The Biochemistry of Chromium[1,2]

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ABSTRACT Chromium has been known to be a micronutrient for mammals for four decades, but progress in elucidating the role of chromium has proceeded slowly. However, recent studies have shed light on a potential role of chromium in maintaining proper carbohydrate and lipid metabolism at a molecular level. The oligopeptide chromodulin binds chromic ions in response to an insulin-mediated chromic ion flux, and the metal-saturated oligopeptide can bind to an insulin-stimulated insulin receptor, activating the receptor's tyrosine kinase activity. Thus, chromodulin appears to play a role in an autoamplification mechanism in insulin signaling. The molecular agent responsible for transporting chromium from mobile pools to insulin-sensitive cells is probably the metal transport protein transferrin. Chromium from the popular dietary supplement chromium picolinate enters cells via a different mechanism. Release of chromium from chromium picolinate for use in cells requires reduction of the chromic center, a process that can lead potentially to the production of harmful hydroxyl radicals. J. Nutr. 130: 715–718, 2000.

KEY WORDS: • chromium • chromodulin
• low-molecular-weight chromium-binding substance
• insulin receptor • transferrin

The last five years have seen a flurry of activity in the elucidation of a potential role for trivalent chromium in mammalian carbohydrate and lipid metabolism at a molecular level. In contrast, the previous 35 years of study, starting from the elucidation of an essential role of chromium(III) in mammals, had resulted in numerous studies of the physiology of chromium deficiency and the effects of chromium supplementation, but little on the structure, function and mode of action of the biologically active form of chromium (1,2). The difficulty lies in the chemistry of the biologically relevant form of chromium, substitutionally inert Cr(III), and the low concentrations of the element in tissues. The understanding of chromium metabolism has been so poor that no reliable method for diagnosing chromium deficiency exists other than observing beneficial changes during chromium supplementation; thus, it is not readily possible to determine the chromium status of an individual (2). As a result, studies on the effects of supplementation of normal diets of healthy patients or diabetic subjects with chromium have been inconclusive; the results obtained have been widely disparate among studies. Chromium levels in tissues and body fluids are so low that analytical research to measure chromium concentrations before ~1980 was unreliable because only chromium from sample contamination was being detected (3).

The Essentiality of Chromium(III). In the late 1950s, Schwarz and Mertz (4) demonstrated the existence of a new dietary factor, which was absent in the diet of rats fed *Torula* yeast as their sole protein source. Rats consuming the diet developed an inability to remove glucose efficiently from the bloodstream, which was reversed by adding foods rich in chromium or by adding synthetic inorganic chromium(III) complexes to the diet. Subsequent studies with epididymal fat tissue from Cr-deficient rats suggested that chromium action was dependent on insulin (5,6). Evidence for an essential role for chromium in humans comes from patients receiving total parenteral nutrition (7). Some patients have developed diabetic symptoms that were refractory to insulin but reversed by addition of chromium. Absorption of chromium has also been shown to be inversely proportional to chromium intake, although at any intake level, the body absorbs dietary chromium poorly, i.e., ~0.5–2% (8). Yet, identifying and isolating the naturally occurring, biologically active form of chromium has proved difficult (9,10).

Low-Molecular-Weight Chromium-Binding Substance/Chromodulin. A breakthrough in establishing the mechanism of chromium action at a molecular level appears to have occurred starting in the 1980s when Wada, Yamamoto, and co-workers reported the isolation and characterization of a unique chromium-binding oligopeptide named low-molecular-weight chromium-binding substance (LMWCr) or chromodulin (11). The oligopeptide possesses a molecular weight of ~1500 Da and is comprised of only four types of amino acid residues, i.e., glycine, cysteine, glutamate and aspartate (12,13). Despite its small molecular weight, it binds four equivalents of chromic ions, apparently in a tetranuclear assembly, as necessitated by charge balance arguments. To date, the oligopeptide has been isolated and purified from rabbit liver (12), porcine kidney and kidney powder (14), bovine liver (13) and colostrum (15), and dog liver (16); it has also been isolated from mouse and rat liver (17), Thus, chromodulin appears widely distributed in mammals. There have been no reported efforts to isolate the oligopeptide from animals other than mammals. The most novel attribute of chromodulin is its ability to potentiate the effects of insulin on the conversion of glucose into carbon dioxide or lipid by isolated rat adipocytes (9,5,18). This stimulation of insulin activation occurs without changing the concentration of insulin required for half-maximal activity, suggesting that chromodulin plays an intrinsic role in the adipocytes. The stimulation is also directly dependent on the chromium content of chromodulin (19). No other naturally occurring chromium-containing species potentiates insulin action in this manner.

