[CANCER RESEARCH (SUPPL.) 50, 804s–808s, February 1, 1990]

Disulfide Bond-targeted Radiolabeling: Tumor Specificity of a Streptavidin-biotinylated Monoclonal Antibody Complex[1]

Renato B. del Rosario and Richard L. Wahl[2]

Division of Nuclear Medicine, Department of Internal Medicine, University of Michigan Medical Center, Ann Arbor, Michigan 48109-0028

Abstract

A site-specific labeling method was developed in which sulfhydryl groups of a murine IgG2a anti-ovarian monoclonal antibody, 5G6.4, were biotinylated with *N*-iodoacetyl-*N'*-biotinylhexylenediamine (Compound *1*) following partial reduction of disulfide bonds with dithiothreitol. Reaction of *1*-alkylated 5G6.4 with ^{125}I-streptavidin gave immunoreactive streptavidin-*1*-biotinylated complexes. Radio-fast protein liquid chromatography data were consistent with the formation of a stable monovalent streptavidin-half-antibody complex as the major species. *In vivo* specific localization of these radioantibody conjugates to human tumor xenografts of ovarian carcinoma was confirmed by a comparative biodistribution study in nude mice using as a control the nonspecific ^{125}I-streptavidin-*1*-alkylated UPC-10 (an irrelevant IgG2a monoclonal antibody) complex prepared analogously as described above. Tumor uptake for radiolabeled 5G6.4 [0.279 ± 0.041% (SE) kg injection dose/g) was significantly greater [$P < 0.025$] than for UPC-10 [0.165 ± 0.027% kg injection dose/g]. The tumor:blood ratio (7.38 ± 1.285) for 5G6.4 was ≈3 times that for UPC-10 (2.48 ± 0.708, $P < 0.01$). This sulfhydryl site-directed approach demonstrated that reduced disulfides of monoclonal antibodies are viable sites for attaching labels without significant loss of *in vitro* and *in vivo* immunoreactivity.

Introduction

Iodoacetyl-type reagents which site-specifically alkylate immunoglobulin interchain sulfhydryls have long been recognized as a tool for studying antibody structure (1). As a new radiolabeling method for monoclonal antibodies, sulfhydryl site-directed alkylation has the potential of directing the label distant from the antigen-binding sites (2, 3). This approach may therefore have the advantage of minimizing loss of immunoreactivity resulting from the nonselective chemistry of other random labeling techniques currently in wide use (4). Recently we reported our preliminary findings on the site-specific biotinylation of monoclonal antibodies using *N*-iodoacetyl-*N'*-biotinylhexylenediamine (5) [Compound *1* (Fig. 1)] and their ability to form immunoreactive complexes with streptavidin (6). In the current paper we provide details on the preparation and characterization of these complexes, as well as evidence for their specific tumor localization to human ovarian carcinoma xenografts. Our radiolabeling strategy is outlined in Scheme 1. The rationale of the method draws primarily from the well known high avidity and tetravalency of avidin and streptavidin (M_r ≈60,000–65,000) towards biotin (7). Following reduction, the ensuing thiols are trapped by *1 in situ* which site-specifically attaches biotin to the antibody. The resulting biotin "bridge" then serves to anchor streptavidin (which can be radiolabeled with high efficiency in a variety of ways) to yield streptavidin-*1*-biotinylated antibody complexes (6, 8, 9). Two attractive features of this procedure are (*a*) radioactivity is introduced distant from the antibody itself and (*b*) chemistry performed on the antibody is solely limited to the sulfhydryl site-specific reduction and alkylation thus avoiding unnecessary side reactions, and thus introducing the label at some distance from the antigen binding sites.

Materials and Methods

Monoclonal Antibodies. The murine anti-ovarian IgG2a monoclonal antibody 5G6.4 was produced as previously described (10) and purified by staphylococcal protein A chromatography (11). UPC-10 was purchased from Bionetics Research (Charleston, SC) as lyophilized ascites and was similarly purified. Antibody concentrations were determined using the method of Bradford (12) using a commercially available bovine γ-immunoglobulin IgG standard (Bio-Rad).

Reduction and Biotinylation of Monoclonal Antibodies with *1*. The procedure is exemplified for 5G6.4. To 500 μl of ≈4.4×10^{-5} (≈2.2×10^{-2} μmol) of 5G6.4 in ≈0.1 M sodium phosphate buffer (pH ≈8) were added by syringe 14 μl of a 3.89×10^{-2} M (DTT[3] (Aldrich or Sigma) solution in 0.2 M Na_2HPO_4 ([DTT]/[IgG2a] ≈25). After standing for 5 h at 37°C, 43 μl of a 1.27×10^{-2} M DMSO (Sigma) of *N*-iodoacetyl-*N'*-biotinylhexylenediamine(*1*) (5) (Pierce Chemical Co.) solution were added, and the cloudy emulsion was kept at 37°C overnight in the dark. The mixture was allowed to stand at 4°C for an additional 12 days. It was then chromatographed through Sephadex G-25-150 (9 ml) using 0.1 M phosphate buffer. The protein was collected in 2 × ≈300–400-μl fractions, pooled together, and filtered through a 0.22-μm filter to remove residual insoluble material.

