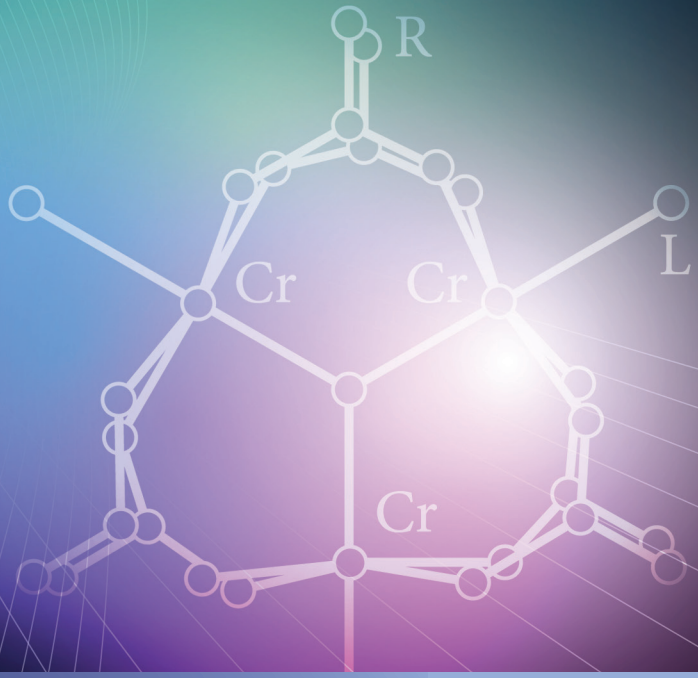
JOHN B. VINCENT

THE BIOINORGANIC CHEMISTRY OF CHROMIUM



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The Bioinorganic Chemistry of Chromium

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Contents

Preface

Two oxidation states of chromium, Cr^{3+} and Cr^{6+}, are generally considered biologically and environmentally relevant and stable, that is, they are stable in the presence of air and water. Chromium(III) complexes are both kinetically and thermodynamically stable. However, chromium(VI) complexes are kinetically stable but unstable thermodynamically. In the presence of appropriate reducing agents, Cr^{6+} can readily be reduced via Cr^{4+} and/or Cr^{5+} intermediates ultimately to Cr^{3+}.

The biochemistries of both Cr^{3+} and Cr^{6+} have controversial histories. The public is generally more familiar with the chemistry of Cr^{6+} (or chromate) because of its toxicity. Chromium(VI), d^0, is most commonly encountered as the intensely coloured chromate, $[CrO_4]^{2-}$, or dichromate, $[Cr_2O_7]^{2-}$, anions. These two species are interconvertable in water. Chromate occurs at basic pH values and has a distinctive yellow colour; $PbCrO_4$ has been used as the pigment in paint used for yellow highway lines. Below pH 6, chromate is in equilibrium with yellow–orange dichromate. Acidic dichromate solutions are potent oxidants. The coordination environment of chromium in both the chromate and dichromate anions is tetrahedral. The intense colour of both anions results from ligand to metal charge transfer bands. Mixed ligand complexes of Cr^{6+} with oxides and halides or oxides and amines are well known, as are Cr(VI) peroxo complexes. The diamagnetic Cr^{6+} centre does not give rise to ESR (electron spin resonance) spectra, while NMR (nuclear magnetic resonance) studies of Cr(VI) complexes with oxo, peroxo and halo ligands are of limited utility.

While Cr(VI) complexes are known to be potent carcinogens and mutagens when inhaled, a serious debate has arisen with regards to the effects of the oral intake of these complexes, as illustrated in recent years by the popular movie *Erin Brokovich*. Chromium(VI) complexes could

give rise to these effects through a number of mechanisms, including oxidation by the complexes or the subsequently generated Cr^{4+} and Cr^{5+} intermediates, reactions of reactive oxygen species (ROS) generated as by-products of these oxidations, reactions of organic radicals generated in these processes and the binding of the ultimately generated Cr^{3+} to biomolecules. The relative importance of these mechanisms is far from being explained.

However, while the chemistry of Cr^{6+} and Cr^{3+} may be intertwined to some degree and this intertwining cannot simply be dismissed, this book focuses on the biochemistry of Cr^{3+}, particularly in terms of its potential use as a nutritional supplement, nutraceutical agent or pharmaceutical agent. (The coordination of Cr^{3+} ions to DNA as a result of Cr^{6+} reduction is beyond the scope of this work, and the nature and significance of this binding is a current topic of much debate.)

