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Site-specific Modifications of Light Chain Glycosylated Antilymphoma (LL2) and Anti-Carcinoembryonic Antigen (hImmu-14-N) Antibody Divalent Fragments[1]

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Abstract

Site-specific introduction of metal-chelating groups into $F(ab')_2$ fragments of an antilymphoma antibody (LL2) possessing a natural Asn-linked light chain carbohydrate and an anti-carcinoembryonic antigen antibody (hImmu-14-N) grafted with a light chain carbohydrate site is described. For this purpose, four yttrium- (and indium)-chelating agents were used, containing a primary amino group for antibody binding and 1-(4-substituted benzyl)diethylenetriaminepentaacetic acid as the metal-chelator, separated by structurally different additional linkers. Conjugates were prepared by reacting excess chelator with oxidized carbohydrate of $F(ab')_2$ fragments, with or without a subsequent reduction step. The conjugates, with up to an average of 5.5 chelating groups attached to a $F(ab')_2$ fragment, were readily labeled with ^{90}Y and ^{111}In and were found to retain antigen-binding ability in *in vitro* assays. Tumor targeting was demonstrated using a ^{88}Y-labeled hImmu-14-N $F(ab')_2$ carbohydrate-modified conjugate. 2-Pyridyldithiopropionic hydrazide was conjugated to the carbohydrate region, and the disulfide was selectively deprotected to the thiol group, which is reactive with reduced ^{99m}Tc. These initial experiments establish that light chain carbohydrate modification of $F(ab')_2$ is as facile as with the Fc-region carbohydrate of intact IgG, and thereby offer the possibility of designing site-specifically substituted $F(ab')_2$ fragments with favorable pharmacokinetic properties.

Introduction

For RAID[3], the use of the preferred ^{99m}Tc isotope necessitates the use of antibody monovalent or divalent fragments in view of the parity between the *in vivo* half-life of the fragments and the 6-h half-life of the radioisotope. Similarly, a $F(ab')_2$ fragment is pharmacokinetically perhaps the ideal agent to use for RAIT with relatively short-lived isotopes, such as ^{90}Y (1). The use of antibody fragments, rather than whole IgG, for RAID/RAIT with these isotopes is particularly indicated with humanized antibodies, which probably will stay longer in circulation than murine antibodies (2) and, therefore, could lead to increased systemic whole-body radiation.

Invariably, prior modification of $F(ab')_2$ with a bifunctional chelating agent is required for labeling with metallic radionuclides. Random modification of $F(ab')_2$ fragments, such as at lysine residues, has the potential to compromise the antigen-binding site, whereas simple site-specific modification is nearly impossible due to the lack of heavy-chain carbohydrate as well as the lability of the hinge region disulfide bonds. The discovery and reengineering, in our laboratories, of an unusual light chain glycosylation site in certain antibodies offers a practical solution to these problems.

LL2 (3), a non-Hodgkin's B-cell lymphoma antibody (anti-CD22), was discovered to possess a natural Asn-linked glycosylation site (asparagine-valine-threonine) in the framework 1 region of its κ light chain variable (VK) domain. Glycosylation at this site does not interfere with immunoreactivity. Molecular modeling studies demonstrated that the carbohydrate moiety forms a "cap" around the base of the VK domain, away from the antigen-binding site (4). Using LL2 as a prototype, the same Asn-glycosylation has been reengineered (4) onto the corresponding position on the light chain of a clinically useful humanized version of an anti-CEA monoclonal antibody, Immu-14 (5, 6).

The purpose of this study was to introduce metal-chelating groups onto the light chain carbohydrate of the $F(ab')_2$ fragments derived from these antibodies. This exercise was undertaken not only to provide a complementary chemical proof as to the presence of light chain carbohydrate but also to develop the carbohydrate site for hapten conjugations and radiochemistries.