Combined with studies of diet supplementation with chromium on glucose transport by rat adipocytes (20), the insulin

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dose-response studies suggest a role for chromium as chromodulin in signal transduction. In the last five years, systematic examinations of the activation or inhibition of phosphatase and kinase activity in rat adipocytes by chromodulin revealed two effects, i.e., a small activation of a membrane phosphotyrosine phosphatase (21) and, most significantly, an insulin-sensitive stimulation of insulin receptor tyrosine kinase activity (13,22). Specifically, addition of chromodulin to rat adipocytic membranes or isolated rat insulin receptor in the presence of 100 nmol/L insulin resulted in a concentration-dependent stimulation of protein tyrosine kinase activity up to eightfold; fitting the concentration dependence gave a dissociation constant of 250–875 pmol/L for binding of chromodulin to the insulin receptor. The site of activation appears to be located at or near the kinase active site; addition of chromodulin to a fragment of the β subunit of the insulin receptor that contains the active site (but does not require insulin to be active) resulted in a similar stimulation of kinase activity. Just as with the studies with intact rat adipocytes, stimulation of insulin-dependent insulin receptor kinase activity by chromodulin is dependent on the chromium content of the oligopeptide. Titration of the metal-free or apo-form of the oligopeptide with chromic ions revealed that four equivalents of chromic ions are required for maximal activity. Other transition metals that are commonly associated with biological systems were ineffective in restoring the ability of apochromodulin to stimulate the kinase activity, indicating that the reconstitution is chromium specific. (Similar results have been obtained in titration studies examining the reconstitution of stimulation of phosphatase activity.)

Recently, these results, combined with the results of studies of chromium homeostasis in response in glucose and insulin challenges, have been incorporated into a proposal for the mode of action of chromodulin as part of an autoamplification system for insulin signaling (**Fig. 1**). In response to increases in blood sugar levels, insulin is released rapidly into the bloodstream. Insulin binds to an external α subunit of the transmembrane protein insulin receptor, bringing about a conformation change of the receptor. The receptor autophosphorylates tyrosine residues on the internal portion of its β subunit, turning the receptor into an active kinase (23). Chromodulin is stored in its apo-form in the cytosol (19) and nucleus (Ramirez and Vincent, unpublished results) of insulin-sensitive cells. Increases in plasma insulin concentrations have been found to result in a movement of chromium from the blood to insulin-dependent cells (24,25). This transfer is likely mediated by the metal transport protein transferrin (see below). Apochromodulin possesses a large chromic ion binding constant ($K \approx 10^{21}$) (26) such that it should sequester chromium in response to this chromic ion flux. The newly generated holochromodulin (i.e., Cr_4-chromodulin) can then bind to the insulin-stimulated insulin receptor, helping to maintain its active conformation and amplifying insulin signaling.

When blood concentrations of insulin diminish and receptor signaling must be terminated, chromodulin may be eliminated from cells. The large chromium binding constant indicates that chromium is not likely to be lost from chromodulin to regenerate the apo-form; generation of apo-oligopeptide from Cr_4-chromodulin requires treatment with chelating agents at low pH levels and elevated temperatures, which are not physiologically relevant (27). The chromic centers of chromodulin are not readily reduced by biological reducing agents to labile chromous ions (28), which could be removed more easily from the oligopeptide. This loss of chromodulin from cells is consistent with increased urinary chromium concentrations after carbohydrate and sugar intake (29–32) and with chromodulin potentially representing the major form of chromium(III) in urine (16). The manner in which apochromodulin is replaced is unknown; presumably, the oligopeptide is synthesized as a proprotein which is modified post-translationally to give the oligopeptide (as with insulin and proinsulin). Similarly, nothing is known of the mechanism of regulation of chromodulin levels; however, the potential existence of a chromium-binding translation factor similar to the metal-binding factors that regulate the production of other metalloproteins (e.g., iron-responsive element binding protein) is intriguing.

