Chemical Properties and Toxicity of Chromium(III)

ARTICLE *in* CHEMICAL RESEARCH IN TOXICOLOGY · APRIL 2008

Impact Factor: 3.53 · DOI: 10.1021/tx700385t · Source: PubMed

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Perspective

Chemical Properties and Toxicity of Chromium(III) Nutritional Supplements

Aviva Levina and Peter A. Lay*

School of Chemistry, The University of Sydney, NSW 2006, Australia

Received October 26, 2007

The status of Cr(III) as an essential micronutrient for humans is currently under question. No functional Cr(III)-containing biomolecules have been definitively described as yet, and accumulated experience in the use of Cr(III) nutritional supplements (such as $[Cr(pic)_3]$, where pic = 2-pyridinecarboxylato) has shown no measurable benefits for nondiabetic people. Although the use of large doses of Cr(III) supplements may lead to improvements in glucose metabolism for type 2 diabetics, there is a growing concern over the possible genotoxicity of these compounds, particularly of $[Cr(pic)_3]$. The current perspective discusses chemical transformations of Cr(III) nutritional supplements in biological media, with implications for both beneficial and toxic actions of Cr(III) complexes, which are likely to arise from the same biochemical mechanisms, dependent on concentrations of the reactive species. These species include: (i) partial hydrolysis products of Cr(III) nutritional supplements, which are capable of binding to biological macromolecules and altering their functions; and (ii) highly reactive Cr(VI/V/IV) species and organic radicals, formed in reactions of Cr(III) with biological oxidants. Low concentrations of these species are likely to cause alterations in cell signaling (including enhancement of insulin signaling) through interactions with the active centers of regulatory enzymes in the cell membrane or in the cytoplasm, while higher concentrations are likely to produce genotoxic DNA lesions in the cell nucleus. These data suggest that the potential for genotoxic side-effects of Cr(III) complexes may outweigh their possible benefits as insulin enhancers, and that recommendations for their use as either nutritional supplements or antidiabetic drugs need to be reconsidered in light of these recent findings.

Contents

1. Chromium(III) in Nutrition

Chromium is probably the most controversial of the transition metal ions in terms of its biological activities (*1–4*). The highest oxidation state of this element, Cr(VI), is a well-established human carcinogen and one of the most commonly encountered occupational hazards (*3, 5*). The chemical mechanisms of Cr(VI)-induced cytotoxicity and genotoxicity, including its efficient cellular uptake and reduction by cellular constituents with the formation of highly reactive Cr(V/IV) intermediates and kinetically inert Cr(III) products, are generally well understood (*3, 6–8*), although many biochemical details remain to be elucidated (*9*). On the contrary, the most stable oxidation state of chromium, Cr(III), is still regarded by many nutritionists as an essential micronutrient for humans (involved in glucose metabolism), although this opinion has been questioned (*2, 10*); a comprehensive review on the subject is provided in a recent book (*11*). The controversy is enhanced by the wide use of Cr(III)-based nutritional supplements, particularly Cr(III) picolinate ($[Cr(pic)_3]$, where pic = 2-pyridinecarboxylate) (*12*), despite the growing concern over the low efficacy and the potential toxicity of such supplements (*13–16*). The following discussion is a brief summary of our current understanding of the role of Cr(III) in nutrition (*11*).

The first suggestion that a biological Cr(III) compound could act as a "glucose tolerance factor" or GTF was made in the 1950s by Schwarz and Mertz, on the basis of experiments with nutrient-deficient rats (*17*). Numerous attempts in the following decades to establish the chemical nature of GTF (e.g., a Cr(III) complex with amino acid and nicotinamide ligands) led to the GTF hypothesis being discarded as a product of experimental artifacts (*18*). More recent efforts to isolate and characterize a natural Cr(III)-containing biologically active substance were centered on the low-molecular-weight chromium-binding substance (LMWCr), originally described by Yamamoto and co-workers (*19*), and later renamed chromodulin (by analogy with a Ca(II) binding protein, calmodulin) by Vincent and co-workers (*20, 21*). This substance, isolated either from the livers of Cr(VI)-treated animals (*19*) or from the in vitro reactions of Cr(VI) with liver homogenates (*20*), has been described as a small (~1.5 kDa) peptide, selectively binding four Cr(III) ions in a multinuclear assembly (*22*) and capable of enhancing the tyrosine kinase activity of isolated β-subunits of human insulin receptors (*23, 24*). These results were disputed on the basis of

* Corresponding author. E-mail: p.lay@chem.usyd.edu.au.

