

In Vivo Transchelation of Copper-64 from TETA-Octreotide to Superoxide Dismutase in Rat Liver

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An understanding of the metabolic fate of radiometal-labeled peptides is important due to their application in the areas of diagnostic imaging and targeted radiotherapy. Radioisotopes of copper (^{64}Cu , $T_{1/2} = 12.7$ h; ^{67}Cu , $T_{1/2} = 62$ h) have been labeled to monoclonal antibodies (mAbs) and peptides and have applications in the areas of PET imaging and targeted radiotherapy of cancer. Copper-64-TETA-D-Phe¹-octreotide ($[^{64}\text{Cu}]\text{TETA-OC}$) has been shown to bind to the somatostatin receptor, both in vitro and in vivo, and this agent inhibited the growth of somatostatin-receptor positive tumors in rats. Copper-64-TETA-OC, however, showed a retention of activity in the blood, liver, and bone marrow, suggesting possible dissociation of ^{64}Cu from TETA-OC in vivo. The purpose of this study was to determine if ^{64}Cu dissociates from $[^{64}\text{Cu}]\text{TETA-OC}$ and binds to the protein, superoxide dismutase (SOD) in rat liver. The liver metabolism of $[^{64}\text{Cu}]\text{TETA-OC}$ was examined in normal rats using a gel-electrophoresis assay specific for SOD and size-exclusion chromatography. The major metabolite in rat liver at 20 h postinjection had a molecular weight of 32 kDa as shown by size-exclusion chromatography. A gel electrophoresis assay specific for the detection of SOD [nitro-blue tetrazolium (NBT)] showed that a ^{64}Cu -labeled protein isolated from rat liver homogenates comigrated with SOD. Evaluating the metabolic fate of copper radiopharmaceuticals demonstrated that Cu(II) dissociates from macrocyclic chelators such as TETA and binds to proteins in high concentrations, namely SOD in rat liver.

INTRODUCTION

An understanding of metabolism can aid in the design of radiopharmaceuticals for optimal retention in target tissues (such as tumors) and clearance through nontarget organs (such as the liver or kidneys). The information that can be obtained through in vivo metabolism studies includes the identification of the radiolabeled metabolites, which accumulate in both target and nontarget organs (1–6). An understanding of the metabolic fate of ^{64}Cu - and ^{67}Cu -labeled monoclonal antibodies (mAbs) and peptides is of importance due to their applications in the areas of PET imaging (7–10) and targeted radiotherapy of cancer (11–14). The metabolites of radiometal-labeled radiopharmaceuticals determine the retention and clearance of the radionuclide in the target tissue(s) as well as the clearance organs.

The biodistribution and metabolism of several radiometal-peptide/mAb compounds have previously been investigated (15–19). These studies demonstrate that the in vivo metabolism of ^{111}In -labeled proteins and peptides is very different from that of analogous ^{64}Cu -labeled agents. Whereas ^{111}In -labeled peptides and mAbs are metabolized in the liver primarily to $[^{111}\text{In}]\text{chelate-amino acid moieties}$, the analogous ^{64}Cu -labeled chelator-biomolecule conjugates show significant dissociation of ^{64}Cu from the chelator followed by binding of the ^{64}Cu to 30–35 kDa proteins.

Superoxide dismutase (SOD) is a homodimeric enzyme of ~ 32 kDa with each subunit containing one copper and one zinc atom believed to be bridged by an

imidazole group (20). SOD is distributed widely in the cytosol of eukaryotic cells. This enzyme is especially abundant in the liver, kidney, adrenal, and red blood cells. SOD provides a defense mechanism against the potential toxicity of oxygen radicals by catalyzing the disproportionation of the superoxide ion to hydrogen peroxide and oxygen.

