Chromium(III) complexes from CrO_4^{2-} reduction at pH 7

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Abstract

Chromate was reduced by L-cysteine ethyl ester at neutral pH in the presence of potential ligands. Reduction products were isolated and compared to complexes produced from the direct thermal reaction between Cr(III) and the same ligand. Ligands used in the reduction studies were AMP, aspartic acid and aspartame. Results showed that the better the ligand, and the higher the concentration of the ligand, the more products similar to known Cr(III) complexes were produced. New Cr(III) complexes which were synthesized by direction reaction for this study were $Cr(aspartame)_2^+$, $Cr(aspartame)^{2+}$ and $Cr(2,5\text{-}PDCA)_3$.

Introduction

Cr(VI) is classified as a priority pollutant and has been shown to be a carcinogen for workers who are exposed occupationally [1, 2]. Its adverse biological effects result from DNA damage and/or the formation of DNA–protein crosslinks [3–6]. These crosslinks contain chromium and are not easily repaired by the cell [5, 6]. Earlier work has shown that Cr(VI) acts through an 'uptake reduction' model where Cr(VI) enters the cell but is reduced by cellular species to Cr(III). During the reduction chromium may be incorporated into cellular components [3, 7]. Potential reducing agents within cells are both complexing and non-complexing species [3, 8]. A kinetic study which surveyed a variety of reducing agents *in vitro* at neutral pH showed that an ionizable hydrogen on the reducing agent is necessary for reduction. Rate constants were measured and mechanisms proposed, but no product analyses were done [9]. In another study, analysis of cellular components after exposure to Cr(VI) showed the presence of chromium in a wide variety of cellular fractions, but the exact cellular reducing agents were not known [10]. However, the most likely cellular reducing agent is glutathione while other important reducing agents are ascorbate, hydrogen peroxide, glutathione reductase, DT diaphorase, cytochrome p-450 reductase and aldehyde oxidase [3, 8, 11, 12]. A further study identified chromium(III) product formation with di- and trinucleotides after reduction of Cr(VI) with glutathione, but products were not characterized [13]. Product characterization was performed in acidic solution in work where Cr(VI) was reduced in the presence of an excess of potential ligands with both one-electron and two-electron non-coordinating reducing agents [14]. Reduction of Cr(VI) at neutral pH with coordinating reducing agents has been compared with that using the same ligands in acidic solution [15]. One of these coordinating reducing agents, L-cysteine, formed a complex in neutral solution which was different from products formed in acidic solution [15].

It was our aim to investigate what Cr(III) species might form at pH 7–8 after the reduction of Cr(VI) with a non-coordinating one-electron reducing agent in the presence of potential ligands, and then compare these products to complexes that were formed with the same ligands in thermal substitution reactions with Cr(III) at lower pH. Ligands chosen were those which might have biological relevance to the DNA–Cr(III)–protein crosslinks. These ligands were AMP (adenosine monophosphate), aspartic acid, aspartame (L-aspartyl-L-phenylalanine methyl ester) and 2,5-PDCA (2,5-pyridinedicarboxylic acid). The thermal substitution products with both AMP and aspartic acid are well known [16, 17].

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Experimental

Materials and equipment

All materials were reagent grade and used without further purification. UV–Vis spectra were recorded on either a Varian DMS-100 spectrophotometer or a Hewlett Packard 8452A diode array spectrophotometer. IR spectra were recorded on a Perkin-Elmer 392 infrared spectrophotometer.

Thermal syntheses

For the synthesis of $Cr(2,5\text{-PDCA})_3 \cdot 4H_2O$, 0.37 g (2.2 mmol) of 2,5-pyridinedicarboxylic acid (2,5-PDCA) was dissolved in 10 ml of deionized water by adding 0.10 g of solid NaOH (2.5 mmol). After the addition of 0.20 g of $CrCl_3 \cdot 6H_2O$ (0.75 mmol) the solution was boiled for 10 min. During that time, the solution changed from green to purple with the formation of a precipitate and finally to wine red with no precipitate. The volume was maintained at approximately 10 ml during the heating process. The wine red solution was cooled in ice and the red powdery product was removed by filtration. The pH of the filtrate was 3.0. (Lowering the pH of the filtrate with 6 M HCl caused further precipitation.) The product was recrystallized from boiling water. Yield 0.24 g (50%). *Anal.* C, 40.5; H, 3.73; N, 6.60*; Cr, 8.56%.