^{125}I-Streptavidin. ^{125}I-Streptavidin (≈20–30 μCi/μg) was purchased from Amersham (Arlington Heights, IL) and used without further purification. Alternatively, unlabeled streptavidin (Molecular Probes, Eugene, OR) was labeled using the Iodo-Gen method (13) as follows. To a reaction vial containing 12 μl of ^{125}I (5.05 mCi; ICN Biomedicals, Lisle, IL) and Iodo-Gen (≈20 fold molar excess over protein) was added 100 μl of a 10-μg/ml solution of streptavidin (Molecular Probes) solution in 0.05 M sodium phosphate buffer (pH ≈7). After standing for 20 min, the labeled protein was passed through an anion-exchange column (Bio-Rad AG-1-X8, 200–400 mesh) and collected in a total volume of ≈1.5 ml (3.3 mCi). Cell binding assays on product mixtures purified by FPLC upon reaction with *1*-biotinylated 5G6.4 were carried out based on a published procedure with the use of ≈10×10^6 77IP3 ovarian carcinoma target cells/tube (14, 15).

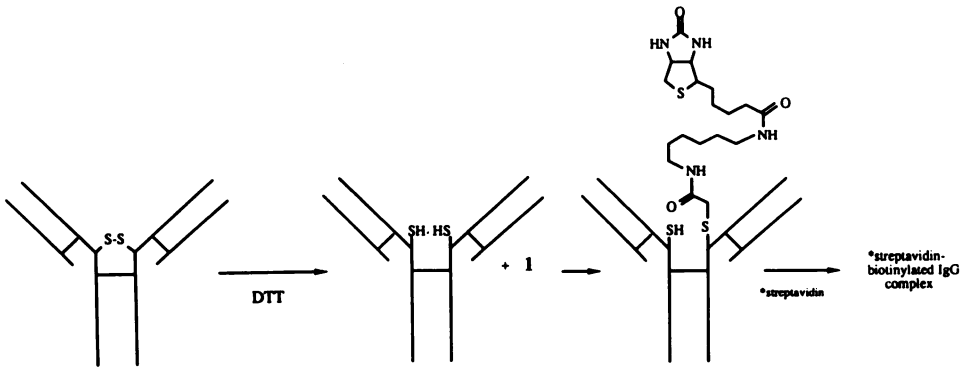
Native and SDS-PAGE, Autoradiography, and FPLC Analysis. Native and SDS-PAGE were routinely performed using commercially available Pharmacia 8–25% polyacrylamide gradient gels on a Pharmacia Phast System in the absence or presence of DTT (16). Samples were typically heated for 2–3 min in a boiling water bath prior to electrophoresis. For native-PAGE/autoradiography, developed gels were covered with a thin plastic film and the activity was measured without delay by immediate exposure to X-ray film or counted using a Bioscan System 200 Imaging Scanner. FPLC analyses were conducted on a Pharmacia P-500 system equipped with a series of two Superose size exclusion columns with 0.1 M phosphate buffer (pH ≈7) as eluting buffer and a flow rate of 24 ml/h. For molecular weight calibrations, commercial standard proteins (Fig. 6) were used. For radio-FPLC studies, 1-min fractions were collected and the activity per tube was measured on Packard Minaxi 5000 Auto-Gamma counter.

Animal Experiments. Details on the animal model utilized have been published previously (17). Each s.c. tumor-bearing nude mouse (≈25–30 g) was given an (17) i.p. injection of the following preparation. To ≈120 μl of *1*-alkylated 5G6.4 (≈1.7×10^{-3} μmol, ≈1.4×10^{-5} M, ≈17

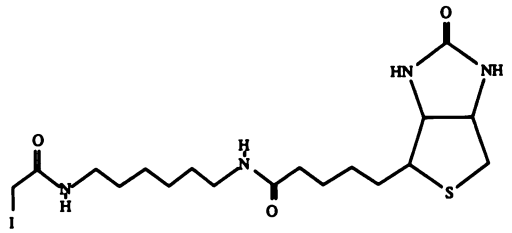
[1] Presented at the "Second Conference on Radioimmunodetection and Radioimmunotherapy of Cancer," September 8–10, 1988, Princeton, NJ. Supported in part by NIH Grants ROI CA41531-02 and ROI CA42768-01A1.

[2] To whom requests for reprints should be addressed.

[3] The abbreviations used are: DTT, dithiothreitol; DMSO, dimethyl sulfoxide; FPLC, fast protein liquid chromatography; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis.



Scheme 1. Preparation of radiolabeled streptavidin-*1*-biotinylated antibody complexes.