Rogers et al. analyzed the liver metabolism in rats of anti-colorectal carcinoma mAb 1A3 radiolabeled with ^{67}Cu through three different macrocyclic bifunctional chelators (BFCs) (18). Intact ^{67}Cu -labeled mAb BFC-1A3 conjugates and two other metabolites of the ^{67}Cu mAbs were observed. One of these metabolites was identified as $[^{67}\text{Cu}]\text{BFC-lysine}$ by reversed-phase HPLC, while from size-exclusion chromatography, the higher molecular weight non-mAb species (~ 35 kDa) was suggested to be the result of in vivo transchelation of ^{67}Cu from the BFC to superoxide dismutase (SOD). Since there are many biological proteins with a wide range of molecular weights to which copper can bind (20), this work was carried out to confirm transchelation of ^{67}Cu from $[^{67}\text{Cu}]\text{BFC}$ conjugates of biological molecules to SOD in vivo in the rat liver.

The current focus of our research is the development of radiometal-labeled peptides for targeting tumor receptors for PET imaging and targeted radiotherapy (8, 14). In the development of $[^{64}\text{Cu}]\text{TETA-D-Phe}^1\text{-octreotide}$ ($[^{64}\text{Cu}]\text{TETA-OC}$, Figure 1) as an agent for targeted radiotherapy of somatostatin-receptor positive tumors, biodistribution studies in a tumor-bearing rat model demonstrated that there was a 10-fold greater concentration of $[^{64}\text{Cu}]\text{TETA-OC}$ in the blood at longer time points (24 h) compared to $[^{111}\text{In}]\text{DTPA-OC}$ (8). This observation,

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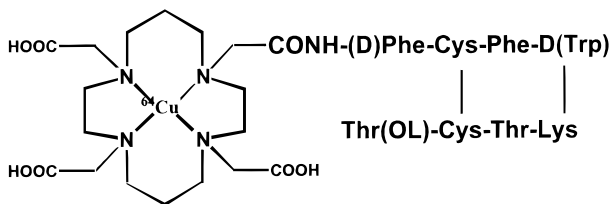


Figure 1. Structure of [^{64}Cu]TETA-OC.

along with the lack of liver clearance and increasing marrow uptake of [^{64}Cu]TETA-OC over time (out to 48 h) (14), suggests possible dissociation of ^{64}Cu from TETA-OC in vivo. To further examine the potential exchange of radioactive copper from BFC-biological molecule conjugates to SOD, we examined the liver metabolism of [^{64}Cu]TETA-OC in normal rats using gel-electrophoresis techniques, assays specific for SOD along with size-exclusion chromatography (21).

MATERIALS AND METHODS

Reagents and Instrumentation. Ultrapure ammonium dihydrogen phosphate was purchased from Johnson Matthey (Royston, England), and ultrapure ammonium acetate and ammonium citrate was from Fluka (Ronkonkoma, NY). TETA-OC was synthesized as previously described (14). Sephadex G-25/50, riboflavin, SOD from bovine liver, and human erythrocytes and bovine serum albumin (BSA) were purchased from Sigma (St. Louis, MO). C-18 Sep-Pak cartridges were from Waters/Millipore (Burlington, MA). Mature female Sprague-Dawley rats were from Sasco (Omaha, NE). Copper-64 was produced at Washington University on a biomedical cyclotron (22). Bio-Spin-6 chromatography columns, Native Tris-Glycine Ready gels (10% resolving gel, 4% stacking gel), TWEEN-20, TEMED (*N,N,N,N* tetramethylethylenediamine), Tris/glycine running buffer (10 \times), Tris/glycine/SDS running buffer (10 \times), native/denaturing sample buffer and nitrocellulose/PVDF membrane (0.2 μm) were purchased from Bio-Rad (Hercules, CA). Nitro-Blue tetrazolium (NBT) was purchased from Aldrich (Milwaukee, WI).

