A newly synthetic chromium complex – chromium(phenylalanine)$_3$ improves insulin responsiveness and reduces whole body glucose tolerance

Xiaoping Yang$^{a,b}$, Kamalakannan Palanichamy$^a$, Allyn C. Ontko$^a$, M.N.A. Rao$^c$, Cindy X. Fang$^{a,b}$, Jun Ren$^{a,b}$, Nair Sreejayan$^{a,b,*}$

$^a$ Division of Pharmaceutical Sciences, School of Pharmacy, University of Wyoming, Laramie, WY 82071, USA
$^b$ Center for Cardiovascular Research and Alternative Medicine, University of Wyoming, Laramie, WY 82071, USA
$^c$ Divis Laboratories Limited, Hyderabad, Andhra Pradesh, India

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Abstract Low-molecular-weight organic chromium complexes such as chromium picolinate are often used as dietary supplements to improve insulin sensitivity and to correct dyslipidemia. However, toxicity associated with such chromium compounds has compromised their therapeutic value. The aim of this study was to evaluate the impact of a newly synthesized complex of chromium with phenylalanine, Cr(pa)$_3$ on insulin-signaling and glucose tolerance. Cr(pa)$_3$ was synthesized by chelating chromium(III) with p-phenylalanine ligand in aqueous solution. In mouse 3T3-adipocytes, Cr(pa)$_3$ augmented insulin-stimulated glucose-uptake as assessed by a radioactive-glucose uptake assay. At the molecular level, Cr(pa)$_3$ enhanced insulin-stimulated phosphorylation of Akt in a time- and concentration-dependent manner without altering the phosphorylation of insulin receptor. Oral treatment with Cr(pa)$_3$ (150 μg/kg/d, for six weeks) in ob/ob (+/+) obese mice significantly alleviated glucose tolerance compared with untreated obese mice. Unlike chromium picolinate, Cr(pa)$_3$ does not cleave DNA under physiological reducing conditions. Collectively, these data suggest that Cr(pa)$_3$ may represent a novel, less-toxic chromium supplement with potential therapeutic value to improve insulin sensitivity and glycemic control in type II diabetes.

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1. Introduction

Insulin resistance is comitant with type II diabetes, obesity, hypertension, and other features of the metabolic syndrome [1]. It is the major risk factor for cardiovascular diseases and one of the leading causes of mortality and morbidity. Proper management of insulin resistance has been shown to play a pivotal role in the reduced risk for cardiovascular diseases. However, compounds which improve the sensitivity of insulin are somewhat limited. These compounds such as pioglitazone augment the action of insulin by increasing insulin sensitivity and may be of benefit for the long-term treatment for type II diabetes. One such compound is the mineral chromium. Chromium is thought to play a key role in normal carbohydrate metabolism by potentiating the action of insulin, leading to increased insulin sensitivity in type II diabetes and obesity [2]. Dietary deficiency of chromium has been shown to increase the risk of developing diabetes [3]. Clinical trials have demonstrated that supplementation with chromium chloride or chromium picolinate can lower blood glucose levels in diabetic patients [4].

Better bioavailability of low-molecular-weight organic chromium complexes as compared to chromium salts (2–5% versus 0.5–2%) has led to the development of low-molecular-weight organic complexes of chromium as therapeutic agents to counter the diminished insulin effect under type II diabetes [5]. Emerging evidence has shown that the biologically active form of chromium is a chromium-oligopeptide complex, which further justifies the use of organic-chromium-complexes as biomimetic chromium supplements [6]. The chromium complex of picolinic acid, the most popularly used dietary supplement has been shown to modulate intracellular pathways of glucose metabolism and improve comorbidities associated with insulin resistance in several animal and human studies [7,8]. However, recent reports have indicated that the picolinate ligand may shift the redox potential of chromium in the complex such that it can be reduced by biological reductants to generate hydroxyl radicals causing deleterious DNA mutations [9,10]. The pro-oxidant nature of chromium picolinate, greatly limited its therapeutic applications and prompted us to seek other safe synthetic chromium complex with a natural amino acid ligand [10]. The objective of this study was therefore to evaluate the ability of a newly synthesized complex of chromium with the amino acid p-phenylalanine, Cr(pa)$_3$, on insulin-signaling transduction and glucose uptake in 3T3-adipocytes, and glucose clearance in a ob/ob (+/+) mouse model of insulin resistance/type II diabetes. It was also of interest to test the hydroxyl radicals generating capacity and DNA damage of Cr(pa)$_3$ since such is the case for the toxicity of chromium picolinate.

