A Chromium(III) Complex of Oxidised Glutathione Isolated from the Reduction of Chromium(VI) with Glutathione

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(Received May 5, 1989; revised June 19, 1989)

Abstract

A chromium(III) complex of oxidised glutathione has been isolated from the reaction of glutathione with chromate in neutral aqueous solution. The properties of this complex are consistent with N_3O_3 coordination at chromium(III) and the compound may be similar to one form in which chromium is excreted by animals challenged with chromate.

Introduction

Chromate is well known to be both carcinogenic and mutagenic [1], and there is considerable current interest in the mechanisms by which this toxicity is expressed [2–9]. Chromium(VI) damages DNA in a number of ways including intrastrand crosslinking, crosslinking to proteins and by causing strand breaks [10–12]. Some years ago Cupo and Wetterhahn observed that chromate caused more DNA strand breaks in chick embryo hepatocytes in which glutathione had been induced [13]. A number of groups have hence become interested in substantiating the hypothesis that the interaction of chromate with GSH can generate species capable of damaging DNA [4–9, 14]. Within cells chromate will ultimately be reduced to chromium(III) [1] and although, we, and others, have studied models for such reactions there is little or no information concerning the nature of any final chromium(III) containing reaction products. Wetterhahn and Connett briefly mentioned the isolation of a chromium(III) complex of GSSG [1] but no experimental details were reported.

The purpose of this paper is to provide more details concerning one final chromium(III) containing product of the reaction between glutathione and chromate at neutral pH. The compound is unable to generate strand breaks in PM2 bacterial DNA [14] and the present paper defines the material used in our earlier study. There is also a second interest in chromium(III)/GSSG complexes as a number of workers have reported the isolation of low molecular weight chromium(III) species, suggested to be GSH or GSSG complexes, either from animals, or perfused organ models, challenged with chromium(VI) [15–22]. The spectroscopic properties of the complex described in the present study, especially the circular dichroism, may be useful in the identification of low-molecular weight chromium(III) complexes from such *in vivo* or *in vitro* studies [9–16].

Results and Discussion

Chromate (0.1 mol dm^{-3}) and glutathione (1.0 mol dm^{-3}) were allowed to react together for one to two hours; the resulting purple solution was reduced in volume and repeatedly chromatographed on Sephadex SP-25 anion exchange resin and eluted with increasing concentrations of sodium chloride; the columns were protected from light.

The chromium(III) containing fraction was readily identified as a single purple/red band which we were unable to resolve into more than one component. The final eluate was concentrated to a small volume and chromatographed twice on Sephadex G50 in an attempt to desalt the mixture. We were never able to separate the final fraction from all salt. A purple, solid material was obtained by evaporating the final solution to dryness under reduced pressure.

Analytical results for the compound are summarized in Table 1. The ratio of C:N:S confirms that these elements are present in the mole ratio expected for GSH or GSSG. The analytical figures are in reasonable agreement (Table 1) with the overall formula $Na_2[Cr(GSH)_2]\cdot 2H_2O\cdot 0.7NaCl$ involving the coprecipitation of about 0.7 mol of sodium chloride. The molecular weight of this species is *c.* 780 for the monomeric species. Yamamoto *et al.* have reported that a chromium(III) complex, containing amino acids, of RMM *c.* 1600, can be isolated from mice given intra-peritoneal injections of chromate [18], this could suggest a dimeric formulation for such complexes. Wiegand *et al.* have reported much higher

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0020-1693/89/$3.50

TABLE 1. Analytical results[a]

	Found	Mole ratio	Ratio CNS[b] (for GSH)	Calculated for (A)
Cr	5.9	1		6.65
Na	8.09	3		7.94
C	30.58	23	10(10)	30.71
H	4.17	37		4.12
N	10.49	7	3(3)	10.49
S	8.14	2	2(3)	8.21
Cl	2.80	0.7		2.8
O	29.83	16		28.65

[a]The analytical results presented above can be interpreted in terms of an empirical formula involving two moles of glutathione and one chromium atom $Na_2[Cr(GSH)_2]\cdot 2H_2O\cdot 0.7NaCl$ ($CrNa_{2.7}C_{20}H_{37}N_6S_2Cl_{0.7}$ (A) RMM = 781). The sodium chloride is almost certainly a coprecipitated impurity. Despite extensive efforts we have been unable to totally desalt this complex. [b]Mole ratio of C:N:S calculated from the analytical results (required for GSH).

