

## DOCTORAL THESIS

### Insulin-potentiating effects of chromium(III): a mechanistic study

Xu, Minghua

*Date of Award:*  
1999

[Link to publication](#)

#### General rights

Copyright and intellectual property rights for the publications made accessible in HKBU Scholars are retained by the authors and/or other copyright owners. In addition to the restrictions prescribed by the Copyright Ordinance of Hong Kong, all users and readers must also observe the following terms of use:

- Users may download and print one copy of any publication from HKBU Scholars for the purpose of private study or research
- Users cannot further distribute the material or use it for any profit-making activity or commercial gain
- To share publications in HKBU Scholars with others, users are welcome to freely distribute the permanent URL assigned to the publication

15634279

TH  
PH.D  
1999X2

**Insulin-Potentiating Effects of Chromium(III):  
A Mechanistic Study**

XU Minghua

**A thesis submitted in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy**

August 1999

Hong Kong Baptist University

## ABSTRACT

Chromium, a trace nutrient essential for maintaining a normal carbohydrate and lipid metabolic status, has been shown to exert its effects via its insulin-potentiating activity. A dietary agent known as the glucose tolerance factor (GTF), which alleviates the diabetic-like symptoms, such as glucose intolerance, associated with chromium deficiency, was shown to depend on Cr(III) for its biological activity. However, despite four decades of research, neither the structure of this Cr(III)-containing GTF nor the mechanism by which Cr(III) potentiates the actions of insulin has been elucidated.

In this study, 11 structurally characterized Cr(III) complexes and a synthetic analog of the GTF, referred to as the synthetic GTF, were prepared and their potentiating activities towards insulin-induced lipogenesis, anti-lipolysis, total glucose uptake and glucose transport were measured using isolated rat adipocytes. The Cr(III) complexes studied were chosen in such a way that some of their ligand compositions closely resembled the chemical composition of the GTF while others bore no such resemblance at all. Only the synthetic GTF was shown to display a strong insulin-potentiating effect towards these four insulin-induced activities. Furthermore, in the presence of the synthetic GTF, an increase in insulin responsiveness, but not in insulin sensitivity, was observed in the insulin dose-response curve towards lipogenesis. This observation suggested that the Cr(III) most likely exerts its potentiating effect at a postreceptor-binding event along the insulin signal transduction pathway. This notion was reinforced by an observed lack of effect of these Cr(III) compounds on insulin-receptor binding. Trypsin pretreatment of the adipocytes, which resulted in a significant attenuation of the lipogenesis activity stimulated by both insulin and the synthetic GTF, showed that a structurally intact insulin receptor is a prerequisite for the observed insulin-potentiating effect. Experiments conducted in the presence of specific inhibitors for the insulin receptor protein tyrosine kinase (IR-PTK), cytosolic PTK and protein tyrosine phosphatase (PTPase) activities strongly suggested that the IR-PTK must be involved in this process. A detailed investigation of the effects of the Cr(III) compounds on the IR-PTK activity of partially purified insulin receptor preparation from rat skeletal muscle revealed that the  $[\text{Cr}(\text{L-cys-}N,O,S)_2]$  complex, at 1 mM concentration, was the only compound which induced a significant enhancement (ca. 2-fold) of the IR-PTK activity over the insulin control. PTK assays conducted in the presence and absence of 1 mM  $\text{NaVO}_3$ , a potent PTPase inhibitor, indicated further that the Cr(III) potentiates insulin actions via a *direct* activation of the IR-PTK activity.

The distinct structure-activity profile obtained in assays using intact cells, such as lipogenesis where only the synthetic GTF was found to be active, versus those using a cell-free system, such as the PTK assay where only the  $[\text{Cr}(\text{L-cys-}N,O,S)_2]$  complex was shown to be active, suggested the following: (i) the GTF probably represents a readily absorbable form of Cr(III) which can only potentiate insulin action after being transported into the cell, and (ii) the intracellularly active Cr(III) is likely to contain cysteine as a key ligand.