$^*$Corresponding author.
E-mail address: sreejayan@uwyo.edu (N. Sreejayan)

2. Materials

2.1. Insulin Receptor beta polyclonal antibody was obtained from Santa Cruz Biotechnology (Santa Cruz, CA). All other antibodies used in this study were from Cell Signaling Technology Inc (Beverly, MA). Dulbecco's Modified Eagle Medium (DMEM), penicillin-streptomycin liquid, insulin/transferrin/selenious acid (ITS), fetal bovine serum (FBS), newborn calf serum (CS) were from Invitrogen Corporation (Carlsbad, CA). Micro BCA protein assay kit was from Pierce Chemical (Rockford, IL). pUC19 DNA was obtained from New England Biolabs (Beverly, MA). Chromium picolinate was a kind gift from Dr. J.B. Vincent (The University of Alabama, Tuscaloosa, AL). All other chemicals were from Sigma–Aldrich Chemical Co (St. Louis, MO). Doubly deionized water was used to prepare stock solutions of chromium complex for all experiments unless otherwise indicated.

2.2. Synthesis and characterization of \( \text{Cr}(@)_{4} \)

Aqueous solutions of \( \text{CrCl}_{3} \cdot 6\text{H}_{2}\text{O} \) (2.6 g; 10 mmol in 50 ml water) and \( \text{n}-\text{phenylalanine} \) (4.8 g, 30 mmol in 50 ml water) were mixed at 80°C and refluxed for 4 h. The homogenized green reaction mixture was freeze-dried. The greenish-violet solid obtained was washed with acetone and dried in air oven. Yield: 81%, m.p. > 300°C. Found: C, 47.84; H, 5.60; N, 5.92. The stoichiometry \( \text{CrCl}_{3} \cdot 4\text{H}_{2}\text{O} \cdot \text{HCl} \cdot 2\text{H}_{2}\text{O} \) requires C, 47.00; H, 5.40; N, 6.09. The ESMS of the complex in methanolic solution registers signals at 545.1 and 165.9 representing, respectively, the tris chelate and the deprotonated ligand. Formation of the complex was associated with \( \nu(C-H) \) (1563 cm\(^{-1}\)) and \( \nu(C=O) \) (1355 cm\(^{-1}\)) shifts in the IR-spectrum by about 40 and 30 cm\(^{-1}\), respectively. The broadening of the moderately sharp absorption band in the free ligand (2900–3100 cm\(^{-1}\)) to about 600 cm\(^{-1}\) may be attributed to the reorganization in intramolecular hydrogen bonding after chelation. New absorption bands in the far IR region around 370–310 cm\(^{-1}\) are attributed to the reorganization in intramolecular hydrogen bonding after chelation. New absorption bands in the far IR region around 370 and 310 cm\(^{-1}\) can be assigned to the Cr-O and Cr-N bonds. The UV–Vis spectrum of the methanolic solution of the complex registered bands at 22 075 cm\(^{-1}\) \( (\epsilon) \) and 22 075 cm\(^{-1}\) \( (\epsilon) \). The complex being green in color, the above two bands are due to the absorption in yellow and blue parts of the spectrum. These absorptions are due to the spin allowed transitions \( T_{\Delta g}^{\text{v}} \leftrightarrow A_{\Delta g}^{\text{v}} \) \( (\epsilon) \) and \( T_{\Delta g}^{\text{F}} \leftrightarrow A_{\Delta g}^{\text{F}} \) \( (\epsilon) \). The third band \( \epsilon \) overlaps with UV absorption of the ligand. These observations suggest a hexa-coordinate environment around chromium (III). The pH of the aqueous solution of the complex is 4.7, and the presence of chloride demonstrates the presence of HCl in the lattice. Based on the stoichiometry, elemental analysis and spectral studies, the product obtained is a complex containing a 1:3 ratio of chromium to phenylalanine.

