ABSTRACT

Cobalt(III), Chromium(III), Glutathione, and Their Relevance to the Glucose Tolerance Factor

Matthew E. Naumann

Director: David E. Pennington, Ph.D.

The so-called glucose tolerance factor (GTF) is believed to be a biological complex of chromium that works alongside insulin to enhance the body's ability to metabolize sugar. Chromium and cobalt complexes with glutathione were synthesized as potential analogues to the GTF. These complexes were then analyzed via mass spectrometry and UV-visible absorption for potential clues to the composition and connectivity of the complexes. Upon analysis, the cobalt complex with glutathione appeared to have an uncoordinated sulfhydryl group, consistent with the hypothesis that the GTF interacts with insulin or its receptor via a sulfhydryl-disulfide exchange. Such a complex could theoretically be tested for GTF activity.

APPROVED BY DIRECTOR OF HONORS THESIS:

_____

Dr. David E. Pennington, Department of Chemistry &

Biochemistry

APPROVED BY THE HONORS PROGRAM:

_____

Dr. Andrew Wisely, Director

Date: _____

COBALT(III), CHROMIUM(III), GLUTATHIONE, AND THEIR RELEVANCE TO THE GLUCOSE TOLERANCE FACTOR

A Thesis Submitted to the Faculty of

Baylor University

In Partial Fulfillment of the Requirements for the

Honors Program

By

Matthew E. Naumann

Waco, Texas

May 2012

TABLE OF CONTENTS

ACKNOWLEDGMENTS

This thesis not only represents my work, but also the work of all those who have supported me and stood by my side throughout my time at Baylor.

I would like to thank Dr. David Pennington for his tremendous support and guidance, not only with regards to my thesis work, but also my four years here at Baylor. Since before I even became a college student, he helped to guide me in many of the decisions I would face concerning my college education. I am eternally grateful to him for being my mentor, even through the times when he felt like there weren't enough hours in the day.

I, of course, would also like to thank my parents for their continued support throughout my time in college (and the eighteen years before that). They have done nothing but encourage me to excel in every challenge that is and was set before me. Their support helped me make it through the times when giving up was a very appealing option. I am eternally grateful that God chose to give me such amazing parents, and I look forward to what the future holds for us.

In addition, I want to thank Drs. Sarah-Jane Murray, Sung-Kun Kim, and Sung Joon Kim for agreeing to be members of my defense committee. Their feedback on this work helped me to make it into this final product.

Finally, my thanks go out to all of the other amazing professors I've had the pleasure of knowing during my college education. They have not only given me the intellectual tools I will use for the rest of my life, but they have also shown me new and intriguing ways to look at the world around me.

Now, as I move on to the next chapter of my life, the names of those around me go with me. For it is only because of them that I stand where I do today.

CHAPTER ONE

Introduction

It is a well-known fact that the human body requires essential elements to function properly. Among these are calcium, iron, and magnesium, though there is currently some debate concerning the existence of a role for chromium in the body.[1] Though some continue to question chromium's role, studies have been performed in an effort to search for evidence of chromium's necessity. Levander reported that experimentally-induced chromium deficiency in rats resulted in impaired growth, decreased life expectancy, and hyperglycemia (high blood glucose) among other side effects, indicating that chromium does indeed play some important role in the human body.[2]

In the search for chromium's purpose in biological systems, the 1950s and 1960s produced research in the area concerning the necessity of chromium in rats, as mentioned previously. Mertz, a pioneer in early biologically active chromium research, proposed that the so-called glucose tolerance factor (GTF), a substance containing chromium, plays an important role in the body's reaction to high glucose levels.[3]

Since the discovery of the so-called glucose tolerance factor, researchers have developed several hypotheses concerning the makeup of this important complex. One of these proposed structures suggests that the GTF is composed of the chromic ion, nicotinic acid, glycine, glutamic acid, and cysteine.[1] A second proposal suggests that the GTF is simply made up of the chromium(III) ion and picolinate.[1] Finally, in 2000, John Vincent[1]

suggested possible structures completely different from the previous two, $[Cr_3O(O_2CCH_3)_6(H_2O)_3]^+$ and $[Cr_3O(O_2CCH_2CH_3)_6(H_2O)_3]^+$. He and his colleagues coined the term Chromodulin as a name for this substance.[1]

In addition to the apparent disagreement concerning what the true "glucose tolerance factor" is, there currently exists some uncertainty about the mechanism by which this factor increases the body's tolerance to high levels of blood glucose. In the same article, Vincent proposed that insulin induces the cell's uptake of chromium, thus activating Chromodulin and resulting in the uptake of glucose from the blood.[1] Similar structures of insulin and its receptor, having disulfide bridges in both structures, suggest a possible direct interaction between GTF and insulin or its receptor).

