ISOLATION AND PARTIAL CHARACTERIZATION OF A ^{51}Cr COMPLEX FROM BREWERS' YEAST[1]

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Summary

A butanol:H_2O extract of Brewers' yeast grown in a medium which contained ^{51}Cr was analyzed by gel-filtration chromatography. A single radioactive peak was eluted at an elution volume which suggested a molecular weight of approximately 400-600 daltons. Subsequent examination of pooled radioactive fractions obtained from gel-filtration chromatography demonstrated that the ^{51}Cr complex was eluted in a single peak from both cation- and anion-exchange resins. The elution characteristics of the ^{51}Cr complex indicated that the compound is a single anionic species. The ^{51}Cr complex was purified by a combination of gel-filtration chromatography and ion-exchange chromatography and subsequently analyzed by thin-layer chromatography. The results indicated that the ^{51}Cr complex from Brewers' yeast is a peptide that contains at least six amino acids. When a partially purified preparation of the ^{51}Cr complex from yeast was administered orally to rats, the absorption and retention of ^{51}Cr was significantly greater than that in rats given ^{51}Cr in the form of $CrCl_3 \cdot 6H_2O$. These experiments demonstrate that chromium is associated with a single metal-binding peptide in Brewers' yeast and indicate that the chromium in this complex is absorbed and retained more efficiently than chromium salts.

Chromium is apparently poorly absorbed from the gastrointestinal tract of both humans (1) and rats (2). Since chromium is an essential element in mammalian metabolism (3), efforts in our laboratory have been directed toward identifying compounds, both naturally occurring and synthetic, which will improve the intestinal absorption of this element. Mertz and Roginski (4) have demonstrated that ^{51}Cr extracted from

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Brewers' yeast is absorbed and retained in rats to a much greater extent than labeled chromium chloride. To date, no reports have appeared which describe the chemical characteristics of a chromium complex or complexes obtained from Brewers' yeast. The following manuscript describes the isolation and partial characterization of a chromium-binding peptide from Brewers' yeast which improves ^{51}Cr absorption and retention in rats.

Methods

The ^{51}Cr complex reported here was extracted from Brewers' yeast *(Saccharomyces cerevisiae)* grown in 1000-ml batches of autoclaved Sabouraud's medium (Difco Laboratories)[3] supplemented with 5% (w:v) glucose (3). The yeast cells were introduced into the medium from a yeast-culture agar slant after which 4 μg ^{51}Cr-$CrCl_3 \cdot 6H_2O$ (International Chemical and Nuclear; spec. act. = 257 Ci/g) were added to the medium. The inoculated yeast media in 3000-ml Erlenmeyer flasks were placed in mechanical shakers in a disinfected media room which was maintained at a constant temperature of 28°C. The yeast were allowed to grow for 6 days until they reached their apparent stationary phase (5) after which the cells were harvested by centrifugation at 10,000 x g for 10 min. The supernatant was poured off and discarded.

Yeast pellets of approximately 7-10 g were extracted by stirring for 4 hours in a mixture of butanol (200 ml) and distilled H_2O (200 ml). The mixture was separated into the organic phase and the water phase while standing in a separatory funnel for one hour. Thereafter, the water phase was removed and freeze-dried. The butanol:H_2O extraction procedure accounted for 100% of the radioactivity in the yeast pellet and of this, 90% was recovered in the water phase. Generally 20-30%

[3]Mention of a proprietary product does not necessarily imply endorsement by the USDA.

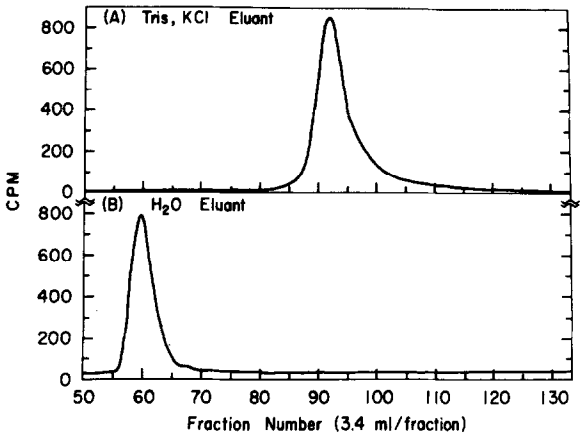

Figure 1. Elution of the ^{51}Cr extract from Brewers' yeast on Sephadex G-25. For details, see text.

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of the radioactivity in the water phase was lost during the freeze-drying procedure.

