Studies on the interaction of gold(I) thiomalate ('myochrysine') with 2-thiouracil in aqueous solution followed by ^{13}C NMR spectroscopy

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Abstract

The interaction of gold(I) thiomalate (Autm) ('Myochrysine') with 2-thiouracil (2-TUH) has been studied in aqueous solution by ^{13}C NMR spectroscopy. 2-TUH is in slow exchange at a 1:1 ratio of Autm:2-TU, but at higher ratios, fast exchange occurs with the tm^- of Autm. No free tm^- ^{13}C NMR resonances were observed. When L-cysteine and CN^- were added sequentially to the 1:3 ratio of Autm:2-TU solution, the cysteine did not remove 2-TUH from the complexed 2-TU–Autm, however, CN^- bound strongly to Au(I) forming $Au(CN)_2^-$ and released 2-TUH and tm^- as free ligands.

Introduction

2-Thiouracil (2-TUH) has been identified in t-RNA [1–3] and is known to possess important biological activity, e.g. mutagenic and anticancer [4, 5]. Despite the importance of this ligand, studies on its interaction with gold(I) thiolate drugs have not been reported. The complexation of this ligand with many transition metal ions has been reported, including the X-ray crystal determination of $Et_3PAu(2\text{-TU})$, $[Au(dppe)_2][(2\text{-TU})\text{-}(2\text{-TUH})]$, $Ph_3PAu(2\text{-TU})$ [6, 7], $Cu(2\text{-TUH})_2ClDMF$ [8] and $[Pt(2\text{-TU})_2I]_2$ [9] (where 2-TU is deprotonated and 2-TUH is protonated 2-thiouracil). In this paper we report on ^{13}C NMR studies of the interaction of 2-TUH with gold(I) thiomalate (Autm) and compare the data with another series of thione ligands such as imidazolidine-2-thione (Imt), ergothionine and 1,3-diazinane-2-thione (Diaz). 2-Thiouracil is known to exist as thione in the solid state [10], and in solution it exists in three forms [6, 11]. Imt and Diaz also exist as two tautomers [12, 13] (see Scheme 1).

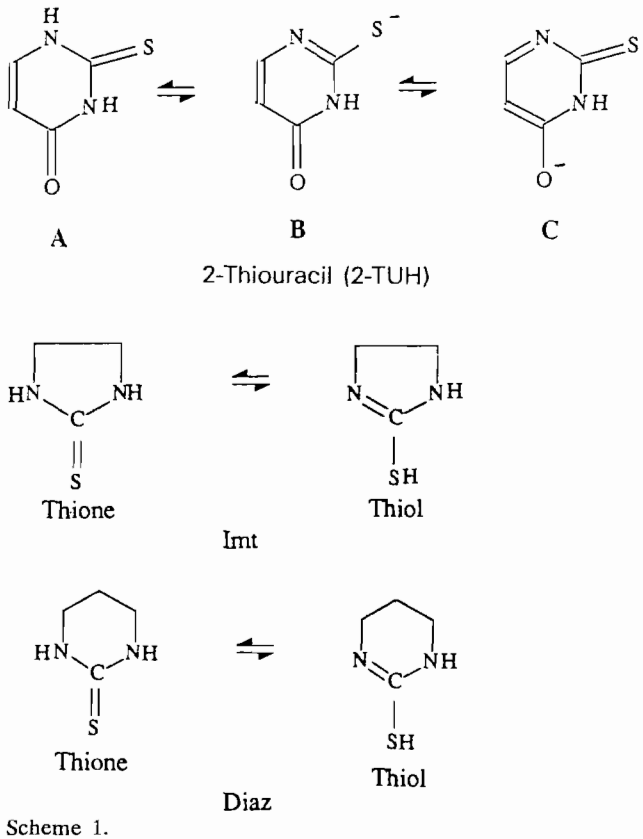
In this paper we show that 2-TUH binds to Autm more strongly than Imt and Diaz as observed by ^{13}C NMR spectroscopy.

Experimental

Chemicals

Gold(I) thiomalate (Autm) was obtained from ICN K and K Laboratories, Plainview, New York. The Autm was analyzed as Autm ($\frac{1}{8}$glycerol·H_2O) [14–16].

L-Cysteine (cys), thiomalate (tmH), KCN, 99.7% D_2O, 40% NaOD in D_2O and 35% DCl in D_2O were purchased from Fluka Chemical Company and 2-thiouracil (2-TUH) was purchased from Aldrich Chemical Company.



Scheme 1.