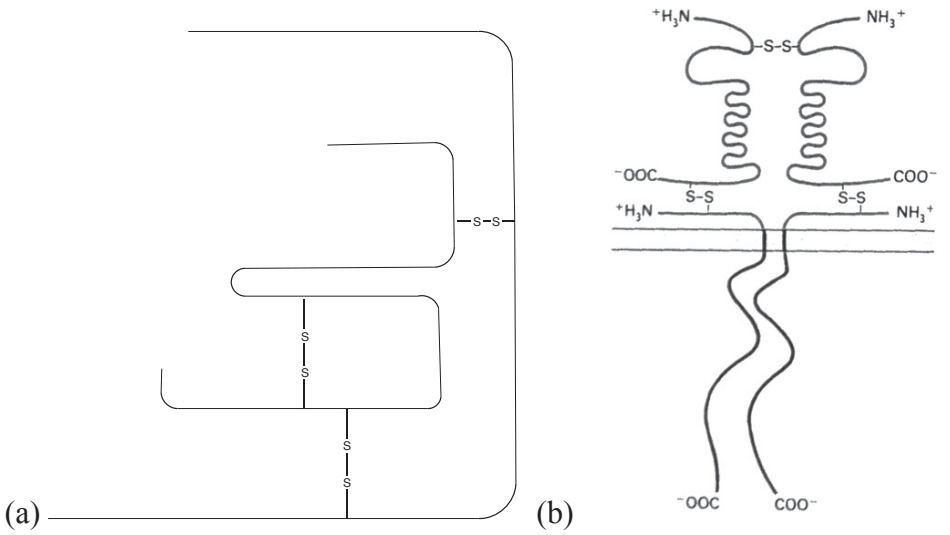
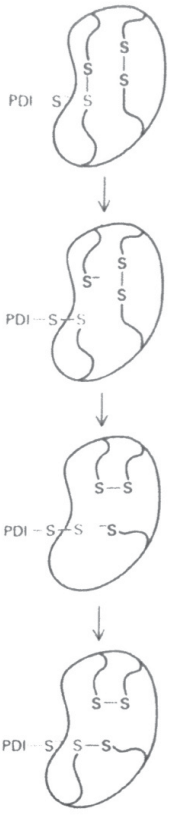
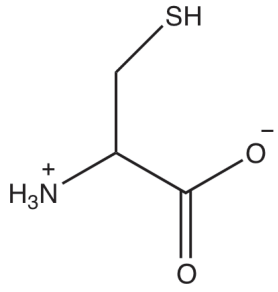


Figure 1 – (a). Simple representation of the insulin protein with disulfide bridges shown. (b). The Insulin Receptor. The portion containing the disulfide bridges is extracellular.[4] (Copyright holder is Cold Spring Harbor Laboratory Press. Used with permission from Axel Ullrich, the primary author of the original source [A. Ullrich, H. Reidel, Y. Yarden, L. Coussens, A. Gray, T. Dull, J. Schlessinger, M.D. Waterfield, and P.J. Parker. *Cold Spring Harbor Symp. Quant Biol.* 51(1986):715.], cited in Fig. 13-52 of Stryer's *Biochemistry, 4th Edition*, W. H. Freeman, 1995, p.352). The disulfide bridges in either the receptor or the insulin protein may be reduced by the GTF.

We hypothesize that the multiple disulfide linkages present in both insulin and its receptor are not coincidental and that an enhancement by a chromium-based glucose tolerance factor could initiate a sulfhydryl-disulfide exchange (see below), a common reaction in biological systems. The GTF's direct interaction with insulin becomes all the more intriguing when considering the proposed structure mentioned earlier, which contains cysteine (see below).



(a)



(b)

Figure 2 – (a) Example of a sulfhydryl-disulfide exchange using protein disulfide isomerase (PDI)[4] (Used with permission from W.H. Freeman and Company; Fig 16-22 of Stryer's *Biochemistry, 4th Edition*, W. H. Freeman, 1995, p.430). (b) Structure of the amino acid cysteine.

As a sulfhydryl-disulfide exchange is commonplace in biological systems, we hypothesize that such a reaction may occur with the GTF. Such a reaction would include

the GTF undergoing a sulfhydryl-disulfide exchange with either insulin or its receptor. This would free up a sulfhydryl group on the protein with which the reaction takes place, allowing insulin and its receptor to undergo a second sulfhydryl disulfide exchange, thereby inducing the uptake of sugar from the blood stream.

A 1985 article by Connett and Wetterhahn[5] postulated the importance of gluthathione (the ligand to be studied in this paper) in relation to chromium. Their paper suggested that glutathione, by coordinating around a chromium(VI) center, serves to shield chromium from the reducing agents present within the human body.[5] Connett and Wetterhahn also assert glutathione's importance as indicated by the fact that it is the most abundant (intracellular) thiol in the human body.[5]

In a 1984 article by Cooper, Blackwell, and Buckley,[6] the GTF activity is measured for several synthetic chromium complexes with amino acids. Of those tested, the most active were $Cr(gly)_n(H_2O)_{6-n}^{3+}$, $Cr(gln)_2(H_2O)_2^{+}$, and a chromium-nicotinic acid-glycine complex of unknown structure. Though some of the amino acids by themselves showed a certain level of GTF activity, said activity was seemingly enhanced by the coordination of the molecules around a chromium center.

A recent article by Maciejewska and Cieslak-Golonka[7] detected the presence of bridged complexes in the synthesis of various chromium complexes with amino acids. It may be that the true GTF is a bridged chromium complex. Whether or not this is the case is unclear, but the possibility of bridged complexes resulting in any synthesis must always be considered, and any data obtained must be interpreted while keeping such possibilities in mind.

Chromium is the most likely metal contained in the GTF, but it should be noted that some studies have suggested the presence of chromium is not essential. In a summary article by Barrett, O'Brien, and de Jesus, a study by Merck is referenced.[8] During Merck's extraction of the GTF, it was found that some fractions that were extracted from yeast contained no chromium(III) but retained a certain level of biological activity.[8] Barrett *et al.* make it a point to draw attention to the fact that this was a yeast extract and may not actually apply to humans.[8] In addition, when one takes into account the previously discussed work by Cooper *et al.*, it becomes apparent that GTF activity may simply be enhanced, rather than generated, through coordination around chromium.