10.1021/tx700385t CCC: $40.75
Published on Web 02/01/2008

the reported isolation method of LMWCr (involving exogenous Cr(VI)), the absence of definitive structural information for this compound, and the inability of several research groups to reproduce the data of Vincent and co-workers (*3, 25, 26*). Thus, unlike for all the other transition metals that are regarded as essential for some form of life (V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W), no Cr-containing biomolecules have been definitively characterized as yet in terms of their structures or the mode of action (*18*).

The most often cited evidence of Cr(III) essentiality in humans is the reported alleviation of glucose intolerance symptoms in total parenteral nutrition (TPN) patients following the addition of a Cr(III) salt ($CrCl_3 \cdot 6H_2O$) to the TPN solutions (*27*), but the data accumulated in this field over the last 30 years have proven to be inconclusive (*2, 26*). Similar uncertainty is related to the use of Cr(III) complexes with organic ligands as insulin-enhancing nutritional supplements. Data published in the early 1980s suggested that the safe and adequate dietary intake of Cr(III) for adults is 50–200 μg/day and that 50–90% of diets in developed countries were Cr(III)-deficient, which led to the wide promotion of Cr(III)-based nutritional supplements (particularly $[Cr(pic)_3]$) (*18*). Subsequent developments in analytical techniques led to decreases in the determined values of Cr content in food (in the early studies, samples were typically contaminated with Cr from stainless steel appliances used for sample preparation) (*28*). Accordingly, the estimates of adequate dietary Cr(III) intake decreased (the currently accepted values are 25–35 μg/day for adults) (*29*). However, no clinical manifestations of Cr(III) deficiency in humans have been established (*4, 26*). Accumulated experience in the use of Cr(III) nutritional supplements showed that there are no measurable benefits (such as the popularly promoted conversion of fat into muscle) of taking Cr(III) supplements for people with normal glucose metabolism (*18, 30*). In the opinion of an independent nutrition expert (not involved in Cr research), on the basis of the data available to date, Cr(III) should not be classified as an essential nutrient and should not be recommended as a nutritional supplement for the general population (*31*).

In recent years, the focus in nutritional Cr(III) studies has switched to the potential use of Cr(III) complexes as insulin-enhancing pharmaceuticals for the treatment of type 2 diabetes, with recommended doses of 0.2–1.0 mg of Cr per day (at least 6–40 times of the typical dietary intake of Cr) (*29*) for 1–10 months (*16, 32*). While several studies reported positive effects of high doses of $[Cr(pic)_3]$ ($\geq$0.4 mg Cr per day) on glucose metabolism parameters in type 2 diabetics (*32*), the use of this compound has caused increasing concern because of the growing evidence of its genotoxicity, which is enhanced by the picolinato ligand (see sections 2 and 3) (*16*). In search of safer Cr(III)-based antidiabetics, complexes with propionato (*33*), L-histidinato (*34*), D-phenylalaninato (*35*), and nicotinato (niacinato or 3-pyridinecarboxylato) (*36*) ligands as well as Cr(III)-enriched yeast (*37*) have been proposed. Marked antidiabetic activities and low toxicities of these compounds in animal models have been reported (*34–36, 38, 39*), while both positive (*40*) and negative (*41*) results were observed in recent human trials with Cr(III)-enriched yeast. A trend emerging from numerous studies of Cr(III) supplementation in farm animals is that beneficial effects of Cr(III) (such as improvements in glucose metabolism and immune status) are only observed when the animals are subjected to unusual stress conditions (*42*).

Controversial data on antidiabetic effects of Cr(III) may be explained on the basis of the concept of hormesis (*26, 43*), that is, a stimulatory or beneficial effect of subtoxic doses of a toxic chemical. For instance, low concentrations of highly toxic Cd(II) or Hg(II) ions are known to induce stress-related increase in glucose uptake in cultured cells (*44*), similar to that observed for Cr(III) complexes (and used as a proof of Cr(III) essentiality) (*45, 46*). A recent hypothesis that the antidiabetic activity of Cr(III) is caused by its biological oxidation to Cr(VI/V) species (*7, 25, 47*) (see sections 3 and 4) is also in line with the concept of hormesis. Relatively low doses of Cr(VI) are known to decrease blood glucose levels in experimental animals (*48*) and to stimulate plant growth (*49, 50*), while higher doses cause acute toxicity, and chronic exposure to Cr(VI) leads to increased incidence of cancers (*3*). Thus, beneficial and toxic effects of Cr complexes in various oxidation states are likely to be intimately linked (*47*). The main aim of this perspective is to show the interrelationships of these effects and their link to the chemical properties of chromium.