0020-1693/93/$6.00

NMR measurements

^{13}C NMR spectra were measured at 50.3 MHz on a Varian XL-200 spectrometer operating in the pulsed Fourier transform mode. The ^{13}C NMR measurements were made with coherent off-resonance ^{1}H decoupling or with broad-band ^{1}H decoupling. ^{13}C NMR chemical shifts were measured relative to the CH_2 resonance of internal glycerol g_2 which occurs at 63.33 ppm from $SiMe_4$.

pH measurements

All pH measurements were made at 22 °C with a Fisher Accumet pH meter, model 620 equipped with Fisher microprobe combination pH electrode. The pH* was used to indicate the actual meter reading for D_2O solutions with no correction for deuterium isotope effects [17].

Results

Figure 1(A) shows the ^{13}C NMR spectrum of Autm in D_2O solution at pH* 7.40. When 0.5 equiv. of 2-TUH was added as a solid to the 0.20 M Autm solution, it did not dissolve until the pH* was increased to 10.60 by adding NaOD. The solution of Autm was yellow; it became very pale yellow after the addition of 2-TUH. None of the 2-TUH resonances appeared and the Autm resonances became broad as shown in Fig. 1(B). This broadening may be due to the slow exchange between tm^- and 2-TUH with gold(I).

When 1 equiv. (0.2 M total) of 2-TUH was added as a solid to the above solution, the pale yellow solution became colorless. As shown in Fig. 1(C), the ^{13}C NMR resonances of Autm remained broad. The b_2 resonance of Autm was shifted from 47.81 to 48.60 ppm and the b_1 resonance shifted from 47.81 to 43.69 ppm. The most shifted resonance for the 2-TUH was >C=S and C-6 (see Table 1).

When second and third equivalents of 2-TUH were added to the 1:1 ratio of the Autm:2-TU solution the resonances of 2-TUH and Autm became very sharp and all the 2-TUH resonances were shifted toward the chemical shifts of the free 2-TUH because of excess 2-TU per gold(I), i.e. 3:1 2-TU:Au(I). Figure 1(E) shows the ^{13}C NMR spectrum of 2-TUH at pH* 10.50.

Figure 2(A) shows the ^{13}C NMR spectrum of Autm:2-TU at a 1:3 ratio. Note that only very small thiomalate disulfide resonances were observed at 54.35 and 41.19 ppm. To see whether L-cysteine (cys) complexed more strongly than 2-TU, 1 equiv. of cys was added as a solid to the solution at a ratio of 1:3 of Autm:2-TU and the pH* was decreased from 10.60 to 8.20. The cys resonances remained almost unshifted. When a second equivalent of cys was added, the pH* decreased to 7.10 and the solution remained colorless. The b_1 resonance of Autm became broad due to the slow exchange with cys, and a small shift was observed for