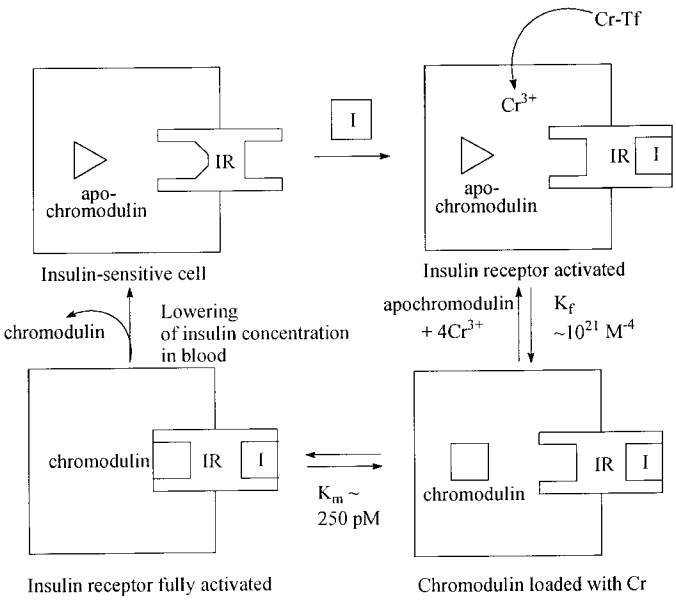

FIGURE 1 Proposed mechanism for the activation of insulin receptor kinase activity by chromodulin in response to insulin. The inactive form of the insulin receptor (IR) is converted to the active form by binding insulin (I). This triggers a movement of chromium (presumably in the form of Cr-transferrin, Cr-Tf) from the blood into insulin-dependent cells, which in turn results in the binding of chromium to apochromodulin (triangle). Finally, the holochromodulin (square) binds to the insulin receptor, further activating the receptor kinase activity. Apochromodulin is unable to bind to the insulin receptor and activate kinase activity. When the insulin concentration drops, holochromodulin is released from the cell to relieve its effects.

Chromium Absorption and Transport. The mechanisms of absorption and transport of chromic ions are still uncertain. In vivo administration of chromic ions to mammals orally or by injection results in the appearance of chromic ions in the iron-transport protein transferrin. This 80,000-Da blood serum protein tightly binds two equivalents of ferric iron at neutral and slightly basic pH levels; it is maintained only ~30% loaded with iron on average and consequently has been proposed to potentially carry other metal ions (33). In vitro studies of the addition of chromium sources to blood or blood plasma also result in the loading of transferrin with Cr(III), although under these conditions, albumin and some degradation products also bind chromium (34). Thus, it has been assumed that transferrin was involved in chromium transport, although it has never been demonstrated in vivo. Transfer of chromium from transferrin to apoLMWCr has been demonstrated in vitro (26). Recent reports on the effects of insulin on iron transport and the relationship between hemochromatosis and hepatic iron overload and diabetes suggest that transferrin may actually be the major physiologic chromium transport agent. Plasma membrane recycling of transferrin receptors is

sensitive to insulin; increases in insulin result in a stimulation of the movement of transferrin receptor from vesicles to the plasma membrane (35). The receptors at the cell surface can bind metal-saturated transferrin, which subsequently undergoes endocytosis with accompanying metal release at the acidic pH of the newly formed vesicles. On the basis of these results, a mechanism for chromium transport is proposed (**Fig. 2**). Increases in insulin levels should result in increased transport of transferrin, including the portion containing bound chromium, culminating in chromium transport from the blood to insulin-sensitive cells and ultimately chromodulin. In adult-onset diabetics, in which blood chromium levels are reduced and urinary chromium losses are increased (36), this transport system may be exceeding normal operation. This may be related to patients with unexplained hepatic iron overload (characterized by a nearly constant association with diabetes) whose transferrin-bound iron levels are greatly increased (37). Could increased loading of transferrin with iron prevent adequate chromium binding and transfer by transferrin, resulting in insulin resistance and diabetes? The hemochromatotic diabetic condition is certainly exacerbated by reduced chromium retention (38), as observed in patients with adult-onset diabetes (36).