Organs were prepared using a Tekmar tissue homogenizer (Cincinnati, OH), a Branson Sonifier 185 cell disrupter, and a Sorvall RC2-B centrifuge. Pharmacia Superose 12 gel filtration columns were used in conjunction with a Pharmacia/LKB fast-protein liquid chromatography (FPLC) system (Piscataway, NJ) equipped with a UV-vis detector for analysis of liver homogenates. A set of eight calibration standards, with molecular masses ranging from 246 Da (cytidine) to 669 kDa (thyroglobulin), were used to calibrate the columns. A Beckman Gamma 8000 automated well-type γ counter was used to quantitate radioisotope in FPLC fractions. Radio-TLC was carried out using a Bioscan System 200 Imaging Scanner (Washington DC). Radioactive gels were analyzed via an InstantImager Electronic Autoradiography System from Packard Instrument Co. (Meriden, CT). BCA protein determination kit was obtained from Pierce (Rockford, IL).

Preparation of [^{64}Cu]TETA-OC, [^{64}Cu]SOD, and [^{64}Cu]BSA. Copper-64 chloride ($^{64}\text{CuCl}_2$) was diluted greater than 10-fold with 0.1 M NH_4OAc , pH 5.5. The [^{64}Cu]acetate was added to TETA-OC (1–10 μg) or SOD (1 mg), and the final volume adjusted to 1.0–1.5 mL with buffer. After incubation for 45 min at room temperature, [^{64}Cu]SOD (60 μCi) was purified using a G25/50 sephadex gel filtration column prepared in 0.1 M NH_4OAc . After a 60 min incubation at room temperature, [^{64}Cu]TETA-OC

was purified using a Sep-Pak cartridge as previously described (8). Radiochemical purity was assayed by FPLC for [^{64}Cu]SOD and by HPLC and radio-TLC for [^{64}Cu]TETA-OC.

For labeling to BSA (1 mg), $^{64}\text{CuCl}_2$ (60 μCi) was added to 0.1 M H_3PO_4 , pH 8.3, and incubated for 15 min at room temperature. Purification was performed using a G25/50 sephadex gel filtration column prepared in 0.1 M H_3PO_4 , pH 8.3. Radiochemical purity was assessed by FPLC.

In Vivo Metabolite Analysis in Sprague-Dawley Rats. Female Sprague-Dawley rats ($n = 3-4$) were injected via the tail vein with [^{64}Cu]TETA-OC (3–13 mCi; 5 μg) or [^{64}Cu]acetate (1–13 mCi), and livers were harvested 20 h postinjection. Prior to injection, the dose was diluted in 0.9% NaCl (saline). The liver was rinsed with precooled saline, blotted dry and treated with 5 mL of precooled 0.1 M $\text{NH}_4\text{OAc}/\text{EtOH}$ mixture (35:65), homogenized (1 min), and sonicated (45 s). After centrifugation at 23500g for 30 min at 5 $^\circ\text{C}$, the supernatants were removed. Organ blanks, where the [^{64}Cu]acetate or [^{64}Cu]TETA-OC was added directly to the liver prior to homogenization and centrifugation, were performed as controls. The total protein content of the liver supernatants was determined using the BCA method. All liver supernatants were analyzed by FPLC and gel electrophoresis (<200 μg of total protein loaded/lane). Some samples were also analyzed by radio-TLC on silica gel in 1:1 MeOH:10% NH_4OAc . For FPLC analysis, a 0.1–0.2 mL aliquot of the supernatant was injected on a Superose 12 gel filtration column. This column was eluted with 20 mM Hepes and 300 mM NaCl (pH 7.3) buffer at a flow rate of 0.4 mL/min, and the fractions were counted on a γ counter.