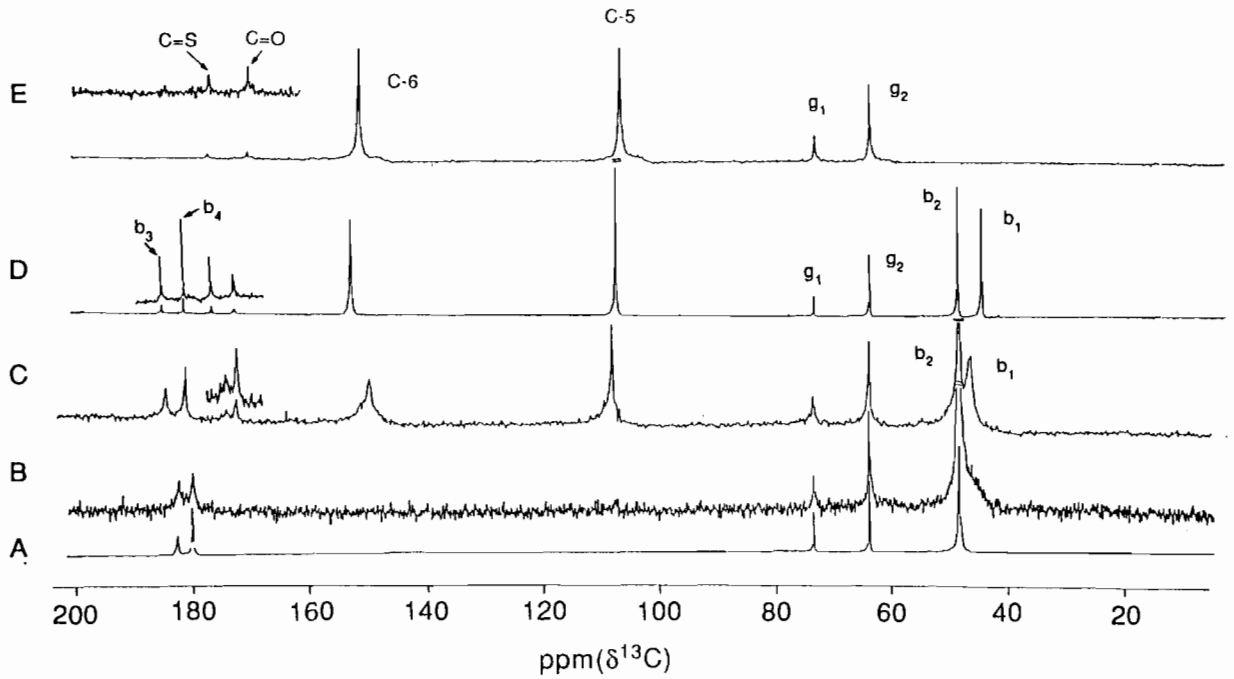


Fig. 1. The 50 MHz ^{1}H noise decoupled ^{13}C NMR spectra of Autm:2-thiouracil at various molar ratios: (A) 0.20:0, (B) 0.20:0.10, (C) 0.20:0.20, (D) 0.20:0.40, (E) 0.00:0.10. g_1 and g_2 are the –CH and $-CH_2$ resonances of glycerol. (For resonance assignments see Fig. 2).

TABLE 1. ^{13}C NMR chemical shifts of Autm:2-TU[a] at various molar ratios. The values are taken from Fig. 1; some of the spectra are not shown in Fig. 1

Spectrum	pH*	Autm:2-TU	Autm				2-TUH			
			b_2	b_1	b_3	b_4	C=S	C=O	C-5	C-6
A	7.40	1:0	47.81	47.81	181.98	179.56				
B	10.60	1:0.5	47.81[b]	47.81[b]	181.45	179.40				
C	10.50	1:1	48.02	46.66	184.04	180.68	173.54	171.59	107.54	149.12
D	10.50	1:2	48.00	43.96	185.00	181.12	176.37	172.50	106.90	152.48
	10.60	1:3	48.60	43.69	185.16	181.18	177.23	172.50	106.57	152.63
E	10.50	0:1					177.17	170.34	106.25	151.04
	DMSO	0:1					175.90	160.90	105.20	142.00

[a]2-TUH resonances are assigned as in ref. 18. [b]Very broad resonances.

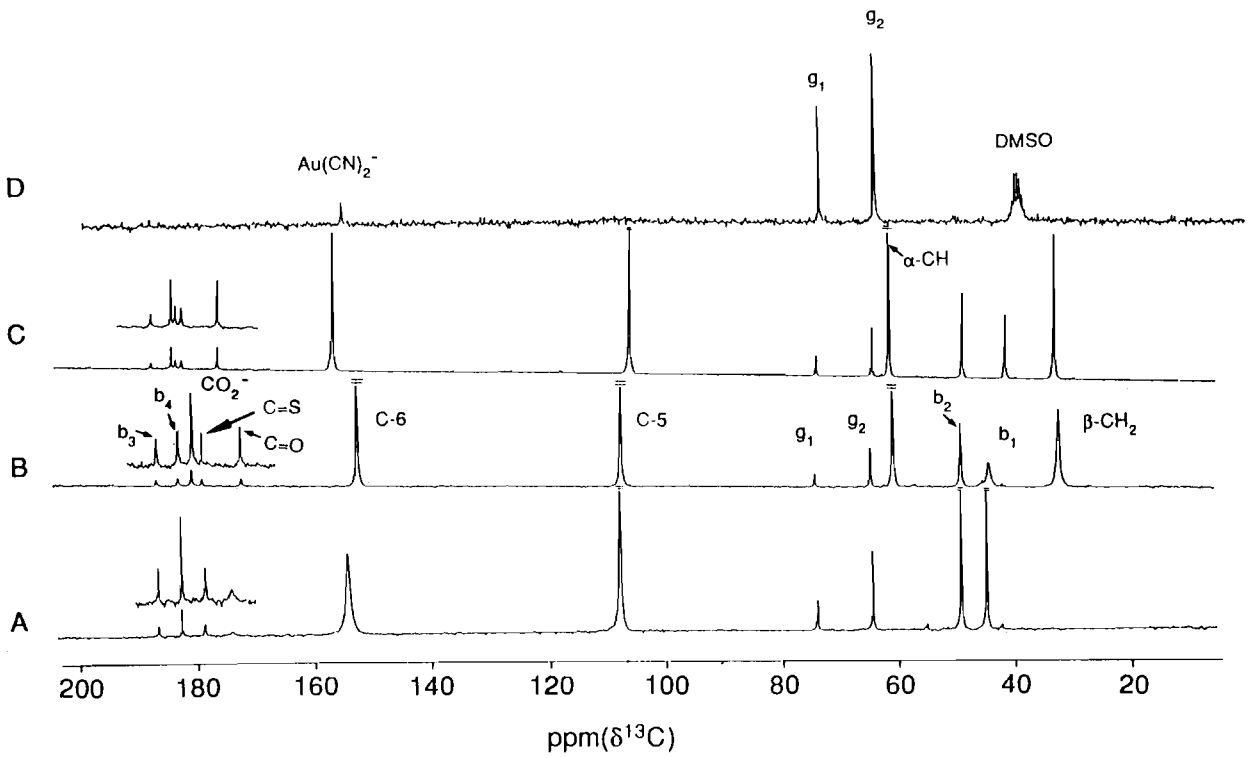
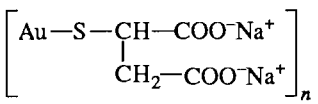
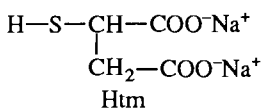