Materials and Methods

General

HPLC Analyses. For antibody conjugates, an analytical Bio-Sil 250 size-exclusion column, provided with a guard column, was used; 0.2 M phosphate buffer, pH 6.8 (1 ml/min flow rate), was used as the mobile phase, with in-line UV (280 nm) and/or radioactivity detection. Nonantibody preparations were analyzed on an analytical reverse-phase C_{18} column (250 mm × 4.6 mm outside diameter), fitted with a guard column, using gradient elution (0.1 M sodium acetate/methanol gradient; 1 ml/min) and UV (254 nm) detection.

ITLC Analyses. Silica gel-impregnated glass fiber sheets (Gelman Sciences, Ann Arbor, MI) were cut into strips (0.8 cm × 10 cm long). The radioactive preparation was spotted onto one end of the strip and developed, then the strip was cut into 10 sections and counted in a γ counter. For ^{111}In- and ^{90}Y-labeled antibodies, a 10-min "EDTA chase" (using 10 mM EDTA) was carried out on an aliquot of the labeling mixture, which was then examined by ITLC using 10 mM EDTA as developer. Antibody-bound radioactivity remained at the origin. Colloidal precipitation of metal ion in the labeling mixtures was determined by co-spotting an aliquot of EDTA-chased labeling mixture with 1% human serum albumin on an ITLC strip and developing with a 5:2:1 water:ethanol:ammonia mixture. In this system, all but colloidal radioactivity moved to the solution front. In all of the preparations, colloid was less than 2%.

Radioisotopes. ^{90}Y chloride and ^{111}In chloride were purchased from Dupont NEN (Boston, MA) and buffered to pH 6 prior to labeling. ^{99m}Tc, as ^{99m}Tc pertechnetate, was purchased as unit doses from Syncor (Fairfield, NJ). ^{88}Y chloride was obtained from Los Alamos National Laboratory (Los Alamos, NM). Reagents and solvents, from commercial sources, were of high purity grade and were used as such. Metal-free conditions were used for all work involving antibody-conjugate preparations and radiolabeling.

Preparation of Amine-terminating Bifunctional DTPAs

Aminobenzyl DTPA (I) was available from stock (I-IV; Fig. 1). For II, 1-(4-isothiocyanatobenzyl)-DTPA (IA; 42.3 mg; 41% metal-binding; 0.032 mmol) was dissolved in a mixture of 400 μl of 0.1 M aqueous sodium bicarbonate and 40 μl of DMF. A solution of 1,10-diaminodecane (50 mg; 0.29 mmol) in 300 μl of 0.1 M aqueous sodium bicarbonate and 100 μl of DMF

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[3] The abbreviations used are: RAID, radioimmunodetection; RAIT, radioimmunotherapy; ITLC, instant thin-layer chromatography; DMF, dimethylformamide; HPLC, high pressure liquid chromatography; SDH, succinic dihydrazide; SEC, size-exclusion chromatography; CEA, carcinoembryonic antigen; DOTA, 1,4,7,10-tetraazacyclododecane *N,N′,N″,N‴*-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; hImmu-14-N, humanized (complementarity determining region-grafted) Immu-14 with light chain carbohydrate; PDPH, 2-pyridyldithiopropionic hydrazide.

