^{197}Au Mössbauer Characterization of the Noncovalent Adducts Formed between Serum Albumin and Dicyanoaurate(I), a Gold-Drug Metabolite

Annapurna Canumalla,[†] C. Frank Shaw III,*[,†,§] and Friedrich E. Wagner[‡]

Department of Chemistry, University of Wisconsin−Milwaukee, P.O. Box 413, Milwaukee, Wisconsin 53201-0413, and Department of Physics, Technische Universität München, D85747 Garching, Germany

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Chrysotherapy is an important treatment for rheumatoid arthritis.[1,2] Metabolic transformations of the first- and second-generation gold drugs (Myochrysine, Solganol, and auranofin) begin with the displacement of their thiolate ligands via ligand exchange reactions with serum proteins and may culminate at inflammatory sites where dicyanoaurate(I) is formed.[3] It is a common metabolite of all three gold drugs, found in the serum and urine of patients,[4] and must be transported from the inflamed sites to the kidneys. Serum albumin carries up to 95% of the serum gold, and may be a transport agent for $[Au(CN)_2^-]$.

The dicyanoaurate(I) ion is linear with d(Au−C) = 197.1(1) pm, d(CN) = 146(1) pm, and ∠C−Au−C = 179(2)° for three independent ions in $Tl[Au(CN)_2]$[5] and has an extremely large binding constant, $\log \beta_2 = 36.6$.[6] ^{13}C NMR studies of albumin−dicyanoaurate(I) complexes, their equilibrium binding constants ($K_1 = 5.5 \times 10^4$ and $K_2 = 5.5 \times 10^3$), and the similarity of reactions of native and sulfhydryl-modified albumin all provide indirect evidence that multiple, intact dicyanoaurate(I) ions bind to albumin,[7,8] unlike gold drugs which undergo ligand exchange reactions preferentially or exclusively at cysteine 34.[9−11]

Mössbauer spectroscopy using the 77 keV resonance of ^{197}Au is a suitable technique for characterizing gold complexes[12−17] and is applicable to noncrystalline materials. It provides a method to distinguish among (i) adduct formation by intact dicyanoaurate(I); (ii) cyanide displacement at cysteine 34 to form Alb−S−Au−CN; and (iii) formation of three-coordinate complexes at cysteine 34 or several of the 17 histidine side chains, $[Alb-S-Au(CN)_2]^{2-}$ or $[Alb-N_{His}-Au(CN)_2]^{2-}$, respectively. From the extensive isomer shift (IS) and electrical quadrupole splitting (QS) data available for well-characterized complexes,[12−14] one can also derive information on the bonding state of gold. So far, various gold drugs and analogues of such drugs,[15,16] but only one protein complex, albumin−gold−thiomalate,[17] have been studied by Mössbauer spectroscopy. In this work, we report on a Mössbauer study of adducts of dicyanoaurate(I) bound to serum albumin.

Experimental Section

BSA (bovine serum albumin, fatty acid free) was obtained from Sigma Biochemicals; $K[Au(CN)_2]$ was synthesized as previously described.[18] Gold was measured by atomic absorption spectroscopy; albumin thiol content with Ellman's reagent ($\epsilon_{412} = 13\,600\ M^{-1}\ cm^{-1}$); and albumin by its UV absorbance ($\epsilon_{278} = 39\,600\ M^{-1}\ cm^{-1}$). Adducts of albumin with bound dicyanoaurate(I) were prepared by incubating $Au(CN)_2^-$ and albumin (2−5 g batches) dissolved in double-distilled water at 25 °C. The incubations were performed with twice the desired ratio of gold to albumin, since preliminary studies demonstrated that ca. 55% of the gold added is bound in the final product, multiple batches being reproducible ±0.1 Au/BSA. Equilibration is rapid,[7,8] and after incubation for 10 min, the protein and associated gold were precipitated with 3.1 M $(NH_4)_2SO_4$ at pH 4.5. The precipitates, which contained excess salt and were difficult to dry, were redissolved and desalted over Penefsky spun columns (8 mL bed volume of Sephadex G-50; 300−350 μM albumin)[7,8,19] and finally lyophilized to yield adducts with Au:BSA ratios 1.03 (5.20 g) and 2.91 (6.40 g), designated hereafter as 1:1 and 3:1 adducts. Neither the precipitation nor the lyophilization of albumin, a robust protein stabilized by 17 disulfide bonds, should alter the dicyanoaurate(I) binding mechanism, since the protein structure is not irreversibly altered by these processes.

