Identification of Metabolites of $^{111}$In-Diethylenetriaminepentaacetic Acid-
Monoclonal Antibodies and Antibody Fragments in Vivo

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Abstract

The in vivo fate of various $^{111}$In-labeled polypeptides has been the subject of many investigations. Intracellular metabolism has been studied through the use of $^{111}$In-labeled glycoproteins that are concentrated in the lysosome by receptor-mediated endocytosis. This study has demonstrated that the main lysosomal metabolite is $^{111}$In-chelate-ε-lysine, both in vitro and in vivo (Y. Arano et al., J. Nucl. Med., 35: 890–898, 1994; F. N. Franano et al., Nucl. Med. Biol., 21: 1023–1034, 1994). Since the vast majority of radiolabeled antibodies do not localize within the target tissue, an understanding of the metabolism of $^{111}$In-labeled antibodies in nontarget tissue is important for the rational design of future radiolabeled antibodies.

We investigated the in vivo metabolism of $^{111}$In-DTPA$\textsuperscript{\textgamma}$-conjugated antibody in female Sprague-Dawley rats using the anticolorectal carcinoma monoclonal antibody (MAb) 1A3 and MAb 1A3-F(ab$'$)$_2$. Livers and kidneys were harvested from rats injected with either intact MAb or MAb fragments and analyzed by gel filtration chromatography. Thirty-five % of the radioactivity from $^{111}$In-DTPA-1A3 MAb present in the liver was in the form of a low molecular weight species at 1 through 5 days. In contrast, $^{111}$In-DTPA-1A3-F(ab$'$)$_2$ was >98% degraded to a low molecular weight species in the kidney after 1 day. In each case, the low molecular weight metabolites were collected and further analyzed by silica gel thin-layer chromatography, reversed phase high-performance liquid chromatography, and ion-exchange chromatography and compared to $^{111}$In-DTPA and $^{111}$In-DTPA-ε-lysine standards. In each system, the major metabolite co-eluted with $^{111}$In-DTPA-ε-lysine, similar to the results obtained with $^{111}$In-labeled glycoproteins that are delivered to lysosomes by receptor-mediated endocytosis. A minor metabolite that was more highly charged than $^{111}$In-DTPA was also observed. Analysis of urine and feces demonstrated that the main excretory product of both $^{111}$In-labeled intact 1A3 and 1A3-F(ab$'$)$_2$, was $^{111}$In-DTPA-ε-lysine. Based on this data, we propose that $^{111}$In-DTPA-antibodies are degraded within lysosomes of nontarget organs such as the liver and kidneys.

Introduction

The identification of in vivo metabolites of antibodies, proteins, and polypeptides radiolabeled with metal radionuclides is important for understanding the mechanisms involved in the uptake and retention of activity in nontarget organs. These molecules were initially labeled with isotopes of iodine ($^{123}$I, $^{125}$I, or $^{131}$I); however, conventional labeling with these isotopes results in poor target retention (1, 2). This lack of retention most likely results from the rapid egress of the radionuclide, thereby decreasing radiometal accumulation in nontarget tissues. The success of this strategy has been limited (8–11). These results reflect, in part, our uncertainty as to what metabolites are produced in vivo. By determining the structures of the various metabolites, we hope to learn the mechanisms of radiolabel retention as well as develop possible methods of circumventing the high hepatic and renal background of $^{111}$In-labeled molecules.

Much data implicate lysosomes in the metabolism of MAbs at both target and nontarget sites. Several studies have shown that radiolabeled antibodies bound to cell surface antigens are internalized and delivered to the lysosome for degradation (4, 12–14). For nontargeted antibodies, the evidence is more indirect. Intact antibodies and their fragments could be internalized and delivered to lysosomes via three different endocytic mechanisms: absorptive endocytosis, fluid phase endocytosis, and receptor-mediated endocytosis. Absorptive endocytosis is exemplified by the renal tubal reabsorption of proteins (15). Fluid phase endocytosis is well studied in the liver, where hepatocytes internalize 20% of their volume per hour, and 18% of that volume is transported to intracellular storage compartments such as lysosomes (16). Receptor-mediated endocytosis of antibody-antibody and antibody-antigen complexes by the Fc receptor could also deliver radiolabeled antibodies to hepatic lysosomes (17–19).