Coordination complexes of Cr^{3+} are nearly always octahedral. Consequently, the chromic centre has a d^3 electron configuration with three unpaired electrons ($S = 3/2$) in each of the t_{2g} orbitals. This configuration is responsible for the kinetic inertness of Cr(III) complexes, where ligand exchange half-times are generally in the range of hours. The hexaaquo ion of chromium, $[Cr(H_2O)_6]^{3+}$, is purple in aqueous solution. Solutions of the ion are acidic; at neutral and basic pH the ion readily oligomerizes to give hydroxo-bridged species starting with the $[(H_2O)_5Cr(\mu\text{-}OH)_2Cr(H_2O)_5]^{4+}$ ion. The commonly used commercial form of $CrCl_3{\cdot}6H_2O$ is actually *trans*-$[Cr(H_2O)_4Cl_2]Cl{\cdot}2H_2O$. Dissolution of this green solid initially yields green solutions of the $[Cr(H_2O)_4Cl_2]^+$ cation. The Cr^{3+} ion has a large charge to size ratio and is considered as a hard Lewis acid, preferring oxygen and nitrogen coordination. With common biomolecules, coordination to anionic oxygen-based ligands such phosphates and carboxylates would be expected.

The magnetic and spectroscopic properties of chromium(III) complexes do not readily lend themselves to providing much information on the coordination environment of chromic centres in biomolecules. For mononuclear complexes, a magnetic moment close to the spin-only value for an $S = 3/2$ centre (3.88 BM) is generally observed. While ^1H and ^{13}C nuclear magnetic resonance spectra can be obtained on Cr(III) complexes, the spin 3/2 centre results in greatly broadened and shifted resonances in NMR spectra. The structure of the complex must generally be known in order to interpret the NMR spectra, rather than the reverse. In contrast, Cr(III) complexes can give rise to sharp features in ESR spectra (ESR is also known as electron paramagnetic resonance

(EPR) spectroscopy); however, the ESR spectra of biomolecules have often proved to be quite broad, providing limited information. ESR spectroscopy is probably a significantly underutilized technique in characterising chromium in biological systems. Cr^{3+} as an impurity in the Al_2O_3 matrix of emeralds and rubies gives rise to the green and red colour of these gems; yet, the electronic spectra of chromium-containing biomolecules are usually very simple. Three spin-allowed d→d transitions are expected; two usually occur in the visible region, while the third is expected in the ultraviolet region (where it can be hidden by ligand based features). No charge transfer transitions generally occur while the visible absorption bands have extinction coefficients of typically less than 100 M^{-1} cm^{-1}. Thus, only relatively concentrated solutions of Cr^{3+} have appreciably observable colour. Cr(III) complexes are generally stable against oxidation or reduction.

Although chromium as the Cr^{3+} ion was proposed to be an essential element about 50 years ago, its status is currently in question, as recent experiments appear to demonstrate that the element can no longer be considered essential. Supplemental nutritional doses of Cr^{3+} have been proposed to result in body mass loss and lean muscle mass development, leading to an appreciable nutraceutical industry being built around chromium. However, these claims have been thoroughly refuted. Chromium has also been suggested to be a conditionally essential element whose supplementation could lead to improvements in carbohydrate and lipid metabolism under certain stress situations, including type 2 diabetes and the effects of shipment of farm animals; this is currently an area of intense and hotly debated research with recent findings suggesting that beneficial effects from Cr^{3+} supplementation are pharmacologically, not nutritionally, relevant. At the same time, supplementation of the diet with at least certain Cr(III) complexes has been proposed to have potentially deleterious effects.

Chapter 1 examines the current status of chromium as defined by various government agencies or public foundations. Chapter 2 reviews the evidence that chromium is an essential trace element. Chapter 3 explores the history of nutritional studies on chromium(III) complexes. The ability of chromium(III) complex supplementation to generate body composition changes is covered in Chapter 4, while potential pharmacological effects of chromium supplementation, particularly for type 2 diabetic subjects, is reviewed in Chapter 5. Chapter 6 explores the mechanisms by which chromium might have pharmacological effects. Chapters 7 and 8 review chromium supplements that are commercially available or under development and the use of chromium supplements in farm animal

nutrition, respectively. The potential toxicity of chromium supplementation is examined in Chapter 9.