In an article by Freeman *et al.*,[9] a crystal structure for $[Co(en)_2(R)cysS]^+$ is reported. The connectivity of the cysteine residue is asserted to be N,S-bonded to the cobalt center (Figure 3)[9]. If glutathione behaves like cysteine when coordinating to a metal center, then a sulfhydryl-disulfide exchange such as that discussed earlier would be highly unlikely due to the sulfur being coordinated to the metal center and, therefore, unable to undergo such a reaction.

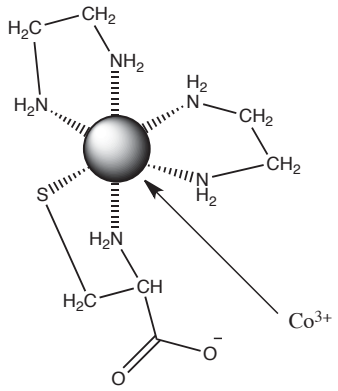


Figure 3 – Structure of the cysteinatobis(ethylenediamine)cobalt(III) cation as determined by X-ray crystallography (after Figure 7 in Freeman *et al*.). In this structure, the sulfhydryl group of the cysteine was observed to coordinate to the cobalt center.

Sulfhydryl coordination has also been observed in some chromium(III) complexes with glutathione (among other ligands). Maciejewska and Cieslak-Golonka[8] reported the successful synthesis of $[Cr_2(\mu\text{-O-Glu})_2(GSH)(OH)_2(H_2O)]\bullet 3H_2O$, $Cr_4(\mu\text{-OH})_6Nic(GSH)_2(OH)(H_2O)_4]$, and $Cr_2(\mu\text{-O-Asp})_2(GSH)(OH)_2(H_2O)]\bullet 5H_2O$. In all of these complexes, the researchers reported coordination by the sulfydryl group to the chromium center as detected spectrophotometrically.

Coordination by the sulfhydryl group has been observed in studies such as the preceding two, but the complexes to be synthesized in this paper used different accompanying ligands. This may result in a differing connectivity around the metal center.

Chromium, though not generally thought of in such a fashion, plays a vital role in the human body. Chromium deficiency can mirror diabetes, resulting in mistreatment and misdiagnosis. Further research to elucidate chromium's role in the body (perhaps via this "glucose tolerance factor") could help the medical community better understand the importance of chromium, possibly resulting in more widely available chromium supplements consisting of chromium clothed with amino acids to increase its

biocompatibility. Because of the importance of chromium, this study will try to model the hypothesized cysteine-containing complex using simpler chromium and cobalt complexes with the tripeptide, glutathione, in order to clarify the hypothesized interaction of the GTF with insulin and/or its receptor.

We expect that a chromium or cobalt complex with the glutathione ligand(s) has the potential to model the so-called glucose tolerance factor. If the molecule is successful in modeling the GTF, we expect that the synthesized analogue(s) will be capable of undergoing a sulfhydryl-disulfide exchange with either insulin or its receptor, mirroring the hypothesized mode of action by which the GTF enhances sugar metabolism in the human body.

CHAPTER TWO

Experimental Procedure

Sources and Instrumentation

The hydrogen peroxide used in the synthesis of $[Co(en)_2Cl_2]Cl$ was purchased from Mallinckrodt Chemicals. The $Cr(NO_3)_3 \bullet 9H_2O$ was purchased from Fluka Chemika. The $[Co(en)_2(CO_3)_2]$ was a complex previously synthesized in the laboratory. Samples were analyzed on an LTQ Orbitrap Discovery mass spectrometer (Thermo Electron, Bremen, Germany) using positive and negative spray ionization (+ESI/-ESI). Full-scan accurate mass spectra of direct infused samples (flow rate, 10 μL/min) were obtained at high resolution (30,000 FWHM) on the Obitrap mass analyzer using internal calibration (accuracy of measurements < 2 ppm) and processed using Xcalibur v.2.0.7 software. Electrospray source conditions were: sheath and auxiliary gas flow 50 and 5 arbitrary units (a.u.), respectively; heated capillary temperature 300 °C; electrospray voltage 4.5 kV for +ESI and 5.0 kV for -ESI; capillary voltage 43 V for +ESI and -43 V for -ESI; tube lens voltage 205 V for +ESI and -148 for -ESI.

Synthesis of Cobalt complex with Glutathione

Synthesis from Carbonatobis(ethylenediamine)cobalt(III) chloride

The reaction was performed at a 1:2:1 molar ratio of reduced glutathione, HCl, and $Co[en_2CO_3]Cl$, respectively. The three reactants were combined in a beaker with approximately 25 mL of DI water, resulting in an almost immediate change in color to a

dark, brick red. HCl was then added dropwise in an attempt to react the remaining solid present at the bottom of the beaker. To remove any H_2O that may have coordinated around the cobalt center, the reaction solution was heated at 50°C for 30 minutes and was subsequently filtered. After one week, the reaction was noted to have turned completely brown, with no red color readily noticeable. The reaction solution was run through an approximately 1.5 cm diameter and 3.0 cm in height column using Sephadex C-25 (setup shown in Figure 4). One compound was observed to pass through the column using only DI water as an eluent. Two other bands were observed to become adsorbed onto the column. A solution of 0.1 M NaCl was then applied to the column resulting in the movement of both initial bands, but leaving a third residual band on the top of the column. The two initial bands were completely eluted with 0.5 M and 1.0 M NaCl, respectively. The residual band at the top of the column could not be removed with a saturated NaCl solution. This is believed to be the result of either reaction with the column or the possible presence of solid in the solution.