2.3. Cell culture and differentiation

The 3T3-L1-pre-adipocytes were obtained from the American Type Culture Collection and were propagated at 37°C in DMEM with 10% newborn calf serum plus 50 U/ml penicillin and 50 µg/ml streptomycin in an incubator under a humidified atmosphere of 5% CO\(_2\)/95% air. Induction of differentiation was done one day post-confluence. Cells were maintained in differentiation medium of the following composition: DMEM, 10% FBS, 0.2 µg/ml dexamethasone, 0.5 mM 1-isobutyl-3-methylxanthine (IBMX) and ITS for 3 days. At day 3, the dexamethasone and IBMX were removed with ITS remaining on the cells for an additional two days. Differentiation was allowed to continue in DMEM supplemented with 10% FBS, ITS and the adipocytes were used between day 9 and 14 post-induction. Prior to the cellular assays, cells were serum starved by overnight incubation in DMEM (containing 0.2% serum).

2.4. Gel electrophoresis and Western blotting

Cells were lysed in RIPA buffer (150 mM NaCl, 0.25% sodium deoxycholate, 1% NP-40, 0.1 mM EDTA and 50 mM Tris, pH 7.2) containing 2 mM sodium vanadate, 1 µM PMSF, 1 µM sodium fluoride, 1 µg/ml aprotinin, 1 µg/ml leupeptin, and 1 µM pepstatin and sonicated to reduce the sample viscosity. The lysate was centrifuged at 15 000 x g for 15 min at 4°C and the protein concentration in the supernatant was determined by the bicinchoninic acid method. Equivalent amounts of proteins were boiled in Laemmli sample buffer. Proteins were separated on a 10%–20% polyacrylamide gel, and electrophoretically transferred to a nitrocellulose membrane. The membranes were incubated at 1 h at room temperature in blocking buffer (5% w/v non-fat dry milk in tris buffered saline containing 0.1% Tween 20). Membranes were incubated in appropriate phospho-specific primary antibody for insulin receptor beta and Akt at 4°C overnight. The horseradish peroxidase-coupled secondary antibodies were visualized using enhanced chemiluminescence reagents (Cell Signaling Technology, Inc, Beverly, MA). Blots were then stripped and re-probed with antibodies directed against insulin receptor beta and Akt.

2.5. Animals and treatment protocol

All animal treatment procedures described in this study were approved by the animal Care and Use Committee at University of Wyoming (Laramie, WY). Homozygous B6.V-<sup>ob</sup>/ob male mice purchased from the Jackson Laboratory (Bar Harbor, ME) at age 5 weeks were divided randomly into two weight-matched groups, marked as ob/ob(+/-) control and ob/ob(+/-) treatment (n = 10). Number, age and weight matched normal C57 mice were used as lean control. All of these animals were maintained on conventional laboratory diet under well-controlled conditions of temperature (22 ± 2°C), humidity (55 ± 5%) and 12 h/12 h light–dark cycle and had ad libitum access to water and standard rodent chow. Rodent diet and tap water were consumed by mice ad libitum. Cr(III) was provided in the drinking water and, on the basis of water intake, was administered to provide an intake of about 150 µg/kg/day corresponding to about 10–15 µg elemental Cr/kg/day for ob/ob (+/−) and lean treatment groups. The dosage of chromium used was based on earlier animal studies reported with chromium picolinate [11].

