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Molecular Basis of Chromium toxicity: the Role of Glutathione

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Molecular Basis of Chromium Toxicity:
The Role of Glutathione

A thesis submitted to
Regis College
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in partial fulfillment of the requirements
for Graduation with Honors

by

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Chapter I: Chromium in Today’s World

The Los Angeles Times cites the hexavalent chromium as one of the most dangerous toxic contaminants in California’s air.¹ Estimates from the Occupational Safety & Health Administration (OSHA) indicate that every year 380,000 U.S. industrial workers are exposed to hexavalent chromium on the job, mainly through inhalation. Hexavalent chromium, in the form of dichromate, is used in chrome plating, stainless steel welding and the production of chromate pigments and dyes. In these settings, chromium dust is released into the air where it can be inhaled by workers. The presence of these chromium dust particles result in the air becoming toxic in areas where many of these plants are present. This dangerous hexavalent molecule is also often recognized in popular culture as a toxic metal, partly due to the 2000 Oscar-award winning film Erin Brockovich.

In February 2006, the Los Angeles Times reported that the chromium industry and their consulting scientists withheld and skewed data in an attempt to make the findings more favorable to industry.¹ This article cites a report written by Michaels et al., published in Environmental Health: A Global Access Science Source, which closely examined the questionable activities of scientists working for the industrial consulting firm, ENVIRON. Both resources suggest that the unchanged data showed that workers exposed to any level, even low levels, of chromate were dying from lung cancer

Chromium (VI) is classified as a human carcinogen by the National Toxicology Program and the International Agency for Research on Cancer, and has been documented as a cause for lung cancer for over fifty years. Given this knowledge, the U.S. federal agency that regulates the workplace—the Occupational Safety and Health Administration
(OSHA) — casually set a standard permissible exposure limit (PEL) of 52 micrograms of chromium per cubic meter of air for an 8-hour time-weighted average. This limit was based on a standard level which had been suggested in 1971. In 1993, a petition to lower the level of acceptable exposure was submitted to OSHA by the Oil, Chemical, and Atomic Workers International Union (OCAW) (which is now a part of the United Steelworkers).\textsuperscript{2} This request resulted in two lawsuits. Both challenged OSHA’s delay (which was felt to be unreasonable) in implementing a higher standard. The chromium industry-backed OSHA in this. On April 2, 2003 the U.S. Court of Appeals for the Third Circuit ordered that the final rule on the reduction of occupational exposure be decided by January 18, 2006.

In 2004, OSHA suggested that the standard of 52 micrograms of chromium per cubic meter of air be lowered to 1 microgram. The OSHA proposal received negative comments from industry, despite the benefits that lower to this level would provide for workers health. Industry groups claimed that making this change would “bankrupt businesses and cost the metal-finishing industry $380 million annually.”\textsuperscript{3} Industry representatives also challenged the new standard with the findings of a 2002 study.

As Michaels \textit{et al}\textsuperscript{2} explain, industry consultants working for ENVIRON conducted a 2002 study in four separate plants, two German and two in the U.S. In the proposal for the study, the scientists said that they would look at the results from the four plants as a single group. This would give their report significance through the combined cohort. Upon completing the study and discovering that the combined results indicated elevated cancer risks at low levels of exposure, the scientists changed their tune and
submitted the data in two separate pieces, separating the plants by country. In making this change the scientists were able to manipulate their study in a manner that allowed for them to disguise the cancer rate. The separated results, which indicated no cancer link in the U.S. plants and elevated cancer risk in the German plants, were presented to OSHA in an attempt to show that the recommended strict standard was unwarranted. The results of this study were never published together, but instead were only published as separate documents. 2 The fact that both the scientists and the industry representatives were willing to alter results in their benefit brings forth the question of ethics and vested interests. Michaels et al. suggest that industry scientists were looking out more for the bottom line than for human health and well-being.