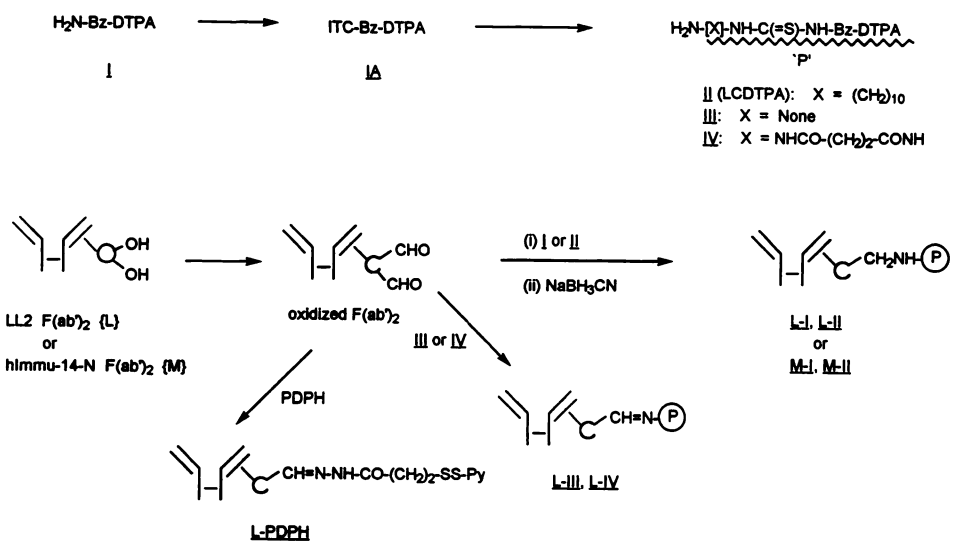

Fig. 1. Conjugations at the light chain carbohydrate of F(ab')$_2$ fragments with amino-DTPAs (I-IV) or PDPH.

were added. The pH of the solution was adjusted to 9.53, and the reaction mixture was incubated at 37°C for 2.5 h. The pH was readjusted to 12.5 using 6 M sodium hydroxide, and the mixture was extracted with chloroform to remove excess diamine. The aqueous layer was evaporated, and the residue was further extracted with THF and chloroform. The final residue was redissolved in milliQ water, the pH was adjusted to 6.22, and stored frozen. HPLC analysis (C_{18} column) showed a single peak at 10.5 min, different from that of IA at 6 min. III was prepared using 19.7 μmol of IA and 6.6 μl of a 55% aqueous solution of hydrazine hydrate (six equivalents) at a concentration of 60 μmol/ml (HPLC, C_{18} column: 2.45 min). For IV, a solution of IA at a pH of 6.1 was prepared at a concentration of 63.4 μmol/ml. An aqueous solution of SDH at a pH of 9–9.5 was prepared at 182.4 μmol/ml. Reaction of IA with an 8-fold excess of SDH at pH 8.5 gave a product with a HPLC retention time of 2.7 min, whereas reaction using a 4-fold excess of IA gave a product with a retention time of 2.3 min. Reaction using a 1:1 ratio of the reactants furnished a 2:3 ratio of mono- and di-DTPA substituted products. For conjugation purposes, product IV was prepared as a mixture, of which only the mono-DTPA product contains an aldehyde-reactive amino group, and the pH of the solution was adjusted to 6.5.

Conjugation of Amine-terminating DTPAs with Oxidized LL2 F(ab')$_2$ or Oxidized hImmu-14-N F(ab')$_2$

LL2 F(ab')$_2$ (0.8 ml; 1.95 mg/ml) in 50 mM acetate-buffered 150 mM saline (pH 5.3) was treated with sodium periodate at a final concentration of 14.3 mM and incubated in an ice-bath for 1 h. Excess periodate was reacted with glycerol (20 μl), and the oxidized antibody was purified on a centrifuged-SEC (7) equilibrated in 0.1 M phosphate buffer (pH 7.0). The solution of oxidized antibody was made 150 mM with respect to sodium chloride, the pH was adjusted to the 6.0–6.2 range, and then the solution was treated with a 500-fold (I), a 300-fold (III), or a 200-fold (II or IV) molar excess of the amino-DTPAs. In each case, the reaction mixture was vortexed and incubated in the dark at room temperature for 18 h. In the case of conjugations using I or II, a reduction step involving an additional incubation for 2.5 h with aqueous sodium cyanoborohydride (10 mM) was used. A control experiment showed that there was no fragmentation of F(ab')$_2$ to Fab' during the reduction. Each conjugate was purified by a centrifuged-SEC in acetate buffer (0.1 M sodium acetate, pH 6.5) and concentrated in a Centricon-30 concentrator. The acylhydrazone derivative (L-IV) was buffer-exchanged with 0.1 M acetate (pH 7.2). HPLC analyses in each case showed a single peak with a retention time similar to that of unmodified antibody, with less than 5% aggregate in the preparation. The procedures were adapted for the preparation of conjugates M-I and M-II of hImmu-14-N F(ab')$_2$.