## TABLE OF CONTENTS

DECLARATION	i
ABSTRACT	ii
ACKNOWLEDGMENTS	iii
TABLE OF CONTENTS	iv
LIST OF TABLES	viii
LIST OF FIGURES	x
LIST OF ABBREVIATIONS	xvi
<b>Chapter I Introduction</b>	<b>1</b>
1.1 Definition and Function	1
1.2 Nutritional Role of Chromium in Animals	1
1.3 Discovery of Chromium's Role in Human Health	2
1.4 Chromium and Insulin Resistance	3
1.5 Chromium and Diabetes Mellitus	6
1.6 Biologically Active Chromium	10
1.7 Assays for Measuring the Biological Activity of Cr(III)	14
1.8 Mechanism of Chromium Action	15
1.9 Major Insulin Actions	17
1.10 Structure of the Insulin Receptor	19
1.11 Insulin Signal Transduction Cascade	22
1.12 Insulin Receptor Substrates	22
1.13 Protein Tyrosine Phosphatases	24
1.14 Objectives of the Study	25
1.15 Outline of the Study	25
<b>Chapter II Materials and Methods</b>	<b>27</b>
2.1 Materials	27
2.2 Isolation of the Natural GTF from Brewer's Yeast	28
2.3 Preparation and Partial Purification of the Synthetic GTF	28
2.4 Preparation of the $K[Cr(L-cys)_2] \cdot 2H_2O$	30
2.5 Preparation of the $K_2[Cr(glutathione)_2(H_2O)] \cdot 2H_2O$	30
2.6 Preparation of the $K[Cr(ida)_2] \cdot 3H_2O$	31
2.7 Preparation of the $K[Cr(mida)_2]$	31
2.8 Preparation of the $K[Cr(L-asp)_2] \cdot 3H_2O$	31
2.9 Preparation of the $[Cr(L-his)_2]NO_3$	32
2.10 Preparation of the $Na[Cr(2,6-dipic)_2] \cdot 2H_2O$	32
2.11 Preparation of the $Cr(pic)_3 \cdot 1.5H_2O$	33
2.12 Preparation of the $[Cr(pic)_2(H_2O)_2]^+$	33
2.13 Preparation of the $K_3[Cr(C_2O_4)_3] \cdot 3H_2O$	33
2.14 Preparation of the $K_2[Cr(C_2O_4)_2(L-cys)] \cdot 2H_2O$	34
2.15 Preparation of the $Cr(L-ala)_3$	34
2.16 Preparation of the $Na[Cr(glygly)_2] \cdot H_2O$	34
2.17 Preparation of the $NH_4[VO(O_2)_2(bpy)] \cdot 3H_2O$	35

2.18	Estimation of the Overall Charge of Complex	35
2.18.1	Anion-Exchange Column Chromatography	36
2.18.2	Cation-Exchange Column Chromatography	36
2.19	Chromium Analysis by Spectrophotometry	37
2.20	Chromium Analysis by Atomic Absorption Spectrophotometry	37
2.21	UV-Visible Absorption Spectroscopy	37
2.22	Elemental Analysis	37
2.23	IR Spectroscopy	38
2.24	NMR Spectroscopy	38
2.25	Ellman's Assay	39
2.26	Diabetic Animal Model	40
2.27	Chromium Administration	41
2.28	Glucose Assay	41
2.29	Preparation of Rat Adipocytes	42
2.30	Trypsin Pretreatment of Adipocytes	42
2.31	Lipogenesis Assay	43
2.32	Lipolysis Assay	43
2.33	Total Glucose Uptake Assay	44
2.34	3-O-methyl-glucose Transport Assay	44
2.35	Purification of Insulin Receptor from Skeletal Muscle	45
2.36	Preparation of Cytosolic and Membrane-Associated Protein Tyrosine Phosphatase	46
2.37	Insulin Receptor Protein Tyrosine Kinase Assay	47
2.38	Protein Tyrosine Phosphatase Assay	48
2.39	<i>In vivo</i> Protein Phosphorylation in Rat Adipocytes	48
2.40	SDS Polyacrylamide Gel Electrophoresis	48
2.41	Western Blotting	49
2.42	Iodination of Insulin	50
2.43	Preparation of Human Placental Membranes	51
2.44	Insulin-Receptor Binding Assay	51
<b>Chapter III Characterizations of Cr(III) Complexes</b>		<b>53</b>
3.1	Isolation and Characterization of the Natural GTF	53
3.2	Purification and Characterization of the Synthetic GTF	53
3.3	Characterization of the Cr-Cysteine Complex	55
3.4	Characterization of the Cr-Glutathione Complex	56
3.5	Characterization of the Cr-Iminodiacetic Acid Complex	57
3.6	Characterization of the Cr-Methyliminodiacetic Acid Complex	58
3.7	Characterization of the Cr-Aspartic Acid Complex	59
3.8	Characterization of the Cr-Histidine Complex	59
3.9	Characterization of the Cr-2,6-Dipicolinic Acid Complex	60
3.10	Characterization of the Cr-Tris(picolinic acid) Complex	61
3.11	Characterization of the Cr-Bis(picolinic acid) Complex	62
3.12	Characterization of the Cr-Tris(oxalic acid) Complex	62
3.13	Characterization of the Cr-Bis(oxalic acid)cysteine Complex	63
3.14	Characterization of the Cr-Alanine Complex	64
3.15	Characterization of the Cr-Glygly Complex	65
3.16	Characterization of the $\text{NH}_4[\text{VO}(\text{O}_2)_2(\text{bpy})]$ Complex	66