During the 2004-2005 rulemaking proceedings, OSHA requested information like the data collected by in the ENVIRON study, yet those researchers choose once again to not release their information in one report. 2 Instead of protecting their employees and the employees of other companies working with dichromate, the chromium industry chose to use altered results, to reduce its regulatory burden. The honesty of researchers is counted upon in making decisions, as “many U.S. regulatory agencies, including the EPA and the Food and Drug Administration (FDA) rely heavily on unpublished studies, submitted by study sponsors, in reaching regulatory decisions.” 2 If consultants choose not to be truthful in the results they share, they hurt more people than they help financially.

Situations like this are not unique to the chromium industry, as examples are found in many industrial settings. The Michaels et al. report challenged what they referred to as the faulty, biased handling of a cancer study by ENVIRON. Michaels, the
director of the Project on Scientific Knowledge and Public Policy at George Washington University’s School of Public Health, said in an February 2006 interview, “This was a 10-year campaign to shape the science to fit the industry’s agenda rather than shape the regulation to fit the science,” ¹. In response to Michaels’ assessment, Joel Barnhart the vice president of technical issues at Elementis Chromium’s plant in Corpus Christi, Texas said, “I would categorically say there was not an orchestrated effort to hide anything. What I can say is that it certainly may have not been handled well.” His lawyerly words suggest that he is attempting to avoid criminal liability by suggesting that the actions taken were not done so in an attempt to cause harm, or deceive anyone. In stating that it was not “handled well,” he is suggesting that it was nothing more than an act of mismanagement and negligence. ³ By carefully choosing his statement he makes it appear that there was not a criminal act committed.

At the end of their report, Michaels et al. report recommend that: “Parties in regulatory proceedings should be required to disclose whether the studies they submit were performed by researchers who had the right to present or publish their findings without the sponsor’s consent or influence.” ² This would allow the agencies to weigh the submitted information accordingly.

On May 30, 2006 the new Permissible Exposure Limit (PEL) set by OSHA went into effect. Significantly lower than the prior PEL of 52 µg/m³, the new rule limits exposure of workers to 5 µg/m³ over an 8-hour time-weighted average. For the companies that feel they cannot meet this new standard OSHA requires a compliance alternative. Not only did the new rule significantly lower the amount of chromium that workers can be exposed
to, it also called for a number of changes for worker protection. These changes include requirements for “exposure determination, protective clothing and equipment, hygiene areas and practices, medical surveillance, record keeping, and start-up dates that include four years for the implementation for engineering the controls to meet the PEL.”

The connection between the development of illness and cancers following continuous related chromium (VI) exposure has been known for over 50 years.² The best estimates of cancer cases due to industrial chromium exposure suggest that eight cancer cases occur for every one hundred workers, who have worked 40 years.⁴ The recent changes in PEL should help to lower the number of these cases, but it is likely that all the cases will not be eliminated. Further research into the biological details of chromium cancers is needed, as well as enforcement of the new allowable exposure levels.
Chapter II: Chemistry of Chromium: an Introduction into the Nature, Biological Role, and Toxicity of Chromium

Chromium is a biologically important metal that is commonly found in II, III, and VI oxidation states, although transients states, such as IV and V also exist. Chromium II is strongly reducing and is easily susceptible to air oxidation. As detailed in chapter one, two of these states, VI and III, are known within popular culture, due to news coverage and the film Erin Brockovich. Chromium (III) will be discussed further, as it is an important as a dietary supplement in metabolism.

Chromium (VI) — now recognized as a hazardous, carcinogenic— can only be understood clearly through further studies of chromium’s various oxidation states. Cr (VI), as well as Cr (III) are known to be the products of the disproportionation of both transient species, chromium IV and V. Chromium (V), as an intermediate of chromium (VI), has been suggested to be a major player in chromium related cancers.