2. Toxicological Studies of Cr(III) Nutritional Supplements

Representative results of recent toxicological studies of Cr(III) complexes (most of them concerning $[Cr(pic)_3]$) in animal or cell models are summarized in Table 1 (*39, 51–64*), and comprehensive reviews of earlier studies are available in the literature (*16, 65*). Several new manifestations of genotoxicity of $[Cr(pic)_3]$ have been reported, including skeletal and neurological defects in the offspring of mice receiving $[Cr(pic)_3]$ during pregnancy or lactation (*52, 53*), and *hprt* locus mutations in $[Cr(pic)_3]$-treated cultured cells (*55*). Other animal studies, using either lower doses of the compound (*56*) or short treatment times (*51*), reported the absence of toxic effects. Negative results of some cellular genotoxicity studies with $[Cr(pic)_3]$ (*58*) were explained (*16, 55*) with short treatment times and the presence of dimethylsulfoxide (DMSO, used to prepare stock solutions of $[Cr(pic)_3]$), which acts as a free-radical trap. Many studies of $[Cr(pic)_3]$ toxicity reported similar but lower toxic effects of equivalent doses of the ligand (picolinic acid) and no significant toxicity of equivalent doses of Cr(III) complexes with other ligands (*52–54, 57*).

Complexes of Cr(III) with organic ligands other than picolinate generally showed low toxicities (Table 1) (*39, 59*), except for one study that reported significant cytotoxicities of a wide range of Cr(III) complexes with common O/N-donor ligands (*60*) (in apparent contradiction with a study performed under similar conditions on a different cell type) (*59*). Aquated $CrCl_3$ (predominantly *trans*-$[CrCl_2(OH_2)_4]^+$) (*66*) was reported to promote protein oxidation and nitration in human macrophages (in a study modeling toxic effects of metal ions generated by endoprostheses) (*61, 62*). Probably, the most intriguing reports related to Cr(III) toxicity were those that detailed increased cancer rates in the progeny of male mice that received a single ip injection of aqueous $CrCl_3$ at two weeks before mating (*63, 64*). These results were attributed to hormonal disfunctions caused by Cr(III) exposure rather than to direct effects of the metal ion since no detectable levels of Cr were found in the sperm of treated mice (*63, 64*). Irrespective of the mechanism by which these disfunctions occur, they point to a serious possible complication of Cr(III) supplementation.

In summary, an overview of recent toxicological studies (Table 1) shows that the nature of the ligand and the treatment conditions are among the crucial factors that determine the toxicity of Cr(III) nutritional supplements. Therefore, the toxic effects of Cr(III) complexes have to be studied in relation to their general chemistry (including ligand-exchange and redox

Table 1. Representative Recent Studies on the Toxic Effects of Cr(III) Complexes

compd[a]	test system	conditions	results	year	ref
Cr-pic	mice	single ip injection, 0.75–3.0 mg/kg, dissolved in saline	no genetic DNA damage (comet and micronuclei assays) for 16–42 h	2007	(*51*)
Cr-pic	primary human lymphocytes	0.50 mM in cell culture medium (directly dissolved or from DMSO solution) for 3 h	DNA damage (comet assay); inhibited by DMSO	2007	(*51*)
Cr-pic	pregnant mice	200 mg solid per kg food per day for 10 days	increased skeletal and neurological defects in the offspring[b]	2006	(*52*) (*53*)
Cr-pic	fruit fly	260 μg Cr per kg of feeding medium	retarded development[c]	2006	(*54*)
Cr-pic	Chinese hamster ovarian AA8 cells	80 mg/cm^2 (from solid suspended in acetone) or 1.0 mM (from DMSO solution) for 48 h	*hprt* locus mutations; inhibited by DMSO	2006	(*55*)
Cr-pic	uninephrectomized rats	5 mg solid per kg food per day for 60 days	no adverse effects on renal function	2005	(*56*)
Cr-pic	mouse lymphoma cells	60–120 μg Cr per mL medium (from DMSO solution) for 4 h[d]	increase in mutant colonies (trifluorothymidine assay)[b]	2005	(*57*)
Cr-pic	Chinese hamster ovarian K1 cells	96–770 μg per mL medium (from DMSO solution) for 4–20 h[d]	no significant increase in chromosome damage	2005	(*58*)
Cr-pic Cr-His Cr-Cl	human keratinocytes (HaCaT)	50–250 μM in cell culture medium for 24 h	no significant cytotoxicity (formazan blue assay); no DNA damage (comet assay)	2007	(*59*)
Cr-niacin	rats	50–125 ppm Cr (solid complex in food) per day for 90 days	no adverse health effects	2005	(*39*)
Cr-niacin	mouse lymphoma cells	1–200 μg/mL (from DMSO solution) for 3–24 h	no significant increase in mutations	2005	(*39*)
various Cr(III)[e]	primary human dermal fibroblasts	1–100 μM in cell culture medium for 24, 48, or 72 h	cytotoxicity (formazan blue assay)	2005	(*60*)
Cr-Cl	human macrophages (U937)	0–250 ppm Cr for 0–72 h	induction of protein oxidation and nitration	2005–2006	(*61*) (*62*)
Cr-Cl	male mice	1.0 mmol/kg, single ip injection (in H_2O, pH 4.0), 2 weeks before mating	increased cancer rates in offspring	1999–2004	(*63*) (*64*)