For the synthesis of Cr(III)–aspartame complexes, crystalline aspartame was dissolved in deionized water to give a 60 mM solution and the pH of the solution was adjusted to 5.5. An equal volume of 20 mM $Cr(H_2O)_6(NO_3)_3$ or $CrCl_3 \cdot 6H_2O$ solution was added with mixing to give a final solution that was 30 mM in aspartame and 10 mM in chromium. The pH of the solution was 3.7. The solution was heated at 80 °C for 20 min. During heating the color of the solution changed from blue–violet to red–violet and the final pH of the solution was 2.3. Other heating times and different ratios of reactants (aspartame to chromium) were tested. Products were separated on a Sephadex SP C-25 cation exchange column in the Na^+ form at pH 5. A red–violet fraction eluted with 0.2 M NaCl and a violet fraction with 0.5 M NaCl. All products were characterized in solution.

Chromium–aspartic acid complexes and Cr–AMP complexes were synthesized as described previously by heating at 80 °C using different ligand to Cr(III) ratios [16, 17]. The Cr–AMP product was isolated by adjusting the pH to 4.5 and collecting the precipitate by filtration.

Synthesis by reduction of CrO_4^{2-}

A stock solution that was 10 mM in chromium was prepared from $K_2Cr_2O_7$ and used in all reduction experiments. In each case the appropriate weight of ligand was added to 10 ml of stock chromium solution and the pH of the solution adjusted to 7.4 with 0.1 M NaOH. Ten ml of a freshly prepared 30 mM solution of L-cysteine ethyl ester at pH 7.4 was added dropwise to the Cr(VI)–ligand solution. The pH of the reaction solution was monitored and kept between 7.2 and 7.8 by the addition of 0.1 M HCl or 0.1 M NaOH as needed.

For reaction solutions containing AMP, the temperature was maintained between 35 and 40 °C and the dropping rate was about 8 drops per min. After all of the L-cysteine ethyl ester solution had been added, the reaction was allowed to stir for an additional 15 min and left at room temperature overnight. The solution was then refrigerated overnight and if a precipitate was present after refrigeration, the solution was filtered. The spectrum of the solution was recorded. To isolate the product, the pH was adjusted to 4.0 with 0.1 M HCl and the mixture was again refrigerated overnight. The reaction product was filtered and allowed to air dry.

For reduction in the presence of L-aspartic acid the dropping rate was 13 drops per min and the temperature was maintained at 40 °C. The reaction solution was left at room temperature overnight. The solution was then filtered, 1 ml of 1 M HCl was added to the filtrate and the solution diluted to 50 ml and refrigerated. The spectrum of the solution was recorded and then the products were separated on a Dowex 50X-2 100–200 mesh ion exchange column in the H^+ form using 0.5 and 6 M HCl. An anion exchange column (Dowex 1X-2 100–200 mesh in Cl^- form) was also used and eluted with 0.5 M HCl. Products were not isolated from solution but were identified spectrophotometrically.

In the presence of aspartame the dropping rate was 4 drops per min and the temperature was maintained at 30 °C for another hour and then left at room temperature overnight. A light purple powder formed after 24 h. The solution was then refrigerated. The powder was filtered and redissolved in 3 M HCl.

Chromium analyses were performed by heating samples of the complexes in basic H_2O_2 and reading the absorbance at 372 nm [18]. Amino acid and aspartame analyses were done by reaction with ninhydrin [19]. Analysis for AMP was done spectrophotometrically at $\lambda = 260$ nm ($\epsilon = 1.5 \times 10^4$ M^{-1} cm^{-1}) based on free AMP, assuming that coordination through phosphate does not affect ligand transitions.

*Analysis carried out by Galbraith Laboratory, Inc., P.O. Box 51610, Knoxville, TN 37950.