2.6. Intraperitoneal glucose tolerance test

At the end of the treatment schedule, mice were subjected to the intraperitoneal glucose tolerance test (IPGTT) as described previously [12]. Briefly, the mice were fasted for 12 h and then given intraperitoneal injection of glucose (2 g/kg body weight). Glucose levels were determined in blood drops obtained by clipping the tail of the mice immediately before glucose challenge, as well as at 15, 60 and 120 min thereafter. Serum glucose levels were determined using ACCU-CHEK* Advantage* Glucose Analyzer (Roche Diagnostics Corporation, IN). Results of IPGTT are also expressed as integrated areas under the curves (AUC) over 120 min for glucose calculated by using the WinNonlin software (Pharsight Corporation, Mountain View, CA).

2.7. Assay for generation of hydroxyl radicals

The potential of chromium compounds to generate hydroxyl radicals in vitro was assessed by the method of Gutteridge et al. [13]. Briefly, a reaction mixture containing either chromium picolinate or Cr(III), 2-deoxyribose (4 mM), ascorbic acid (100 µM) and hydrogen peroxide (100 µM) in potassium phosphate buffer (pH 7.4, 10 mM) was incubated at 37°C for 30 min. An aliquot of the mixture was treated with 1% (w/v) thiobarbituric acid and heated at 90°C for 10 min, rapidly cooled and the amount of chromogen formed in the sample was measured by its absorption at 532 nm. Ferric-EDTA (100 µM) was used as a positive control.

2.8. DNA cleavage reactions

DNA cleavage-stimulated by chromium compounds was assessed as described previously [14]. All solutions were prepared using Chelex-100 treated water to remove any traces of metal ions. Aliquots of pUC19 (about 40 µM in base pairs in 5 mM Tris, 500 µM EDTA buffer, pH 8.0) were mixed with ascorbic acid (5 mM) in the presence of the test compounds in phosphate buffered saline (pH 7.4), to give a final volume of 15 µL. Reactions were allowed to proceed 60 min followed by quenching with 2 µl of nucleic acid sample loading buffer. The mixtures were loaded directly onto a 1% agarose gel pre-stained with ethidium bromide and electrophoresed at 60 V. The gels were photographed on a UV transilluminator.
2.9. Glucose uptake assay

Glucose uptake activity was analyzed by measuring the uptake of 2-deoxy-D-[3H] glucose as described previously [15]. Briefly, confluent 3T3-L1 adipocytes grown in 6-well plates were washed twice with serum-free DMEM and incubated with 2 ml of the same medium at 37 °C for 2 h. The cells were washed 3 times with Krebs-Ringer-HEPES (KRH) buffer and incubated with 2 ml KRH buffer at 37 °C for 30 min. Insulin (6 nM) and/or Cr(pa)3 (0.25-25 μM) were then added to adipocytes accompanying the procedure of differentiation. Glucose uptake was initiated by the addition of 0.1 ml KRH buffer and 2-deoxy-D-[3H] glucose (0.2 μCi/ml) and 5 mM glucose as final concentrations. Glucose uptake was terminated by washing the cells three times with cold PBS. The cells were lysed overnight with 1 ml 0.5 M NaOH and 0.1% SDS (w/v). The radioactivity retained by the cell lysates was determined by a scintillation counter (Beckmann LC 6000IC) and normalized to protein amount measured with a Micro BCA Protein Assay Kit (Pierce Chemical, Rockford, IL).

2.10. Data analysis

Data are expressed as means ± S.E.M. and statistically evaluated using Student’s paired t test using Sigma Plot statistical software (Jandel Scientific, San Rafael, CA). A P value of less than 0.05 was considered to be statistically significant.