[a] Designations of the compounds: Cr-pic is $[Cr(pic)_3]$ (pic = 2-pyridinecarboxylato(-)); Cr-His and Cr-niacin are Cr(III) complexes of L-histidine or niacin (3-pyridinecarboxylic acid), which are not fully characterized (*39*); and Cr-Cl is *trans*-$[CrCl_2(OH_2)_4]^+$ (commercial $CrCl_3{\cdot}6H_2O$) (*66*). [b] Similar but lower toxicities were observed for equivalent doses of picolinic acid; no toxicities were observed for *trans*-$[CrCl_2(OH_2)_4]^+$. [c] Similar effect of the equivalent dose of picolinic acid; no effects of Cr(III) nicotinato or propionato complexes. [d] Either in the presence or absence of metabolic activation (rat liver S9 fraction). [e] Cytotoxicity decreased in the following order: $[Cr(en)_3]^{3+}$ > *trans*-$[Cr(salen)(OH_2)_2]^+$ > $[Cr(ox)_3]^{3-}$ > $[Cr(edta)(OH_2)]^-$ > $[Cr(pic)_3]$ > *trans*-$[CrCl_2(OH_2)_4]^+$ (where en = 1,2-ethanediamine; salen = 1,2-bis(salicylideneamino)ethane(2-); ox = oxalato(2-); and edta = *N*,*N*-ethanediaminetetraacetato(4-)).

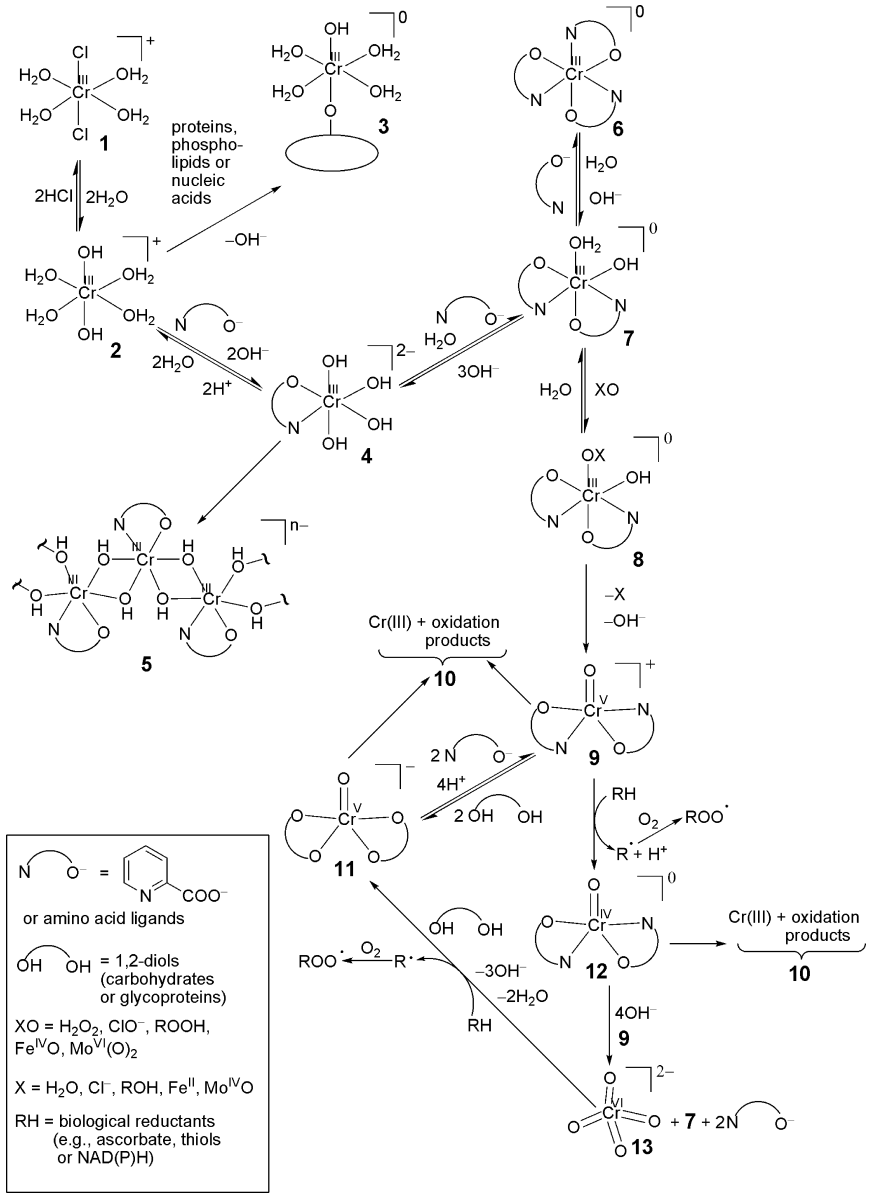
reactions; section 3) and, in particular, to their reactivities in biological media (section 4).

