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. These studies have indicated that the main lysosomal metabolite is $^{111}$In-chelate-$\epsilon$-lysine, both in vitro and in vivo (Y. Arano et al., J. Nucl. Med., 35: 890–898, 1994; F. N. Frano 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 tissues is important for the rational design of future radiolabeled antibodies.

We investigated the in vivo metabolism of $^{111}$In-DTPA-$\epsilon$-conjugated antibody in female Sprague-Dawley rats using the anticolorectal carcinoma monoclonal antibody (MAb) 1A3 and MAb 1A3-F(ab$'\prime$)$_2$. 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$'\prime$)$_2$ 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 gel thin-layer chromatography, reversed phase high-performance liquid chromatography, and ion-exchange chromatography and compared to $^{111}$In-DTPA and $^{111}$In-DTPA-$\epsilon$-lysine standards. In each system, the major metabolite co-eluted with $^{111}$In-DTPA-$\epsilon$-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$'\prime$)$_2$ was $^{111}$In-DTPA-$\epsilon$-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 ($^{125}$I, $^{123}$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 radiolabeled metabolites (3, 4). Antibodies labeled with $^{111}$In (through the use of bifunctional chelates) frequently demonstrate higher uptake and slower clearance of radioactivity at target sites when compared to radiolabeled molecules. However, $^{111}$In MAb's demonstrate a large amount of nontarget uptake in the liver and kidneys (5–7).

Many 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.

Much data implicate lysosomes in the metabolism of MAb's 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 tubular 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-$\epsilon$-lysine (20, 22). This result indicates that the chelate-$\epsilon$-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-$\epsilon$-lysine. Therefore, we studied the metabolism of the 1A3 MAb and its F(ab$'\prime$)$_2$ fragments. This antibody is specific for a human colorectal carcinoma antigen and thus should behave as a nontargeted antibody in the rat. We compared the radiolabeled metabolites prepared from urine, feces, liver extracts, and renal homogenates to a series of standards including $^{111}$In-DTPA-$\epsilon$-lysine.

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

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3 The abbreviations used are: MAb, monoclonal antibody; DTPA, diethylenetriaminepentaacetic acid; cDTPA, diethylenetriaminepentaacetic dihydrate; TLC, thin-layer chromatography; RP-HPLC, reverse phase high pressure liquid chromatography.

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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 $\varepsilon$-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-$\varepsilon$-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 anticoagulant carcinoma MAb (39), was labeled with $^{111}$In through the use of the bifunctional chelate DTPA. DTPA was conjugated to the $\varepsilon$-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-$\varepsilon$-lysine was identified as the major metabolite in the glycoprotein studies, we synthesized $^{111}$In-DTPA-$\varepsilon$-lysine as a standard. The isolated metabolites were then compared with $^{111}$In-DTPA-$\varepsilon$-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-$\varepsilon$-lysine was synthesized as described previously (22). RP-HPLC column (201 HS 104; 4.6 × 250 mm, C$_{18}$) was from Vydu (Hesperia, CA). Silica gel 60 F-254 TLC plates were from EM Science (Gibbstown, NJ). Chelex-100 was from Bio-Rad (Richmond, CA). Centricom-30 concentrators were purchased from Amicon, Inc. (Beverly, MA). Ultrafree-MC 0.22 μm filter units were from Millipore (Bedford, MA). Bio-Spin 6 chromatography columns were 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 experiments. Materials and Methods. DTPA conjugation to 1A3 and 1A3-(Fab')$_2$. The intact MAb 1A3 and its (Fab')$_2$ fragments were diluted to 50–65 μM in 0.1 M NaH$_2$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 Centricom-30 concentrator. The DTPA-1A3 and DTPA-1A3-(Fab')$_2$ conjugates were stored at −80°C until ready for use.

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), liver 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 ($M_r \le 5000$). 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-Hennessey et al. (23), who found metabolites of different molecular sizes when studying $^{111}$In-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 $^{111}$In activity after injection of $^{111}$In-DTPA antibody fragments. Kidneys were harvested at 1, 3, and 5 days after injection from rats injected with $^{111}$In-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 $^{111}$In-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 $^{111}$In-DTPA-$\varepsilon$-lysine or $^{111}$In-DTPA standards and applied to glass-backed silica gel plates. Both liver and kidney metabolites comigrated with the $^{111}$In-DTPA-$\varepsilon$-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, $^{111}$In-DTPA. These metabolites were easily separated from the $^{111}$In-DTPA standard.

Urine metabolites of both $^{111}$In-DTPA-1A3 and $^{111}$In-DTPA-1A3-F(ab')$_2$ also comigrated with the $^{111}$In-DTPA-$\varepsilon$-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 $^{111}$In-DTPA-$\varepsilon$-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 $^{111}$In-DTPA-$\varepsilon$-lysine, and a minor metabolite that eluted between the solvent front and $^{111}$In-DTPA was also observed (Fig. 3). The fecal data for $^{111}$In-DTPA-1A3 metabolites showed a broad major peak centered around the $^{111}$In-DTPA-$\varepsilon$-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 $^{111}$In-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 $^{111}$In-DTPA-$\varepsilon$-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 $^{111}$In-DTPA-1A3-F(ab')$_2$ urine sample also contained a major metabolite that behaved similarly to $^{111}$In-DTPA-$\varepsilon$-lysine by RP-HPLC (Fig. 3). The slight discrepancy in retention times of the major metabolite and the $^{111}$In-DTPA-$\varepsilon$-lysine standard may result from the differences in sample preparation, and this possibility is being investigated. The fecal data for $^{111}$In-labeled fragments showed that the unidentified metabolite was the major metabolite, and the minor...
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The data indicate that $^{111}$In-DTPA-eps-lysine was the major low molecular weight metabolite produced from $^{111}$In-DTPA-conjugated, intact antibodies and F(ab$^\prime$)$_2$ antibody fragments administered in vivo. Liver, kidney, and excretory samples all contained $^{111}$In-DTPA-eps-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-eps-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$^\prime$)$_2$ 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$_x$ fragments (40). The uptake of the F(ab$^\prime$)$_2$ 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 the 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.