Results

The thermal reaction between $CrCl_3$ and AMP at 80 °C was performed as described previously [16, 20]. In the thermal reaction of AMP with Cr, ratios of Cr to AMP from 1:1 to 1:10 were used in the reaction solution, but in all cases the analysis of the solid product showed that the Cr/AMP ratio was 0.9. The light green product when dissolved in 0.5 M HCl had λ_{max} of 593 ($\epsilon = 28\ M^{-1}\ cm^{-1}$) and 425 ($\epsilon = 28\ M^{-1}\ cm^{-1}$) nm for the complex from the 1:1 solution which agrees with previous results [15], and 604 ($\epsilon = 25\ M^{-1}\ cm^{-1}$) and 436 ($\epsilon = 23\ M^{-1}\ cm^{-1}$) nm for the product from the 1:10 reaction solution. The amount of water in the solid depended on the method of drying. When the product was allowed to air dry, approximately ten waters were found per chromium.

When the reaction of chromium with AMP occurred from the reduction of CrO_4^{2-}, the resulting product was blue with varying Cr/AMP ratios (Table 1), although the Cr to AMP ratios in the reaction solutions were similar to those used in the thermal experiments. The product in each case was redissolved in 0.05 M HCl and the visible spectrum was found to vary for each ratio (Table 1), although the absorption maximum for all of these solutions before precipitation was 566 nm. The absorption maximum for the solutions from the substitution experiments before precipitation was 595 nm except for the solution where the ratio was 1:1 and the λ_{max} was 585 nm. Yields were about 40% based on the initial amount of chromium for the thermal experiments and about 10% for the reduction experiments.

$Cr(asp)_2^-$ and $Cr(asp)^+$ were prepared as described previously [17]. Variation of the chromium to aspartic acid ratio from 1:1 to 1:6 changed the relative amounts of these two products. For $Cr(asp)_2^-$ the λ_{max} values were 515 ($\epsilon = 68\ M^{-1}\ cm^{-1}$) and 382 ($\epsilon = 39\ M^{-1}\ cm^{-1}$) nm which agreed with previously reported values and showed no change with pH [17, 21]. $Cr(asp)^+$ was separated from the other products by cation exchange but not isolated from solution. λ_{max} for this product was 545 ($\epsilon = 62\ M^{-1}\ cm^{-1}$) and 402 ($\epsilon = 34\ M^{-1}\ cm^{-1}$) nm at pH 5. However, the spectrum did change as the pH was increased.

In the reduction experiments with CrO_4^{2-} at pH 7.4 with L-cysteine ethyl ester, ratios of chromium to aspartic acid were varied from 1:2 to 1:10. The spectrum of the product solution after acidification and filtration showed λ_{max} at 551 for the 1:2, 1:4 and 1:6 solutions with increasing absorbances. The 1:10 solution had λ_{max} at 540 nm with an even higher absorbance. Chromium analysis of the final product solutions showed that the 1:10 solution had the most chromium remaining in solution (5% loss) after the reaction while the least remained in the 1:2 solution (15% loss). Ion exchange separation of these product solutions showed that in the 1:10 solution 15% of the chromium formed $Cr(asp)_2^-$ (identified spectrophotometrically and by ion exchange behavior); 53% formed $Cr(asp)^+$ (again identified spectrophotometrically and by ion exchange behavior) and 17% polymeric material which could only be removed from a cation exchange column with 6 M HCl. (The remaining chromium could not be recovered from the column.) This polymeric material had λ_{max} at 552 nm ($\epsilon = 34\ M^{-1}\ cm^{-1}$). In the 1:2 aspartic acid to chromium solution no $Cr(asp)_2^-$ was produced (all the product came through an anion exchange column). The cation exchange column results showed that about half the chromium recovered from the column was $Cr(asp)^+$ (23% of the original) and that the other half was the less mobile polymeric material (22%). When the pH of a solution of $CrCl_3$ and a ten fold excess of aspartic acid was adjusted to 7.4 and left for 24 h at room temperature, only about 10% of the chromium was in solution at the end of that period and about 95% of that was in the $Cr(asp)_2^-$ form.

The thermal reaction between aspartame and chromium produced two complexes which could be separated by cation exchange chromatography. (Neither product was ever isolated from solution.) The complex which eluted first from the column behaved as though it had a +1 charge and had a ratio of 2 aspartames to 1 chromium. The product displayed absorption maxima at 525 ($\epsilon = 42\ M^{-1}\ cm^{-1}$) and 385 ($\epsilon = 32\ M^{-1}\ cm^{-1}$) nm. The second complex eluted from the column displayed maxima at 550 ($\epsilon = 33\ M^{-1}\ cm^{-1}$) and 405 ($\epsilon = 29\ M^{-1}\ cm^{-1}$) nm. The charge of the complex appeared to be 2+ and the aspartame to chromium ratio was 1:1. From the spectra of the final reaction solutions and spectra of the separate products, β_1 and β_2 were determined to be 2.0×10^2 and 3.2×10^4, respectively.