Recent studies with $^{111}$In-chelate-glycoprotein have shown that these molecules are delivered to lysosomes by receptor-mediated endocytosis (20, 21). Once in lysosomes, the glycoprotein polypeptide backbone is rapidly degraded, yielding low molecular weight radiolabeled metabolites, and these metabolites are retained within the lysosome (21). Studies with several different bifunctional chelates have found that the predominant metabolites are of the general structure, $^{111}$In-chelate-lysine (20, 22). This result indicates that the chelate-lysine bond is inefficiently hydrolyzed within the lysosome, and the $^{111}$In-chelate bond is also stable in the acidic environment of the lysosome.

Prior studies on the metabolism of $^{111}$In-chelate-antibodies have found that they were degraded to low molecular weight metabolites (23, 24). This result, together with the above data implicating lysosomes in antibody metabolism and the identification of metabolites produced by lysosomal metabolism, suggested that $^{111}$In-DTPA-antibodies would be degraded to $^{111}$In-DTPA-ε-lysine. Therefore, we studied the metabolism of the 1A3 MAb and its F(ab$'$)$_2$ fragments.

Different investigators have varied the bifunctional chelates and chemical linkers between the radiometal and protein in attempts to decrease radiometal accumulation in nontarget tissues. The success of these efforts has been limited (8–11). These results reflect, in part, our uncertainty as to what metabolites are produced in vivo. By determining the structures of the various metabolites, we hope to learn the mechanisms of radiolabel retention as well as develop possible methods of circumventing the high hepatic and renal background of $^{111}$In-labeled molecules.

Prior studies on the metabolism of nontargeted antibodies have found that the radiolabel was recovered in a low molecular weight form. Metabolism studies of antibodies labeled with $^{111}$In have indicated that they are catabolized to small molecular weight compounds in the liver and kidneys (25–27). Studies with other radiometals have...
identified radiometal-chelate-lysine metabolites in the urine after administration of whole antibody and antibody fragments (28–30).

The \(^{111}\)In complex and the amide bond formed when the chelate is linked to the \(\epsilon\)-amine of lysine are both stable to the low pH of the lysosome and to lysosomal hydrolases (24, 26, 31–35). If \(^{111}\)In dissociates from the complex, \(^{111}\)In reassociation with the chelate is unlikely because of the low concentrations occurring with carrier-free radionuclides. If decomplexation occurred, most likely the \(^{111}\)In would bind some other protein. This other protein could be observed by size-exclusion chromatography and would likely have a biodistribution pattern which differed from chelate-associated \(^{111}\)In. If the amide bond joining the chelate to the protein was not stable, \(^{111}\)In-labeled chelate-amino acid(s) would not have been seen in previous studies (24, 26, 27).

Intracellular metabolism of \(^{111}\)In-labeled polypeptides has been largely attributed to internalization and degradation within lysosomes. These polypeptides are delivered to the lysosome through receptor-mediated endocytosis (36, 37). A single receptor can deliver many polypeptide molecules to the lysosome, where the polypeptide is catabolized to amino acids in a low pH environment (33–35). Through the use of glycoproteins targeted to cell surface receptors in the liver, it has been shown that \(^{111}\)In-DTPA-labeled polypeptides are hydrolyzed to small molecular weight compounds in the lysosome that are slowly released from the cell (21). Similar studies have identified the small molecular weight compound as \(^{111}\)In-chelate-\(\epsilon\)-lysine (20, 22, 38).

We were interested in identifying the exact structure of the major metabolite produced when whole antibody and antibody fragments labeled with \(^{111}\)In were administered in vivo and seeing if this structure was consistent with the metabolite identified in the glycoprotein studies. MAb 1A3, an anticolorectal carcinoma MAb (39), was labeled with \(^{111}\)In through the use of the bifunctional chelate DTPA. DTPA was conjugated to the \(\epsilon\)-amino groups of lysines available in both MAb 1A3 and MAb 1A3-F(ab')2 to form amide linkages. The labeled conjugates were administered to mature, female Sprague-Dawley rats, and metabolites were isolated from the liver, kidneys, urine, and feces at various time points. Because \(^{111}\)In-DTPA-\(\epsilon\)-lysine was identified as the major metabolite in the glycoprotein studies, we synthesized \(^{111}\)In-DTPA-\(\epsilon\)-lysine as a standard. The isolated metabolites were then compared with \(^{111}\)In-DTPA-\(\epsilon\)-lysine, \(^{111}\)In-DTPA, and \(^{111}\)In-acetate standards.