SOD Activity Gels. Fifty micrograms of total protein from the supernatant of liver homogenate samples or 1.5 μg of SOD standards (human and bovine) or BSA standard was applied to a 10% Tris/glycine Ready Gel and subjected to native polyacrylamide gel electrophoresis (PAGE). Radiolabeled agents and liver homogenates were analyzed by PAGE prior to analysis of radioactivity on the gel using the InstantImager. A separate native PAGE gel was run where the gel was submerged in 50 mL of 2.5 mM NBT at room temperature and shaken gently for 30 min. The gel was then transferred to a solution containing 28 mM TEMED, 2.8 mM riboflavin, and 36 mM potassium phosphate (pH 7.8) and shaken gently at room temperature for 20 min. The gel was wrapped in plastic wrap and placed on a light source for 15–30 min. The gel stained blue at sites of $\text{O}_2^{\cdot-}$, yet, staining was absent at sites of $\text{O}_2^{\cdot-}$ scavenging, thus, white bands on a blue background indicated the presence of Cu/Zn-SOD (21).

RESULTS

Radiolabeling and Chromatography. TETA-OC was labeled with ^{64}Cu with a specific activity of ~ 1500 mCi/ μg . The radiochemical purity of ^{64}Cu -labeled TETA-OC was >95% as determined by HPLC and/or radio-TLC. The radiochemical purity of [^{64}Cu]SOD and [^{64}Cu]BSA was >95% as determined by FPLC. A radioactivity balance was performed for each sample run on the FPLC to determine if any activity remained trapped on the column. For all samples analyzed by FPLC, including standards and liver homogenates, there was little or no remaining activity left on the columns.

Analysis of Liver Extracts. Standards. A standard curve was constructed for each Superose column using

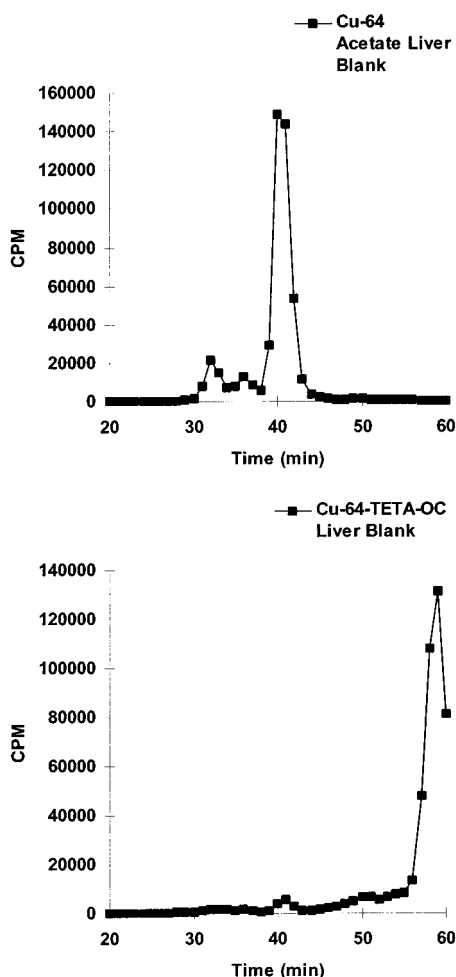


Figure 2. FPLC chromatograms of liver "blanks" of ^{64}Cu -acetate and ^{64}Cu]TETA-OC added to liver homogenate ex vivo followed by homogenization and chromatographic analysis of the liver supernatant. (Top panel) ^{64}Cu]Acetate: peak 1, 32 min (10.7%), molecular mass ~ 150 kDa; peak 2, 36 min (7.5%), molecular mass ~ 45 kDa; peak 3, 40 min (81.8%), molecular mass ~ 11 kDa. (Bottom) ^{64}Cu]TETA-OC: peak 1, 41 min (3.4%), molecular mass ~ 8 – 10 kDa; peak 2, 50 min (10.6%), molecular mass < 2 kDa; peak 3, 59 min (84%) molecular mass < 2 kDa.

proteins of molecular masses ranging from 246 Da to 669 kDa. From this curve, the molecular masses of the ^{64}Cu]containing species formed in vivo were estimated. ^{64}Cu -labeled bovine SOD and ^{64}Cu -labeled human SOD standards eluted with the same retention time by FPLC (37 min). A ^{64}Cu]TETA-OC standard had a retention time of 58 min and was well-resolved from ^{64}Cu]SOD.