1

Fig. 1. *N*-Iodoacetyl-*N'*-biotinylhexylenediamine (Compound *1*).

fold molar excess) was added 1.5 ml (≈195 μCi, ≈4.5 × 10^{-3} μg/μl as specified by manufacturer) ^{125}I-streptavidin (Amersham, ≈28 μCi/μg). After overnight standing at 4°C, the solution was further diluted with ≈3 ml 0.1 M sodium phosphate (pH ≈7–8) and used directly for IP delivery (≈32–33 μCi in ≈0.5 ml/mouse). The radiolabeled UPC-10 was prepared in exactly the same manner. Native-PAGE and quantitative scanning autoradiography showed that both preparations were contaminated by streptavidin plus other radiolabeled impurities (18.9% for UPC-10 and 14.8% for 5G6.4 relative to total sample activity). In calculating for tissue activity, the initial dose activity of radiolabeled complex/animal was corrected to account for the purity of the reagents. Animals were sacrificed after 5 days and activities for liver, kidney, spleen, intestine, muscle, tumor, and blood were measured.

Results

Partial reduction of the interchain disulfides of the IgG2a antibodies 5G6.4 and UPC-10 was readily accomplished using DTT (6). Since *1* was completely insoluble in aqueous media, it was added as a solution in DMSO and the amount of DTT used was adjusted such that minimal precipitation of *1* occurred in the resulting DMSO (8–13% by volume) emulsion (see "Materials and Methods"). Fig. 2 (*lanes B* and *C*) shows the nonreducing SDS-PAGE profile of the chromatographed product mixture upon quenching the reduction mixture of 5G6.4 (Fig. 2, *Lane A*) and UPC-10 (Fig. 2, *Lane B*) with a stoichiometric quantity of *1* with respect to DTT. As a molecular weight reference, the migration profile of a mixture of H2L2 (intact, M_r ≈150,000), H2L (≈125,000), H2 (≈100,000), HL (≈75,000), H (heavy chain, ≈50,000) and L (light chain, ≈25,000) fragments of a typical IgG2a antibody is included in Fig. 2, *Lane A*.[4] Analysis of the *1*-alkylated 5G6.4 product (Fig. 2, *Lane B*) mixture by size exclusion FPLC revealed one major peak with elution time identical with that of unreduced antibody plus a small quantity of low molecular weight species (Fig. 3).

[4] R. B. del Rosario, R. L. Wahl, S. J. Brocchini, R. G. Lawton, and R. H. Smith. Sulfhydryl site-specific labeling of a monoclonal antibody by a fluorescent equilibrium transfer alkylation cross-link reagent, submitted for publication.

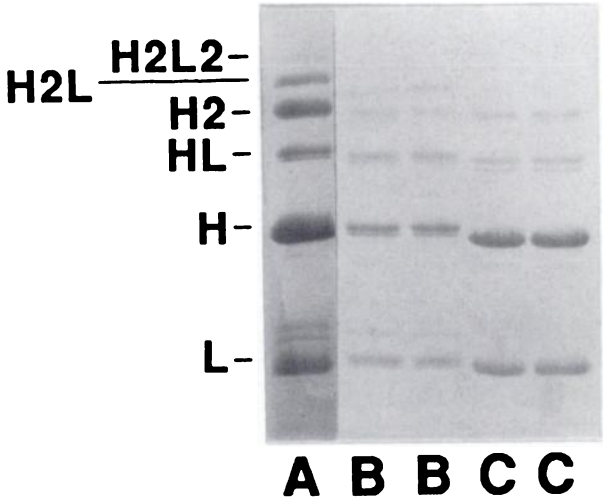


Fig. 2. Nonreducing SDS-PAGE analysis of *1*-alkylated 5G6.4 (*A*) and UPC-10 (*B*).

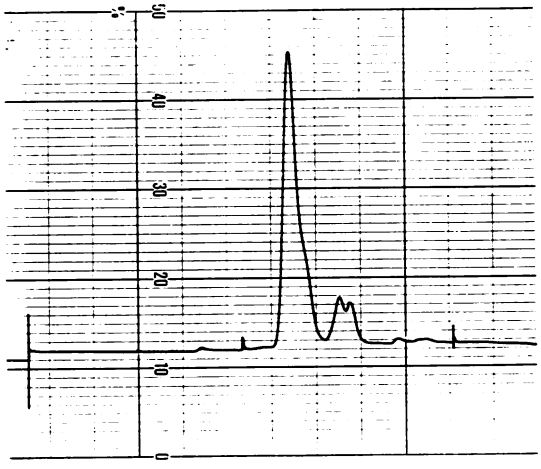


Fig. 3. FPLC analysis of *1*-alkylated 5G6.4 (elution time, 58 ± 2 min).

Comparison of Fig. 2, *Lane B* and Fig. 3 suggests that the product mixture consisted predominantly of *1*-alkylated H and L fragments which remained strongly associated by noncovalent interactions in the absence of denaturing reagents. This observation is in agreement with the hypothesis that reduced heavy and light chains of an IgG molecule do not undergo complete dissociation even after alkylation (1, 18). The site specificity of *1* thiols over other nucleophilic residues was previously confirmed by flow cytometric experiments (6).