Materials and Methods

Materials. DTPA and the cyclic anhydride of cDTPAA were purchased from Sigma Chemical Co. (St. Louis, MO). DTPA-\(\epsilon\)-lysine was synthesized as described previously (22). RP-HPLC column (201 HS 104; 4.6 X 250 mm, C\(_{18}\)) was from Vydac (Hesperia, CA). Silica gel 60 F-254 TLC plates were from EM Science (Gibbstown, NJ). Chelex-100 was from Bio-Rad Laboratories (Hercules, CA). A Tekmar tissue homogenizer (Cincinnati, OH), a Branson Sonifier 185 cell disrupter, and a Sorvall RC2-B centrifuge were used for the metabolism experiments. \(^{111}\)InCl\(_3\) (4.2 X 10\(^{-4}\) Ci/\(\mu\)g) was provided by Mallinckrodt, Inc. (St. Louis, MO). Strong cation exchange column (Mono Q HR 5/5) and Superose 12 size exclusion column were from Pharmacia Biotech, Inc. (Piscataway, NJ). MABs 1A3 and 1A3-F(ab')2 were purified from serum-free medium by Invitron (St. Louis, MO) using proprietary methods. Mature, female Sprague-Dawley rats were from Sasco (Omaha, NE). All animal experiments were performed in compliance with guidelines specified by the Washington University Animal Studies Committee.

The Mono Q column was used with a Spectra-Physics SP8700XR HPLC pump. All ion exchange chromatography used an isotonic eluant of 100% buffer A for 5 min, and then a gradient was started which went from 100% buffer A to 0% buffer A over the next 20 min (A = 0.0183 m Na\(_2\)OAc, pH = 9.2; B = 1.0 m NH\(_4\)OAc, pH = 9.2). Flow rate for this system was 1.0 ml/min, with each fraction collected for 24 s. RP-HPLC was performed with a Waters 600E system controller from Millipore. The RP system used an isocratic run of 99% 100 mm Na\(_2\)OAc, 150 mm NaCl, pH 5.5, with 1.1% methanol at 1.0 ml/min, collecting 2-drop fractions. TLC plates were eluted with 1:1 methanol:10% NH\(_4\)OAc in water and analyzed using a Bicusan System 200 imaging scanner for detecting radioactive peaks. Fast protein liquid chromatography was performed on a Pharmacia/LKB system using a Superose 12 gel filtration column with 20 ml HEPES-300 mm NaCl, pH 7.3, at a flow rate of 0.4 ml/min, and with fractions collected every minute. All radioactive fractions were counted on a Beckman Gamma 8000 automated well-type gamma counter.

**DTPA Conjugation to 1A3 and 1A3-(Fab')2.** The intact MAB 1A3 and its (Fab')\(_2\) fragments were diluted to 50–65 mM in 0.1 m Na\(_2\)OAc, pH 5.5, and then added to a 10-fold molar excess of cDTPAA and mixed at room temperature for one h. The reaction mixtures were then purified by gel filtration chromatography, and the appropriate fractions were collected and concentrated using a Centricon-30 concentrator. The DTPA-1A3 and DTPA-1A3-(Fab')\(_2\) conjugates were stored at ~80°C until ready for use.

**Radiolabeling.** \(^{111}\)In-acetate complexes were prepared by diluting \(^{111}\)InCl\(_3\) in 0.4 m Na\(_2\)OAc, pH 5.5. \(^{111}\)In-DTPA, \(^{111}\)In-DTPA-\(\epsilon\)-lysine, \(^{111}\)In-DTPA-1A3, and \(^{111}\)In-DTPA-1A3-F(ab')2 were prepared by incubating the standards or conjugates with \(^{111}\)In-acetate for 1 h at room temperature. Free metal was removed from \(^{111}\)In-DTPA and \(^{111}\)In-DTPA-\(\epsilon\)-lysine complexes by adding 200 mg of Chelex-100 metal binding resin for 50 min and filtering through a 0.22 \(\mu\)m spin filter. Uncomplexed \(^{111}\)In(III) was removed from \(^{111}\)In-DTPA-1A3 and \(^{111}\)In-DTPA-1A3-F(ab')2 by gel filtration using a Bio-Spin 6 column that had been equilibrated with 0.4 m Na\(_2\)OAc, pH 5.5, according to the manufacturer's instructions. Radiochemical purity was determined using silica gel radio-TLC for the small molecular weight standards and gel filtration for the antibody conjugates. \(^{111}\)In-DTPA and \(^{111}\)In-DTPA-\(\epsilon\)-lysine showed single radioactive peaks at Rd of 0.56 and 0.40, respectively, by TLC with 1:1 methanol:10% NH\(_4\)OAc in water as the eluant. Gel filtration of \(^{111}\)In-DTPA-1A3 and \(^{111}\)In-DTPA-1A3-(Fab')\(_2\) demonstrated a single radioactive peak for each sample.