A second control experiment involved subjecting unoxidized LL2 F(ab')$_2$ to reaction conditions used for preparing L-I. Two nonselectively modified LL2 F(ab')$_2$ conjugates were prepared (for comparative *in vitro* experiments) by reacting the antibody with 12 or 20 equivalents of IA, at pH 8.5 or 8.8, to obtain conjugates containing an average of 2.2 or 4.3 DTPA groups, respectively. A known procedure (8) using either Co/^{57}Co or In/^{111}In was adopted to determine the chelator/F(ab')$_2$ ratios for the conjugates (Table 1).

Yttrium and Indium Radiolabelings

Labeling with either ^{90}Y acetate or ^{111}In acetate was carried out by incubating the conjugate with the activity needed for the desired specific activity. The pH of labeling was in the 6–6.5 range, except in the case of acylhydrazone (L-IV), for which the pH was 7.2. Table 1 details the radiolabeling data. Radiolabeling with ^{88}Y for animal biodistributions was carried out by incu-

Table 1 *Chelator:antibody ratios and radiolabeling*

Site-specific F(ab')$_2$ conjugate:antibody/ chelator[a]	No. of chelators[b]	Radiolabeling data			
		Isotope	μCi/μg	% Inc.[c] (HPLC)	% coll[c] (ITLC)
LL2/DTPA	5.5	^{90}Y	4.3	91.0	0.5
		^{111}In	7.5	92.5	ND
LL2/LCDTPA	4.6	^{90}Y	1.6	91.3	1.5
		^{111}In	12.7	94.0	ND
LL2/TSC-DTPA	1.3	^{90}Y	1.9	64.8	1.2
		^{111}In	2.1	86.0	<1
LL2/AH-DTPA	ND	^{90}Y	1.2	60.8	0.3
hImmu14-N/DTPA	3.1	^{90}Y	1.9	57.6[d]	0.6
hImmu14-N/LCDTPA	2.1	^{90}Y	1.3	91.6	0.7

[a] DTPA, aminobenzyl-DTPA; LCDTPA, aminodecylthioureatobenzyl-DTPA; TSC-DTPA, thiosemicarbazidylbenzyl-DTPA; AH-DTPA, acylhydrazine-containing DTPA.
[b] Number of chelators per antibody fragment in the conjugate, determined by metal-binding assays (8). ND, not determined.
[c] *Inc.*, incorporation; *coll*, colloid.
[d] Based on one labeling; the material was subsequently repurified and used for a ^{88}Y labeling for an animal experiment.