Ex Vivo Analyses. Organ blanks were carried out for ^{64}Cu]acetate and ^{64}Cu]TETA-OC to determine whether the species seen after i.v. injection were produced by the techniques used to extract the metabolites from the organs. In the case of "free" ^{64}Cu , we sought to determine whether ^{64}Cu would form ^{64}Cu]SOD in liver cell homogenates. FPLC (Figure 2, top) showed three species present in the liver blank: 11% eluted with a retention time of 32 min (molecular mass ~ 110 kDa), 8% eluted with a retention time of 36 min, which corresponded with ^{64}Cu]SOD, and 80% eluted at a longer retention time (40 min). Thus, the addition of free ^{64}Cu to liver tissue only forms a very small amount of ^{64}Cu]SOD. In the case of ^{64}Cu]TETA-OC (Figure 2, bottom), the organ blank eluted with the same retention time as the standard solution of ^{64}Cu]TETA-OC (58 min), demonstrating that the tissue homogenization process did not dissociate the ^{64}Cu from the TETA-OC.

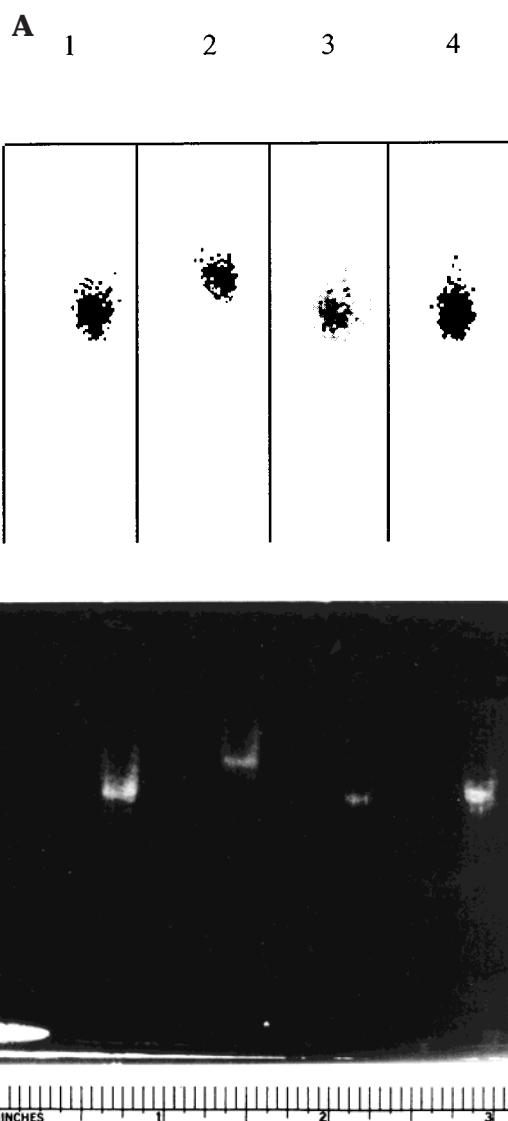


Figure 3. (A) Autoradiography showing ^{64}Cu -labeled bands of liver homogenate samples from rats injected with either ^{64}Cu]TETA-OC or ^{64}Cu]acetate on native polyacrilamide gel electrophoresis. (B) Native polyacrilamide gel electrophoresis of the liver homogenate samples in A stained with NBT, riboflavin, and *N,N,N,N*-tetramethylethylenediamine for SOD activity. Lane 1, SOD from human erythrocytes ($3.33 \mu\text{g}$ protein); lane 2, SOD from bovine liver ($1.67 \mu\text{g}$ of protein); lane 3, liver homogenates from the rat injected with ^{64}Cu]TETA-OC ($38.6 \mu\text{g}$ protein); lane 4: liver homogenates from the rat injected with ^{64}Cu]acetate ($52 \mu\text{g}$ of protein).