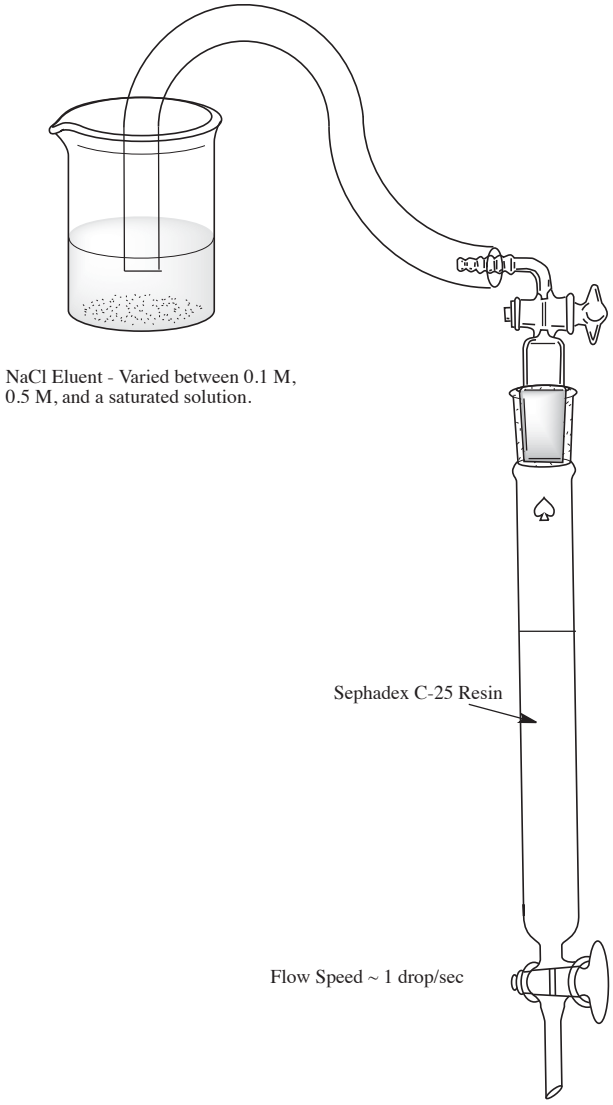


Figure 4 – Column chromatography apparatus used to separate reaction mixture for the synthesis of $[Co(en)_2(GSSG)]^{?+}$; a siphon was set up to provide a continuous flow of eluent.

Attempts to obtain crystals out of the reaction solution resulted in merely a glassy substance remaining on the bottom of the beaker. Once this layer had formed, it was quite resistant to dissolution in any solvent (including H_2O, ethanol, and acetonitrile). What was able to be redissolved in water was then filtered. After non-specific precipitation using ethanol, a solid powder was formed which proved difficult to redissolve.

The mass spectrum that we ran on this compound was unsuccessful, as the solid once again refused to dissolve to an extent detectable by the instrument.

Synthesis of Dichloridobis(ethylenediamine)cobalt(III) chloride

For the synthesis of $[Co(en)_2Cl_2]Cl$, the procedure used was that noted in Dennis Estep's Masters Thesis.[10] Fifty grams of $[Co(H_2O)_6]Cl_2$ dissolved in 50 mL of water was added to 222 mL of 10% aqueous ethylenediamine. Forty milliliters of 30% H_2O_2 was added dropwise, maintaining the reaction temperature within the range of 4-10°C. The reaction was heated at 65°C (approx.) for 20 minutes, after which 100 mL of concentrated HCl was added. The solution was then evaporated with heat and constant stirring until the total volume reached about 150 mL. Once the solution cooled to room temperature, 75 mL of 100% ethanol was added. The solution was then filtered and washed twice with 100% ethanol. The resulting solid was then allowed to air dry. The solid obtained weighed 31.6 g. The compound was then placed in an oven at 130°C to drive off any remaining HCl with no measurable change in mass or color.

Synthesis from $[Co(en)_2Cl_2]Cl$

As an alternate route for the synthesis of our glutathione complex, we also used the compound synthesized above. A 1:1 molar ratio of $[Co(en)_2Cl_2]Cl$ to reduced glutathione was combined in approximately 25 mL of DI water and was heated at 50°C for 30 minutes with stirring. The characteristic brown color was not observed until about 5 minutes of reaction time. The reaction mixture was analyzed by mass spectrometry. It

should also be noted that the solid dissolved much more readily in water than did solid that was obtained from the synthesis from $[Co(en)_2CO_3]Cl$.

The reaction solution was then passed, after filtering through a bed of Celite to remove any solid, through a 1.5 cm x 10 cm column of Sephadex C-25. One substance passed straight through the column using only water as an eluent. Three distinct bands were noted to be adsorbed on the column in the order of brown, pink, and brown. The first brown band and the pink band were eluted with 0.1 M NaCl. The second brown band was then eluted with 0.5 M NaCl. As with the previous column, a residual band was noted at the top of the column and could not be eluted with saturated NaCl solution. Upon running a second column of C-25 (dimensions of 2 cm x 25 cm), the initial band that eluted with water was observed to slow down significantly upon approaching the bottom of the column. Crystals could not be obtained.

Synthesis from $[Co(en)_2Cl_2]Cl$ Under Basic Conditions

To obtain crystals of a cobalt-glutathione complex, the synthesis used was that reported in an article by Liu and Douglas.[11] Reduced glutathione was dissolved in warm water containing an equimolar amount of NaOH. $[Co(en)_2Cl_2]Cl$ was then added to the solution (equimolar amount) at 40°C. Then the mixture was heated over a steam bath for approximately 5 minutes with stirring. The solution was placed in an ice bath until cool, and the resulting solid was filtered off. To the filtrate, a large excesses of NaI followed by 95% ethanol were added to induce crystallization. The resulting solid was filtered off and redissolved in a minimum amount of hot water. However, once the compound came into contact with the air, it was observed to change very rapidly from a dry, brown powder to

a darker brown gunk-like material. Once this change occurred, redissolution proved to be very difficult (as with the solid obtained from the synthesis from $[Co(en)_2CO_3]Cl$).