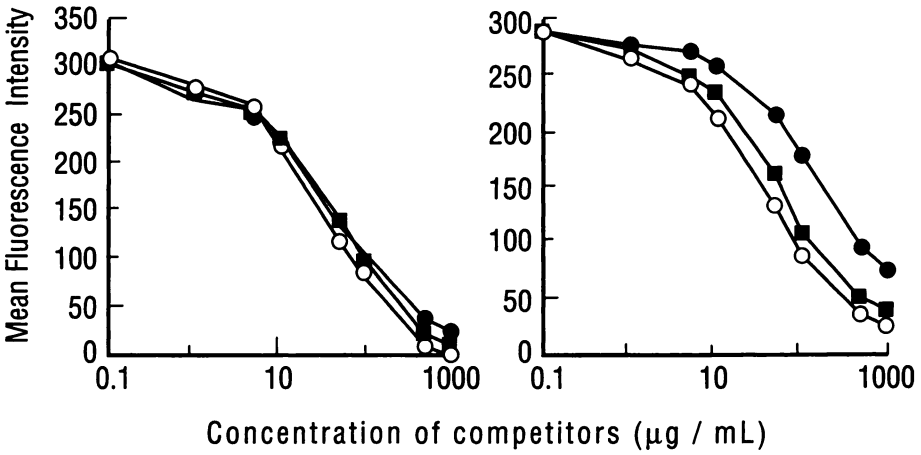

Fig. 2. Comparisons of immunoreactivities of carbohydrate-modified and random lysine-modified conjugates of LL2 F(ab')$_2$ with that of unmodified antibody, determined in competitive binding assays using Raji cells as the source of antigen (13). *Left*, carbohydrate-modified conjugates with 5.5 molecules of DTPA (■), 4.6 molecules of LC-DTPA (●), and unmodified antibody (○). *Right*, lysine-modified conjugates containing 2.2 (■) and 4.3 (●) molecules of DTPA and unmodified antibody (○). Reproduced with permission from the publisher (13). Copyright 1995, The American Association of Immunologists.

bating M-I, M-II, or hMN14 F(ab')$_2$-lysyl-DTPA (control) for 2 h with ^{88}Y acetate at a specific activity of 0.1 to 0.15 μCi/μg. Two successive purifications on a centrifuged-SEC were carried out, and the final eluate (in acetate buffer) was diluted to 1 μCi/100 μl with sterile saline. Purified material had >95% antibody-bound yttrium.

Animal Model

Female athymic mice (20 to 22 g), 4 to 8 weeks of age (Harlan, Madison, WI), developed to bear LS174T human colon carcinoma xenografts (9) were used. For animal experiments, 1 μCi of ^{88}Y-labeled conjugate, in a volume of 100 μl, was administered per mouse.

Introduction of the PDPH Group onto the Carbohydrate of LL2 F(ab')$_2$

The oxidation product of LL2 F(ab')$_2$ (0.5 ml; 4.1 mg/ml), pH 5.7, was mixed with 15 μl of DMF and a DMF solution of PDPH (2.4 mg/150 μl; 50–70 fold molar excess; final DMF concentration, 4–6%) and incubated for 2 h. *In situ* reduction of disulfide to the thiol group, for subsequent ^{99m}Tc labeling, was carried out at pH 4.5 using DTT. To selectively reduce the pyridyldithio group and not the antibody disulfide bonds, various reaction conditions were examined (Table 3). Thiol-containing conjugate, obtained using the optimized reduction conditions, was purified on a centrifuged-SEC equilibrated in ABS buffer. Thiol groups were quantified by Ellman's assay (10).

^{99m}Tc Labeling

A lyophilized formulation of 200 μg of DTT-reduced LL2 F(ab')$_2$-PDPH conjugate with 25 μg of stannous ion, prepared using a documented procedure (11), was reacted with 1 mCi of ^{99m}Tc pertechnetate. HPLC analysis after 1 h of incubation showed 90.2% incorporation. A control experiment involving the lyophilized formulation of the unmodified antibody, which was subjected to the same DTT reduction condition, gave less than 7.6% nonspecific incorporation (Fig. 3). After 18 h, the label was intact for the conjugate, and the nonspecific incorporation in the control did not change appreciably, rising to 10.6% during this time.

Results and Discussion

1-(4-Isothiocyanatobenzyl)-DTPA was modified using various diamines, such as 1,10-diaminodecane, hydrazine, and SDH, to obtain amine-terminating DTPAs II-IV as shown in Fig. 1. The HPLC retention times of the products, on an analytical C_{18} column, were as could be predicted; the relatively lipophilic "long chain" DTPA or 10-aminodecylthioureatobenzyl-DTPA containing a C-10 hydrocarbon spacer had a longer retention time compared to the other three (I, III, and IV). Interestingly, a distinction between mono- and di-DTPA adducts of SDH, prepared either with an excess of diamine or an excess of isothiocyanate, could be obtained on HPLC, although both eluted near the void volume. Reaction using 1:1 molar ratio of the reactants, in this case, furnished a 2:3 mixture of mono- and di-DTPA adducts. Based on this empirical observation, retention times of III and IV were indicative of the amine-terminating nature of these products. More direct proof of structure was obtained through conjugation chemistry achieved with these reagents.