**In Vivo Metabolism of \(^{111}\)In-DTPA-1A3 and \(^{111}\)In-DTPA-1A3-(Fab')\(_2\)-Mature, female Sprague-Dawley rats were injected with 500–1200 \(\mu\)Ci of \(^{111}\)In-DTPA-1A3 (specific activity, 4.9–6.7 \(\mu\)Ci/\(\mu\)g) or \(^{111}\)In-DTPA-1A3-F(ab')2 (specific activity, 2.3–5.4 \(\mu\)Ci/\(\mu\)g). Urine and feces were collected from 72–96 h for intact antibody and 48–72 h for (Fab')\(_2\) fragments. The urine was then passed through a 0.2 \(\mu\)m filter and either directly analyzed, or concentrated and analyzed by TLC, RP-HPLC, and anion exchange chromatography. Feces were suspended in an equal volume of 0.1 m NH\(_4\)OAc, pH 5.5, homogenized, and sonicated with a Branson Sonifier 185 (3 min at power = 4). The suspension was clarified by centrifugation (23,500 X g for 60 min), and the supernatant was passed through a 0.22 \(\mu\)m filter. The fecal sample was concentrated to about 500 \(\mu\)l using a Labconco Centrivap Concentrator before analysis by RP-HPLC. Livers were removed from rats injected with \(^{111}\)In-DTPA-1A3, and kidneys were removed from rats injected with \(^{111}\)In-DTPA-1A3-F(ab')2 at 1-, 3-, and 5-day time points. The organs were rinsed with water to remove as much blood as possible, homogenized in an equal volume of labeling buffer using a Tekmar tissue homogenizer, and sonicated (3 min at power = 4). The samples were clarified by centrifugation (23,500 X g for 60 minutes), and the supernatant was passed through a 0.22 \(\mu\)m spin filter. A 0.2-ml sample was analyzed by gel filtration. Fractions were collected and counted on an automated gamma counter. Low molecular weight fractions were further analyzed by TLC, RP-HPLC, and anion exchange HPLC, as described above.

**Results**

**Gel Filtration Chromatography of Liver and Kidney Metabolites.** Since prior studies indicated that significant amounts of the \(^{111}\)In radiolabel from \(^{111}\)In-1A3 MAB accumulated within the liver (23, 24, 27), livers from Sprague-Dawley rats injected with \(^{111}\)In-DTPA-1A3 were harvested at 1, 3, and 5 days after injection. Between 8–10% of the injected dose was recovered in the liver at these time points. The livers were then processed, and metabolites were analyzed...
by gel filtration (Fig. 1). After 1 day, 65% of the radioactivity co-eluted with the intact antibody. The remainder eluted as a low molecular weight metabolite (Mr <5000). At days 3 and 5, the high molecular weight peak remains, but intermediate molecular weight metabolites appear as a shoulder. The low molecular weight peak continues to account for 35% of the radioactivity. This agrees well with data by Jones et al. (24) and Motta-Hennessy et al. (23), who found metabolites of different molecular sizes when studying 111In-labeled intact antibodies.

The renal metabolites were analyzed since prior work in our lab with 1A3-F(ab')2 and other antibody fragments showed a substantial accumulation of 111In activity after injection of 111In-DTPA antibody fragments. Kidneys were harvested at 1, 3, and 5 days after injection from rats injected with 111In-DTPA-1A3-F(ab')2, and 14–20% of the injected dose was recovered in the kidney. The metabolites were analyzed by gel filtration, and only low molecular weight metabolites (>98%) were detected at each time point.

For rats injected with 111In-DTPA-1A3-F(ab')2, the urine was collected between 48–72 h. Gel filtration demonstrated only low molecular weight metabolites (data not shown). There was not enough radioactivity associated with the fecal matter collected to generate an accurate gel filtration profile.