Glucose Tolerance Factor and Chromium (III)

Trivalent chromium is the most stable oxidation state of chromium and has the largest number of known coordination compounds. It is an essential part of human and animal diets. Chromium (III) is known to be an essential co-factor in the metabolism of glucose and is the active component of the glucose tolerance factor (GTF). The GTF plays a role in binding insulin to receptor sites on membranes. Although this form of chromium is essential to the body in small amounts, large amounts can be harmful and potentially fatal. Although, many aspects of the GTF have yet to be bio-chemically
defined, it has been suggested that the GTF plays an important role in insulin activity, but this too remains undefined. 6

Chromium Toxicity

Hexavalent chromium is a known carcinogen when ingested in large amounts. Cr (VI) is easily ingested by workers in industrial settings, through inhalation or contact. People have also been exposed to Cr (VI) through contaminated food and water. 5 It is a well-established cause for human lung cancer and is potentially widespread due to its solubility in water.

Exposure to chromium (VI) primarily occurs in industrial settings. The predominated chromium compound used in the chrome-industry is sodium dichromate (Na₂Cr₂O₇). At a physiological pH of pH 7, this chromium compound becomes CrO₄²⁻, which is the form that often enters cells through the sulfate transport channel 6. This system is an anion channel that allows for sulfate to pass into the cell. This entry becomes possible because the molecule that usually passes through this system is SO₄²⁻, which is comparable to CrO₄²⁻ in size and charge. The sulfate transport channel provides the opportunity for chromium molecules to enter the cells and mitochondria of a number of cells in mammals. Insoluble chromium compounds, which cannot use the anion channels, enter the cell through phagocytosis. 7 Upon entering the cell, Cr (VI) can cause direct DNA damage. Upon its entering the cells the chromate is reduced by glutathione, ascorbic acid, or cysteine. 6 The intracellular reduction of Cr (VI) to Cr (III) forms reactive Cr (V), Cr (IV), along with other free radical intermediates, all of which are capable of causing DNA damage. Stabilization of Cr (V) and Cr (VI) within the cells can also occur, with glutathione and ascorbate being the most likely cellular reductants. 7
The tripeptide, glutathione (GSH), is found in concentrations up to 10mM intracellularly, and is an essential antioxidant within the body. GSH is found within and outside of the cells and is homeostatically controlled, with its highest concentrations found in the liver. Its functions include involvement in catalysis, metabolism, transport, and detoxification. The interaction between chromium and glutathione in the body is central to being able to understand chromium toxicity, as has been indicated by a number of past experiments. One such experiment looked at the effect on chromium toxicity in chick embryo hepatocytes when GSH was induced into the cells. The results of the study indicated that GSH played an important role, as the total number of DNA strand breaks was found to increase in the cells where the GSH had been added.

Although Cr (VI) is a known human bronchial carcinogen, its mechanism of action is not clearly understood. Cr (VI) is often reduced by glutathione within the body, creating free-radicals or other reaction species including Cr (V). Many previous studies have suggested that the Cr (V) intermediates, formed from interactions between Cr (VI) and GSH, play a direct (and major) role in DNA cleavage.

As will be discussed, the present research was focused on gaining further understanding of the disproportionation and reactivity of synthesized chromium (V)-glutathione molecule in a laboratory setting. The importance of the interaction between chromium and glutathione within the body are known and has been studied, but the details of the chemistry still remain unclear, with many unanswered questions. The Cr (V)-GSH complex is not likely to be formed in large quantities in vivo, as the reduction of Cr (VI) with GSH in the presence of D-glucose or other 1,2-diolatoligands predominantly result in the formation of Cr (V)-carbohydrate complexes. Nonetheless,
the Cr (V)-GSH is formed initially during Cr (VI) intercellular reduction and are important potential sources of reactive species that should be examined.
Chapter III: Chemistry of Chromium (V):

Structure and Reactivity of Chromium (V) – Glutathione Complex

Hexavalent chromium is a potential carcinogen and an occupational hazard in industries (for example, chrome dye, welding and leather tanning). Higher levels of chromium (VI) are responsible for chromium dermatitis, although every oxidation state of chromium is known to be able to react with nucleic acids and proteins. Chromates enter into the cells via the sulfate transport channel and are reduced to genotoxic chromium (V) by a large number of intracellular reductants, such as citrate, lactate, ascorbate, hydrogen peroxide, and glutathione.