^{64}Cu]Acetate. To determine whether the i.v. administration of "free" ^{64}Cu (in the form of Cu-acetate) resulted in the formation of ^{64}Cu]SOD in the rat liver, ^{64}Cu]acetate was injected in the rat, and the rat was sacrificed and the liver was removed 20 h postinjection. The liver was extracted using either phosphate-buffered saline (PBS), NH_4OAc (0.1 M, pH 5.5), or EtOH/PBS (75:25). Size-exclusion chromatography showed one ^{64}Cu -containing species eluting at approximately 35 kDa, independent of extraction media. These results agreed with those reported by Rogers et al. (18). The retention time of this species was equivalent to ^{64}Cu]SOD prepared in vitro using bovine or human SOD (Sigma). The gel electrophoresis of the liver homogenates indicated one radioactive band, which migrated slightly faster than the in vitro prepared ^{64}Cu -labeled bovine SOD, although it migrated identically to ^{64}Cu -labeled human SOD (Figure 3A). The

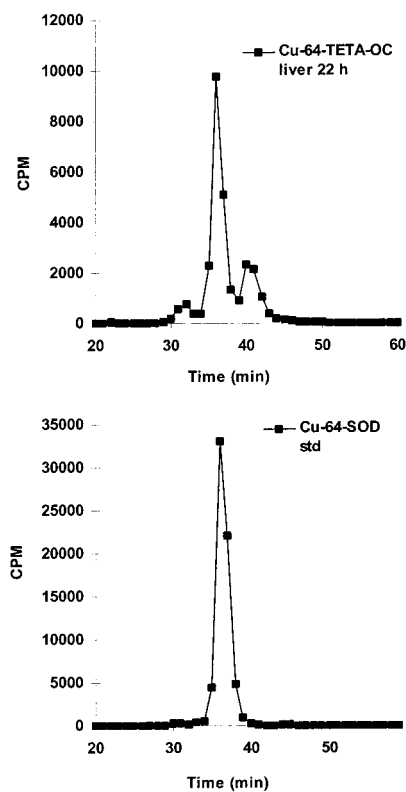


Figure 4. FPLC chromatograms of (top) liver homogenates from rats injected with (top) [^{64}Cu]TETA-OC compared to (bottom) [^{64}Cu]SOD standard. (Top panel) [^{64}Cu]TETA-OC: peak 1, 32 min (6.8%), molecular mass \sim 150 kDa; peak 2, 36 min (68.4%), molecular mass \sim 32 kDa; peak 3, 40 min, molecular mass \sim 11 kDa. (Bottom panel) [^{64}Cu]SOD standard: peak 1, 36 min (100%), molecular mass \sim 32 kDa.

different migration patterns of human SOD1 mutants on native gels have been attributed to differences in charge (23). This is likely why we are seeing a difference between the migration of human vs bovine SOD. The NBT assay was performed on the gels and the migration distance of the radioactive bands corresponded to that for the white bands observed after NBT treatment (Figure 3B). A negative control was also run on a native gel with the NBT assay. Copper-64-labeled BSA was present on the autoradiograph of the native gel; however, a white band was not present after NBT treatment (data not shown).

[^{64}Cu]TETA-OC. In liver homogenates 20 h postinjection of [^{64}Cu]TETA-OC, approximately 69% of the ^{64}Cu eluted by FPLC as a \sim 35 kDa species with 7% eluting as a higher molecular mass (\sim 100 kDa) species and 24% eluting as a lower molecular mass species (11–14 kDa). The majority of radiolabeled metabolites eluted identically to [^{64}Cu]SOD (Figure 4). Analysis of the gel after PAGE showed one ^{64}Cu -containing band, which migrated similarly to ^{64}Cu -labeled human SOD, but slightly faster than the bovine [^{64}Cu]SOD (Figure 3A). This band was also equivalent to the radioactive band observed in the liver homogenate after injection of [^{64}Cu]acetate. Assay of the gel with NBT exhibited white bands at migration distances equal to the radioactive bands, thus indicating the presence of Cu/Zn-SOD and its *in vivo* incorporation of ^{64}Cu (Figure 3B).