Though this synthesis was not necessarily successful, it possibly explains the residual bands on top of the columns. These bands were very likely to be the result of solids that formed on the solution/air interface after filtering.

Synthesis of a Chromium-Glutathione Complex from $Cr(NO_3)_3 \bullet 9H_2O$

A chromium complex with glutathione (reduced) was prepared using the method reported by de Meester et al.[12] First, 2.5 x 10^{-3} moles of $Cr(NO_3)_3 \bullet 9H_2O$ and 5.0 x 10^{-3} moles of reduced glutathione were each dissolved in 15 mL of deionized water. The two solutions were then purged with nitrogen. It should be noted that the reaction was performed under nitrogen for the remainder of the time. The two solutions were then combined. The resulting solution (30 mL) was then boiled for 3 minutes. Three pellets of NaOH were then added to neutralize the solution (simple pH paper was used to determine when the solution reached the neutral point). No color change from red-violet to blue was observed as reported in de Meester's experiment. The reaction solution was then repurged with nitrogen overnight. The resulting solution was a light purple color. This solution was then analyzed by mass spectrometry.

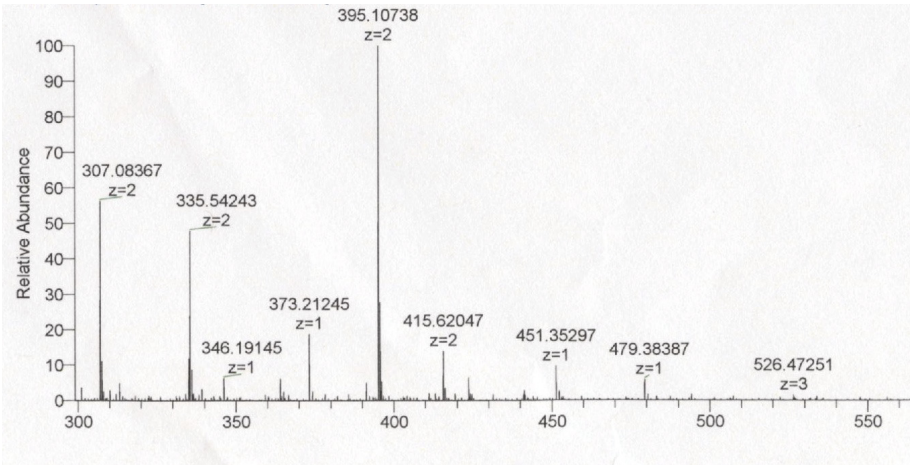
CHAPTER THREE

Results

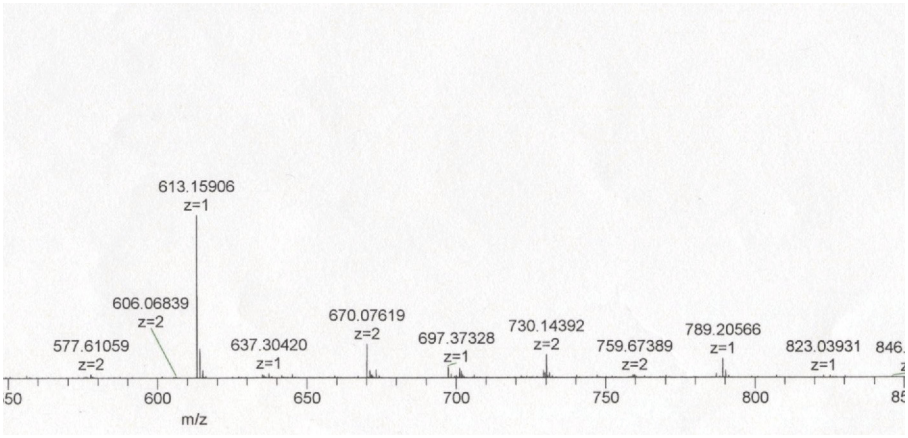
To begin, it is worth discussing the inability to obtain single crystals of any of the synthesized complexes. This is consistent with data reported by Maciejewska and Cieslak-Golonka.[7] In their studies of $[Cr_2(\mu\text{-O-Glu})_2(GSH)(OH)_2(H_2O)]\bullet 3H_2O$, $Cr_4(\mu\text{-}OH)_6Nic(GSH)_2(OH)(H_2O)_4]$, and $Cr_2(\mu\text{-O-Asp})_2(GSH)(OH)_2(H_2O)]\bullet 5H_2O$, no single crystals were able to be obtained.[7] In all cases, the complexes precipitated out of solution to give solids of various colors.

Mass Spectral Data – Co-GSH-ethylenediamine Complex

The mass spectral data of the cobalt complex indicated a peak with an m/z value of approximately 395, with z=2. Further analysis revealed the composition of the substance to be one cobalt, two ethylenediamines, and two glutathiones. Based on the mass detected by the instrument, it is likely that one of the glutathiones became singly deprotonated (most likely one of the carboxylic acids). The peak at m/z=613, most likely corresponds to the singly deprotonated glutathione dimer, giving evidence to redox processes that occurred during the synthesis. Figure 6 below indicates a possible structure for the complex.