**TLC.** Following gel filtration, the low molecular weight metabolites were further analyzed by TLC. Samples were either mixed with 111In-DTPA-ε-lysine or 111In-DTPA standards and applied to glass-backed silica gel plates. Both liver and kidney metabolites comigrated with the 111In-DTPA-ε-lysine standard (Fig. 2). This agrees with Sands and Jones (25), who found that the low molecular weight metabolites were similar, but not identical to, 111In-DTPA. These metabolites were easily separated from the 111In-DTPA standard. Urine metabolites of both 111In-DTPA-1A3 and 111In-DTPA-1A3-F(ab')2 also comigrated with the 111In-DTPA-ε-lysine standard. Fecal samples did not contain sufficient activity for TLC analysis.

**RP-HPLC.** The low molecular weight liver metabolites were purified by gel filtration chromatography and analyzed by RP-HPLC. The major peak co-eluted with 111In-DTPA-ε-lysine. However, a second smaller peak that did not co-elute with any of the standards was also seen. This peak did not increase with time; rather, it decreased during the 5-day period.

In the urine, a major metabolite eluted with 111In-DTPA-ε-lysine, and a minor metabolite that eluted between the solvent front and 111In-DTPA was also observed (Fig. 3). The data for 111In-DTPA-1A3 metabolites showed a broad major peak centered around the 111In-DTPA-ε-lysine standard and a minor peak centered around the unknown metabolite mentioned above. This peak broadening might have been due to hydrophobic materials in the fecal matter that were not separated from the metabolites before the chromatographic analysis; consequently, the presence of a small amount of 111In-DTPA cannot be excluded.

The low molecular weight kidney metabolites were purified by gel filtration chromatography and analyzed by RP-HPLC. Again, the major peak co-eluted with 111In-DTPA-ε-lysine (Fig. 4). A second metabolite was again seen, and in contrast to the pattern seen in the liver, it appears to increase with time.

The 111In-DTPA-1A3-F(ab')2 urine sample also contained a major metabolite that behaved similarly to 111In-DTPA-ε-lysine by RP-HPLC (Fig. 3). The slight discrepancy in retention times of the major metabolite and the 111In-DTPA-ε-lysine standard may result from the differences in sample preparation, and this possibility is being investigated. The fecal data for 111In-labeled fragments showed that the unidentified metabolite was the major metabolite, and the minor...
metabolite eluted with the $^{111}$In-DTPA-€-lysine standard, in contrast to the $^{111}$In-DTPA-1A3-fecal data. It should be noted that the low amount of fecal-associated activity and the broadening of the peaks makes a precise interpretation of this fecal data difficult.

**Anion Exchange HPLC.** Low molecular weight liver metabolites from rats injected with $^{111}$In-DTPA-1A3 were collected from the gel filtration column and analyzed by anion exchange chromatography (Fig. 3). The major metabolite co-eluted with the $^{111}$In-DTPA-€-lysine standard, while one of the minor metabolites co-eluted with the $^{111}$In-DTPA standard; the third metabolite remains unidentified.

Anion exchange chromatography of the $^{111}$In-DTPA-1A3-F(ab')$_2$-kidney samples (Fig. 5) at day 3 confirmed that the major metabolite was $^{111}$In-DTPA-€-lysine. One of the minor metabolites eluted with $^{111}$In-DTPA, and the third, more negatively charged, metabolite remains unidentified.

Analysis of both $^{111}$In-DTPA-1A3 and $^{111}$In-DTPA-1A3-F(ab')$_2$ urine samples showed that the major metabolite in each sample co-eluted with $^{111}$In-DTPA-€-lysine, while one minor metabolite co-eluted with $^{111}$In-DTPA and the third was not identified.

**Discussion**

The data indicate that $^{111}$In-DTPA-€-lysine was the major low molecular weight metabolite produced from $^{111}$In-DTPA-conjugated, intact antibodies and F(ab')$_2$ antibody fragments administered in vivo. Liver, kidney, and excretory samples all contained $^{111}$In-DTPA-€-lysine as the predominant metabolite. The model used in these studies was normal Sprague-Dawley rats and, therefore, assumed to not express any of the antigen for which MAb 1A3 is specific. Since the MAbs are not targeted to cell surface antigens, the mechanism for their internalization and degradation is not well understood. The finding that $^{111}$In-DTPA-€-lysine is the major low molecular weight metabolite in this study suggests that $^{111}$In-chelate-antibodies are also degraded within hepatic and renal lysosomes.