Fig. 2. The 50 MHz ^1H noise decoupled ^{13}C NMR spectra of Autm:2-TU:L-cysteine:CN^- at various molar ratios: (A) 0.20:0.60:0.00:0.00, (B) 0.20:0.60:0.40:0.00, (C) 0.20:0.60:0.40:0.40, (D) $Au(CN)_2^-$ in DMSO-d_6. Resonance assignments: the resonance assignments of L-cysteine (cys), gold(I) thiomalate (Autm) and thiomalate (Htm) are given as follows



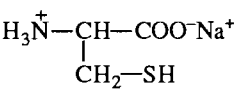
Autm

$-CH = b_1$; $-CH_2 = b_2$; $-CH{-}CO_2^- = b_3$; $-CH_2CO_2^- = b_4$.



Htm

$-CH = f_1$; $-CH_2 = f_2$; $-CH{-}CO_2^- = f_3$; $-CH_2{-}CO_2^- = f_4$,



cys

Reference

$-CH = g_1$ and $-CH_2 = g_2$ for glycerol

TABLE 2. ^{13}C NMR chemical shifts (in ppm) of Autm:2-TU:Cysteine:CN^- at various molar ratios. The values are taken from Fig. 2; some of the spectra are not shown in Fig. 2

Spectrum	pH*	Autm:2-TU: Cys:CN^-	CN^-	Autm				2-TUH				Cys		
				b_2	b_1	b_3	b_4	C=S	C=0	C–5	C–6	α–CH	β–CH_2	–CO_2^-
A	10.60	1:3:0:0		48.60	43.69	185.16	181.98	177.23	172.50	106.57	152.63			
	8.20	1:3:1:0		48.00	43.57	185.03	181.16	177.10	170.33	106.24	150.67	59.39	31.39	181.16
B	7.10	1:3:2:0		47.85	42.95	185.04	181.28	177.10	170.40	106.24	150.63	59.51	31.06	178.96
	7:0	1:3:2:1	153.84	47.85	42.10	185.31	181.55	177.11	170.50	106.27	150.61	59.58	30.70	179.54
C	11:00[a]	1:3:2:2	155.03[b]	47.85	40.51	186.20	181.98	180.93	174.78	105.13	155.03[a]	60.51	32.22	182.77
D	DMSO[c]		153.72											
	7.00	0:0:1:0										59.92	30.66	180.62
	8.20	0:0:1:0										60.55	31.84	182.38
	DMSO	$KAu(CN)_2$	153.67											

[a]Solution was filtered and ran the spectrum. [b]Coalesced at this concentration. [c]Precipitate dissolved in DMSO and the spectrum recorded.

the –CH_2 resonance of cys (see Table 2). However, when 1 equiv. of CN^- was added to the mixture of Autm:2-TU:cys at a 1:3:2 ratio, the b_1 resonance shifted further down field and the $Au(CN)_2^-$ resonance appeared at 153.84 ppm (not shown in the Fig.). When a second equivalent of CN^- was added as a solid, it gave a white precipitate which did not dissolve even at high pH* (i.e. about 11). The solution was filtered and gave the spectra as shown in Fig. 2(C). Note, the 153.84 resonance was not observed. The precipitate was dissolved in DMSO-d_6 and gave the spectrum as shown in Fig. 2(D). One resonance was observed at 153.72 ppm. We dissolved pure $KAu(CN)_2$ in DMSO-d_6 and recorded the spectrum and a resonance appeared at 153.67 ppm which indicates that the precipitate at the 1:3:2:2 ratio of Autm:2-TU:cys:CN^- was the $Au(CN)_2^-$ complex.