In our earlier communication (6), we provided evidence for

the facile preparation of ^{125}I streptavidin-*1*-alkylated 5G6.4 complexes which underwent *in vivo* tumor localization to human ovarian carcinoma xenografts. To test the tumor specificity of these complexes, we examined the uptake of *1*-alkylated 5G6.4 in parallel with *1*-alkylated UPC-10, a nonspecific IgG2a antibody in s.c. ovarian tumor-bearing mice. The corresponding radiolabeled complexes were prepared with the product mixtures of Fig. 2, *Lanes B* and *C* (≈17-fold molar excess) using commercially available ^{125}I-streptavidin (Amersham). Inspection by native-PAGE and autoradiography showed one major radioactive spot for both preparations with molecular mass >streptavidin. Table 1 gives the tissue activity data for ^{125}I-streptavidin-*1*-alkylated 5G6.4 and UPC-10 acquired 5 days post-i.p. (17) injection of ≈28–29 μCi *1*-alkylated UPC-10 (≈8 μCi/μg) and ≈32–33 μCi 5G6.4 (≈0.8 μCi/μg) per mouse. Fig. 4*A* compares the cumulative data along with those for ^{125}I-streptavidin (6), while Fig. 4*B* summarizes the tumor:nontumor ratios for different tissues.

In addition to native-PAGE analysis (6), complexation of streptavidin to *1*-biotinylated 5G6.4 was most clearly demonstrated by size exclusion radio-FPLC. Fig. 5 shows the radio-FPLC plots for ^{125}I-streptavidin (*Trace A*) and ^{125}I-5G6.4 (*Trace B*, Iodo-Gen labeled). Fig. 5*C* gives the radio-FPLC trace of the product obtained when approximately equimolar quantities of *1*-alkylated 5G6.4 and ^{125}I-streptavidin are allowed to stand overnight at 4°C. A nearly identical set of data are obtained when complexation is performed using a ≈7-fold molar excess of *1*-alkylated 5G6.4 to ^{125}I-streptavidin. The complex appears as a broad peak which overlaps both intact antibody and streptavidin (Fig. 5). The molecular mass corresponding to the region about the peak maxima was estimated to be ≈95,000–140,000 Da based on a standard protein curve (Fig. 6) after correcting for the time difference between UV and radioactive peak detection. Cell binding assays performed using eight sample fractions taken from the radio-FPLC analysis (Fig. 5*C*) spanning from the origin of the curve (elution time, ≈50 min) to the region for streptavidin (Fig. 5, *Traces C* and *A*) overlap yielded ≈1–40% specific binding to 77IP3 ovarian carcinoma target cells, with maximum binding (≈50%) being observed at the peak maximum. A similar assay on the ≈7:1 *1*-5G6.4/streptavidin preparation gave ≈58–38% specific binding, with maximum binding (≈60%) again being observed at the peak maximum.

Discussion

Biotinylation of antibodies is a well established protocol in immunohistochemistry (19), protein blotting (20, 21), and immunoassays (22). Biotin conjugation is commonly achieved via reaction of a derivative which reacts with ε-amino groups of IgG lysines (19, 22). The potential of one such derivative for radioimmunolocalization applications was first illustrated by

Table 1 *Biodistribution activity data for ^{125}I-streptavidin plus 1-alkylated 5G6.4 plus UPC-10*[a]

Tissue	UPC-10	5G6.4
Liver	0.282 ± 0.012[b]	0.249 ± 0.022
Kidney	0.918 ± 0.027	0.900 ± 0.063
Spleen	0.203 ± 0.012	0.156 ± 0.031
Intestine	0.037 ± 0.004	0.031 ± 0.003
Muscle	0.017 ± 0.002	0.021 ± 0.004
Tumor	0.165 ± 0.027	0.279 ± 0.041
Blood	0.075 ± 0.005	0.046 ± 0.010

[a] Values are given as % K/g injection dose/g and are the means for four mice. All data were derived after animals were sacrificed 120 h post-i.p. injection.
[b] Mean ± SE.