3. Results

3.1. Synthesis and characterization of Cr(pa)3

A multi-step synthesis of chromium complexes of L-phenylalanine and D,L-phenylalanine using aqua(isothiocyanato)bis-(L-phenylalaninato)chromium and D,L- or L-phenylalanine as starting material has been reported previously [16]. In contrast, a simpler, single step reaction was used in this study to synthesize the complex of chromium(III) with D-phenylalanine in aqueous solution. The synthetic protocol used in the paper was closer to that reported by Abdel-Monem et al. [17] for the synthesis of similar complexes of chromium with L-amino acids. Elemental analysis and spectral studies indicate that the ratio of chromium to D-phenylalanine in the complex is 1:3.

3.2. Cr(pa)3 enhances insulin-stimulated glucose uptake in adipocytes

Chromium has been shown to improve insulin-stimulated glucose uptake in cultured cells sensitive to insulin [18]. The effect of Cr(pa)3 on insulin-stimulated glucose-uptake in 3T3-adipocytes, an insulin-sensitive cell line, was investigated using the 2-deoxy-D-glucose-3H uptake assay. As shown in Fig. 1, incubation with insulin (6 nM) during the course of differentiation of the adipocytes, stimulated a fourfold increase in glucose uptake by adipocytes, consistent with a previous report [15]. Pre-incubation of the cells with Cr(pa)3 (5 and 25 μM) significantly augmented insulin-stimulated glucose uptake in 3T3-adipocytes. In the absence of insulin, Cr(pa)3 did not have any effect on basal glucose uptake (data not shown). These results demonstrate that Cr(pa)3 potentiates insulin-stimulated but not basal glucose uptake.

3.3. Cr(pa)3 does not alter insulin-stimulated tyrosine phosphorylation of insulin receptor beta (IRβ)

Insulin is thought to initiate its signaling cascade via activation and autophosphorylation of IRβ [19]. Once phosphorylated, the insulin receptor functions as a kinase which phosphorylates proteins such as the insulin receptor substrate (IRS) resulting in the downstream propagation of insulin signaling. Since previous studies have shown that chromium complexes may enhance the number [18] and activity [20] of the insulin receptors, subsequent experiments were performed to study the effect of Cr(pa)3 on insulin-stimulated IRβ tyrosine phosphorylation in 3T3-adipocytes. Treatment with insulin resulted in a significant increase in phosphorylation of IRβ as expected, which was not altered by pretreating the cells with Cr(pa)3 for various time or at different concentrations (Figs. 2A and C). Neither did Cr(pa)3 alter IRβ phosphorylation induced by submaximal concentrations of insulin (1 nM) indicating that receptor saturation by the ligand may not be the reason for the lack of effect of Cr(pa)3 (figure not shown). These results indicated that Cr(pa)3 may enhance insulin-stimulated glucose-uptake via a mechanism(s) involving other than at the site of insulin receptor.

3.4. Cr(pa)3 enhances insulin-induced phosphorylation of Akt

Akt has been identified as an important kinase, downstream of insulin receptor necessary for insulin activity [21]. Treatment of adipocytes with insulin resulted in an increase in Akt (thr308) phosphorylation (Fig. 3). Pretreatment of adipocytes with the chromium complex resulted in a further increase in the insulin-stimulated Akt phosphorylation both in a concentration-dependent (Fig. 3A, upper panel) and time-dependent manner (Fig. 3C, upper panel). Cr(pa)3 alone, in the absence of insulin did not alter the phosphorylation levels of Akt (data not shown). These results indicate that Cr(pa)3 may promote insulin signaling and glucose uptake by acting at the post-receptor level.

