxidation of Gly and related amino acids may be important in relation to the severe toxicity of gold drugs. It is possible that Au(III) can also deaminate the amino terminus of peptides and proteins. For example, during the reactions of Au(III) with tripeptides GGH and GGG, the formation of colloidal gold is observed together with other unidentified species.[23] Au(III) modification of peptides is likely to influence MHC peptide presentation and T cell recognition systems, allowing gold to have a major influence on the immune system.

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Notes and references

‡ NMR spectra were recorded at 298 K on a Brüker DMX 500 NMR spectrometer (^{1}H 500.13 MHz, ^{15}N 50.7 MHz), using the procedures described previously.[24] The 1D ^{13}C-{^{1}H} and 2D [^{1}H, ^{13}C] HETCOR NMR spectra were acquired using standard procedures.

§ NMR samples were prepared in 90% H_2O–10% D_2O (600 μl). Fresh solutions of $Na[AuCl_4]\cdot 2H_2O$ (30 mM) and Gly (60 mM) were prepared separately and then mixed in the following molar ratios (Au : Gly) at 298 K: 1 : 1, 2 : 1, 3 : 1, 5 : 1, 8 : 1, 10 : 1, 1 : 2 and 1 : 4. Fresh solutions of DL-Ala and $[AuCl_4]^-$ were mixed in a 2 : 1 molar ratio at 298 K. The concentration of $[AuCl_4]^-$ was 15 mM for all reactions. For reactions at neutral pH, the pH values of the solutions of $[AuCl_4]^-$ and Gly were adjusted separately prior to mixing, and remeasured immediately after mixing.

¶ The same products were observed for the reactions conducted under N_2 and in the absence of light.

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