Ligand exchange reactions are a prerequisite for electron transfer in the oxidation of organic substrates by chromium (V). As chromium (V) has been identified to be the ultimate carcinogen or the primary species acting in chromium related cancers, ligand exchange studies at the chromium (V) center are critical to the understanding of chromium genotoxicity.

The tripeptide glutathione (GSH, γ-Glu-Cys-Gly, LH5, Figure 1) is found both inside and around cells, in concentrations up to 10 mM intracellularly, with the highest in the liver. Glutathione exists in the body in two forms: the reduced form, or antioxidant, which is used in this study, and in the oxidized form known as glutathione disulfide. In the body, GSH has functions during catalysis, metabolism, amino acid transport, and detoxification. GSH is the central molecule in the body’s antioxidant system. As the antioxidant system is the body’s premier source of protection against free radicals and other oxidative stressors, understanding the reaction that occurs between
Cr (V) and GSH to the essential in understanding chromium genotoxicity. 14

**Figure 1: Structure of GSH**

Since Rocek and colleagues discovered bis [2-ethyl-2-hydroxybutanoato (2-) oxochromate (V), [CrVO(ehba) 2]- (Figure 2, 1a) and bis [2-hydroxy-2-methylbutanoato(2-) oxochromate (V), [CrVO (hmba) 2] – (Figure 2, 1b), 15 these complexes have served as model systems for studies of the structure and reactivity of Cr (V) complexes, as well as the mechanism of chromium genotoxicity and chromium-induced cancer. However, the above chromium (V) models have several limitations. As neither ehba (2-ethyl-2-hydroxybutanoic acid) nor hmha (2-hydroxy-2-methylbutanoic acid) is thought to have any cytosolic or mitochondrial biochemistry, their ability to cause in vitro DNA damage may not be physiologically relevant.

**Figure 2: Structure of Chromium (V) complexes with ehba and hmha**

In the most comprehensive structural study of the chromium (V) –glutathione complex to date, the green chromium (V)-GSH complex was shown to involve thiolato
and amido (deprotonated) bonding to the Cr (V) center. The assigned structure (Figure 3) is consistent with EPR, ES-MS, XAFS and XANES data. 7

**Figure 3: Structure of Cr (V)-GSH**

![Diagram of Cr (V)-GSH](image)

The present investigation is concerned with a detailed study of the decomposition of the green Cr (V) complex as a function of the ligand concentration, pH and buffer concentration. The Cr (V)-citrate complex, \([\text{Cr}^V\text{O}(\text{caH}_2)_2]\), first reported by Regis University students, 16 and the relatively stable \([\text{Cr}^V\text{O}(\text{ehba})_2]\), 7 have been studied as a point of comparison for the Cr (V)-GSH complex.

**Materials and Methods**

The known chromium (V) complexes with ehba and hmba were prepared according to the literature methods that can be found in Krumpolc et al. 7 The chromium (V)-GSH complex was synthesized as follows: 7.7g of glutathione (25 mmol) was dissolved in 15 ml of deionized (D.I.) water and titrated to pH 7.0 using 3 M NaOH and brought to a total volume 25 mL using D.I. water. An aqueous solution of sodium chromate (2.5 mmol) was then made by dissolving 0.410 g in 25mL of D.I. water. The
GSH solution was mixed with aqueous sodium chromate solution. The reaction mixture was allowed to stand for 3 minutes. A total of 100 mL of ice cold MeOH was added to the solution. Some precipitate was noted immediately. The reaction mixture was then stored in a deep freeze for 48 hours. The resulting green complex was then collected by suction filtration, dried and stored in a dessicator at 4°C. A Cr (V)-citrate complex was also made according to published methods. 16

The structure and reactivity of these chromium (V) compounds have been studied through the use of EPR, mass Spectra and UV-Vis-spectroscopy.