This work is by far the most exhaustive treatment of the biochemistry and related nutritional and pharmacological effects of Cr^{3+}. It presents the views of the author at the time of writing. Surprisingly after more than two decades of research personally in the field, these views are continually being revised as more experimental results are reported. Much that was learned 20 years ago has had to be 'unlearned' and reassessed. The basics of the field as understood 20 years ago has been entirely inverted by recent experimental results. Clearly while more than five decades old, the field of chromium biochemistry is not a mature field. Major gaps in our knowledge remain to be filled. For example, no biomolecule has been shown unambiguously to bind chromium and be responsible for its effects *in vivo*. Recent research has led to a reassessment of much of what was believed two decades ago and suggests that major advances may be on the horizon. Hopefully this work will inspire additional research that can fill these holes.

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1

Introduction – The Current Status of Chromium(III)

When a member of the general public thinks about chromium and health, unfortunately the first thing to come to mind is probably one or more of the following claims:

- reduces body fat;
- causes weight loss;
- causes weight loss without exercise;
- causes long-term or permanent weight loss;
- increases lean body mass or builds muscle;
- increases human metabolism;
- controls appetite or craving for sugar; or
- 90% of US adults do not consume diets with sufficient chromium to support normal insulin function, resulting in increased risk of obesity, heart disease, elevated blood fat, high blood pressure, diabetes, or some other adverse effect on health.

In other words, most people think of chromium in terms of weight loss and lean muscle mass development as a result of nutraceutical product marketing. Yet the Federal Trade Commission (FTC) of the United States ordered entities associated with the nutritional supplement chromium picolinate to stop making each of the above representations in 1997 because of the lack of 'competent and reliable scientific evidence' [1]. This ruling is now well over a decade old; however, the situation has

The Bioinorganic Chemistry of Chromium, First Edition. John B. Vincent.

changed little. In fact in 2000, products containing chromium picolinate had sales of nearly a half a billion dollars [2]. The FTC currently has pending law suits against entities associated with chromium picolinate-containing products, while the scientific support for most of these claims has completely eroded [3]. For example, recently the National Institutes of Health sponsored a study where male and female rats and mice were given diets containing up to 5% chromium picolinate by mass for up to two years; no effects were observed on body mass or food intake [4]. Studies of the effects of chromium picolinate will be presented in Chapter 4.

The basis for the use of chromium as a nutritional supplement stems from chromium being on the list of essential vitamins and minerals under examination by the National Research Council of the National Academies of Science, USA since 1980 [5], after initially being proposed as an essential element in 1959; (the history of the status of chromium as a trace element is reviewed in Chapter 3) [6]. In 2001, the National Academies of Science established an Adequate Intake (AI) of chromium of 35 μg/day for men and 25 μg/day for women [7]. AI is defined as 'the recommended average daily intake level based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of apparently healthy people that are assumed to be adequate.' The AI 'is expected to cover the needs of more than 97–98% of individuals' [7]. Thus, almost all Americans are believed to be chromium sufficient, and little if any need exists for chromium supplementation. The bases for this determination are rather limited. Anderson *et al.* have established that self-selected American diets contain on average 33 μg Cr/day for men and 25 μg Cr/day for women [8], while nutritionist-designed diets [9] contain on average 34.5 μg Cr for men and 23.5 μg Cr/day for women. Offenbacher *et al.* have found that men (two subjects) could maintain their chromium balance when receiving 37 μg Cr/day [10]. Bunker *et al.* have shown for 22 elderly subjects consuming, on average, 24.5 μg Cr/day that 16 were in chromium balance, 3 were in positive balance and 3 were in negative balance [11]. The situation is likely to be similar in other developed nations; for example, pre-menopausal Canadian women eating self-selected diets have been found to have an average daily intake of 47 μg of chromium [12]. Currently, as discussed in Chapter 2,whether chromium is an essential element is at best an open question, and it probably should not currently be considered to be an essential element. If chromium is an essential element, it must interact specifically with some biomolecules in the body

and serve a specific function; attempts to identify such a molecule and a role in the body will be discussed in Chapter 6.

In addition to the purported use to reduce body mass and build muscle, chromium supplements have also been touted to alleviate the symptoms of type 2 diabetes and related cardiovascular disorders, in addition to other conditions. While administration of chromium(III) complexes has positive effects in rodent models of type 2 diabetes and other conditions, the situation in humans is currently ambiguous (see Chapter 5 for a thorough discussion). According to the American Diabetes Association in its 2010 Clinical Practices Recommendations, 'Benefit from chromium supplementation in people with diabetes or obesity has not been conclusively demonstrated and therefore cannot be recommended' [13]. The American Diabetes Association dropped any mention of chromium in its 2011 and 2012 recommendations.