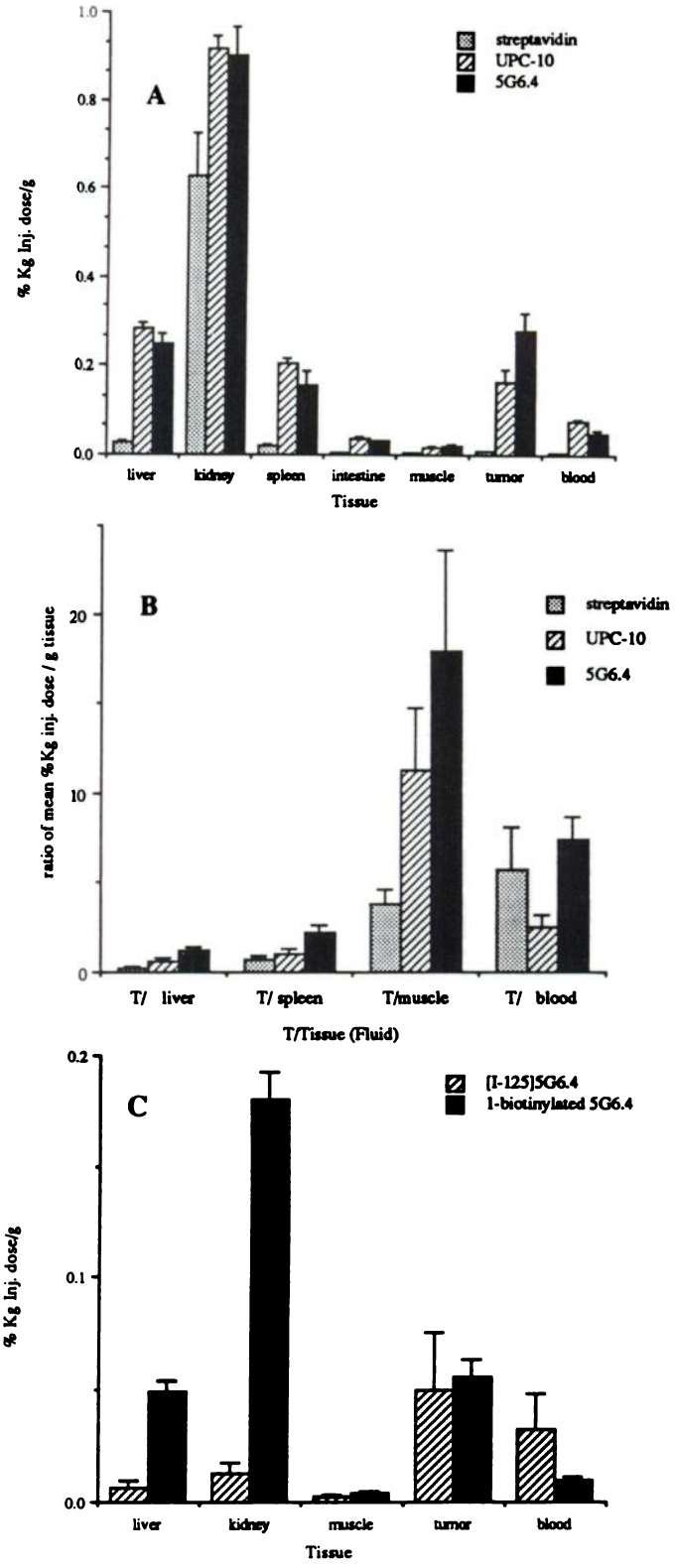


Fig. 4. *A*, biodistribution of ^{125}I-streptavidin and ^{125}I-streptavidin-*1*-biotinylated 5G6.4 and UPC-10; *B*, tumor (*T*):nontumor ratios: *C*, comparative biodistribution of ^{125}I-5G6.4 (Iodo-Gen method) and ^{125}I-streptavidin-*1*-biotinylated 5G6.4.

Hnatowich *et al.* (8) who showed that monomeric streptavidin-biotinylated polyclonal IgG localized to staphylococcal protein A *in vivo* through binding of the antibody Fc portion. In addition to our initial study with *1* (6), a preliminary account describing a somewhat different approach of probing tumor-

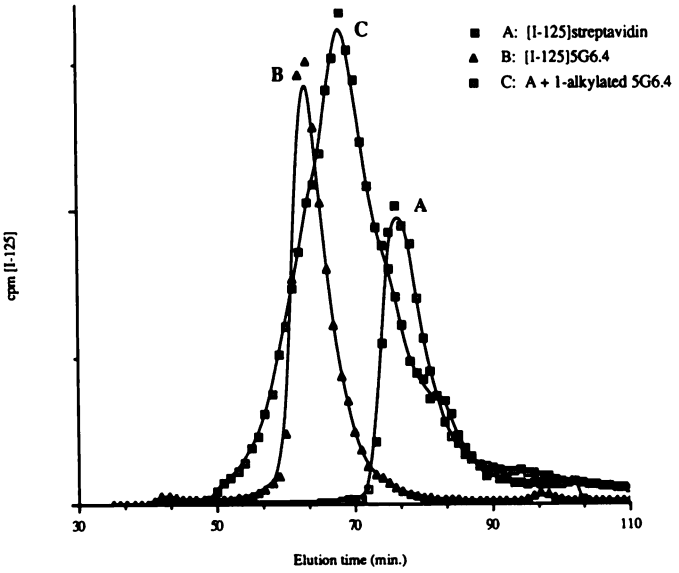


Fig. 5. Radio-FPLC of ^{125}I-streptavidin (*A*), ^{125}I-5G6.4 (*B*), and product of *A* + *1*-alkylated 5G6.4 (*C*).