3. Chemical Properties of Cr(III) Complexes

A summary of the typical reactions of Cr(III) complexes under biologically relevant conditions, on the basis of data from model and in vitro studies (reviewed in refs *7, 47*), is given in Scheme 1. A highly acidic Cr(III) salt, $CrCl_3{\cdot}6H_2O$ (*trans*-$[CrCl_2(OH_2)_4]^+$, **1** in Scheme 1) (*66*) is commonly used as a source of Cr(III) in biochemical studies (*46, 67, 68*) as well as in some nutritional formulations (*69, 70*). This complex undergoes ligand-exchange and deprotonation reactions, leading to Cr(III) aqua-hydroxo complexes, such as **2** (Scheme 1) (*71*). Because of the strong affinity of the Cr(III) ion (a hard acid) to negatively charged O-donor ligands, including carboxylato residues of proteins and phosphato groups of phospholipids or nucleic acids (*72*), **2** can bind easily to biological macromolecules (*73–75*) as well as to the surface of cell membranes (*76*) (**3** in Scheme 1). This property, together with the kinetic inertness of Cr(III) complexes (*72*), forms the basis of the well-known ability of Cr(III) to cross-link protein molecules used in leather tanning (*77*) and in biotechnology (*78*). Alternatively, **2** can undergo further deprotonation and ligand-exchange reactions with low-molecular-mass components of biological media (such as amino acids), leading to mixed-ligand species **4** and eventually to insoluble polymeric complexes **5** (Scheme 1) (*71, 72, 79, 80*).

Chromium(III) complexes with strongly bound chelating ligands, such as picolinate ($[Cr(pic)_3]$, **6** in Scheme 1), are kinetically and/or thermodynamically stable in neutral aqueous solutions in the absence of other strong ligands (e.g., amino acids or proteins) (*79, 81*), but the equilibria can be shifted toward partial hydrolysis products (such as **7** and **4** in Scheme 1) under the conditions when these products undergo further irreversible transformations. The picolinato ligand released during the conversion of **6** to **7** or **4** (Scheme 1), as well as its metabolism products, can act as genotoxins and mutagens on their own right (*13, 52–54, 57, 82*). One possible reaction pathway for **7** is initiated by ligand-exchange reactions with biological oxidants (including H_2O_2, ClO^-, lipid peroxides, and oxidized forms of metalloenzymes, summarily designated as XO in Scheme 1) (*7, 47*). Strong oxidants, such as H_2O_2 or ClO^-, are formed naturally as a part of cell signaling and antimicrobial defense mechanisms in humans, and their concentrations are typically increased under pathological conditions, including inflammation and diabetes (*47*). Short-lived Cr(III)-oxidant complexes (**8** in Scheme 1) undergo intramolecular redox reactions with the formation of Cr(V) oxo species (**9** in Scheme 1) (*7, 47*). Such Cr(V) complexes typically decompose within minutes in neutral aqueous solutions (*83, 84*) through one or several of the following pathways (*7, 8*): (i) two-electron redox reactions (with the ligand or an external reductant), leading to Cr(III) species and organic oxidation products (**10** in Scheme 1); (ii) stabilization of Cr(V) by ligand-exchange reactions with carbohydrate and glycoprotein–ligands (**11** in Scheme 1) (*85*);

Scheme 1. Typical Ligand-Exchange and Redox Reactions of Cr(III) Complexes under Biologically Relevant Conditions (Based on Data from Refs 7, 47)[a]



[a] The oval shape in **3** is any relevant oxygen-donor biomolecule.

and (iii) one-electron transfer reactions with the formation of unstable Cr(IV) complexes (**12** in Scheme 1), which then react with Cr(V) with the formation of $[Cr^{VI}O_4]^{2-}$ (**13** in Scheme 1) and Cr(III) species. The latter pathway is believed to be responsible for the formation of Cr(VI) during the reactions of Cr(III) nutritional supplements with biological oxidants (such as H_2O_2 or oxidase enzymes) in aqueous buffer solutions or in blood serum (*25, 79*). Note that H_2O_2 can also be formed during the reactions of O_2 with strong biological reductants such as ascorbate under physiologically relevant conditions (*86, 87*), which represents an additional safety concern for nutritional formulations that contain both Cr(III) and vitamin C (*88*).

