Aurothionein Formation from Zn, Cd–Thionein and Et_3PAuCl, but not $Et_3PAuSATg$ (Auranofin)

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Received October 2, 1985

Abstract

Et_3PAuCl reacts *in vitro* with horse-kidney zinc, cadmium–metallothionein (Zn, Cd–Th) to displace zinc, but not cadmium. The ratio of gold-bound to zinc displaced is larger than that observed for gold sodium thiomalate (AuSTm), suggesting a different mode of binding. $Et_3PAuSATg$ (Auranofin: 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato(triethylphosphine)gold(I)), a new antiarthritic compound, does not react with Zn, Cd–Th. The significance of the nonreaction of $Et_3PAuSATg$, in contrast to Et_3PAuCl and AuSTm (previously studied), for the *in vivo* pharmacology of gold is discussed.

Introduction

Gold compounds are important treatments for rheumatoid arthritis, and the chemistry and biochemistry of the medicinal compounds are being intensively studied to learn more about the active metabolites and mechanisms of action [1, 2]. Gold sodium thiomalate (AuSTm; Myochrysine) is an open chain oligomer with bridging thiols joining linear, two-coordinate gold(I) ions [3]. $Et_3PAuSATg$ (Auranofin; 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-glucopyranosato(triethylphosphine)gold(I)) is a discrete complex (monomer) with two-coordinate gold(I) ions [4]. The pharmacodynamics of the two drugs are disparate, reflecting differences in composition, structure and modes of absorption [5, 6].

Following AuSTm administration gold accumulates in the kidneys and livers of mammals, where it binds, *inter alia*, to metallothionein [7, 8]. After auranofin administration, much less kidney accumulation of gold occurs, but gold binding to metallothionein has not been documented [1, 2, 5, 6]. Cultured human epithileal cells exposed to auranofin (low concentrations) or AuSTm (high concentrations) accumulate gold, some of which binds to metallothionein in the cytosol [9]. *In vitro*, limiting amounts of AuSTm react with Zn, Cd–Th to displace Zn^{2+}, forming Au, Zn, Cd–Th [11]. Thus, it is of interest to compare the reactions of auranofin and its analogue Et_3PAuCl with purified metallothionein *in vitro*.

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Experimental

Materials

Et_3PAuCl and $Et_3PAuSATg$ were generously provided by Smith Kline and French Laboratories (Philadelphia, Pa.). Trizma base and Sephadex G-50 were obtained from Sigma Biochemical Co. (St. Louis, Mo., U.S.A.).

Analyses

Metal contents of solutions were analyzed on an Instrumentation Laboratory 357 atomic absorption flame spectrophotometer (AAS). All samples were assayed against serial dilutions of reference standards.

UV–Vis spectra were recorded on a Cary 17 UV–Vis spectrophotometer. To quantitate the native metallothionein, the ultraviolet absorption spectrum of thionein in 0.1 M HCl was recorded at 220 nm, and the concentration was calculated from the published absorptivity coefficient of $\epsilon_{220} = 47\,300$ l mol^{-1} cm^{-1} (Buhler and Kagi, 1974). This method systematically overestimates aurothionein concentrations, since the gold-mercaptide absorbance in the 220 nm region is not eliminated by acidification.

Horse Kidney Zn, Cd–Thionein

Using a procedure previously described [11], a preparation containing 6.7 mol of metal ion per mol of protein in a 2:1 Cd, Zn ratio was obtained. It was divided into aliquots (~1.0 ml) containing 170 nmol Cd and 86 nmol Zn in 5.0 nM tris-HCl buffer, pH 8.6, and stored frozen (–20 °C), then thawed and used immediately.

Reactions with Et_3PAuCl or $Et_3PAuSATg$

In a typical reaction gold complex (120 nmol in 0.05 ml EtOH) was added to 1 ml of the protein

0020-1693/86/$3.50

preparation, incubated at 4 °C for one h, then eluted over Sephadex-G50 (1.5 X 40 cm) using 50 mM tris-HCl, pH 8.6. Fractions (3.3 ml) were collected at 20 ml/h flow rate and analyzed for Zn, Cd and Au content by AAS.

Results and Discussion

The chromatograms in the Figure and metal-exchange data in the Table I demonstrate that Et_3-PAuCl reacts with Zn, Cd–Th to form an aurothionein containing Zn, Cd and Au bound to the protein. It eluted at $K_d = 0.42$, as does native Zn, Cd–Th, indicating that there were not gross conformational changes like the unfolding induced by $(TmSAu)_{20}Th$ formation [11]. The small high-molecular-weight peaks in Fig. 1 represent aggregated thionein, which forms after purification. In the calculation of metal exchange ratios, the hmw metals were treated as metallothionein-bound. The displaced zinc was not recovered in the lmw fractions, presumably due to binding to the Sephadex resin.

Zinc displacement and gold binding were incomplete: 48 and 86% respectively, demonstrating a less favorable reaction for Et_3PAuCl than for AuSTm, which displaces zinc almost quantitatively under similar conditions. The ratio of gold bound to zinc displaced was 2.00. If Et_3PAu binds to each sulfhydryl group freed by zinc displacement, the ratio should approach 2.86 (=20/7, the ratio of sulfhydryls to zinc and cadmium). Bidentate chelation by 2 sulfhydryls with displacement of phosphine would give a ratio of 1.43, as found for reactions where AuSTm is the limiting reagent [11]. The simultaneous occurrence of both coordination modes can explain the observed ratio, 2.00.