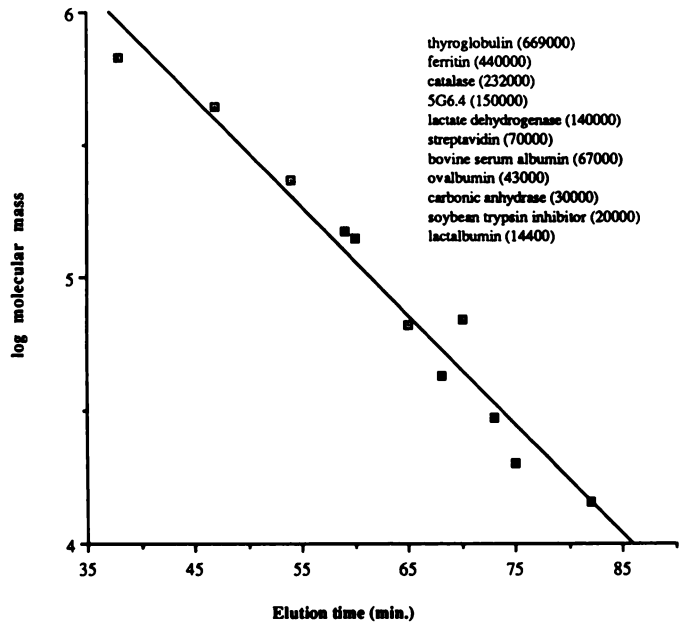


Fig. 6. Protein molecular mass as a function of FPLC elution time (r = 0.96).

bound streptavidin-biotinylated antibody with radiolabeled biotin was also recently presented by the same group (9). In the course of evaluating new methods for sulfhydryl site-specific labeling[4] we became interested in Compound *1* because there was already literature precedence for retention of immunoreactivity following alkylation of reduced disulfides of monoclonal antibodies with iodoacetyl-type compounds (1, 23, 24). Furthermore, the well known high nucleophilicity of IgG interchain thiols (1) should make *1* ideal for biotinylation of antibody fragments.

Present knowledge on immunoglobulin structure (25, 26) suggests that the ideal location for sulfhydryl site-specific incorporation of *1* into an antibody would be via reaction of thiols derived from selective partial reduction of disulfides situated at the hinge region (scheme 1) (18, 27, 28). Reaction conditions for reduction of 5G6.4 prior to reaction with *1* were chosen based on work by Packard *et al.* (29) and our experience with ongoing experiments on the sulfhydryl specific cross-linking reactions of 5G6.4.[4] Total selectivity was not possible and it was found that reactions of reduced disulfides with reagents such as *1* were complicated by a competing reannealing mechanism (30, 31).[4] Nonetheless, the disulfide reduction step of our strategy (Scheme 1) led to a surprising difference in the chemistry of streptavidin-*1*-biotinylated antibody complexation. The radio-FPLC data of Fig. 5*C*, clearly indicated that the major immunoreactive species from the *1*-alkylated 5G6.4 plus streptavidin preparation is not the monomeric streptavidin-biotinylated antibody complex (M_r ≈220,000) observed by Hnatowich *et al.* (8, 9). Instead, the results are more consistent with the major immunoreactive species being a monovalent *1*-alkylated HL-streptavidin complex (molecular mass ≈ streptavidin + half-intact antibody ≈ 60,000 + 75,000 ≈ 140,000). The immunoreactivity of this species at the upper end of Fig. 4*C* was comparable with that obtained from routine random (Iodo-Gen) labeling of 5G6.4 (30–60% using an identical assay). The observation of a broad peak in the radio-FPLC analysis (Fig. 5*C*) is consistent with both the SDS-PAGE (Fig. 2, *Lane B*) and FPLC (Fig. 3) data of *1*-biotinylated 5G6.4 consisting predominantly of noncovalently held IgG2a chain fragments. The correspondence of the radioactive peak maximum (Fig. 5*C*) to presumably a complexed HL fragment may reflect both the steric requirements of streptavidin and the general observation that H-H chain noncovalent interactions are weaker than those for H-L (18). The apparent formation of one major peak in Fig. 5*C* is unusual considering that a wide range of oligomerization is possible in view of the tetravalency of streptavidin. Radio-FPLC analyses of the radiolabeled UPC-10 and 5G6.4 preparations actually used in the animal studies also showed similar peaks with molecular mass ≈<150,000 Da to be major constituents in these product mixtures. However, direct correlation of these earlier FPLC data with Fig. 5*C* was not possible since these were prepared from commercial grade ^{125}I-streptavidin which was found to contain significant quantities of radiolabeled impurities.