TABLE 1. Products of chromium reduction in the presence of AMP

Cr:AMP ratio in reaction solution	Cr:AMP ratio in product	λ_{max} of product in 0.05 M HCl (nm)
1:1–1:10[a]	0.9:1	593–604, 425–430
1:2	2.5:1	555, 417 (shoulder)
1:4	1.7:1	not measured
1:7	1.5:1	566, 417
1:10	1.3:1	570, 419

[a]Thermal substitution reactions.

When CrO_4^{2-} was reduced at pH 7.4 by L-cysteine ethyl ester in the presence of aspartame at either a 3:1 or a 10:1 aspartame to chromium ratio, a fine purple powder precipitated from the solution. When this powder was collected by filtration and redissolved in 1 M HCl, it had λ_{max} at 558 and 403 nm. However, it bound irreversibly to a cation exchange column in the H^+ form. Not even 6 M HCl would remove it. The chromium in the product solution also bound irreversibly to a cation exchange column.

Results of the analysis indicated that the complex produced from the reaction of Cr(III) and 2,5-PDCA was $Cr(2,5\text{-}PDCA)_3 \cdot 4H_2O$. The complex that precipitated from solution had no charge but released three moles of H^+ when titrated potentiometrically between pH 2.5 and 7.5 (approximate pK_a values were 2.5, 3.1 and 4.7). The complex had an absorbance maximum at 520 nm ($\epsilon = 62\ M^{-1}\ cm^{-1}$), a shoulder at 400 nm, and another maximum at 272 nm ($\epsilon = 1.54 \times 10^3\ M^{-1}\ cm^{-1}$) in the fully protonated form and an absorbance maximum at 530 nm at pH 7.4. A comparison of the IR spectrum of the complexed and uncomplexed acid showed a shift in the carbonyl stretch from 1715 to 1635 cm^{-1} which is typical for metal ions coordinated to carbonyls [22]. Although the complex formed with this ligand has the potential to form both geometric and optical isomers no attempt was made to determine if the product was a mixture of isomers or formed one preferentially. No reduction work was done with this ligand.

If CrO_4^{2-} was reduced by L-cysteine ethyl ester at pH 7.4 at 35 °C with no ligand in the solution, a grey precipitate formed immediately and after 24 h all of the chromium was gone from the solution. This grey precipitate was not visible in any solutions containing potential ligands.

Discussion

The kinetics of the reaction between L-cysteine ethyl ester and CrO_4^{2-} at neutral pH has been studied and found to be mixed second and third order [9]. Based on rate constants from this earlier work, calculations showed that the reduction reactions in our work should have been complete minutes after the last drop of L-cysteine ethyl ester was added [9]. However, because the conditions in this work were so different from the earlier work, all reduction solutions were allowed to stand overnight to ensure complete reduction. In all cases, the solutions containing added potential ligands produced different final products from the solution where no added ligand was present. Even the aspartame, which was the poorest ligand, caused some chromium to remain in solution after the reduction, and an entirely different precipitate formed. It appears from these results that this non-coordinating reducing agent allows complex formation in neutral solutions containing molecules with available coordinating groups.

Once it had been established that complexes did form on reduction with L-cysteine ethyl ester, attempts were made to isolate and identify these products and compare them with products that are formed during thermal substitution reactions in solutions containing equivalent amounts of the same ligand.

Our results for the 1:1 thermal synthesis of Cr–AMP agree with previous results and indicate that as the AMP to chromium ratio increases, the phosphate concentration in the coordination sphere increases, because λ_{max} and ϵ increase slightly [20, 23–25]. Although by our method of isolation we could not evaluate how much monomer and how much polymeric material was produced, we did find only green material which shows that the Cr was bonded to the AMP through a phosphate oxygen [20, 23, 26]. Our material from the thermal synthesis showed solubility at both low pH (below 2.5) and high pH (above pH 7.8). The material produced from the reduction did not resemble the thermal product either in solution or in solid form (Table 1), and was probably contaminated with increasing amounts of polymer as the amount of AMP in the solution decreased. However, at pH 7.4 all of the reduction product remained in solution.