Fig. 1 describes the carbohydrate-modification protocol and the conjugates prepared. The first step of periodate oxidation was adapted from a literature procedure (12). Lower pH, lower periodate concentration, and lower temperatures were determined to be crucial factors to minimize aggregate formation and to minimize reduction in antigen-binding affinities (12). In this study, no attempt was made to change the mild oxidation condition used; consequently, this initial oxidation condition (resulting in the aldehydes generated), coupled with the reactivity of the amine used, determined the number of chelators attached. Conjugates derived from I and II were further stabilized by reduction with cyanoborohydride. The number of chelators introduced (Table 1) varied from 5.5 for LL2 conjugate L-I to 1.3 for L-III. Unoxidized LL2 F(ab')$_2$ gave a value of 0.075 for DTPA/F(ab')$_2$, which implied negligible nonspecific conjugation.

In vitro competitive binding assays were carried out to compare the binding to antigen of variously modified LL2 F(ab')$_2$ and hImmu-14-N F(ab')$_2$ conjugates with the corresponding unmodified antibody

Table 2 *Selected biodistributions of ^{88}Y-labeled hMN14-N F(ab')$_2$-carb-DTPA (A) and ^{88}Y-labeled hImmu-14 F(ab')$_2$-lysyl-DTPA (B; control) in LS174T nude mice (%ID/g, n = 4 or 5)*

Tissue	4 h	24 h	48 h
Tumor			
A	14.08 ± 2.91	18.95 ± 4.93	19.07 ± 6.28
B	13.31 ± 3.89	12.97 ± 2.15	18.39 ± 14.57
Liver			
A	11.74 ± 1.86	16.56 ± 2.76	12.37 ± 2.50
B	11.00 ± 2.35	6.00 ± 1.19	6.34 ± 0.78
Spleen			
A	5.67 ± 1.20	9.11 ± 1.60	10.98 ± 3.69
B	10.36 ± 2.27	13.70 ± 4.37	16.59 ± 4.79
L. kidney			
A	10.48 ± 4.08	19.31 ± 2.17	14.56 ± 3.15
B	38.04 ± 14.01	29.89 ± 7.20	21.13 ± 4.54
Lungs			
A	9.66 ± 7.72	3.45 ± 0.54	3.72 ± 1.06
B	5.91 ± 1.79	3.07 ± 1.21	4.65 ± 4.58
Blood			
A	12.44 ± 3.10	1.81 ± 0.37	0.55 ± 0.10
B	9.66 ± 2.97	0.43 ± 0.10	0.32 ± 0.09

Table 3 *DTT reaction conditions: reducing 2-pyridyldithio (PDPH) group of the conjugate versus antibody disulfide (control)*

DTT conc.[a]	Reaction Time (min)	LL2 $F(ab')_2$-carb-PDPH		LL2 $F(ab')_2$ control	
		DTT/$F(ab')_2$[b]	SH/$F(ab')_2$	DTT/$F(ab')_2$[b]	SH/$F(ab')_2$
5 mM	5	114	1.31	108	0.06
10 mM	5	ND	ND	250	0.06
	15	258	2.00	224	0.40
	30	258	2.40	224	0.63
20 mM	5	ND	ND	500	0.22
	10	600	2.61	500	0.34
	15	600	2.40	448	0.58
	30	600	3.23	448	1.27

[a] conc., concentration.
[b] Molar ratio in the reaction; ND, not determined.