**EPR Studies**

X-band EPR spectra of ligand-exchange reactions and solid-state EPR spectra were run on a Varian E9 spectrometer. One mm melting point capillary tubes were used for solution spectra, while solid-state EPR spectra were determined using quartz tubes. The magnetic field modulation frequency was 100 KHz. The uncertainty in g values is about +/- 0.0005. The Cr (V) content of the samples is quantified by comparing the normalized EPR double integral to that of a gravimetrically prepared solution of tempone (Aldrich Chemical, 99%). Ligand exchange reactions initiated by dissolving the complexes in different buffers were examined.

**Spectrophotometric Studies**

A Beckman DU-600 UV-visible spectrophotometer equipped with thermostatted cuvette holders was used to study the complexes. Kinetic studies of the Cr (V)-GSH complex decomposition at neutral pH in a 0.1 M phosphate buffer (pH 7.00; 0.1 M total phosphate; ionic strength 0.22) was monitored for evidence of decomposition at both 372 nm and 610 nm.
Sulphydryl Studies

The total sulphydryl groups in the Cr (V)-GSH complex was determined using Ellman's reagent and a modified version of Ellman's method described in Sedlak and Lindsay. Ellman's method is based on the reduction of 5,5'-dithiobis-(2-nitobenzoic acid) (DTNB, Ellman’s reagent) by SH groups, which forms 1.0 mole of 2-nitro-5-mercaptobenzoic acid per mole of SH. The reaction is shown below:

![Reaction Diagram]

The 2-nitro-5-mercaptobenzoic acid has an intensive yellow color which can be used to measure SH concentration using UV-Vis spectroscopy.

Two standard calibration curves were created, one using DL-cysteine and one using GSH. Standard 2.0 * 10^{-4} M solutions of DL-cysteine and GSH were made using 0.02 M EDTA (the GSH solution was first bubbled in nitrogen), from which aliquots were taken to obtain the desired concentrations (from 1*10^{-6} M to 9*10^{-6} M in 10 mL total volume) within the reaction solutions. All solutions were maintained at a pH of 8.0 or above. The reaction mixtures were made in 15.0 mL test tubes containing 1.5 mL of 0.2 M Tris Buffer pH 8.2, 0.1 mL of 0.01 M DTNB (in MeOH), and a combination of 2.0 * 10^{-4} M stock solution of [Cys] or [GSH] and 0.02 M EDTA totaling 0.5 mL. These were added to achieve the desired concentration of Cys or GSH in a 10 mL sample. The
mixture was then brought to 10.0 mL using 7.9 mL of methanol (MeOH). The tubes were then covered and allowed to react for 30 minutes. The absorbance of each solution was then taken at 412 nm in 1 cm cuvettes. The pH of the reaction mixture was maintained at a minimum of pH of 8.0 through the readings to allow for optimal color.

Two sets of reaction mixtures were made at varying concentrations of Cr (V)-GSH. The first set was made according to the following procedure, with the volume of 0.02 M EDTA and aliquot of Cr (V)-GSH stock solution used, varying according to desired concentration, and always totaling 500 μL.

A typical solution preparation: A reaction mixture containing

\[ 7.0 \times 10^{-6} \text{ M Cr (V)-GSH} \]

1.5 mL of 0.2 M Tris Buffer (pH 8.2), 0.1 mL of 0.01 M DTNB and 150 μL of 0.02 M EDTA were added to a 15 mL test tube. The \(2.0 \times 10^{-4}\) M stock solution of Cr (V)-GSH was then prepared, bubbled. A 350 μL aliquot of Cr (V)-GSH stock solution was then placed in the test tube containing the reaction mixture. The solution was then brought to a total volume of 10 mL using MeOH. The color was then allowed to develop for 30 minutes.

The second set was prepared without the use of Ellman’s reagent, replacing it with 0.1 mL MeOH to maintain the total volume of 10 mL. The second mixture was observed at 372 nm, to allow for the detection of the Cr (VI) species in the solution.

**Results and Discussion**

The EPR signal of the Cr (V)-GSH complex was essentially lost within the time of mixing and transfers into the EPR cavity. The green complex (Figure 3) was relatively more stable in deoxygenated solutions containing 10-50 mM of glutathione (pH 6.8-7.0). At 50 mM GSH, the dominant EPR signal has a g value equal to 1.986, while in 10 mM
GSH, two Cr (V) signals were observed. In addition to the g = 1.984 signal, a second, sharp Cr (V) species (minor) was observed (g = 1.993) (Figure 4).