3.5. Cr(pa)3 improves whole body glucose tolerance

Genetically obese, leptin deficient C57BL/6J ob/ob (+/+) mice and their lean controls were treated with Cr(pa)3 150 μg/kg/d, for 6 weeks. Following acute glucose challenge, the ob/ob (+/+) animals showed poor glucose tolerance compared to the lean control mice as indicated by an increase in the area under the post-treatment glucose concentration curve (Fig. 4A and

![Fig. 1. Cr(pa)3 improves insulin-stimulated glucose uptake in cultured mouse 3T3-adipocytes. Cells were treated with insulin (6 nM) and in the presence or absence of Cr(pa)3 (5 or 25 μM) for 10 days accompanying the procedure of differentiation of the cells. Uptake of radiolabeled glucose in the cells was measured as explained in Section 2. Results are means ± S.E.M. Preincubation with Cr(pa)3 significantly enhanced insulin-stimulated cellular glucose uptake (*P < 0.05, n = 3) as compared to insulin-stimulated glucose uptake in untreated cells.](Image)
B). In both Cr(pa)3-treated and untreated animals, the plasma glucose levels following glucose challenge started to decline after peaking at 15 min and returned to the baseline value after 120 min (Fig. 4A). However, serum glucose levels in the Cr(pa)3 treated ob/ob (+/+ ) mice were significantly lower than that of the untreated control animals at 15 and 60 min.
The integrated AUC over 120 min of glucose, shown in Fig. 4B, shows that the ob/ob (+/++) mice receiving Cr(pa)3 has significantly lower AUC as compared to untreated animals. These effects of Cr(pa)3 were found to be independent of the changes in the body weight as there was no significant difference in the body weight of the ob/ob (+/++) animals treated with Cr(pa)3. Post-treatment weights were 52.2 ± 1.6 g for treated versus 53.8 ± 2.6 g for untreated (P > 0.05 between the two groups, n = 10). Pre-treatment weights were 28.2 ± 1.1 and 28.8 ± 1.0 g for treated and untreated group, respectively. In contrast, in the lean mice, there were no significant changes in the AUC between the Cr(pa)3-treated and untreated group.

3.6. Cr(pa)3 does not cleave DNA under physiologically relevant conditions

Recent studies have indicated that the nutritional supplement chromium picolinate can cause DNA damage by generating hydroxyl radicals [14]. The mutagenic potential of chromium picolinate has been attributed by the picolinate ligand, which primes the redox potential of the chromic center for reduction by biological reductants [22]. In contrast, the phenylalanine ligand is known to scavenge hydroxyl radicals via hydroxylation of the phenyl ring [23] which may be a potential strategy to avert the toxicity. The deoxyribose degradation assay was used to compare the pro-oxidant potential of chromium picolinate and Cr(pa)3. Ferric-EDTA complex in the presence of ascorbate generated hydroxyl radicals causing significant damage to deoxyribose (Fig. 5A). In this model system, chromium picolinate induced a twofold increase (over control) in hydroxyl radical production. In contrast, Cr(pa)3 failed to cause any degradation of deoxyribose, suggesting that this compound may not generate hydroxyl radicals under the conditions tested. The effect of Cr(pa)3 on DNA damage was monitored by observing the conversion of the supercoiled plasmid DNA to circular, nicked form (Fig. 5B). In accordance to previous studies, in the presence of ascorbic acid, chromium picolinate (1.2 μM) caused a nick in the pUC19 DNA. Interestingly, equimolar concentrations of Cr(pa)3 under similar conditions failed to induce any strand breaks in the DNA.

Insulin-stimulated phosphorylation of Akt is enhanced to a greater extent by Cr(pa)3 as compared to that with chromium picolinate. Fig. 6 shows that Cr(pa)3 induces greater stimula-
Our study demonstrates (i) Cr(pa)₃ enhances insulin-stimulated glucose uptake in 3T3-adipocytes. Both the chromium complexes enhanced the insulin-stimulated phosphorylation of Akt as compared to control (figure A, upper panel). The enhancement was more pronounced with Cr(pa)₃ than with chromium picolinate. Figure A lower panel is a blot for total Akt, a control for equal protein loading. Panel B: ratios of gel optical densities of phosphorylated Akt versus total protein. Values are means ± S.E.M., n = 3. *P < 0.05.