**Discussion**

Fig. 3. Reverse phase chromatography of urine and fecal metabolites. A and D. $^{111}$In-DTPA-eps-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$^\prime$)$_2$ in urine and feces collected at a 24-h time interval from 3 to 4 days. $^{111}$In-DTPA-eps-lysine ($\psi$) and $^{111}$In-DTPA ($\varnothing$) elute as indicated by the corresponding arrows.

![Fraction Number](image)

Fig. 4. Reverse phase chromatography of small molecular weight metabolites. A and E. $^{111}$In-DTPA-eps-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$^\prime$)$_2$ kidney metabolites collected from the gel filtration column (fractions 46–48). $^{111}$In-DTPA-eps-lysine ($\psi$) and $^{111}$In-DTPA ($\varnothing$) elute as indicated by the corresponding arrows.
The appearance of 'In-DTPA-€-lysine as the predominant metabolite in the low molecular weight peak strongly suggests that the antibodies are ultimately degraded within the lysosome. This is consistent with the work of Geissler et al. (4), who observed lysosomal degradation of an 125I-labeled intact antibody. The partial degradation 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 111In-DTPA-IA3 (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 IA3 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 111In is either associated with intact or partially degraded antibodies or with small molecular weight metabolites. Antibody associated activity is assumed to still be bound to the DTPA chelate. The high stability of the 111In-DTPA complex is well-established (24, 26, 31, 32). If the 111In 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 111In-DTPA-lysine (7) and 111In-DTPA (8) elute as indicated by the corresponding arrows. Anion exchange results from day 5 are not shown.

and C, after 5 days. This does not agree with the glycoprotein studies that show complete conversion to a low molecular weight species in 1 h once delivered to the lysosomes (21). There are several possible reasons for this. The glycoproteins were targeted to cell surface receptors upon which they were cleared from the blood, internalized, and delivered to the lysosome in a short period of time. Once in the lysosome, the glycoproteins were rapidly degraded to low molecular weight metabolites. 111In-DTPA-IA3 is not targeted to cell surface receptors and remains in the blood longer. Previous data4 from our laboratory using 111In-labeled MAbs show that the liver: blood ratio at 5 days is 1.6 and that by 4 days, only ~15% of the activity is in the blood with the clearance from the blood being only ~2% per day after 4 days. Blood activity and activity deposited in the liver in the previous 24 h will only account for ~30% of the liver activity at 5 days.

The remaining activity must be accounted for by intact or partially degraded radiolabeled antibodies (Mr >50,000) that are deposited within the liver at either intracellular or extracellular sites. Intracellular sites can be divided into the cytoplasm and organelles. It is unlikely that radiolabeled antibodies are found in the cytoplasm due to the lack of a mechanism for transmembrane transport of large polypeptides. Delivery of radiolabeled antibodies to intracellular organelles, including endosomes and lysosomes, is far more likely (16, 43, 44). The appearance of 111In-DTPA-€-lysine as the predominant metabolite in the low molecular weight peak strongly suggests that the antibody is ultimately degraded within the lysosome. This is consistent with the work of Geissler et al. (4), who observed lysosomal degradation of an 125I-labeled intact antibody. The partial degradation 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 111In-DTPA-IA3 (Fig. 1).

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 111In-DTPA-€-lysine is surprising, since it was not observed in the glycoprotein

4 Unpublished data.
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metabolism studies of Franano et al. (22). We have shown that the minor metabolite is not 111In-DTPA-€-lysine, 111In-DTPA, or free 111In-acetate by comparison to known standards (Figs. 2-5). In addition, anion exchange chromatography shows this minor metabolite eluting later than 111In-DTPA-€-lysine and 111In-DTPA, which indicates a compound with a greater negative charge (Fig. 5). Franano et al. (22) show that 111In-DTPA-€-lysine was retained in the kidney more than 111In-DTPA at 1 h after injection (22). This is explained by the more positively charged 111In-DTPA-€-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 111In-DTPA-€-lysine; however, this is unlikely due to the decrease in its size in the liver RP-HPLC traces (Fig. 4). The 111In could be released from the chelate and then to some other small molecule. This is unlikely given the strength of the 111In-chelate bond (24, 26, 31, 32).

Finally, the unknown metabolite could be the result of 111In-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 111In-DTPA-IA3-F(ab’)2 were alkylated in the process of making 1A3-F(ab’)2 from IA3, leaving them unreactive towards cDTPAA. The formation of a sulfhydryl coordinated species is, therefore, excluded. It is most likely that cDTPAA reacted to some extent with an NH2-terminal amino acid. The results of Rana and Meares (52) showed that NH2-termini of Lym-1 were one possible site of attachment of SCN-chelates. Some of the DTPA may have been conjugated to the NH2-termini of IA3 and 1A3-F(ab’), which would account for another 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 NH2-terminal sequencing of IA3 and 1A3-F(ab’)2 that the NH2-terminal amino acid on IA3 and 1A3-F(ab’), is aspartic acid. This agrees with our hypothesis that a fraction of the cDTPA chelates were conjugated to the NH2-terminal amine available on the antibody. The unknown metabolite is less lipophilic and more negatively charged than 111In-DTPA-€-lysine. An 111In-DTPA-aspartic acid metabolite is consistent with both NH2-terminal sequencing and the chromatographic results.

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