**Figure 4: X-Band EPR Spectra Cr (V)-Glutathione Complex in pH 6.8 GSH Buffer (50mM)**

Both the Cr (V) species rapidly decayed within 10 minutes of dissolution in aqueous GSH solutions. In aqueous solution (pH 6-8), the green complex showed two well-defined and relatively stable EPR signals (g = 1.984 and 1.993; Figure 2). The green complex was relatively more stable in deoxygenated solutions containing 10-50 mM of glutathione (pH = 6.8 – 7.0). Kinetics of the decay of chromium (V) were followed by collecting 7-8 spectra during a 10 minute period. First order rate constants were estimated from the ln (height of the EPR signal or the normalized integrals) versus time plots. As the disproportionation reaction of chromium (V)

\[ 3 \text{Cr}(V) \rightarrow 2 \text{Cr}(VI) + \text{Cr}(III) \]

could have a potential second-order dependence on Cr (V), second order kinetic plots were obtained by plotting [1/height of EPR signal] versus time. The first order plots (Figure 5) were consistently better (Correlation coefficient of 0.9945) than the second order plots (Figure 6).
When the Cr (V)-GSH complex was dissolved in ehba buffer (0.1M, pH 3.5), the Cr (V)-GSH signal was lost within the time of mixing (2 minutes) and a new stable signal appeared at g =1.976 which is characteristic of the $\text{[Cr}^\text{V}O(\text{ehba})_2\text{]}^+$ complex (Figure 7).

$$\text{[Cr}^\text{V}O(\text{GSH})_2]^+ + 2\text{ehba} \rightarrow \text{[Cr}^\text{V}O(\text{ehba})_2\text{]}^+ + \text{GSH}^-$$
In a typical experiment using a 25 mM solution of Cr (V)-GSH, approximately 1.15 mM (a typical calculation is shown below) [Cr^V(Oehba)]^− was quantified. This means that the decomposition of Cr (V)-GSH is faster than the ligand exchange with ehba. It is interesting to note that in the work of Levina, et al., there was a near stoichiometric ligand exchange with ehba (50% [Cr^V(Oehba)] and 50% Cr (III)). Interestingly, it was observed that the reaction mixture (resulting from ligand exchange) in the EPR capillary tube is more stable than reaction mixture left outside. It may well be due to some oxygen involvement, which merits further investigation.

A typical calculation is shown below.

\[
\text{Normalized integral for Tempone / 1.25mM} = \frac{\text{Normalized integral for [Cr^V(Oehba)]}}{X \text{ concentration}}
\]

The decomposition of Cr (V) may involve competing disproportionation and ligand oxidation

\[3 \text{ Cr (V)} \rightarrow 2 \text{ Cr (VI) + Cr (III)}\]
In order to determine the mechanism of Cr (V) decomposition versus ligand exchange, we studied the kinetics of the decomposition reaction by Uv-Vis. Spectroscopy (630 nm). Even though the kinetic fits were equally satisfactory for first-order (plot of log $A_{630\,\text{nm}}$ versus time) (Figure 8) and second-order ($1/A_{630\,\text{nm}}$ versus time) (Figure 9), the decomposition reactions became increasingly faster at higher initial concentration, pointing to a second order pathway.

**Figure 8: First Order Plot of Cr (V) decay monitored at 610 nm**

[42 mM Cr(V)-GSH in pH 7.00 Phosphate Buffer (0.1 M)]

![First Order Plot](image)

**Figure 9: Second Order Plot of Cr (V) Decay Monitored at 610 nm**

[42 mM Cr(V)-GSH in pH 7.00 Phosphate Buffer (0.1 M)]

![Second Order Plot](image)
The calibration curves for DL-cysteine (Figure 10) and GSH (Figure 11) both proved to be fairly linear with R² values of 0.9957 and 0.9853.