In December 2003, Nutrition 21, the major supplier of chromium picolinate, petitioned the US Food and Drug Administration (FDA) for eight qualified health claims:

1. Chromium picolinate may reduce the risk of insulin resistance.
2. Chromium picolinate may reduce the risk of cardiovascular disease when caused by insulin resistance.
3. Chromium picolinate may reduce abnormally elevated blood sugar levels.
4. Chromium picolinate may reduce the risk of cardiovascular disease when caused by abnormally elevated blood sugar levels.
5. Chromium picolinate may reduce the risk of type 2 diabetes.
6. Chromium picolinate may reduce the risk of cardiovascular disease when caused by type 2 diabetes.
7. Chromium picolinate may reduce the risk of retinopathy when caused by abnormally high blood sugar levels.
8. Chromium picolinate may reduce the risk of kidney disease when caused by abnormally high blood sugar levels [14].

After extensive review, the FDA issued a letter of enforcement discretion allowing only one (No. 5) qualified health claim for the labelling of dietary supplements [14, 15]: 'One small study suggests that chromium picolinate may reduce the risk of type 2 diabetes. FDA concludes that the existence of such a relationship between chromium picolinate and either insulin resistance or type 2 diabetes is highly uncertain.' The small study was performed by Cefalu *et al.* [16]. This study was a

placebo-controlled, double-blind trial examining 1000 μg/day of Cr as chromium picolinate on 29 obese subjects with a family history of type 2 diabetes; while no effects of the supplement were found on body mass or body fat composition or distribution, a significant increase in insulin sensitivity was observed after four and eight months of supplementation [16]. Mechanisms by which chromium has been proposed to potentially have an effect on type 2 diabetes and associated conditions will be discussed in Chapter 6.

A safety assessment was also part of the FDA evaluation of chromium picolinate [14]. As reviewed in Chapter 9, the safety of chromium picolinate has been questioned after cell culture and developmental toxicity studies in fruit flies have shown that the compound could be mutagenic and carcinogenic. However, the FDA determined that the 'use of chromium picolinate in dietary supplements . . . is safe' [14]. The European Food Safety Authority (EFSA) recently also determined that chromium supplements in doses not exceeding 250 μg Cr per day are safe [17, 18]. The safety of chromium picolinate as a nutritional supplement has been confirmed by a study commissioned by the National Toxicology Program of the National Institutes of Health. The study examined the effects of chromium picolinate comprising up to 5% of the diet (by mass) of rats and mice for up to two years and found no harmful effects on female rats or mice and, at most, ambiguous data for one type of carcinogenicity in male rats (along with no changes in body mass in either sex of rats or mice) [4]. The reasons behind the discrepancies between the toxicology studies will be examined in Chapter 9.

Chromium(III) complexes are often used as animal feed supplements, in addition to being a popular human supplement. The use of chromium as an animal feed supplement was evaluated in the mid-1990s by the Committee on Animal Research, Board of Agriculture of the National Research Council [19]. In general the available data were insufficient for conclusions to be drawn; for example, no conclusions could be reached about the need for supplemental chromium in the diets of fish, rats, rabbits, sheep and horses. Specific recommendations could not be made about the diets of poultry, swine and cattle, although chromium was determined possibly to have a beneficial effect for cattle under stress and improve swine carcass leanness and reproductive efficiency [19]. Chromium was, however, found to be safe as a food additive. As is reviewed in Chapter 8, the situation with regard to chromium dietary supplementation in animals has changed little in the last decade.

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2

Is Chromium Essential? The Evidence

Support for chromium being essential comes primarily from: (i) studies attempting to provide rats with chromium-deficient diets, (ii) studies examining the absorption of chromium as a function of intake, (iii) studies of patients on total parenteral nutrition and (iv) studies looking for an association between insulin action and chromium movement in the body (Table 2.1).

Chromium levels in tissues, food components and other biological samples reported prior to circa 1978 are problematic and should be ignored [12, 13]. Improvements in analytical techniques revealed several problems, including appreciable contamination of biological samples (as these samples were often homogenized in a stainless-steel blender); in fact, measured Cr levels reflected the levels of contamination not the actual tissue or fluid Cr concentrations, which were extremely small. Another major problem in atomic absorption experiments prior to 1978 was that workers were attempting to measure a tiny signal against a large background; a linear correspondence was actually found to exist between background absorbance and the 'apparent Cr content' of samples [12]. Currently, analyses of human blood and urine samples with Cr concentrations above 1 ppb should be considered suspect, unless the subjects are taking chromium supplements. Consequently, studies prior to 1978 utilizing patients who were believed to be Cr deficient based on Cr tissue or fluid concentrations and that reported Cr levels in tissues, foods or fluids of one order to several orders of magnitude too high must be

The Bioinorganic Chemistry of Chromium, First Edition. John B. Vincent.