fragments. Details are reported elsewhere (13). Fig. 2 compares the carbohydrate-modified LL2 F(ab')2 conjugates L-1 and L-2 with two nonspecifically modified conjugates. Using 50% inhibition to binding (ID_{50}) as an example, the carbohydrate modified conjugates (with an average of 5.5 DTPA or 4.6 "long chain" DTPA or 10-aminodecyl-thioureatobenzyl-DTPA groups) had antigen-binding capacity unchanged, whereas lysine-modified conjugates with 2.2 and 4.3 DTPAs had binding affinity reduced by 25 and 79%, respectively. Similarly, although there was no change in the binding to CEA of hImmu-14-N conjugate M-II examined to date, significant reduction in the binding affinity was observed in the case of a conjugate randomly modified with 3 DTPAs.[4]

Radiolabeling incorporations with either ^{90}Y acetate or ^{111}In acetate were generally high (Table 1). Colloidal precipitation of the metal was negligible. Immunoreactivity was determined in the case of ^{90}Y-labeled hImmu-14-N conjugate M-II by treating an aliquot of the purified radioimmunoconjugate with a 80-fold excess of CEA and analyzing on HPLC. Complete shift of the peak due to ^{90}Y-conjugate at 9.63 min to antibody-antigen complex at 7.12 min was seen.

In vivo biodistribution of ^{88}Y labeled hImmu-14-N $F(ab')_2$ conjugates (Table 2) in nude mice bearing human colon carcinoma revealed a pattern different from that for ^{88}Y-labeled hImmu-14$F(ab')_2$, which lacks the light chain carbohydrate group. The presence of carbohydrate increases the *in vivo* residence time, which is favorable for tumor accretion, and decreases kidney accumulation. The latter finding is interesting in that it reduces the customarily high kidney uptake of radioactive metal when using $F(ab')_2$ fragments. The splenic uptake is also reduced, whereas a higher liver uptake is seen, which is not surprising for a glycosylated substance. ^{88}Y-labeled M-II had a biodistribution pattern similar to that for ^{88}Y-labeled MI (data not shown).

Site-specific incorporation of a ^{99m}Tc chelatable group was demonstrated by coupling PDPH to oxidized LL2 $F(ab')_2$. The key to the successful production of this agent depended on selectively reducing the pyridyldithio group in the presence of reduction-sensitive hinge-region disulfide bonds. Although the use of DTT at a low pH of 4.5 is known to bring about facile reduction of the pyridyldithio group (14) and has been advantageously used in the case of an IgG-PDPH conjugate (15), its application to $F(ab')_2$ is nontrivial. A detailed study was, therefore, undertaken to optimize the reaction conditions for selective reduction. Table 3 details the results. The control experiment involved subjecting unmodified $F(ab')_2$ to identical DTT reduction conditions. It is clear that a DTT:$F(ab')_2$ ratio of less than 250, at a DTT concentration of 10 mM or less and a reaction time of 5 min, are optimum reduction conditions. Adjusting for the thiol group generated on the antibody, the number of thiolating agents introduced was determined to range from 1.3 to 2.0, which may be attributable to small differences in the conjugation conditions in different runs. ^{99m}Tc-labeling using lyophilized formulation of the LL2 $F(ab')_2$ conjugate (L-PDPH, reduced with 5 mM DTT in a 5-min reaction) and 1 mCi of ^{99m}Tc pertechnetate generator eluate shows >90% incorporation, whereas the control sample showed less than 8% incorporation into the antibody (Fig. 3). After 18 h, ^{99m}Tc-conjugate maintained its integrity with negligible decomposition, and the control sample did not appreciably improve in incorporation.