$Cr(asp)^+$ had been reported before, but has never been isolated from solution or characterized spectrophotometrically [21]. We were not successful in isolating it from solution, but did spectrophotometrically detect a material with a positive charge. The reduction produced products similar to those from the thermal synthesis as well as highly charged polymeric material. As the ligand to Cr(VI) ratio increased the ratio of $Cr(asp)_2^-$ and $Cr(asp)^+$ to polymeric material increased, but the product distribution gives little indication of intermediates formed during the reduction. Although aspartic acid will form complexes thermally in 24 h at room temperature at low pH 2–3.5, this does not happen at pH 7.4, ruling out the importance of reduction to Cr(III) followed by ligand complexation as a route to product formation [27].

Aspartame had not been used as a ligand for Cr(III) before. Although enough ligand was present under conditions of our thermal synthesis, no $Cr(aspar)_3$ appeared to be formed, but complexes with 2 aspartames and 1 aspartame per chromium did form. Both of these complexes were stable in low pH solutions and appeared to be bidentate.

Because these complexes were not isolated from solution no attempt was made to determine isomerization for the 2:1 complex. The reduction product with aspartame probably included aspartame in its coordination sphere but no attempt was made to characterize the reduction material since it was not similar to the thermal material, and seemed to be highly polymeric.

The Cr(III)(2,5-PDCA)$_3$ complex was also a new complex. By spectral comparisons with other Cr(III) complexes with N_3O_3 coordination sphere, 2,5-pyridinedicarboxylic acid appears to behave as a bidentate ligand coordinating to the chromium through the pyridine nitrogen and the 2-carboxyl group [28, 29]. Further evidence that the last carboxyl group (presumably the 5 group) is not coordinated comes from the fact that 3 mol of H^+ per complex can be titrated potentiometrically. A Cr(III)–2,5-PDCA complex which contained only one molecule of 2,5-PDCA, and was produced from the oxidation of Cr(II), had been reported previously [30]. Under conditions of thermal synthesis, only one carboxyl group of 2,5-PDCA was deprotonated. If more than one was deprotonated, the product was contaminated with a violet material. Since both carboxyls would be deprotonated under conditions of the reduction method, the reduction method was not tried.

Overall, our results show that the presence of ligands in solution prevents the reduced chromium from precipitating and that the better a chelating agent a ligand is, the more closely the reduction products resemble thermal products. β_1 and β_2 for chromium–aspartic acid complexes are 1.4×10^{12} and 1.3×10^{21} compared to 2.0×10^{2} and 3.2×10^{4} for chromium–aspartame complexes [17]. Increased concentration of ligands in reduction solutions also increased the concentration of products which resembled thermal substitution products.

Previous findings showed that in acid solution in the presence of an excess of ligand, a one-electron non-coordinating reducing agent produces a higher substituted product to non-substituted product ratio than a two-electron non-coordinating reducing agent, and the better the ligand the greater the amount of coordinated product that is formed [14]. These results imply that for a one-electron reducing agent a substituted Cr(IV) species is an important intermediate. For a two-electron reducing agent, non-substituted Cr(V) and substituted Cr(IV) intermediates determine product distribution, as long as ligand oxidation does not occur [14]. (We did not investigate the possibility of ligand oxidation.) In neutral solution for work other than kinetic studies, all reducing agents have been coordinating reducing agents [13, 15, 31]. One study showed that excess reducing agent was coordinated, while another showed, if enough excess ligand is present, other complexes will form even if the reducing agent coordinates. EPR studies indicate the formation of a Cr(V) intermediate as the initial reduction product with thiol reducing agents at neutral pH [9, 15, 32]. Both non-substituted Cr(V) and substituted Cr(IV) intermediates could be formed in subsequent steps of the reduction [9, 15]. Certainly, in this work, the production of polymeric Cr(III) containing material and identifiable substituted Cr(III) products is consistent with the formation of these intermediates.

Our results imply (i) that the reduction of Cr(VI) at physiological pH by a non-coordinating reducing agent makes available during the reduction chromium species that can coordinate to biological ligands, and (ii) that the higher the concentration of these ligands, and the better the ligand, the more likely the complexation.

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