Discussion

Gold(I) is found in AuS_2 coordination environments for the various types of gold(I) thiolate complexes [19–23]. The addition of 2 equiv. of thiols and selenol usually ejects thiomalate as a free ligand and the bis complex of $Au(SR)_2^-$ or $Au(SeR)_2^-$ [14–16, 24]. However, when thione such as ergothionine, Imt or Diaz is added to the Autm, usually a ternary complex is formed of the type $>C=S+1/n\ (Autm)_n \rightarrow >C=S–Autm$ without ejecting tm^- as a free ligand [25–27]. This observation suggests that thione binds to Autm rather weakly. In the present study when 2-TUH was added to the Autm solution, no free thiomalate was observed which means 2-TUH is not binding very strongly and releasing free thiomalate but it is in the slow exchange with Autm upto a 1:1 ratio of Autm:2-TU and it is in the fast exchange beyond the 1:1 ratio of Autm:2-TU. As shown in Table 3, where 1:1 ratios of Autm:thione ligands are compared, it is clear that 2-TUH binds more strongly to Autm than other thiones reported in the literature.

TABLE 3. Difference in the ^{13}C NMR chemical shifts (Δ) in ppm of the C-2 resonance of thione at a 1:1 ratio of Autm:L

L	pH*	Δ	Reference
2-Thiouracil	10.50	3.63	this work
Ergothionine	7.40	2.99	25
Imidazolidine-2-thione	7.40	2.55	27
1,3-Diazinane-2-thione	7.40	2.05	27

Harker *et al.* [6] studied the complexation of Et_3PAuCl and Ph_3PAuCl with 2-TUH; they found that although the 2-TUH ligand was in the thione form, after complexing with Et_3PAu^+ and Ph_3PAu^+, it changed to the thiolate form. This indicates that the B tautomer (as shown in Scheme 1) is the predominant binding site for 2-TUH. This observation also explains why the C-6 resonance shifted more than the C-5 one after complexation. The C-6 resonance in the free state has an –NH–C=S bond, which changed to the –N=C–S– form, the thione bond became thiolate, which influenced the C-6 carbon more than the C-5 carbon. The C form tautomer of 2-TUH may be negligible where the negative charge is on the oxygen. Oxygen is a hard base, which does not complex with gold(I) which is a soft acid. The neutral ligand (2-TUH) is known to coordinate as a thione in the Cu(I) complex $Cu(2\text{-}TUH)_2ClDMF$ [8].

In previous studies [14, 28] it was reported that when L-cysteine was added to an Autm solution at a 1:0.5 ratio of Autm:cys, the Au(I)–cys complex precipitated and it was only soluble at pH* 11.30. The chemical shift difference between the free and bound β-CH_2 resonance of cys was 3.2 ppm. At a 1:1 ratio the shift was 1.8 ppm and the pH* was reduced to 9.50. At

pH*·7.00 Au–cys forms a polymer which is not soluble at the neutral pH [14, 28]. This observation suggests that cys binds to Autm releasing tm^- as a free ligand. However, in the presence of 2-TUH, the cys was unable to bind to gold(I). When excess cys was added to the mixture, no significant chemical shifts of the cys resonances were observed. Although when cys was added to Autm itself, it bound to Autm forming the $Au(cys)_2^-$ complex and releasing tm^- as a free ligand [14]. The conditional equilibrium formation constant for $Au(cys)_2^-$ was reported to be $K = 3 \times 10^{28}$ at pH 7.00 [29].

The cyanide ion which has a formation constant for $Au(CN)_2^-$ of 4×10^{28} or 10^{39} [30,31] was able to liberate all thione/thiolate ligands forming the $Au(CN)_2^-$ complex as follows [32–34]

$$2\text{-TU–Autm} + 2CN^- \leftrightharpoons Au(CN)_2^- + tm^- + 2\text{-TU}$$

In this study it is shown that 2-TUH which itself is a biologically active nuclobase ligand can form a strong complex with Autm. 2-TUH exists in the thione $\leftrightharpoons$ thiol tautomer; the thiol form predominates when it is complexed to gold(I). The estimated formation constant for $Au(2\text{-TU})_2^-$ would be similar or larger than that of the $Au(cys)_2^-$ complex.

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