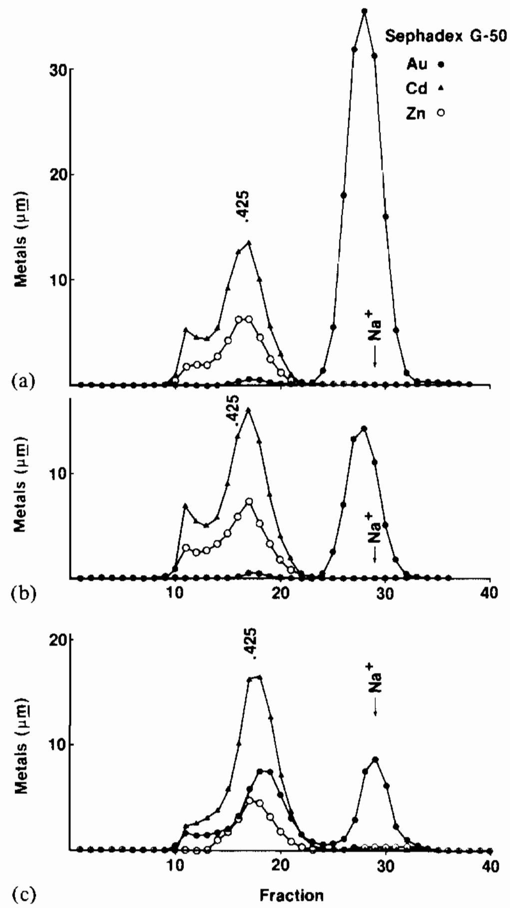

Fig. 1. Et_3PAuCl, but not $Et_3PAuSATg$, reacts to form an aurothionein *in vitro*. Zn, Cd–Th was incubated with Et_3-PAuSATg (A, 3.9 Au/Zn; B, 1.4 Au/Zn) or Et_3PAuCl (C, 1.4 Au/Zn) for 1 h at 4 °C, then fractionated on a Sephadex G-50 column (1.5 x 40 cm): tris-HCl buffer, pH 8.6; 3.3 ml fractions, 20 ml/h flow rate.

TABLE I. Comparison of Metal Exchange with Gold Complexes and Zn, Cd–Thionein

Complex	Metal	Reactant (nmol)	Products (nmol)		Recovery (%)	Au_b/Zn_d[a]
			Thionein	LMW		
Et_3PAuCl	Zn	86	45	<1	52	2.00
	Cd	170	165	<1	97	
	Au	120	82	52	112	
$Et_3PAuSATg$	Zn	86	78	<1	91	–
	Cd	170	172	<1	101	
	Au	120	2	110	93	
$Et_3PAuSATg$	Zn	68	65	<1	96	–
	Cd	136	145	<1	107	
	Au	266	2	276	105	
AuSTm[b]	Zn	200	50	140	95	1.47
	Cd	350	340	43	111	
	Au	300	270	18	96	

[a]Ratio of gold bound to zinc displaced. [b]Data from ref. 11.

$Et_3PAuSTg$ at the same concentration did not displace Zn^{2+} or Cd^{2+} from the protein and the amount of gold bound was negligible (*ca.* 1% of that present). Increasing the concentration of $Et_3PAuSATg$ from 1.5 to 3.9 Au/Zn in the reaction mixture did not change the results. Thus, the reactivity of gold complexes toward Zn, Cd–Th is

$$AuSTm > Et_3PAuCl > Et_3PAuSATg$$

The greater reactivity of the chloride, compared to $Et_3PAuSTg$, results from the weaker bond and greater ease of displacement of chloride compared to acetylthioglucose. Et_3PAuCl is also more reactive than $Et_3PAuSATg$ with bovine serum albumin: $Et_3PAuSATg$ binds only to the fully reduced sulfhydryl group (including that generated by reducing an oxidized form, probably a sulfenic acid), while Et_3PAuCl reacts at that site and additional weak binding sites via unidentified nitrogen-bases on the protein [12]. The reaction of AuSTm and MT has a different stoichiometry (the thiomalates bind to the displaced zinc and cadmium ions) which may make that reaction more favorable than for Et_3PAuCl or $Et_3PAuSATg$ [11].

The non-reaction of auranofin with purified MT contrasts with aurothionein formation in human epithileal cells in culture [9]. This dichotomy demonstrates that metabolite formation via ligand exchange or modification reactions *in vivo* will significantly alter the chemical reactivity and pharmacology of gold. These reactions may occur intracellularly or in body fluids such as serum. Since gold drugs are slow-acting therapeutic agents [5, 6], ligand modification and displacement reactions are comparatively rapid and gold metabolites, not the drugs, circulate in patients. Thus, the metabolites of gold drugs should be identified, isolated, characterized and used for *in vitro* studies of the effects of gold on immune system components, enzymes and other postulated sites of action.

Acknowledgements

This work was sponsored by the National Institutes of Health (GM 29583) and Smith Kline and French Laboratories. We thank Dr. Gian Carlo Stocco (via NATO Grant 890/83) for helpful discussions.

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