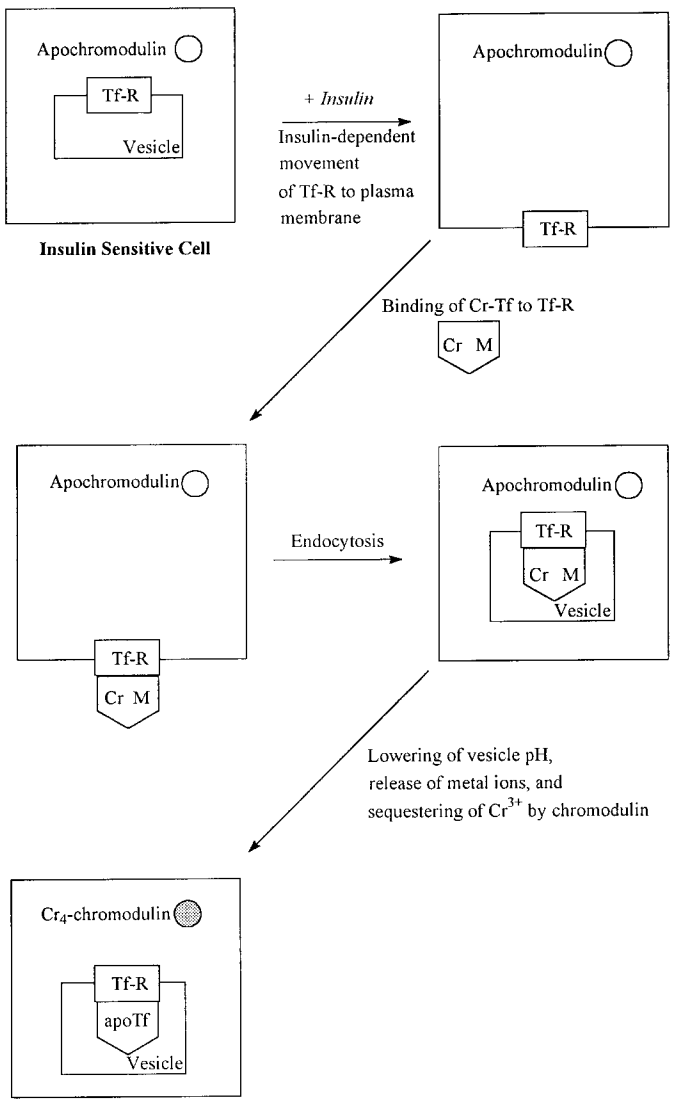

FIGURE 2 Proposed mechanism for the movement of chromium from blood to chromodulin. In response to increases in plasma insulin concentrations, transferrin-receptor (Tf-R) in insensitive cells migrates from vesicles to the plasma membrane. Transferrin (pentagon), which contains two bound metal ions [in this case one chromic ion and one other metal cation (M)], binds to the receptor and is internalized by endocytosis. The pH of the resulting vesicle is reduced by ATP-driven proton pumps, resulting in the release of the metal ions from transferrin. Chromium released from multiple transferrin molecules is sequestered by apochromodulin (open circle) to produce chromium-loaded chromodulin (dark circle).

One note of caution must be addressed. The most popular form of chromium in dietary supplements, chromium picolinate, appears to be absorbed in a different fashion from dietary chromium. Chromium picolinate, $Cr(pic)_3$, is remarkably stable; it remains intact for several hours in synthetic gastric juice (39) and days to weeks under other physiologically relevant conditions (40,41). The complex also appears to pass unhindered through the jejunum (39) and probably migrates to and is incorporated into cells in its original form. Unfortunately, the picolinate ligands shift the redox potential of the chromic center of the complex such that it can be reduced by biological reducing agents such as ascorbate and thiols (40). The resulting chromous complex can interact with oxygen catalytically, generating the hydroxyl radical (26). In vitro studies using concentrations of $Cr(pic)_3$ believed to correspond to those in cells of individuals taking the supplement for prolonged periods of time and typical cellular concentrations of ascorbate found that significant DNA cleavage resulted from the generated hydroxyl radical (40). The release of chromium from $Cr(pic)_3$ (required for it to serve as a source of chromium) requires reduction of the chromium (26), suggesting that the deleterious chemistry and ability of $Cr(pic)_3$ to serve as a more absorbable source of chromium may be inexorably linked. Studies of the long-term effects of $Cr(pic)_3$ usage are required to determine the significance of this chemistry.

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