The gel filtration data of $^{111}$In-DTPA-1A3-F(ab')$_2$ metabolites in kidney homogenates (Fig. 1) showed that >98% of the activity is associated with a low molecular weight species by 1 day. This suggests that the fragments are efficiently deposited within lysosomes by absorptive endocytosis, and subsequent lysosomal degradation is also rapid (15). Yokota et al. (40) have observed similar, rapid renal tubular accumulation with $^{125}$I-labeled F, F' fragments (40). The uptake of the F(ab')$_2$ fragment by the kidney and its apparent lysosomal degradation leads to the following implications: (a) simply using a smaller MAb fragment may not lower the accumulation of radioactivity in the kidney. Similarly, metal conjugates of peptides may fall prey to this same uptake mechanism and lysosomal retention. This has been observed with $^{111}$In-DTPA conjugates of chemotactic peptides and octreotide (41, 42); and (b) metabolizable linkers may not circumvent the problem unless the radiolabel is released prior to endocytosis in a form that is not recaptured by other mechanisms, or the radiolabeled metabolite is rapidly exported from the lysosome.

The gel filtration results of $^{111}$In-DTPA-1A3 liver homogenates (Fig. 1) are not interpreted as easily. These data showed that 65% of the activity is still associated with the MAb or with a lower molecular weight species ($M_r$ >50,000), which is seen as a shoulder in Fig. 1, B

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**Fig. 3.** Reverse phase chromatography of urine and fecal metabolites. A and D. $^{111}$In-DTPA-€-lysine and $^{111}$In-DTPA standards were mixed and injected on RP-HPLC to give standard traces. B and C. $^{111}$In-DTPA-1A3 metabolites in urine and feces collected at a 24-h time interval from 4 to 5 days. E and F. $^{111}$In-DTPA-1A3-F(ab')$_2$ in urine and feces collected at a 24-h time interval from 3 to 4 days. $^{111}$In-DTPA-€-lysine (V) and $^{111}$In-DTPA (△) elute as indicated by the corresponding arrows.

**Fig. 4.** Reverse phase chromatography of small molecular weight metabolites. A and E. $^{111}$In-DTPA-€-lysine and $^{111}$In-DTPA standards were mixed and injected on RP-HPLC to give standard traces. B-D. $^{111}$In-DTPA-1A3 liver metabolites collected from the gel filtration column (fractions 46-48). F-H. $^{111}$In-DTPA-1A3-F(ab')$_2$ kidney metabolites collected from the gel filtration column (fractions 46-48). $^{111}$In-DTPA-€-lysine (V) and $^{111}$In-DTPA (△) elute as indicated by the corresponding arrows.
observed could occur within endosomes since they also contain proteases (16, 43–45) and thus may account for the shoulder that starts to appear in the gel filtration profile of $^{111}$In-DTPA-1A3 (Fig. 1).

Extracellular sites for radiolabeled antibody localization include the interstitial fluid, extracellular antigens, and extracellular surface molecules (4, 13, 14, 16, 40, 43, 44, 46–49). Given the 1.6 liver: blood ratio, it is unlikely that the radiolabeled antibody is in the interstitial fluid and not bound to cell membranes. The presence of cell surface or extracellular antigens specific for the MAb 1A3 in the animal model used in these experiments is also highly unlikely. Thus, the MAb is most likely bound nonspecifically to surface molecules or internalized by specific (i.e., Fc receptor) or nonspecific (fluid phase endocytosis) means. Based on this data, we propose the model illustrated in Fig. 6 to accommodate the various aspects of hepatic retention and metabolism.

The gel filtration data (Fig. 1, A–C) of the liver homogenates show that a majority of $^{111}$In is either associated with intact or partially degraded antibody or with small molecular weight metabolites. Antibody associated activity is assumed to still be bound to the DTPA chelate. The high stability of the $^{111}$In-DTPA complex is well established (24, 26, 31, 32). If the $^{111}$In had dissociated from the chelate, it would be highly improbable that the protein with which it associates would have the same molecular weight. The small molecular weight species were subsequently shown to contain $^{111}$In-DTPA- amino acid(s) by the various chromatographic techniques used. This is in agreement with data that show the transchelation of $^{111}$In from DTPA is slow at physiological and lysosomal pH (7.2 and 5.0, respectively; Refs. 46, 47, and 50).