Evidence for specific tumor uptake comes from the cumulative data of Figure 4, *A* and *B*. Most notable are the differences between the tumor uptake for 5G6.4 and UPC-10 and the tumor:nontumor ratios, particularly for tumor:blood level activities. Tumor uptake for *1*-alkylated 5G6.4 [0.279 ± 0.041% SE kg injection dose/g] was significantly greater ($P < 0.025$] than for UPC-10 [0.165 ± 0.027% kg injection dose/g]. Target:nontarget ratios were highest for *1*-alkylated 5G6.4 with a tumor:blood ratio (7.38 ± 1.285) ≈3 times that for *1*-alkylated UPC-10 (2.48 ± 0.708, $P < 0.010$). Fig. 4*C* gives a qualitative comparison between ^{125}I-streptavidin-*1*-alkylated 5G6.4 and the biodistribution of Iodo-Gen-labeled ^{125}I-5G6.4 in i.p. tumor-bearing mice following i.p. administration taken from an earlier study (17). (Values for *1*-biotinylated 5G6.4 study in Fig. 4*C* were arbitrarily divided by 5 to fit both results to scale for a more graphic comparison.) Although the tumor:blood ratio for *1*-biotinylated 5G6.4 was notably better than Iodo-Gen-labeled 5G6.4, the kidney and liver uptake were significantly higher using the present method. The reasons for these high nontarget uptakes are yet unclear since the procedure is not yet optimized. The unusually high kidney uptake may partly be explained by the fact that the streptavidin-*1*-biotinylated 5G6.4 preparation contained at least 15% unconjugated streptavidin plus other radiolabeled impurities (by native-PAGE and quantitative autoradiography). (Note also that commercial grade ^{125}I-streptavidin localizes almost exclusively in the kidney.) The presence of dissociated *1*-biotinylated low molecular weight fragments in the biotinylated antibody preparation (as in Fig. 3) may have

also produced undesired labeled fragments leading to higher nonspecific tissue uptake. It is likely that target:nontarget ratios could be significantly improved by using high quality ^{125}I-streptavidin. Indeed the specific binding of the preparations rose from 20–25% to 50–60% when Iodo-Gen-labeled streptavidin was substituted for the commercial grade material. Finally, the formation of high molecular weight oligomers arising from the tetravalency of streptavidin which may have undesirable *in vivo* properties could be separated by size exclusion chromatography (8).

The above results with Compound *1* indicate that antibody disulfide bonds are viable sites for attaching labels without loss of *in vitro* and *in vivo* immunoreactivity. The successful trapping of reduced thiols with *1* also implies that introduction of thiols via other methods (*i.e.*, reaction with iminothiolane) is not necessary for sulfhydryl-directed radiolabeling. Furthermore, our experience in the use of a preformed streptavidin-antibody complex suggests that conjugation of a large label carrier does not necessarily lead to loss of immunoreactivity. This may have important implications for other biochemical applications of antibody immunoconjugates.

Acknowledgments

We wish to thank Susan J. Fisher, Gayle A. Jackson, Martin Strnat, Jeffrey S. Barrett, Catherine Voorhees, and Joseph Wissing for valuable technical assistance and helpful discussions.