TABLE 2. Spectral properties[a] of the chromium(III) GSSG complex

Electronic		545(59.9)	395(68.0)
Circular dichroism	580(−1.6)	500(−1.9)	378(−0.5)

[a]Electronic spectra and circular dichroism max./min., $\Delta\epsilon_{Cr}$ or ϵ_{Cr} units (mol^{-1} dm^3 cm^{-1}) all are calculated per chromium atom.

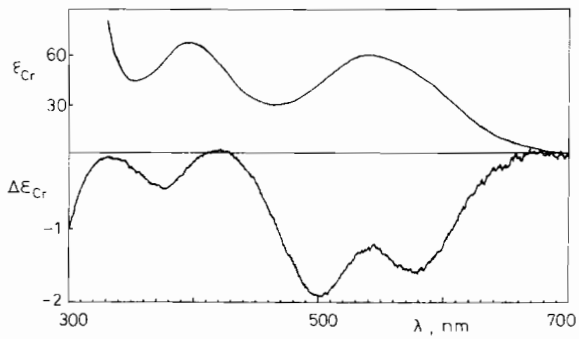

Fig. 1. Electronic spectrum and circular dichroism of the chromium(III) complex.

molecular weights, 5000 and 10000, for chromium(III) complexes isolated from the reaction of GSH and chromate [15]; no spectroscopic properties were given. However, in related work a chromium(III) complex from perfused rat liver was found to have absorptions at 548 and 418 nm and an apparent molecular weight of 6000 [16].

The spectroscopic properties of the complex are summarised in Table 2 and Fig. 1. The ligand field strength, as judged from the lowest energy d–d transition ($^4T_{2g}$ parentage in O_h), is slightly weaker than that of tris amino acidate complexes of chromium(III) [23–25] which may suggest that the coordination sphere is N_3O_3–N_2O_4. The ligand field strength is also similar to that in other GSH and GSSG complexes of chromium(III) [1, 16, 24] some of which have been isolated from biological systems; these data are summarised in Table 3.

TABLE 3. Position of lowest energy ligand field transition in some chromium complexes[a]

Complex	Band	Chromophore/Reference
CrGSSG complex	18.34	this work
CrGSSG	18.25	from perfused rat liver [16]
$Cr(GSH)_2$	18.31	N_2O_3S ? [24]
CrGSSG polymer	18.18	N_3O_3 ? preliminary data only [1]
$[Cr(NH_3)_6]^{3+}$	21.6	N_6 [26]
fac-$[Cr(glyO)_3]$	19.2–19.6	N_3O_3 [23]
$[Cr(L\text{-}Cys^{2-})_2]^-$	16.5	$N_2O_2S_2$ [24]
$[Cr(ox)_3]^{3-}$	17.5	O_6 [25]

[a]All values are $10^3 \times cm^{-1}$.

The circular dichroism of the compound is unusual in that it is dominated by a series of negative absorptions (between 300 and 700 nm). The lowest energy component of the electronic spectrum of a chromium(III) complex ($^4T_{2g}$ in octahedral) usually splits into components of opposite sign in the circular dichroism of complexes of lower symmetry [23, 24]. The reasons for the sign pattern observed for the present complex are unclear, possibilities are that the spectrum is that of a complex mixture of diastereoisomers (not separated by our procedures) or the symmetry of the complex is unusual. In any event the spectrum shown in Fig. 1 is reproducibly observed for the products of the chromium(VI)/GSH reaction. Moreover, the spectrum of the unpurified reaction mixture of chromium(VI) and GSH is also dominated by strong negative Cotton effects.