For the Mössbauer measurements, source and sample were both cooled to 4.2 K in a liquid helium bath cryostat. The ^{197}Pt ($t_{1/2} = 19$ h) source activity was regenerated as necessary by neutron irradiation of isotopically enriched ^{196}Pt metal in the Munich Research Reactor. The low gold content of the samples (1 and 3 Au per 64 kD or approximately 0.3 and 0.9 wt %) required large samples and prolonged accumulation of the Mössbauer spectra (9 and 4 days, respectively) to obtain acceptable signal-to-noise ratios. The spectra were measured with a sinusoidal velocity waveform and fitted with quadrupole doublets consisting of Lorentzian lines of equal width and intensity.[20]

Results and Discussion

The spectra of the two adducts are shown in Figure 1. The line widths, within the limits of statistical error of about 0.1 mm

[†] University of Wisconsin−Milwaukee.
[§] Present address: Department of Chemistry, Eastern Kentucky University, Richmond, KY 40475.
[‡] Technische Universität München.

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(20) The data points between the doublet components in the upper panel of Figure 1 were fitted to a doublet (IS = 5.01 and QS = 5.97 mm s^{-1}) and a singlet (IS = 2.05 mm s^{-1}). Neither value corresponds to a reasonable gold coordination site, since a singlet requires $Au^{I}L_4$ coordination with T_d local symmetry and the doublet falls in the range of gold(III), which is not plausible.

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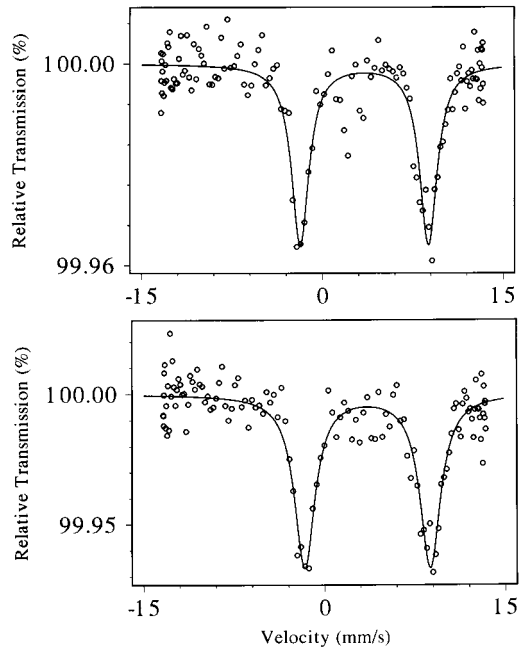

Figure 1. ^{197}Mössbauer spectra of (upper) the 1:1 and (lower) the 3:1 $[Au(CN)_2^-]$·albumin adducts measured at 4.2 K. Velocities are as measured relative to the Pt metal source.

Table 1. ^{197}Au Mössbauer Parameters for Albumin−$Au(CN)_2^-$ Adducts and Model Compounds[a]

compd	IS (mm s^{-1})	QS (mm s^{-1})	ref
1:1 $[Au(CN)_2^-]$·albumin	4.73(8)	10.65(7)	*b*
3:1 $[Au(CN)_2^-]$·albumin	4.78(8)	10.41(6)	*b*
$K[Au(CN)_2]$	4.37(1)	10.05(1)	*b*
albumin−$S(AuSTm)_{1.3}$	3.11(2)	6.68(2)	17
	2.93[c]	6.50[c]	
$[Au(CN)(SCH_2CH(NH_3^+)CO_2^-)]^-$	*3.61*	*8.29*	*calcd*[d]
$[Au(CN)S_2O_3]^{2-}$	*3.74*	*8.65*	*calcd*[d]
$[Au(CN)_2(SCH_2CH(NH_3^+)CO_2^-)]^{2-}$		*8.87*	*calcd*[e]
$[Au(CN)_2S_2O_3]^{3-}$		*9.05*	*calcd*[e]