A sample of the absorbance readings taken for varying concentrations of Cr (V)-GSH are outlined in Figure 12. Using the equation of the line from the GSH calibration curve (y = 0.1393x + 0.0306), the concentration of GSH within the reaction mixtures was
determined using the absorbencies taken at 412 nm. The concentration of GSH within the solutions is outlined in Figure 12.

**Figure 12: Calculated GSH and Cr (VI) Concentrations**

<table>
<thead>
<tr>
<th>Concentration of Cr(V)-GSH in Starting Solution</th>
<th>Absorbance at 412 nm (Ellman’s Reagent Present)</th>
<th>Concentration of GSH</th>
<th>Absorbance at 372 nm (no Ellman’s Reagent)</th>
<th>Concentration of Cr(VI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>7 X 10^{-6} M</td>
<td>0.2799</td>
<td>1.79 X 10^{-6} M</td>
<td>0.0349</td>
<td>7.27083 X 10^{-7} M</td>
</tr>
<tr>
<td>5 X 10^{-6} M</td>
<td>0.1649</td>
<td>9.64 X 10^{-7} M</td>
<td>0.0201</td>
<td>4.1875 X 10^{-7} M</td>
</tr>
<tr>
<td>3 X 10^{-6} M</td>
<td>0.1597</td>
<td>9.27 X 10^{-7} M</td>
<td>0.0158</td>
<td>3.29167 X 10^{-7} M</td>
</tr>
<tr>
<td>1 X 10^{-6} M</td>
<td>0.0649</td>
<td>2.46 X 10^{-7} M</td>
<td>0.0078</td>
<td>1.625 X 10^{-7} M</td>
</tr>
</tbody>
</table>

*Concentration of Cr (VI) was calculated using Beer- Lambert Law and an Extinction Coefficient of 4.8 * 10^4 M^{-1} cm^{-1} at 372 nm.

Since there are two GSH molecules for every one Cr (V) molecule in the starting Cr (V)-GSH complex (Figure 3), if complete disproportionation was occurring the concentration of GSH would be anticipated to be twice that of the starting Cr (V)-GSH concentration. As can be seen in Figure 12, this was not the finding of our experiment. Instead, the studies revealed the presence of only 9-15% the expected concentration of GSH. This finding suggests that majority of the GSH is being converted to the disulfide, GSSH, prior to the readings. The Cr (VI) present as a result of disproportionation was comparable with that found for GSH, with 12-24% of the expected Cr (VI).

When comparing the concentration of GSH determined to be in each solution with the concentration of Cr (VI) in the same starting concentration of reaction mixture, a relationship of 1.5 to 2.8 moles of GSH for every 1.0 mole of Cr (VI) was found. This finding is somewhat consistent with that of previous studies which found that there was 2.0 moles of GSH for every 1.0 mole of Cr (VI). 7
In the recent work of Lay et al, the decomposition kinetics (monitored at 630 nm) of the green chromium (V) complex in water could be fitted to two consecutive first-order processes. Interestingly, the decomposition of oxochromium (V) complexes of hydroxyl-carboxylic acids\textsuperscript{15} is second order in chromium (V), consistent with the disproportionation reaction:

$$3\text{Cr (V)} \rightarrow 2\text{Cr (VI)} + \text{Cr (III)}$$

In our work, both EPR and UV-Vis. kinetics of decomposition of the Cr (V)-GSH complex (in pH 7 phosphate buffer) could not unequivocally establish first-order and second order pathways. As in the pH range 8.0-8.5, disproportionation of chromium (V) is the dominant pathway; we are presently investigating the decomposition kinetics.
Afterward: A Reflection on My Time Spent with Chromium

As I look back over the last few years of research, I realize that the time that I spent working with Dr. Mahapatro and other research students has given me the opportunity to experience science as a part of life. I have come to understand the importance of persistence, collaboration and community.