(a)



(b)

Figure 5 – Mass spectrum for the cobalt complex with glutathione. The peak at m/z=395.11 is believed to correspond to bis(ethylenediamine)(glutathionedisulfide)-cobalt(III); calculated mass=790.77; observed mass=790.22. The peak at m/z=613.16 is believed to correspond to glutathione disulfide (GSSG) plus one proton; calculated mass=613.64; observed mass=613.16.

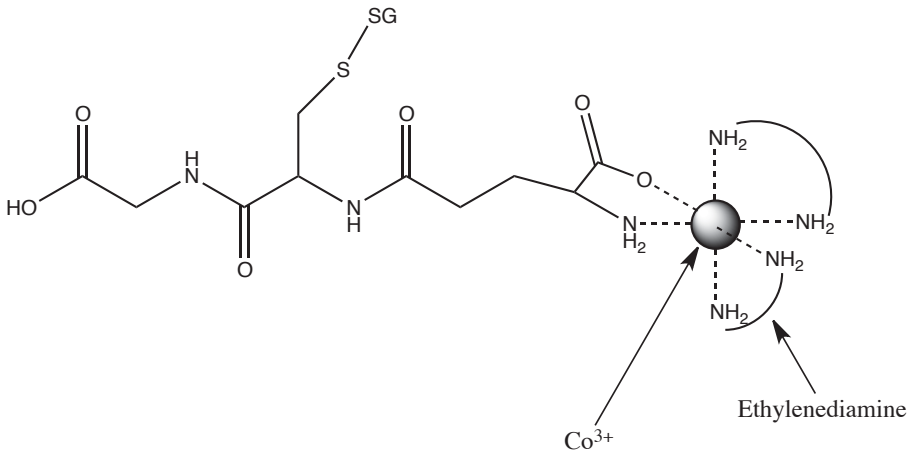


Figure 6 - Possible structure of $[Co(en)_2(GSSG)]^{?+}$ – based on mass spectral data (Figure 5).

The above structure is only one possible structure based on the data. In addition, the connectivity as shown is purely theoretical. At the present time, it is difficult to know with certainty how the glutathione is connected to the cobalt center.

UV-Vis Spectral Data – Co-GSH-ethylenediamine Complex

The UV-Vis absorption spectrum did give some indication as to the potential connectivity around the cobalt center. The spectrum revealed only two apparent shoulders at approximately 353 and 442 nm. These data are somewhat consistent with those reported in Robert Williams' Ph.D. dissertation.[13] He reported peaks around 347 and 486 nm for N,O-bonded aspartate and glutamate cobalt complexes.[13] The differences between this UV-Vis absorption and that obtained by Williams[13] may be due to the difference in the ligands of interest.