4. Discussion

This is the first study to examine the effect of a novel complex of chromium with the amino acid d-phenylalanine on insulin signaling and glucose tolerance. Major findings of our study are (i) Cr(pa)₃ enhances insulin-stimulated glucose uptake in adipocytes, (ii) Cr(pa)₃ improves insulin-signal transduction in cultured mice adipocytes, (iii) oral feeding of Cr(pa)₃ to insulin resistant animals improves glucose tolerance and (iv) Cr(pa)₃ does not generate toxic hydroxyl radicals that cleave DNA under physiological conditions.

The ability of chromium to enhance insulin-stimulated glucose uptake in cultured cells has been reported previously [18]. Though the exact mode of action of chromium is unknown, several mechanisms have been proposed. Chromium is thought to enhance insulin binding to cells, to enhance the insulin receptor number and to potentiate insulin receptor kinase activity [20]. In our studies, however, Cr(pa)₃ did not increase the protein levels of insulin receptor nor did it enhance the tyrosine phosphorylation of insulin receptor indicating that chromium may be acting downstream of the insulin receptor. Phosphorylation of Akt in response to insulin stimulation is a pivotal event in insulin signal transduction that results in the activation and translocation of glucose transporter GLUT4-containing vesicles from the cytosol to plasma membrane leading to cellular glucose uptake [24]. Besides its involvement in the activation of GLUT4 vesicles, Akt can phosphorylate glycogen synthase kinase 3, which is an essential step in the activation of glycogen synthase, the enzyme involved in glycogen synthesis. The present study indicates that Cr(pa)₃ may be improving insulin sensitivity by enhancing the insulin-stimulated phosphorylation of Akt. Interestingly, a recently conducted clinical study demonstrates that individuals with type-II diabetes who supplemented their diet with chromium picolinate had increased activity of Akt (in the skeletal muscles) compared to those who were on placebo [25]. The reported ability of wortmanin, a PI3-kinase inhibitor to inhibit Cr-stimulated potentiation of insulin activity [26] underscores Akt as a potential target for chromium compounds. Furthermore, the results presented here (Fig. 6) suggest that Cr(pa)₃ may function as a more potent enhancer of insulin-stimulated Akt phosphorylation than chromium picolinate. However, the mechanism by which chromium enhances insulin-stimulated Akt phosphorylation is unclear. One potential explanation could be the inhibition of the enzyme phosphotyrosine phosphatase (PTP-1B) by chromium, which is a known negative regulator of insulin signaling [27].

The identification that chromium in the biologically active form is a complex with a 1500 Da polypeptide leads to the synthesis and evaluation of several low-molecular-weight organic chromium complexes amongst which chromium picolinate has been the most extensively studied compound [28]. One problem with chromium picolinate is its poor solubility in physiological buffers that may result in poor bioavailability. Besides, the picolinate ligand has been shown to generate hydroxyl radicals that can cause deleterious DNA damage [10,14]. These observations prompted the use of a non-toxic amino acid such as d-phenylalanine as the ligand to chelate chromium. Phenylalanine besides having better solubility at physiological pH is a known scavenger of hydroxyl radicals [23]. Derivatives of d-phenylalanine such as nateglidine have also shown to have beneficial effects in type II diabetes [29]. Thus, the rationale in synthesizing phenylalanine complex of chromium was to obtain a better bioavailable, less toxic and more effective biomimetic complex of chromium. The present study indicates that Cr(pa)₃ possesses these attributes and therefore would be an ideal candidate for further extensive studies on its insulin-potentiating properties.

5. Conclusion

This is the first study to show that a novel synthetic complex of chromium with the d-phenylalanine improves insulin responsiveness and whole body glucose tolerance. Unlike chromium picolinate, Cr(pa)₃ does not cleave DNA under physiological conditions. Taken together, it is tempting to speculate that chromium complexes of amino acids may represent a novel class of chromium complexes that is less toxic and potentially beneficial in the treatment and management of insulin resistance and glucose intolerance in type II diabetes.

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