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Comment on Purification and Characterization of Chromium-Binding Substances from High-Chromium Yeast

Chromium-enhanced yeast has been studied on multiple occasions as a chromium nutritional supplement, primarily in terms of attempting to improve symptoms of type 2 diabetes.[1] Although the results of these trials have been mixed, these studies have raised the question about the nature of the form of chromium in the yeast. The use of chromium-enhanced yeast stems from earlier research on a purported biologically active form of chromium called glucose tolerance factor (GTF). The isolation and characterization of GTF from brewer's yeast was first reported in 1976.[2] GTF from the yeast was subsequently found to be an artifact of the isolation procedure (involving, for example, refluxing the yeast extract in 5 M HCl for 18 h,[2] which would have hydrolyzed proteins, nucleic acids, and complex carbohydrates).[1] Also, the characterization turned out to have been performed on an impure material;[1,2] paper chromatography yielded multiple bands with the biologically active band possessing only 6% of the chromium.[2] Several groups subsequently separated the component that increases insulin-stimulated glucose metabolism in rat adipocytes from the chromium-containing component.[1] The relative amounts of the proposed components of GTF (chromium, nicotinic acid, and the amino acids glycine, cysteine, and glutamic acid) were not determined.[1,2] The history of GTF and its influence on the fields of chromium nutrition and biochemistry have been reviewed numerous times recently (e.g., see ref 1).

The recent paper by Liu et al.[3] has a commendable goal of isolating the form of chromium in yeast grown in chromium-enriched media. The authors attempt to purify the chromium-containing low-molecular-weight species from an extract of freeze-dried yeast using only G75, G25, and G15 chromatography. Use of only size exclusion chromatography is highly unlikely to give a high-purity material from a complex extract as size exclusion chromatography lacks specific interactions between the solvate and media. The authors name the chromium-containing fraction from the G15 column PP1. Material absorbing at 260 and 280 nm and containing chromium eluted in a single band from the G15 column. This indicates either that the material is pure or that the G15 column provided little additional purification of the Cr-containing band from the G-25 column beyond that from the very similar G25 column. Isolated PP1 was characterized by mass spectrometry, amino acid analysis, nicotinic acid analysis, and biological activity assays. However, the authors' results demonstrate that PP1 is not pure; the authors' flow diagram indicates that HPLC separates PP1 into multiple components and, hence, cannot be pure. The reverse-phase HPLC of PP1 (Figure 6 in the Liu et al. paper) revealed that PP1 contains two major chromium-containing components (and other minor components that absorb in the UV region). The two major components were named peaks I and II. In the HPLC experiment, peak I elutes with the solvent front and potentially is not pure as a result. Peak I has most of the chromium but does not give rise to a negative mode MS signal. In contrast, both peak II and PP1 gave MS signals at m/z 712 and 769. Thus, most of the chromium is not associated with the MS features attributed to PP1. Peak II has a greater absorbance in the UV region than peak I, suggesting most of the organic material is not in the same peak as the chromium. Thus, peaks I and II are distinct components of PP1, indicating PP1 is not pure.

Amino acid analysis also showed that PP1 is not pure as the ratio of GLU/GLY/CYS/ASP is approximately 1:1:1:1/6. These amino acids, which are indicated as the major amino acids, comprise only ~35% of the amino acids according to Liu et al.'s Table 1. The concentration of chromium and the concentrations of the amino acids were not reported. Thus, one cannot determine if the chromium and amino acids occur in stoichiometric amounts. Similarly, one cannot tell if the amount of nicotinic acid (the concentration of which was given) occurs in a stoichiometric ratio to chromium. As PP1 is not pure and the ratios of the potential ligands to chromium were not established, determining what ligands are associated with the chromium is impossible. Thus, one cannot establish what the compositions of the chromium-containing species are.

The biological activity was also assayed using the impure PP1. The rat adipocyte assay is known to respond to numerous compounds. Thus, the biological activity observed cannot be attributed specifically to the chromium; it could potentially be from components of PP1 that lack chromium. The type of rats from which the fat pads were removed is not given, nor are the rats' ages or any other data about the rats, variables that could affect the results of the assays and the ability of other laboratories to reproduce them.

The authors made an error citing the literature on low-molecular-weight chromium-binding substance (LMWCr), the biomolecule that carries Cr from the tissues to the urine. The authors use the facts that GTF and LMWCr were reported to have UV absorption at 262 and 260 nm, respectively, to support the presence of nicotinic acid in PP1. However, LMWCr has been shown not to possess nicotinic acid.[1] Thus, the absorbance at 260 nm in PP1 may not arise exclusively from nicotinic acid.

The isolation and characterization by Liu et al.[3] of the high-molecular-weight chromium-binding substance has the same lack of demonstration of purity and failure to determine the ratios of chromium to potential ligands.

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The authors declare no competing financial interest.

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