The freeze-dried extracts were dissolved in 5 ml distilled H_2O and applied to a 2.5- x 90-cm column packed with Sephadex G-25 which had been equilibrated with 1 mM Tris ((hydroxy methyl) amino methane), pH 7.3, and 0.05 M KCl. The same buffer was used to elute the sample and fractions of 3.4 ml were collected and monitored for radioactivity in a gamma-well counter. The radioactive fractions were then pooled and freeze-dried.

Ion-exchange chromatography was carried out on 0.9- x 15-cm columns packed with either Dowex-50W strongly acid cation-exchange resin in hydrogen form or Dowex-1 strongly basic anion-exchange resin in chloride form. Water and various concentrations of NaCl were used as eluants as described in the next section. Fractions of 1.1 ml were collected and counted for ^{51}Cr radioactivity.

The ^{51}Cr complex was purified by a combination of gel-filtration chromatography and ion-exchange chromatography, the details of which will be described in a separate publication. The purified compound was hydrolyzed with 6 N HCl for 20 hours at $110^{o}C$ in an evacuated tube. Hyrolyzed samples and unhydrolyzed fractions were analyzed by

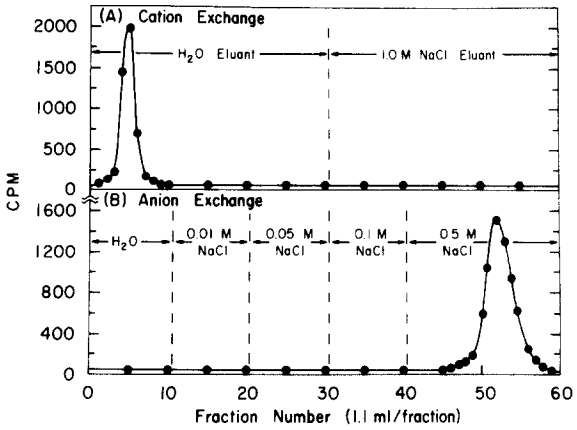

Figure 2. Chromatography of the ^{51}Cr complex from Brewers' yeast on cation- and anion-exchange resins. Freeze dried samples obtained from chromatography on Sephadex G-25 (Tris:KCl eluant) were dissolved in 1.0 ml H_2O and applied to the resins.

thin-layer chromatography on silica-gel impregnated glass paper (Gelman Type ITLC-SA) in two separate solvent systems. One solvent contained butanol:acetic acid:water (4:1:1). The other solvent contained phenol:water (75:25) and the chromatogram was developed in an ammonia-rich atmosphere. The chromatograms were developed twice in both solvents, dried thoroughly and sprayed with ninhydrin.

The absorption and whole-body retention of the ^{51}Cr complex from Brewers' yeast was examined in 120-day-old male rats which had been fed a diet of Purina Lab Chow and tap water. All animals were fasted for 18 hours prior to being used in experiments. Each of four rats was stomach-tubed with 2 ml of a solution which contained the ^{51}Cr complex that had been partially purified by extraction and gel-filtration chromatography *(vide supra)*. The solutions contained 2 ng ^{51}Cr-Cr. Similarly, each of four rats was stomach-tubed with 2 ml of a solution that contained 2 ng ^{51}Cr-$CrCl_3 \cdot 6H_2O$. Following oral administration of the isotope, the animals were monitored for radioactivity in a whole-body counter over a period of 210 hours.

Results and Discussion

A chromatogram of the yeast-extract sample eluted from a Sephadex

G-25 column with the Tris, KCl solvent system is shown in Figure 1A. A single peak of ^{51}Cr radioactivity was observed near fraction 92, corresponding to an elution volume (V_e) of 313 ml. This elution volume is 50 ml ahead of that of organic cobalt complexes with an average molecular weight of 350 daltons (unpublished data) which suggests that the ^{51}Cr complex has a molecular weight in the range of 400-600 daltons. Typically, 80-90% of the radioactivity applied was recovered in the single peak of radioactivity shown in Figure 1A. In contrast, when ^{51}Cr in the form of $^{51}CrCl_3 \cdot 6H_2O$ was applied to the column described above and eluted in an identical manner, less than 1.0% of the applied radioactivity was recovered in the eluted fractions. This observation was an assurance that the ^{51}Cr extracted from Brewers' yeast was not a simple ^{51}Cr salt. Moreover, we examined a portion of the labeled Sabouraud's medium and found that the ^{51}Cr in the medium retained the properties of uncomplexed $^{51}CrCl_3 \cdot 6H_2O$ which indicated that the ^{51}Cr complex described in this manuscript is synthesized by yeast cells.