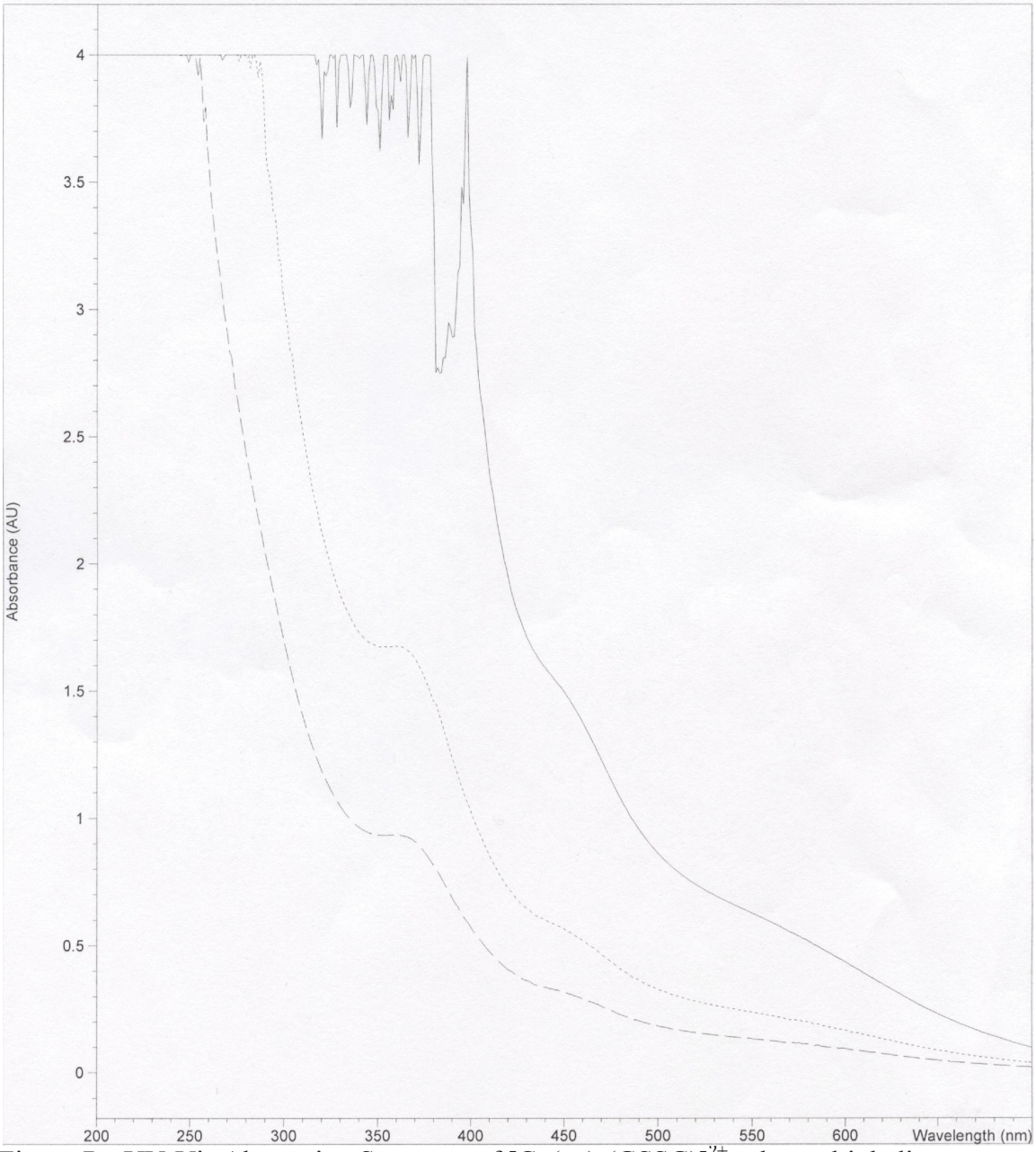


Figure 7 – UV-Vis Absorption Spectrum of $[Co(en)_2(GSSG)]^{?+}$ - the multiple lines represent varying solution concentrations. Shoulders are apparent at 353 and 442 nm.

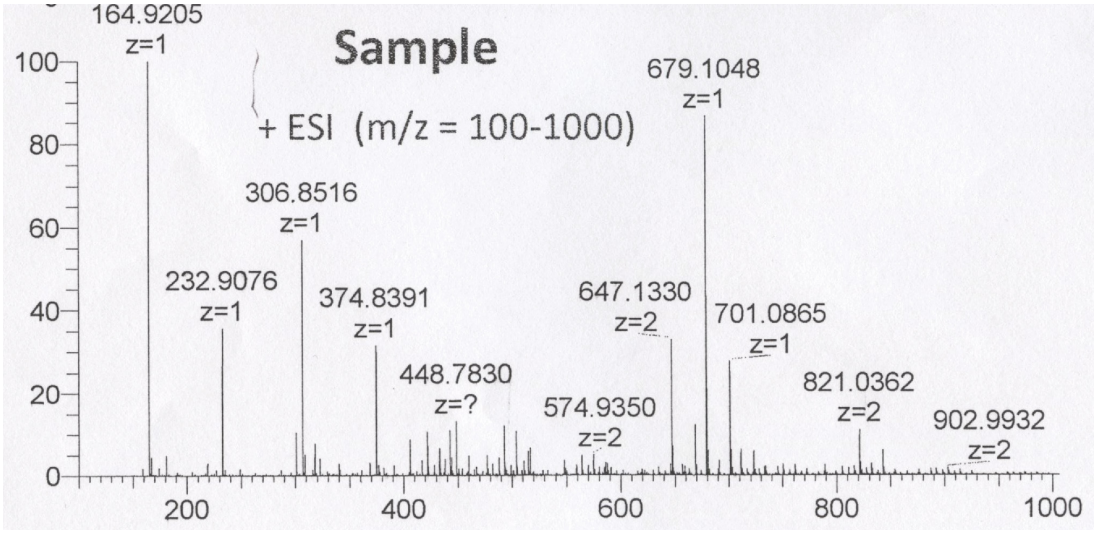
On a side note, the cobalt complex showed some strange properties. During a second synthesis using the same method as Liu and Douglas[11], the solid was filtered and washed with ethanol. Once the ethanol passed through the filter, the remaining solid demonstrated a noticeable change in properties over the course of about 15 seconds. The

previously brown powder rapidly became a thick, brown, sticky substance (as observed in a previous experiment that attempted recrystallization through slow evaporation of the solvent). Though the cause of the change is as of yet unknown, one possible explanation may be the sensitivity of the glutathione ligand to oxidation by atmospheric oxygen.

There is also evidence for further oxidation of glutathione by the Co(III) present in the sample. The light pink band that eluted from the column indicated the very probable presence of Co(II) in solution. This would also explain the strong presence of the uncoordinated glutathione dimer in the solution (mass spectrum; m/z=613.15) as well as the mass spectral results that imply coordination of the dimer by the $Co(en)_2^{3+}$ moiety. It is not clear whether the glutathione first coordinated to $Co(en)_2^{3+}$, replacing the two chloride ions, and was then oxidized, or whether $Co(en)_2^{3+}$ coordinated the GSSG species directly.

Mass Spectral Data – Cr-GSH Complex

(a)



(b)