<b>Chapter IV</b>	<b>Insulin-Potentiating Activity of Cr(III)</b>	<b>68</b>
4.1	Blood Glucose-Lowering Effect of Cr(III) in Diabetic Mice	68
4.2	Effect of the Synthetic GTF and Cr(III) Complexes on the Insulin-Receptor Binding in Human Placental Membrane	69
4.3	Effect of the Natural and Synthetic GTF on Lipogenesis	70
4.4	Effect of the Cr(III) Complexes on Lipogenesis in the Presence of Insulin	72
4.5	Effect of the Cr(III) Complexes on Lipogenesis in the Absence of Insulin	73
4.6	Effect of the Synthetic GTF on the Insulin Dose-Response Curve of Lipogenesis	74
4.7	Effect of Trypsin Pre-Treatment on Lipogenesis	76
4.8	Effect of Quercetin on Lipogenesis Activity	79
4.9	Effect of Staurosporine on Lipogenesis Activity	81
4.10	Effect of the Synthetic GTF on Lipolysis	81
4.11	Effect of the Cr(III) Complexes on Total Glucose Uptake in Adipocytes	84
4.12	Effect of the Synthetic GTF on 3- <i>O</i> -Methyl-glucose Transport Rate	85
4.13	Concentration Dependence of 3- <i>O</i> -Methyl-glucose Transport	87
<b>Chapter V</b>	<b>Cr(III) and Insulin Receptor PTK</b>	<b>89</b>
5.1	Effect of the Cr(III) Complexes on Insulin-Induced Receptor PTK Activity	89
5.2	Effect of Staurosporine on PTK Activity	90
5.3	Concentration Dependence of the Effect of [Cr(cys- <i>N,O,S</i> ) <sub>2</sub> ] on IR-PTK	91
5.4	Effect of NaVO <sub>3</sub> on Insulin-Stimulated IR-PTK	92
5.5	Effect of NaVO <sub>3</sub> on PTK Activity Induced by Vanadium(V)-peroxo Complexes	93
5.6	Effect of the [Cr(cys- <i>N,O,S</i> ) <sub>2</sub> ] on the Insulin Dose-Response Curve of IR-PTK	95
5.7	Effect of the Synthetic GTF on Protein Tyrosine Phosphatase Activity	96
5.8	Effect of the [Cr(cys- <i>N,O,S</i> ) <sub>2</sub> ] on PTPase from Rat Adipocytes	99
5.9	Effect of the Cr(III) Complexes on Cytosolic PTPase from Rat Adipocytes	100
5.10	Effect of the Cr(III) Complexes on Membrane-Associated PTPase from Rat Adipocytes	101
5.11	Effect of the Synthetic GTF on the Phosphorylation of Endogenous Proteins in Intact Rat Adipocytes	102

<b>Chapter VI</b>	<b>General Discussions and Conclusions</b>	105
6.1	Purification and Characterization of the Synthetic GTF and the Cr(III) Complexes	105
6.2	Insulin-Potentiating Activity of Cr(III)	109
6.3	Effect of Cr(III) on Insulin-Receptor Binding	110
6.4	Effect of Cr(III) on Lipogenesis Activity	111
6.5	Effects of Cr(III) on Insulin-Stimulated Anti-Lipolysis	115
6.6	Effect of Cr(III) on Glucose Transport	116
6.7	Effect of Cr(III) on Insulin Receptor PTK	117
6.8	Effect of Cr(III) on Protein Tyrosine Phosphatase (PTPase)	121
6.9	Effect of Cr(III) on Endogenous Protein Phosphorylation	123
6.10	Conclusions	124
<b>Chapter VII</b>	<b>References</b>	126
<b>APPENDIX 1</b>	<b>UV-Visible Absorption Spectra of the Cr(III) Complexes</b>	139
<b>APPENDIX 2</b>	<b>IR Spectra of the Cr(III) Complexes and the <math>\text{NH}_4[\text{VO}(\text{O}_2)_2(\text{bpy})]</math> Complex</b>	149
<b>APPENDIX 3</b>	<b>Published Papers</b>	157
<b>APPENDIX 4</b>	<b>Curriculum Vitae</b>	158