DISCUSSION

In the design of radiopharmaceuticals with optimal targeting for either specific organ systems or tumors, it is important to understand their metabolism *in vivo*. Duncan and Welch reported that [^{111}In]DTPA-conjugated

glycoproteins were metabolized to [^{111}In]DTPA-lysine in the rat liver, confirming that transchelation of ^{111}In to proteins was not the major factor for the accumulation of ^{111}In in the liver (15). Studies carried out in our lab with ^{64}Cu -labeled chelator-protein and peptide conjugates showed a very different pattern of liver metabolism than what occurs with similar [^{111}In]labeled conjugates. Rogers et al. demonstrated that in rats [^{64}Cu]chelate-mAb conjugates were metabolized to [^{64}Cu]chelate-lysine conjugates in the kidney, but in the liver, transchelation of ^{64}Cu to a 32 kDa protein was the predominant metabolite (18). Due to our current interest in ^{64}Cu -labeled somatostatin analogues for diagnostic imaging (8, 10) and targeted radiotherapy (14, 24), we focused on the metabolism of [^{64}Cu]TETA-OC to determine if similar transchelation occurred *in vivo* in the rat liver.

Analyses were performed where the [^{64}Cu]acetate or [^{64}Cu]TETA-OC was added to whole livers *ex vivo* to differentiate between [^{64}Cu]SOD formed from *in vivo* processes vs that formed from extracellular incorporation of ^{64}Cu into SOD released upon lysing the cells. Addition of [^{64}Cu]acetate to rat liver *ex vivo* resulted in the formation of only a small amount of [^{64}Cu]SOD (Figure 2), whereas the [^{64}Cu]TETA-OC liver blank showed only intact agent. Seo and Ettinger reported that when $^{64}\text{Cu}(\text{NO}_3)_2$ was added to mouse hepatic cytosols, three major ^{64}Cu -binding fractions were detected (25). These fractions corresponded to molecular masses of 130 kDa and \sim 50 kDa and metallothionein (MT) (\sim 17 kDa). Although we observed a similar chromatographic profile with rat whole liver homogenate, the amounts of each ^{64}Cu -labeled protein present were different than what Seo and Ettinger observed. The major peak at 40 min was possibly [^{64}Cu]MT. A potential reason for the lack of [^{64}Cu]SOD formation in rat liver *ex vivo* may be slow kinetics. Our *in vivo* data was obtained at 22 h postinjection, whereas the *ex vivo* homogenates were analyzed shortly after [^{64}Cu]acetate was added to the liver homogenates. Although these control experiments showed that [^{64}Cu]SOD does not form rapidly in rat liver homogenates *ex vivo*, they also demonstrated that the homogenization and sonication processes used to analyze the liver samples did not cause dissociation of ^{64}Cu from TETA-OC to form [^{64}Cu]SOD.

After the injection of either [^{64}Cu]acetate or [^{64}Cu]TETA-OC, supernatants from rat liver homogenates were analyzed by FPLC and PAGE. The FPLC chromatograms of liver homogenates from rats injected with [^{64}Cu]acetate showed one ^{64}Cu -labeled protein present in the liver homogenate supernatant with a molecular mass of 32 kDa. These results are very similar to those of Terao and Owen (26), where they found that when ionic ^{67}Cu was injected into rats, 92.8% of the ^{67}Cu was bound to a protein with a molecular mass of 31 kDa (suggested to be either erythrocyte, cytochrome or SOD). Terao and Owen also found small amounts of ^{67}Cu labeled to proteins with molecular masses of 150 kDa [5.1%; probably ceruloplasmin (Cp)] and 11 kDa (2.2%; a sulfhydryl containing protein) (26), which we did not observe.