Unlike for Cr(III) complexes, the extracellularly formed Cr(VI) is efficiently taken up by cells, where it reacts with cellular reductants (such as ascorbate or glutathione) in the presence of carbohydrates with the formation of Cr(V) intermediates **11** (Scheme 1). These intermediates are further reduced to Cr(III)-biomolecule complexes (**10** in Scheme 1), thus closing the redox cycle (*7, 47*). Highly reactive Cr(V/IV) complexes, such as **9**, **11**, or **12**, are known to cause oxidative DNA damage and Cr(III)-DNA binding in vitro (*3, 7*), the latter lesions being the most likely cause of mutations in Cr(VI)-treated cells (*89–91*). The same complexes as well as **13** are also likely to react with cysteine residues of regulatory enzymes (such as protein tyrosine prosphatases), causing alterations in cell signaling, including the enhancement of insulin action (*7, 25, 47, 92*).

Another redox reaction pathway, proposed for $[Cr(pic)_3]$ and other Cr(III) complexes with aromatic ligands, involves the formation of highly reactive Cr(II) species during the reactions with strong biological reductants such as ascorbate (*93, 94*). Rapid reactions of the resultant Cr(II) complexes with molecular oxygen in this postulated scheme would lead to Cr(V/IV) complexes and organic radical species, which were hypothesized to be responsible for the oxidative DNA damage induced by $[Cr(pic)_3]$ in the presence of ascorbate (*94*). No direct evidence for the formation of Cr(II) species in these systems has been obtained as yet because of their extremely high reactivity in neutral air-saturated aqueous solutions (*7, 47*). High negative

redox potentials for the $[Cr^{III}(pic)_3]/[Cr^{II}(pic)_3]^-$ couple (−1.23 V vs the Ag(I)/Ag(0) couple in *N*,*N*-dimethylformamide solutions; no data for aqueous solutions are available) (*95*) suggest that the direct reduction of $[Cr(pic)_3]$ by biological reductants (typical redox potential range, from 0 to −0.4 V) (*96*) is extremely unlikely. The in vitro oxidative DNA damage by the $[Cr(pic)_3]$ + ascorbate + O_2 system (*94*) is more likely to be caused by the formation of H_2O_2 during the reaction of ascorbate with O_2 (catalyzed by the traces of Fe(III) and Cu(II) ions) (*86, 87*).

In summary, Scheme 1 illustrates two main chemical properties of Cr(III) complexes that can lead to toxicity: (i) ligand-exchange reactions with the formation of kinetically inert Cr(III)-biomolecule complexes that can alter the structures and functions of biological membranes, proteins, and nucleic acids (*3, 91*); and (ii) redox reactions that lead to genotoxic Cr(V/IV) and organic radical species (*7, 47*). Scheme 1 also points to the existence of a redox balance between the different oxidation states of Cr in biological systems, which may provide a unified explanation for both the beneficial and toxic effects of Cr compounds (*47*). Finally, Scheme 1 shows the scope of potential reactions of Cr(III) nutritional supplements with various types of biomolecules, which are considered in detail in the next section.

4. Biotransformations of Cr(III) Complexes

Understanding the coordination chemistry of transition metal complexes in biological systems is a highly challenging research area, primarily because of the low concentrations of metals involved (*97, 98*). Prior to 2005, studies of Cr(III) metabolism in humans and experimental animals used mostly ^{51}Cr-labeled compounds and gel-filtration chromatography (a comprehensive review of this research is available in ref *99*). A general conclusion drawn from these studies was that Cr(III) can bind to both high- and low-molecular-mass ligands (believed to be transferrin and chromodulin, respectively) (*24*) in biological fluids, but no direct chemical characterization of these species was possible. Limitations of these techniques include a large uncertainty in the determination of molecular masses by gel-filtration chromatography (*3*) and the high costs and health hazards associated with the use of radiolabeled Cr(III) compounds.

Recently, a combination of size-exclusion chromatography with inductively coupled plasma mass spectrometry (ICPMS, used for Cr detection) and electrospray mass spectrometry (ESMS) was used for the detection and preliminary characterization of Cr-binding biomolecules in Cr(III)-enriched yeast (*100*). Numerous Cr(III)-binding proteins were identified in yeast extracts, which suggests that the metal binding was less specific than assumed previously (*24, 99*). Similar conclusions were drawn from a study of subcellular Cr(III) distribution in liver cells of Cr(III)-treated rats using ^{50}Cr-enriched samples and neutron activation analysis (*101*). A problem inherent to such studies is the possibility of hydrolysis and redistribution of Cr(III) species during cell lysis, extraction, and chromatography procedures (*80, 102*). Application of X-ray absorption near-edge structure (XANES) spectroscopy (*103*) provides a unique opportunity to compare the coordination environments of Cr in intact Cr-treated cells or tissues and in subcellular or chromatographic fractions and thus detect possible changes in the chemical state of Cr during sample processing (*80*).