The composition of the complex can also be commented on from the infrared spectra. The spectrum is broad and generally uninformative, but the antisymmetric and symmetric stretches of the carboxylates can be identified at 1637 and 1389 cm^{-1}, clearly indicating that carboxylate functions are coordinated to chromium(III) [26]. Some information may be obtained from the region 4000–2200 cm^{-1}; there is no obvious –SH stretch (usually seen at *c.* 2500 cm^{-1}) and secondly there is a peak at 523 cm^{-1}, the region in which an S–S stretch might be expected [27].

We have also sought to distinguish between GSH and GSSG in two further experiments. The formation of a sulfur to chromium(III) bond is usually characterized by an intense S–Cr charge transfer transition

at *c.* 250 nm [24], no such absorption is observed. The complex also gave no indication of a positive reaction with Ellman's reagent [28], which is a sensitive test for free thiol functions.

Conclusions

The properties of the material we have isolated are summarised below.

(1) The complex is a readily soluble ionic material, suggesting a monomeric material (RMM *c.* 800), but it is difficult, on the basis of our results to rule out some degree of polymerisation.

(2) The complex has a ligand field strength similar to that of the tris amino acidates of chromium(III) suggesting an N_3O_3 chromophore.

(3) The complex contains carboxylate functions coordinated to chromium(III).

(4) There is no absorbance in the position associated with the S–Cr charge transfer transition. The complex contains no free –SH groups, as judged from infrared spectroscopy of the solid and Ellman's assay.

The spectral properties of this complex may be of use in elucidating chromium speciation in samples isolated from *in vitro* and *in vivo* experiments. The complex described above has similar spectroscopic properties to the final product of the reduction of chromate by GSH either, from the intermediate complex we have described [9], or from solutions of chromate and GSH [1, 29]. In particular the strong negative Cotton effect at 500 nm seems to be present in all such species. This is quite different from the optical properties of tris amino acidate [23, 25] and glutathione [23] complexes at these wavelengths.

The fact that this complex does not cause strand breaks in PM2 DNA lends further support to the idea that reactive intermediates generated in the reduction of chromate are responsible for any damage to DNA. Although chromium(III) crosslinks may be implicated as one way in which DNA is ultimately damaged by chromate [30, 31], these are probably generated during the reduction process. The toxicity of most chromium(III) complexes appears to be low, and chromium, in this oxidation state, may be required as a trace nutrient [32]. Chromium(III) complexes of GSH/GSSG may be important in the excretion of chromium by animals treated with chromate.

Experimental

Materials and Methods

Potassium dichromate was BDH AnalaR grade; ion exchange and gel permeation resins were purchased directly from Pharmacia and glutathione from Sigma; all other chemicals were purchased from BDH Chemicals. Electronic spectra were measured with a Perkin-Elmer 330, circular dichroism with a JASCO J600 instrument and infrared spectra as either nujol mulls or KBr discs (1%, 200 mg) between 4000 and 400 cm^{-1} using a Mattson Polaris FTIR. Chromatography was performed using a Pharmacia low pressure system consisting of a Pharmacia peristaltic pump and a Fracevap collector. Micro analyses (Cr, Na, C, H, N, S) were determined by Butterworths Laboratories, and free chloride ion by titration with silver nitrate [32].

Preparation and Purification of the Complex

In a typical experiment a solution 0.1 mol dm^{-3} sodium dichromate and 1.0 mol dm^{-3} GSH at pH 7.0 (±0.1) were allowed to react together for a minimum of 2 h. The solution was then reduced in volume and chromatographed, twice, on Sephadex Sp-25 anion exchange resin, with sodium chloride of increasing ionic strength. The chromium(III) complex eluted as a single band. The final eluate was concentrated to a small volume *c.* 5 ml and applied to a column of Sephadex G-50 (0.5 m) and eluted with water. Two passes through this column removed most of the salt contaminating the complex. A small amount of sodium chloride persisted even after more exhaustive chromatography. Throughout all operations the columns were protected from light with aluminium foil.

Acknowledgements

This work has been supported by a grant from the Cancer Research Campaign with some additional support from The University of London Central Research Fund. We thank Dr Alex Drake of the U.L.I.R.S. circular dichroism service at Birkbeck College for assistance with the CD spectroscopy.

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