[a] Experimental values were measured at 4.2 K; calculated values are italicized; IS relative to gold metal; obtained by adding 1.23 mm s^{-1} to the values measured relative to the Pt metal source. [b] Experimental values, this work. [c] Fixed for a two-site fit of the data. [d] IS and QS values obtained as averages of the AuL_2 and AuL'_2 values.[12] [e] Calculated using the partial quadrupole splitting parameters ^{3}L,[26] as described in the text.

s^{-1}, equal the natural width of the ^{197}Au Mössbauer resonance, $W_0 = 1.89$ mm s^{-1}. The IS and QS values observed for the two adducts (Table 1) do not differ significantly, but are larger by about 0.5 mm s^{-1} than the corresponding values for crystalline $K[Au(CN)_2]$.[21] Compared to the IS and QS values for Au(I) complexed to other ligands,[12−14] the values for the albumin adducts are so close to those for $KAu(CN)_2$ and other $[Au(CN)_2^-]$ salts and adducts[21−24] that they establish unambiguously that intact ions bind to albumin as essentially linear $[NC-Au-CN]^-$ entities. In fact, the IS and QS values observed for $[Au(CN)_2^-]$ salts as well as for the albumin adducts are among the largest observed for linear Au(I) complexes. Hence, they represent a unique fingerprint for the linear dicyanoaurate(I) entity. Both IS and QS are substantially larger than values reported earlier for gold thiomalate complexed at cysteine 34 in an S−Au−S coordination environment[17] (Table 1).

It is of interest to compare the actual IS and QS values with values expected for other gold environments. IS values (±0.3 mm s^{-1}) and QS values (±0.5−0.8 mm s^{-1}) for mixed-ligand $[RSAuCN^-]$ complexes, which have been characterized in situ[7] but not isolated and subjected to Mössbauer spectroscopy, can be estimated as the average values for the homoleptic $[Au(CN)_2^-]$ and $[Au(SR)_2^-]$ species.[12,13,25] QS values for hypothetical three-coordinate $[Au(SR)(CN)_2^{2-}]$ species which have never been observed can be calculated by the method of partial quadrupole splittings.[12,25] The values for the mixed 2- and 3-coordinate complexes derived from data for $[Au(S_2O_3)^{3-}]$ and $[Au(SCH_2CH(NH_3^+)CO_2^-)_2^-]$ given in Table 1 are smaller than the experimental values and, thus, rule out the alternative models, consistent with binding of intact $[Au(CN)_2^-]$ to form adducts by hydrogen bonding and van der Waals interactions.

$[Au(CN)_2^-]$, used as a crystallographic heavy-atom probe, binds intact to haloalkane dehydrogenase (HD),[26] human carbonic anhydrase I,[27] horse liver alcohol dehydrogenase,[28] and human liver interleukin-1 receptor antagonist protein.[29] In HD, iodide can bind at the same site.[26] These structures support the proposed model of noncovalent binding to albumin. The free energies of binding, $\Delta G_1 = -6.5$ kcal/mol and $\Delta G_2 = -5.3$ kcal/mol, calculated from the published binding constants[8] given above, also support the binding model. The existence of multiple albumin binding sites for anions such as Cl^-, Br^-, and SCN^- [30,31] is consistent with the two classes of binding sites identified by equilibrium binding studies[7,8] and with NMR studies showing that at least seven dicyanoaurate(I) ions bind to albumin.[8]

The present study is significant for the role of $[Au(CN)_2^-]$ as a gold metabolite. First, $[Au(CN)_2^-]$ in the ultrafiltrates (mw < 10 000 Da) of blood and urine from chrysotherapy patients accounts for about 0.1−1% of the total gold present in these fluids.[4] The ability of albumin, and perhaps other proteins, to bind intact dicyanoaurate(I) suggests that larger quantities may remain protein-bound in the retentates (mw > 10 000 Da). Second, the definitive Mössbauer evidence that dicyanoaurate(I) binds predominantly as intact ions confirms the ability of albumin to function as a transport mechanism and explains the previous findings[7,8] that the binding is labile and easily reversed.

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