The main advantages of XANES spectroscopy for studies of metal speciation in biological samples are (i) its specificity toward the studied metal ion, regardless of the physical state and chemical composition of the sample; and (ii) its high sensitivity to small changes in the coordination environments of metal ions (*103*). In recent XANES spectroscopic studies of Cr(III) speciation in biological systems, the samples were spiked with relatively high concentrations of Cr (0.10–1.0 mM), and the mixtures were freeze-dried prior to spectroscopy (*79, 80*), but developments in the sensitivity of metal detection are likely to lead to the use of more physiologically relevant conditions in the near future. Another direction for future development is the use of microprobe XANES techniques (coupled to X-ray fluorescence mapping), which enable the determination of the oxidation states and coordination environments of metal ions in single mammalian cells (*104*). Average coordination environments of Cr(III) after reactions with biological systems can be deduced from multiple linear regression analyses of XANES spectra, using libraries of model Cr(III) compounds (*79, 80*). Future studies on the biotransformations of Cr(III) and other metal ions in biological systems are likely to combine the chromatography/mass spectrometry approach (*98, 100, 102*) with X-ray absorption spectroscopic techniques (*79, 80, 103*).

Recently (*79*), XANES spectroscopy has been used for detailed reactivity studies of $[Cr(pic)_3]$ (the most widely used nutritional supplement) (*12*) and $[Cr_3O(OCOEt)_6(OH_2)_3]^+$ (a proposed safer alternative) (*33*) in a range of natural and simulated biological media, including artificial digestion systems, blood and its components, cell culture medium, and cultured rat skeletal muscle cells. All of the studied conditions led to the partial or complete disappearance of the initial Cr(III) complexes to produce ligand-exchange products with amino acids, proteins, and aqua/hydroxo ligands (*79*), in agreement with the chemistry described in Scheme 1. As expected (*47, 72*), the extent of ligand exchange was generally higher for the relatively labile $[Cr_3O(OCOEt)_6(OH_2)_3]^+$ complex compared with the more stable $[Cr(pic)_3]$ chelate (*79*). Similar differences in reactivity can be expected for Cr(III) niacinate (*39*) (a mixture of polynuclear Cr(III) carboxylates, containing $[Cr_3O(OCOR)_6(OH_2)_3]^+$ as a major component) (*105*) and more stable Cr(III) complexes with chelating amino acid ligands (such as L-histidine or D-phenylalanine) (*34, 35*). Importantly, ligand-exchange reactions of $[Cr(pic)_3]$ in biological media dramatically increased its susceptibility to reactions with biological oxidants (such as H_2O_2 or the glucose oxidase system) with the formation of potentially genotoxic Cr(VI) species (*79*). Such an observation increases the current concern over the safety of this nutritional supplement (*16*). The formation of Cr(VI/V) species in cultured rat adipocytes, treated with $[Cr_3O(OCOEt)_6(OH_2)_3]^+$, has been recently detected by microprobe XANES spectroscopy (*106*).

The neglect of ligand-exchange reactions of Cr(III) complexes, particularly of highly reactive salts such as $CrCl_3 \cdot 6H_2O$ (*trans*-$[CrCl_2(OH_2)_4]^+$, **1** in Scheme 1) (*63*), in biological media is a persistent deficiency in the relevance of many biochemical and toxicological studies that use **1** as a representative Cr(III) compound (*46, 61–63, 67, 68, 74, 75*). In agreement with the predictions made from model studies (Scheme 1) (*7, 47*), XANES spectroscopy has shown that **1** was converted into a mixture of amino acid and aqua/hydroxo complexes in cell culture medium (*79*). The addition of a freshly prepared aqueous solution of **1** to cell medium containing cultured cells led to a significant amount of Cr(III) binding to the cell surfaces (as opposed to true cellular uptake of Cr(III)), which was completely negated when **1** was pre-equilibrated with the cell culture medium (*79, 107*). The ability of Cr(III) to cross-link proteins (*77*) (including the enhancement of antibody–antigen interactions) is a likely source of artifacts in antibody-based tests of Cr(III) biological activity (*107*), such as the reported ability

of some Cr(III) complexes to enhance tyrosine kinase activity of insulin receptors (*23, 108*). It is clear that toxicological results obtained by the treatments of experimental animals with **1** (*63, 64*) have to be regarded with caution, as this source of Cr(III) is not biologically relevant. Nevertheless, the reported teratogenicity of **1** (*63, 64*), probably caused by the ability of this complex to bind tightly to biological macromolecules (**3**, Scheme 1), should prompt similar studies for other Cr(III) complexes. Indeed, the ability to bind to blood serum proteins was demonstrated experimentally (*79*) even for the relatively nonreactive $[Cr(pic)_3]$ complex (probably through its partial hydrolysis, Scheme 1), and recent studies (*52, 53*) point to the possible teratogenicity of this complex in mammals.