The ionic properties of the ^{51}Cr complex from yeast are depicted in Figures 2A and 2B. As shown in Figure 2A, when pooled fractions obtained from chromatography on Sephadex G-25 were applied to a cation resin, the ^{51}Cr was eluted in a single peak with H_2O eluant. In contrast, 0.5 M NaCl was required to elute similar fractions from an anion resin (Figure 2B). Invariably, 100% of the radioactivity applied to either the cation or the anion resin was recovered in the single peaks described above. These results indicate that the ^{51}Cr complex from Brewers' yeast is a single, anionic species.

During our attempts to desalt the ^{51}Cr complex for further purification, we observed an interesting phenomenon which is depicted in Figure 1B. When a fraction of the ^{51}Cr complex which had been partially purified was applied to the Sephadex G-25 column and eluted

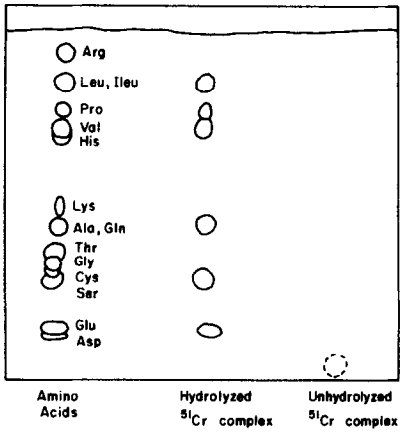

Figure 3. Thin layer chromatography of the ^{51}Cr complex from Brewers' yeast. The figure represents the average of results obtained from several analyses in two different solvent systems as described in the text.

with H_2O, a single radioactive peak was eluted near the void volume of the column, far removed from the elution volume observed when Tris: KCl was used as an eluant. Moreover, when the fractions eluted from Sephadex G-25 with water were concentrated and reapplied on a column which had been equilibrated with the Tris:KCl solvent, the radioactivity was recovered in a peak identical to that shown in Figure 1A. Apparently, the ^{51}Cr complex from yeast undergoes reversible aggregation in an unbuffered medium with low ionic strength.

Throughout the analysis and purification of the ^{51}Cr complex we observed that the fractions which contained the isotope consistently produced a positive reaction when treated with ninhydrin. Furthermore, infrared analysis of a purified sample of the ^{51}Cr complex showed the presence of a carbonyl peak at 1630 cm^{-1} which was shifted to 1725 cm^{-1} by the addition of acid. These observations suggested that the compound may be a peptide which contains at least one carboxylate group. When a highly purified fraction of the ^{51}Cr complex was analyzed by thin-layer chromatography, the results indicated that the chromium is bound to a peptide ligand that contains at least six amino

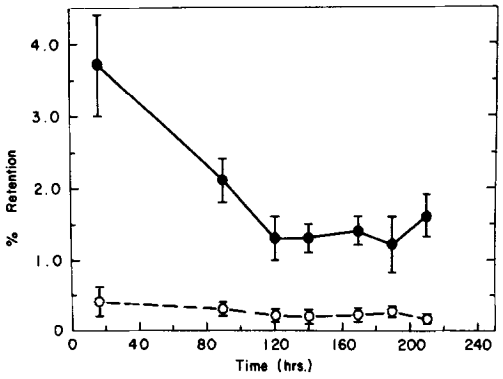

Figure 4. Absorption and net retention of ^{51}Cr in rats stomach-tubed with either the ^{51}Cr complex from Brewers' yeast or ^{51}Cr-$CrCl_3 \cdot 6H_2O$. The closed circles connected with the solid line represent animals given the ^{51}Cr complex from yeast. The open circles connected with the broken line represent the animals given the ^{51}Cr salt. Each value represents the mean $\pm$ SD of four animals.

acids (Figure 3). We have tentatively designated the following amino acids as being constituents of the chromium-binding peptide from Brewers' yeast: leucine or isoleucine, proline, valine, alanine, serine and either glutamic acid or aspartic acid.

The absorption and retention of a partially purified ^{51}Cr complex from Brewers' yeast is illustrated in Figure 4. At each time period, the whole-body retention of ^{51}Cr in animals which had been given the yeast ^{51}Cr complex was significantly greater ($P < 0.01$) than that in animals given ^{51}Cr in the salt form. These results substantiate the observations of Mertz and Roginski (4) and indicate that Brewers' yeast cells synthesize a peptide which effectively increases the absorption and net retention of ^{51}Cr in the rat.

During the last decade, several experiments have suggested that chromium is essential for maintaining normal carbohydrate metabolism in man and other mammalian species (3). More specifically, Mertz (3) has suggested that a chromium complex from yeast, referred to as the "glucose tolerance factor", may potentiate the action of insulin.

Thus, the chromium-binding ligand described in this report may prove to be extremely beneficial in human and animal nutrition.

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