Table 2.1 Evidence used to support an essential role for chromium.

Evidence	Ref.	Complications
Rats fed a Cr-deficient, high-sucrose or high-fat diet develop resistance, reversed by Cr administration	1	Diets not shown to be deficient in Cr; insulin pharmacological doses of Cr utilized
Patients on TPN develop diabetes-like symptoms, which are responsive to Cr	4 (review), 5 (review)	TPN solutions often rich in Cr; pharmacological doses of Cr utilized
Absorption of dietary Cr is inversely proportional to intake	6	Highly suggestive, requires reproduction
Factors that affect glucose metabolism alter urinary Cr loss	7–10, 11 (review)	May reflect insulin-sensitive movement of Fe(III), also may simply reflect increases in absorption associated with diabetes and insulin-resistance

discarded. Thus, with the exception of some ^{51}Cr-labelled tracer studies, the field of chromium nutritional biochemistry really began in the late 1970s. At present, chromium levels in tissues and biological fluids are usually determined by graphite furnace atomic absorption spectrometry, although neutron activation analysis and inductively coupled plasma-mass spectrometry(ICP-MS) can also be used [12]. Neutron activation and ICP-MS have been utilized with stable isotopes of Cr for determining Cr levels in tracer studies, in addition to the continuing use of radioactive ^{51}Cr.

Chromium is ubiquitous in foods but at very low concentrations, however, while processing, particularly in stainless-steel equipment, the concentration appears to increase; in fact, most of the Cr in some foods may come from processing [14]. Foods particularly rich in Cr (i.e. >100 ppb) include broccoli and black pepper [14] and certain beers [15]; however, values for vegetables must be considered carefully because of the variable amount of chromium that comes from soil contamination [16]. The low concentrations of chromium in food, the ease of contamination and the low adequate intake(AI) established for chromium make preparation of a low-chromium (or chromium-deficient if chromium is essential) diet difficult.

2.1 'CHROMIUM-DEFICIENT' DIET STUDIES WITH RATS

Prior to 2011, the most notable efforts of work with rats to generate a chromium-deficient diet had been reported by Anderson and co-workers. Rats in plastic cages (with no access to metal components) were given a diet consisting of 55% sucrose, 15% lard, 25% casein and vitamins and minerals, and providing 33 ± 14 μg Cr/kg diet [1]. The sucrose levels were provided in a theoretical attempt to induce Cr deficiency; dietary carbohydrate stress leads to increased urinary chromium loss (see below). To compromise pancreas function, low copper concentrations (1 mg/kg) were employed for the first 6 weeks; high dietary iron concentrations were used throughout to potentially aid in obtaining Cr deficiency. A supplemented pool of rats were given water containing 5 ppm $CrCl_3$; unfortunately, the volume of water consumed was not reported so that the Cr intake of the rats cannot be determined. Over 24 weeks, body masses were similar for both groups. At 12 weeks, Cr-deficient rats had lower fasting plasma insulin concentrations and similar fasting plasma glucose levels compared with supplemented rats; yet, both concentrations were similar after 24 weeks. In intravenous glucose tolerance tests after 24 weeks on the diet, plasma insulin levels tended to be higher in Cr-deficient rats; rates of excess glucose clearance were statistically equivalent. Glucose area above basal was reported to be higher in Cr-deficient rats; however, at every time point in the glucose tolerance test, the plasma glucose concentrations of each pool of rats were statistically equivalent, suggesting that the difference in area arises from a mathematical error. (These workers reported another study utilizing a high-sucrose diet in 1999, in which the plasma insulin levels were again observed to be elevated; however, the plasma glucose area was not [2].) Thus, a high-sucrose diet can lead to hyperinsulinemia, possibly reflecting defects in peripheral tissue sensitivity to glucose [1]. This research group also obtained similar results using a high-fat diet that contained 33 mg Cr/kg diet [3]. This diet also contained an altered copper content in the first six weeks. After 16 weeks on the diet alone, rats had higher fasting plasma insulin levels, but not fasting glucose levels, compared with rats also receiving drinking water containing 5 ppm Cr [3]. Similar results were obtained when the fasting insulin and glucose levels of the rats on the diet alone were compared with rats on a normal chow diet. Insulin and glucose areas after a glucose challenge were equivalent [3]. Thus, the high-fat diet appears to induce increased fasting insulin levels, which can be corrected with chromium administration.