Several methods have been attempted to increase the clearance of radiolabeled metabolites of intact MAbS and fragments from nontarget organs and thus increase the target to nontarget ratio. Different linking groups have been used to connect bifunctional chelates to proteins. These groups contained metabolizable sites and have generally lowered the retention of activity in nontarget organs (8–11). It appears that metabolites that are more lipophilic and less charged are more readily released (51).

The appearance of a minor metabolite that is not $^{111}$In-DTPA-lysine is surprising, since it was not observed in the glycoprotein

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4 Unpublished data.
metabolism studies of Franano et al. (22). We have shown that the minor metabolite is not \(^{111}\)In-DTPA-\(\varepsilon\)-lysine, \(^{111}\)In-DTPA, or free \(^{111}\)In-acetate by comparison to known standards (Figs. 2-5). In addition, anion exchange chromatography shows this minor metabolite eluting later than \(^{111}\)In-DTPA-\(\varepsilon\)-lysine and \(^{111}\)In-DTPA, which indicates a compound with a greater negative charge (Fig. 5). Franano et al. (22) show that \(^{111}\)In-DTPA-\(\varepsilon\)-lysine was retained in the kidney more than \(^{111}\)In-DTPA at 1 h after injection (22). This is explained by the more positively charged \(^{111}\)In-DTPA-\(\varepsilon\)-lysine being bound to the luminal surfaces of proximal tubular cells in the kidney. Therefore, the higher concentration of the more negative minor metabolite in the kidney over time appears to be a function of the metabolism within the kidney and not due to kidney uptake after metabolism at another site.

The structure of this more negatively charged minor metabolite has not been determined, but there are several possibilities that may explain it. It could be the product of further metabolism of \(^{111}\)In-DTPA-\(\varepsilon\)-lysine; however, this is unlikely due to the decrease in its size in the liver RP-HPLC traces (Fig. 4). The \(^{111}\)In could be released from the chelate and then to some other small molecule. This is unlikely given the strength of the \(^{111}\)In-chelate bond (24, 26, 31, 32).

Finally, the unknown metabolite could be the result of \(^{111}\)In-DTPA bound to an amino acid other than lysine. Under the conjugation conditions, it is unlikely that available hydroxyl groups are reactive with cDTPAA. Also, any available sulfhydryl groups on \(^{111}\)In-DTPA-\(\lambda\)3-\(\text{F(ab')}^2\) were alkylated in the process of making \(\lambda\)3-\(\text{F(ab')}^2\) from \(\lambda\)3, leaving them unreactive towards cDTPAA. The formation of a sulfhydryl coordinated species is, therefore, excluded. Is it most likely that cDTPAA reacted to some extent with an \(\text{NH}_2\)-terminal amino acid. The results of Rana and Meares (52) showed that \(\text{NH}_2\)-termini of \(\lambda\)3 were one possible site of attachment of amino acid. The results of Rana and Meares (52) showed that \(\text{NH}_2\)-termini of \(\lambda\)3-F(\(\text{ab')}^2\) which would account for an other low molecular weight metabolite.

From this discussion, it is clear that radiolabeled antibody metabolism encompasses a wide variety of issues. Fundamentally, the process is dynamic. The observed levels of various intermediates are governed by the rate constants of each step (Fig. 6). To begin deciphering this complex biochemical pathway, we must identify the intermediates and start fitting them into the known biochemical pathways. Then we can begin varying the radiolabel-antibody linkages to improve target to background ratios.

Note Added in Proof

Since this paper was submitted, our group has shown by \(\text{NH}_2\)-terminal sequencing of \(\lambda\)3 and \(\lambda\)3-\(\text{F(ab')}^2\) that the \(\text{NH}_2\)-terminal amino acid on \(\lambda\)3 and \(\lambda\)3-\(\text{F(ab')}^2\) is aspartic acid. This agrees with our hypothesis that a fraction of the cDTPAA chelates were conjugated to the \(\text{NH}_2\)-terminal amine available on the antibody. The unknown metabolite is less lipophilic and more negatively charged than \(^{111}\)In-DTPA-\(\varepsilon\)-lysine. An \(^{111}\)In-DTPA-aspartic acid metabolite is consistent with both \(\text{NH}_2\)-terminal sequencing and the chromatographic results.

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References


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