References

1. Nisonoff, A., Hopper, J. E., and Spring, S. B. Properties and interactions of the light and heavy chains of immunoglobulins. *In:* F. J. Dixon and H. G. Kunkel (eds.), The Antibody Molecule, pp. 238–261. New York: Academic Press, 1975.
2. Srivastava, P. C., Knapp, F. F., Dickson, D. R., and Allred, J. F. Design and synthesis of a new *N*-(*p*-[^{125}I]iodophenyl)maleimide "kit" for labeling monoclonal antibodies. J. Nucl. Med., *28:* 726, 1987.
3. Srivastava, P. C., Knapp, F. F., Allred, J. F., and Buchsbaum, D. J. Monoclonal antibodies radiolabeled with *N*-(*p*-[^{125}I]iodophenyl)-maleimide retain tumor uptake and show insignificant *in vivo* deiodination compared with [^{125}I]ICl radiolabeled MoAb. J. Nucl. Med., *29:* 836, 1988.
4. Rodwell, J. D., Alvarez, V. L., Lee, C., Lopes, A. D., Goers, J. W. F., King, H. D., Powsner, H. J., and McKearn, T. J. Site-specific covalent modification of monoclonal antibodies: *in-vitro* and *in-vivo* evaluations. Proc. Natl. Acad. Sci. USA, *83:* 2632–2636, 1986.
5. Sutok, K., Yamamoto, K., and Wakubayashi, T. Electron microscopic visualization of the SH_1 thiol of myosin by the use of an avidin-biotin system. J. Mol. Biol., *178:* 323–339, 1984.
6. del Rosario, R. B., and Wahl, R. L. Site-specific radiolabeling of monoclonal antibodies with biotin/streptavidin. J. Nucl. Biol. Med., in press, 1988.
7. Green, N. M., Avidin. Adv. Protein Chem., *29:* 85–133, 1975.
8. Hnatowich, D. J., Virzi, F., and Ruschowski, M. Investigations of avidin and biotin for imaging applications. J. Nucl. Med., *28:* 1294–1302, 1987.
9. Rowlinson, G., Ruskowski, M., Gionet, M., Siebecker, D., Snook, D., Epenetos, A., and Hnatowich, D. J. Animal tumor localization studies with streptavidin-conjugated antibody and labeled biotin. J. Nucl. Med., *29:* 762, 1988.
10. Wahl, R. L., Liebert, M., Biesman, B., Roberts, J., Jackson, G. A., Kronberg, S., and Laino, L. Production and characterization of a murine monoclonal antibody reactive with ovarian and other epithelial carcinomas. Proc. Am. Assoc. Cancer Res., *27:* 355, 1986.
11. Ey, P., Prowse, S. J., and Jenkins, C. R. Isolation of pure IgG1, IgG2a and IgG2b immunoglobulins from mouse serum using protein A Sepharose. Immunochemistry, *15:* 429–436, 1978.
12. Bradford, M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., *72:* 248–255, 1978.
13. Fraker, P. J., and Speck, J. C. Protein and cell membrane iodinations with a sparingly soluble chloroamide, 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril. Biochem. Biophys. Res. Commun., *80:* 849–857, 1978.
14. Wahl, R. L., Liebert, M., Carey, J. E., and Jackson, G. A. Quality control of radiolabeled monoclonal antibodies: immunologic and radiochemical. Cancer Drug Delivery, *2:* 236, 1985.
15. Wahl, R. L., Wilson, B. S., Liebert, M., and Beierwaltes, W. H. High dose, unlabeled, nonspecific antibody pretreatment: influence on specific antibody localization to human melanoma xenografts. Cancer Immunol. Immunother., *24:* 221–224, 1987.
16. Laemmli, V. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (Lond.), *227:* 680–685, 1970.
17. Wahl, R. L., Barrett, J. S., Geatti, O., Liebert, M., Wilson, B. S., Fisher, S. J., and Wagner, J. G. The intraperitoneal delivery of radiolabeled monoclonal antibodies: studies on the regional delivery advantage. Cancer Immunol. Immunother., *26:* 187–201, 1988.
18. Palmer, J. L., Nisonoff, A., and Van Holde, K. E. Dissociation of rabbit γ globulin into subunits by reduction and acidification. Proc. Natl. Acad. Sci. USA, *50:* 314–321, 1963.
19. Guesdon, J. L., Ternynck, T., and Arrameas, S. The use of avidin-biotin in immunoenzymatic techniques. J. Histochem. Cytochem., *27:* 1131–1138, 1979.
20. Bayer, E. A., Zalis, M. G., and Wilchek, M. 3(*N*-Maleimido-propionyl)biocytin: a versatile thiol specific biotinylation reagent. Anal. Biochem., *149:* 529–536, 1985.
21. Bayer, E. A., Safars, M., and Wilchek, M. Selective labeling of sulfhydryls and disulfides on blot transfers using avidin-biotin technology: studies on purified proteins and erythrocyte membranes. Anal. Biochem., *161:* 262–271, 1987.
22. Bayer, E. A., and Wilchek, M. The use of the avidin-biotin complex as a tool in molecular biology. Methods Biochem. Anal., *26:* 1–45, 1980.
23. Roholt, O., Onoue, K., and Pressman, D. Specific combination of H and L chains of rabbit γ globulins. Proc. Natl. Acad. Sci. USA, *51:* 173–178, 1964.
24. Sutton, J., Alden, J. R., and Easterbrook-Smith, S. B. The effects of cleavage of the inter-chain disulfide bonds of rabbit IgG on its ability to bind C1q. Biochim. Biophys. Acta, *787:* 39–44, 1984.
25. Amzel, L. M., and Poljak, R. J. Three dimensional structure of immunoglobulins. Annu. Rev. Biochem., *48:* 961–967, 1979.
26. Davies, D. R., and Metzger, H. Structural basis of antibody function. Annu. Rev. Immunol., *1:* 87–117, 1983.
27. Hong, R., and Nisonoff, A. Relative labilities of two types of interchain disulfide bond of rabbit γG-immunoglobulin. J. Biol. Chem., *240:* 3883–3891, 1965.
28. Mage, M. G., and Harrison, E. T. A comparison of the labile disulfide bonds of rabbit γG-immunoglobulin fragments. Arch. Biochem. Biophys., *113:* 709–717, 1966.
29. Packard, B., Edidin, M., and Komoriya, A. Site-directed labeling of a monoclonal antibody: targeting to a disulfide bond. Biochemistry, *25:* 3548–3552, 1986.
30. Sears, D. W., Mohrer, J., and Beychok, S. A kinetic study of the reoxidation of interchain disulfide bonds in a human immunoglobulin IgG1κ. Correlation between sulfhydryl disappearance and intermediates in covalent assembly of H2L2. Proc. Natl. Acad. Sci. USA, *72:* 353–357, 1975.
31. Brennan, M., Davison, P. F., and Paulus, H. Preparation of bispecific antibodies by chemical recombination of monoclonal immunoglobulin G1 fragments. Science (Wash. DC), *229:* 81–83, 1985.