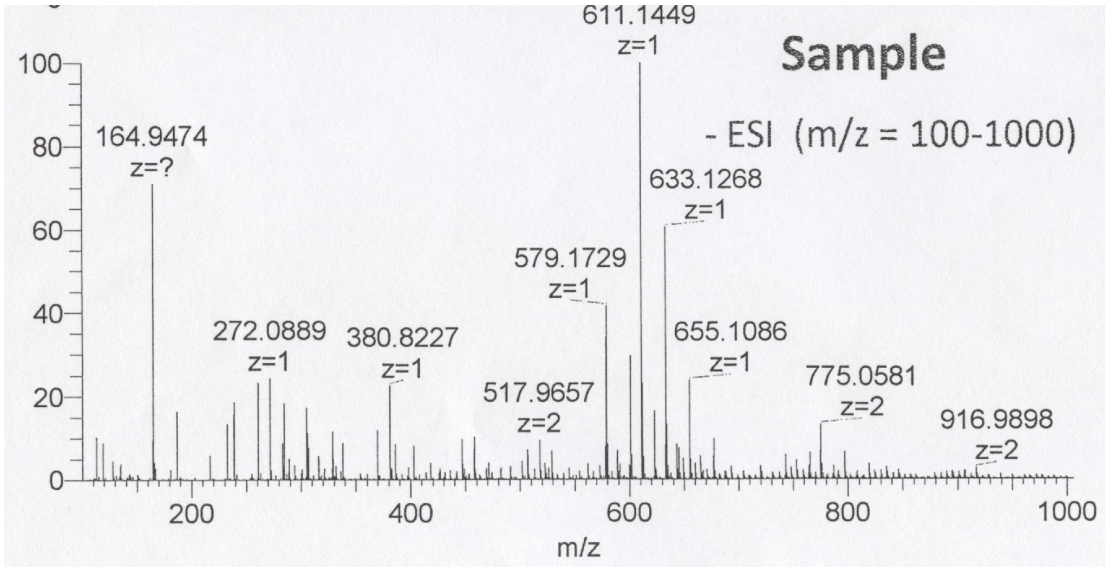


Figure 8 – Mass Spectral Data for the Synthesis from $Cr(NO_3)_3 \bullet 9H_2O$. None of the peaks could be assigned to a possible chromium complex with glutathione.

The mass spectral data for the chromium complex with glutathione gave no indication as to the compound that was synthesized. Analysis of the spectra revealed no peaks that could reasonably be expected based on the reactants used in the synthesis. There are two possible explanations. First, it may be the reaction was unsuccessful in

forming a glutathione complex. Second, it is possible that the complex was not robust enough to withstand the treatment it received within the mass spectrometer. However, due to the color change that was observed during the reaction, one would think that the reaction was successful. Which explanation is the correct one is difficult to determine without further analysis for which there was not sufficient time.

CHAPTER FOUR

Conclusion

We have successfully synthesized a potential cobalt analogue to the chromium-containing glucose tolerance factor. Mass spectral and UV-Vis spectral analysis suggested that the sulfhydryl group of the cysteine residue in glutathione remains free upon glutathione's coordination with the metal. Our synthesis resulted in the oxidation of the sulfhydryl group to form the glutathione dimer. However, the resulting disulfide bridge could be selectively reduced to give a sulfhydryl group capable of interacting with either insulin or its receptor.

The synthesis of a chromium complex, on the other hand, was slightly more elusive in terms of its success. Though a color change was observed upon the mixing of the reactants, data obtained on the complex was no help whatsoever in determining its composition or structure.

In all syntheses, obtaining crystals of the complexes was not achieved. Perhaps a crystallization method other than those attempted would be capable of achieving the desired goal. Crystal structures of the complexes would be highly valuable in terms of determining the connectivity and composition of the complexes that were synthesized.

Other than obtaining crystals of the complexes, the next step in this experiment would be to reduce the disulfide bridge in the glutathione dimer of $[Co(en)_2(GSSG)]^{?+}$ (as was previously mentioned) in order to test the complex's GTF activity. This could be tested against the oxidized form to lend support to the hypothesis that GTF interacts with either insulin or its receptor via a sulfhydryl-disulfide exchange.

It is our hope that a better understanding of how the GTF plays a role in sugar metabolism will, in a more broad sense, help with our understanding of how the human body reacts to high levels of blood sugar.

REFERENCES

1. Vincent, John B. Elucidating a Biological Role for Chromium at a Molecular Level. *Acc. Chem. Res.* 2000, *33*, 503-510.

2. Levander, O. A. Selenium and Chromium in Human Nutrition. *Journal of the American Dietetic Association*. 1975, *66*, 338-344.

3. Mertz, W.; Schwarz, K. Relation of glucose tolerance factor to impaired intravenous glucose tolerance of rats on stock diets. *J. Physiol.* 1959, *196*, 614-618.

4. Stryer, L. 1995. *Biochemistry, Fourth Edition*. New York: W. H. Freeman and Company.

5. Connett, P. H.; Wetterhahn, K. E. In Vitro Reaction of the Carcinogen Chromate with Cellular Thiols and Carboxylic Acids. *J. Am. Chem. Soc.* 1985, *107*, 4282-4288.

6. Cooper, J. A.; Blackwell, L. F.; Buckley, P. D. Chromium(III) Complexes and their Relationship to the Glucose Tolerance Factor, Part II. Structure and Biological Activity of Amino Acid Complexes. *Inorganica Chimica Acta*. 1984, *92*, 23-31.

7. Maciejewska, G.; Cie´slak-Golonka, M. Homo- and Hetero-nuclear Chromium(III) Complexes with Natural Ligands. Part I. Spectroscopic and Mass Spectra Studies on Ternary [M-L_1-L_2] Systems. *Transition Metal Chemistry*. 2001, *27*, 473-480.

8. Barrett, J.; O'Brien, P. O.; de Jesus, J. P. Chromium(III) and the Glucose Tolerance Factor. *Polyhedron*. 1985, *1*, 1-14.

9. Freeman, H.C.; Moore, C.J.; Jackson, W.G.; Sargeson, A.M. Synthesis, Structure, and Stereochemistry of Some Cysteine- and Penicillaminecobalt(III) Complexes. *Inorganic Chemistry*. 1978, *17*, 3513-3521.

10. Estep, D. Baylor University Masters Thesis, 1982.

11. Liu, C.T.; Douglas, B.E. Circular Dichroism of Coordination Compounds. III. Cobalt(III) Complexes of Optically Active Amino Acids. *Inorganic Chemistry*. 1964, *3*, 1356-1361.

12. De Meester, P.; Hodgson, D.J.; Freeman, H.C.; Moore, C.J. Tridentate Coordination by the L-Cysteine Dianion. Crystal and Molecular Structure of Sodium Bis(L-cysteinato)chromate(III) Dihydrate. 1977, *16*, 1494-1498.

13. Robert Williams. Baylor University Ph.D. Dissertation, 1980.