In the beginning working independently seemed like a very scary idea. I felt like I was going to mess things up, as I had never previously had the opportunity to do laboratory-type work without supervision. In high school we completed experiments in our various science classes, like chemistry, but always under the close watch of a teacher. I was not confident in my ability to make wise decisions in an independent situation. But, to my surprise, being pushed to work in this manner taught me more than I would have learned otherwise. Instead of being lost and clueless, I searched for answers and procedures. For example, I remember having a procedure question on a day when Dr. Mahapatro was out of town and needing an immediate answer. My first instinct was to give up and go home for the day, but instead I decided to search for the answer to my question and found it in one of my textbooks.

I gained more than just lab experience working with Dr. Mahapatro and the other research students. That time allowed me to develop a comfort zone on campus that I may have not have found otherwise, as I never lived on campus. It also allowed me to make close friends, with whom I came to understand that in science (and the world), we encounter numerous failures for every single success. This lesson came after we conducted a variety of various experiments within laboratory class and research settings; some which were finished very quickly, and others which required two or three hours, or
even an entire days time. All of these experiments were conducted with a goal in mind, like synthesizing a molecule, and time devoted, just to discover that nothing came of the time spent. This is the point where we were faced with the question of whether to quit, or start over, or try again. It is at these moments (and there were many) that I asked myself why I was doing the work in the first place; was it worth it? And it was during one of these more recent moments that I realized it was worth it. Whether I got useable results or not, I have gained so much from this trial and error process. In this work I have gained confidence in my ability to search for answers and to work as an individual within a very interdependent world. This process of trial and error taught me the importance of persistence, which is an important life lesson.

The interplay of independence and interdependence within the scientific community is not something that I came to understand easily. Working independently within a lab setting is important, but I always felt comfort in knowing that I was never truly working alone. In an undergraduate setting research often begins by building on the previous work of others, attempting to answer new questions or to clarify topics and understanding, as was the case in with my research. Specifically, our research team at Regis built on the previous work of O’Brien et al.12, and Levina et al.7 Through publishing, conferences, and interpersonal communication, different perspectives and ideas are shared within the larger community to come to some answers over time.

Second, I have experienced how most scientific understanding is not completed in one place, but is done in pieces and stages, which are later combined to come to a final understanding. In the case of research on Chromium V, we collaborated with Drs. Sandra and Gareth Eaton of the University of Denver. Our collaboration extends further than our
use of their equipment (EPR), to the combination of ideas and answering of questions. We have often come to points where we have questions that we could not answer, but perhaps someone else could.

Having recently attended the annual American Chemical Society conference in Chicago, I have had the chance to see the community of scholars in action. At first the conference was a little overwhelming with its large program book, variety of event venues (some things took place at the conference center, while others were held at the many ACS hotels), and an interesting shuttle schedule. But once I figured out which events I wanted to attend, and what set of shuttles I could use to get there, I was much more comfortable. With a number of talks all occurring at once it was sometimes difficult to decide which to attend, but I found that the various talks and workshops organized specifically for undergrad students provided me the opportunity to watch as ideas and work were shared. The undergraduate section of the conference was organized in a way that allowed for the students to become acquainted with the Chemical community, while learning information that could be useful to them in the future should they choose to move on to graduate school. During the poster session I overheard a conversation between two University professors who were discussing how the conference experience is valuable for students, and that this is something that they want to share with their respective administrations. They stressed that conferences give students the opportunity to meet other students with similar interests and to network with other people within the chemical community who may be important to them later in their career.

Ultimately the question is why does all of this matter? All the time I spent participating in undergraduate research at Regis and working with Dr. Mahapatro has
given me a number of opportunities that I may not have otherwise encountered. So, ultimately, for me, it comes down to the importance of undergraduate research. I feel that it is important for schools to offer programs like this, which allow students to develop an understanding of independence and persistence within an encouraging environment. For me, the encouragement and support that I have found through the research and thesis process has allowed for me to grow both as academically and personally.
References


   <http://criminal.findlaw.com/articles/1383/>  


Appendix I: Pictures

American Chemical Society National Conference
March 2007

Laboratory Research Fall 2006