5. Conclusions

Accumulated experience in the use of Cr(III) supplements in human and animal nutrition (*15, 42*) as well as failed attempts to isolate and characterize functional Cr(III)-containing biomolecules (*18, 24, 26*) point to the absence of specific biological functions of Cr(III) (*2, 10, 31*) at this stage of our understanding of Cr biochemistry. In this respect, Cr(III) appears to be similar to other nonbiological trivalent metal ions such as Al(III) (*10, 109*). Among the most prominent toxic effects of Cr(III) complexes are genotoxicity (particularly for $[Cr(pic)_3]$) (*16*) and teratogenicity (particularly for $CrCl_3 \cdot 6H_2O$) (*63*). Beneficial (insulin-enhancing) and toxic effects of Cr(III) complexes are likely to arise from the same biochemical mechanisms, which are dependent on the concentrations of the reactive species (hormetic effect) (*26*). These species can include (i) partial hydrolysis products of Cr(III) nutritional supplements (e.g., **2**, **4**, and **7** in Scheme 1), capable of binding to biological macromolecules and altering their functions (*10, 26, 47, 91*), and (ii) highly reactive Cr(VI/V/IV) species (e.g., **9**−**13** in Scheme 1) and organic radicals, formed in the reactions of Cr(III) complexes with biological oxidants (including the reductant/O_2 systems). Recent studies by X-ray absorption spectroscopy (*79*) confirmed the ability of Cr(III) nutritional supplements, including $[Cr(pic)_3]$ and $[Cr_3O(OCOEt)_6(OH_2)_3]^+$, to exchange ligands with amino acids and proteins in biological fluids. Products of these reactions can be oxidized by H_2O_2 or by oxidase enzymes to potentially genotoxic Cr(VI) species under physiologically relevant conditions (*25, 79, 106*).

Oxidation of Cr(III)-biomolecule complexes by biological oxidants in extracellular fluids is likely to be followed by the efficient cellular uptake of the resultant Cr(VI) species, and their reactions with cellular reductants with the formation of strongly oxidizing Cr(V/IV) and free radical intermediates (*3, 6, 47*). One of the likely targets of these strong oxidants in the cell membrane, or in the cytoplasm, is cysteine residues in the active centers of regulatory enzymes, such as protein tyrosine phosphatases (*7, 25*). The resultant inhibition of these enzymes will lead to alterations in cell signaling, including the enhancement in insulin signaling (*7, 25*). Larger amounts of Cr(VI), taken up by cells, will overwhelm the cellular defense mechanisms and penetrate the cell nucleus (*104, 110*), leading to oxidative DNA damage and the formation of genotoxic Cr(III)-DNA lesions (*9, 91*). Since chronic oxidative stress is typical for type 2 diabetes (*111*), prolonged consumption of large amounts of Cr(III) complexes by diabetics may lead to conditions whereby sufficient amounts of Cr(VI) are generated to cause genotoxicity (*47*). In a manner similar to the patterns observed in occupational exposure to Cr(VI) (*5*), cancers caused by the consumption of large amounts of Cr(III) nutritional supplements may take decades to develop because of the slow reoxidation of Cr(III) complexes accumulated in the body (*3, 7*). Taken together, these data suggest that all Cr(III) complexes are potentially genotoxic and recommendations for their use as either nutritional supplements or antidiabetic drugs need to be reconsidered after the proper evaluation of emerging data.

Acknowledgment. This research has been supported by the Australian Research Council (ARC) Large and Discovery grants (to P.A.L., including an ARC Professorial Fellowship). X-ray absorption spectroscopy was performed partially at the Australian National Beamline Facility (Photon Factory, Tsukuba, Japan) with support from Australian Synchrotron Research Program, which is funded by the Commonwealth of Australia under the Major National Research Facilities program, and partially at the Stanford Synchrotron Radiation Laboratory (SSRL), which is operated by the Department of Energy, Office of Basic Energy Sciences. The SSRL Biotechnology Program is supported by the National Institutes of Health, National Center for Research Resources, Biomedical Technology Program, and by the Department of Energy, Office of Biological and Environmental Research. We wish to acknowledge the many contributions of our co-workers and collaborators, which are listed in the references.

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TX700385T