Rat liver homogenate supernatants from rats injected with [^{64}Cu]TETA-OC showed approximately three metabolites by FPLC, with the major metabolite having a molecular mass of 32 kDa (69%). The other two peaks corresponded to molecular masses of \sim 150 kDa (7%) and \sim 11 kDa (24%), which most likely correspond to ceruloplasmin and a sulfhydryl-containing protein such as metallothionein (26). Our results with [^{64}Cu]TETA-OC are very consistent with what has been reported with ionic ^{67}Cu by Terao and Owen and strongly suggest that

^{64}Cu dissociated from the TETA chelator and bound to proteins, primarily SOD.

The NBT assay was used to verify that the major radioactive band containing ^{64}Cu was associated with SOD. In the NBT method, riboflavin is used to generate O_2^- , which is illuminated in the presence of TEMED and oxygen. NBT is photochemically reduced by O_2^- producing a blue color. SOD inhibits this reduction and thus is detected by a colorless band on a blue background. In rats injected with either [^{64}Cu]acetate or [^{64}Cu]TETA-OC, the radioactive bands comigrated with the colorless bands on the NBT assay, strongly suggesting that $^{64}\text{Cu}(\text{II})$ bound to SOD in rat liver.

Further verification of the binding of ^{64}Cu to SOD in rat liver was attempted using Western blot analysis (data not shown). The use of SDS (non-native gel) causes proteins to unfold, and in our experiments, this appeared to cause the dissociation of ^{64}Cu from SOD. Therefore, we could not see a radioactive band for [^{64}Cu]SOD on a Western blot gel when SDS was present. Using native gel techniques (without SDS), we did not detect any binding of the SOD antibody to either human bovine SOD standards or to proteins in liver homogenates. With native gel techniques, the tertiary structure of proteins is intact. It is possible that in their intact state, there is no interaction between SOD and the anti-SOD antibody, and the antibody is only active against a denatured form of SOD.

DeNardo and colleagues have evaluated [^{67}Cu]BAT-2IT-Lym-1 in patients as an agent for RIT of lymphoma (13). They observed a flat, slow phase of blood clearance at about 3 days postinjection of [^{67}Cu]BAT-2IT-Lym-1, which was not observed with [^{131}I]Lym-1, suggesting that ^{67}Cu was incorporated into another protein. There was no evidence for binding of ^{67}Cu to albumin; however, ^{67}Cu was precipitated by anti-human Cp, with a maximum amount of [^{67}Cu]Cp observed on day 4 postinjection. It was hypothesized that ^{67}Cu was released from BAT-2IT-Lym-1 in the liver with subsequent formation of [^{67}Cu]Cp, and then [^{67}Cu]Cp was secreted back into the blood, which has also been suggested by Terao and Owen (26). These data agree with our results demonstrating that copper radionuclides dissociate from macrocyclic chelators in the liver and bind to proteins. The abundance of proteins such as SOD or Cp in the liver varies significantly between species, and rat liver has high concentrations of SOD (27). It has also been demonstrated that Cp can deliver copper to copper proteins such as SOD1 (28). We also observed a small amount of [^{64}Cu]Cp from the metabolism of [^{64}Cu]TETA-OC (~7%).

Possible explanations for the dissociation of copper radionuclides from bifunctional chelator-biomolecule conjugates are that the Cu(II) bifunctional chelator complexes may not be thermodynamically stable, or they may not be kinetically stable. Jones-Wilson and colleagues studied a series of six Cu(II) macrocyclic complexes and showed that some of the most thermodynamically stable complexes appeared to be the least stable in vivo (29), which argues for kinetic stability being more important.

In conclusion, using an assay specific for SOD, we have confirmed the formation of [^{64}Cu]SOD in the liver upon injection of [^{64}Cu]acetate and [^{64}Cu]TETA-OC into rats. Evaluating the metabolic fate of copper-radiopharmaceuticals has demonstrated that Cu(II) dissociates from macrocyclic chelates such as TETA and binds to proteins in high concentrations, namely SOD. Methods for circumventing the in vivo exchange of Cu(II) from such complexes is currently under investigation.

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