Site-specific modification of intact antibodies at the Fc portion

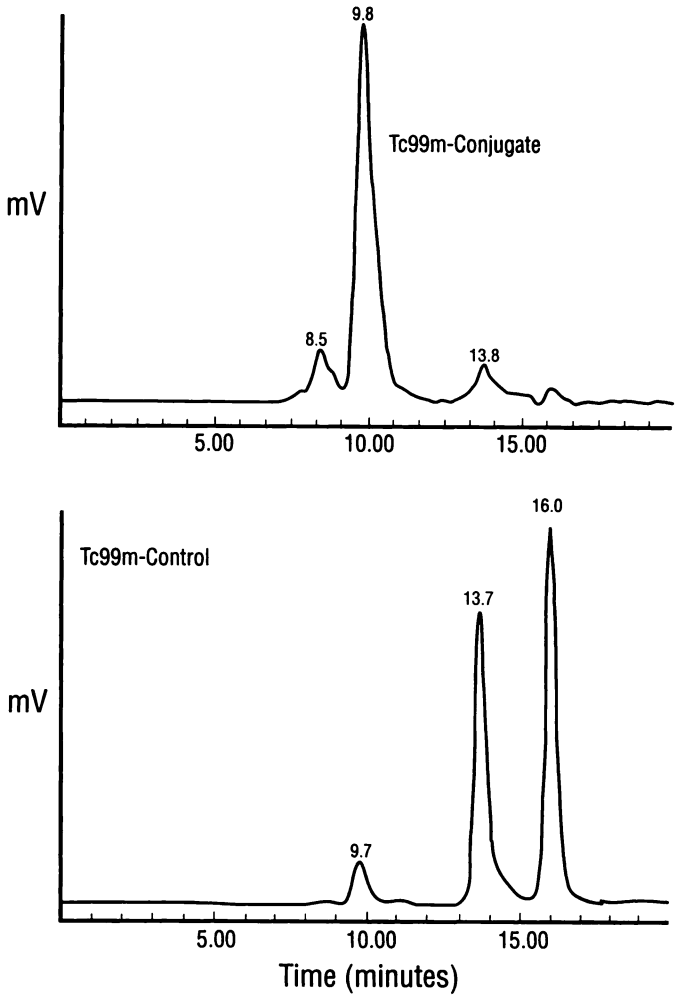

Fig. 3. HPLCs of ^{99m}Tc labelings of the LL2 $F(ab')_2$ conjugate (carbohydrate-modified with PDPH, and DTT-reduced; *top*) and of control LL2 $F(ab')_2$ subjected to identical DTT-reduction condition (*bottom*). The peaks are of ^{99m}Tc-aggregate (8.5 min), ^{99m}Tc-$F(ab')_2$ (9.8 or 9.7 min), reduced ^{99m}Tc-tartrate (13.8 or 13.7 min), and ^{99m}Tc pertechnetate (16.0 min).

[4] S. O. Leung and M. J. Losman, unpublished data.

carbohydrate, with radioisotopes (16) or drug molecules (17, 18), is well documented. Glycosylation of antibodies at the light chain (4) offers the possibility of extending such modifications to the corresponding divalent antibody fragments. To this end, carbohydrate modification, subsequent radiometal labeling, and tumor targeting were demonstrated using divalent fragments of LL2 and hImmu-14-N, both of which possess light chain glycosylation sites. Immunoreactivity is not compromised by the presence of as much as 5.5 chelators, and therefore, light chain carbohydrate has all of the advantages of an IgG Fc-region carbohydrate in the pharmacokinetically more favorable $F(ab')_2$ fragment.

The methodology of light chain carbohydrate modification can, in principle, be extended to the introduction of other chelating agents such as DOTA. For the preparation of ^{90}Y-labeled DOTA-monoclonal antibody immunoconjugate quickly and in high yield, one suggested solution (19) involves attaching 10–11 DOTA molecules to IgG through an intermediate carrier, a generation-2 dendrimer. This procedure enabled a great enhancement in the rate of formation of the ^{90}Y-labeled DOTA complex in the conjugate, and resulted in a specific activity of >45 μCi/μg of ^{90}Y-labeled DOTA-dendrimer-monoclonal antibody, due to the large amount of DOTA chelate. Extension of this methodology to $F(ab')_2$ via random lysine modification could potentially diminish immunoreactivity. The work described here could allow for a parallel approach with $F(ab')_2$ fragments by the attachment of a large number of DOTA molecules, directly or through an intermediate carrier (18), to oxidized light chain carbohydrate.

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