

# Novel unconventional binding site in the variable region of immunoglobulins

(antibody/nucleotide binding site/affinity linker/computer model)

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Communicated by Lewis H. Sarett, Viola, ID, February 12, 1996 (received for review January 11, 1996)

**ABSTRACT** The variable immunoglobulin (Ig) domains contain hypervariable regions that are involved in the formation of the antigen binding site. Besides the canonical antigen binding site, so-called unconventional sites also reside in the variable region that bind bacterial and viral proteins. Docking to these unconventional sites does not typically interfere with antigen binding, which suggests that these sites may be a part of the biological functions of Igs. Herein, a novel unconventional binding site is described. The site is detected with 8-azidopurine nucleotide photoaffinity probes that label antibodies efficiently and under mild conditions. Tryptic peptides were isolated from photolabeled monoclonal antibodies and aligned with the variable antibody domains of heavy and light chains. The structure of a variable Ig fragment was used to model the binding of the purine nucleotide to invariant residues in a hydrophobic pocket of the Ig molecule at a location distant from the antigen binding site. Monoclonal and polyclonal antibodies were biotinylated with the photoaffinity linker and used in fluorescence-activated cell sorter and ELISA analyses. The data support the utility of this site for tethering diagnostic and therapeutic agents to the variable Ig fragment region without impairing the structural and functional integrity of antibodies.

The immunoglobulin (Ig) molecule consists of two variable domains and several constant domains mediating biological effector mechanisms (1). The variable domains contain hypervariable regions that are involved in the formation of the antigen binding site. Biochemical and structural studies have shown that antigen binding is mediated by the assemblage of six so-called complementary determining regions (CDRs). Framework regions (FRs) interspersed between CDRs are believed to maintain the overall fold of the variable Ig domains; however, crystallographic studies have shown that framework regions also are involved in antigen contact and thus participate in the generation of antibody specificity (2). For years antigen binding has been considered the only function associated with the variable domains of antibodies.

Recently, additional sites for binding biologically active molecules have been discovered in the Ig molecule. Besides the canonical antigen binding site, these so-called unconventional sites (3–9) also reside in the variable domain and can bind pathogens, B-cell superantigens, the T-cell coreceptor CD4 and the human immunodeficiency virus type 1 envelope. In addition, the variable domain is involved in Ig self-binding (10) and contains epitopes (idiotypes) recognized by anti-antibodies (11). Similar to the joint involvement of CDR and FR regions in antigen binding, the unconventional binding sites also draw on CDR and FR regions. Docking to these

unconventional sites does not typically interfere with antigen binding, which suggests that these sites may be a part of the biological functions of Igs.

Nucleotide photoaffinity probes, such as 8-azidoadenosine triphosphate (8-N<sub>3</sub>ATP), have been used to characterize nucleotide binding sites in a variety of proteins (12). During photoaffinity labeling of the immunoprecipitate of several ATP binding proteins using [ $\gamma$ -<sup>32</sup>P]8-N<sub>3</sub>ATP, incorporation of radiolabel into the Ig chains was observed. This led to a detailed study of the photomodification of antibodies by using 8-N<sub>3</sub>ATP to determine the biochemical nature of this interaction and to establish the topography of the nucleotide binding site.

Herein, a novel site in the variable Ig domains is described that involves invariant residues. The site binds 8-azidopurine nucleotide photoaffinity probes with high affinity, which allows very efficient photomodification under mild conditions. Furthermore, the computer-modeled insertion of the ligand into a three-dimensional variable Ig fragment (Fv) structure shows that access to the site does not cause steric interference with antigen-contacting CDRs. Because the site is an integral part of the Ig structure, insertion of its ligand is expected not to change or distort the overall structure, or that the ligand would impair the antigen binding site. These predictions are confirmed by showing that affinity-modified antibodies retain full antigen binding. Collectively, the data support the utility of this site for tethering diagnostic and therapeutic agents to the Fv region of antibodies by photoaffinity linkers and suggest the existence of a biological activity mediated by this site.

## MATERIALS AND METHODS

**Photoaffinity Labeling.** Antibodies (3  $\mu$ g) were incubated with 200  $\mu$ M [ $\gamma$ -<sup>32</sup>P]8-N<sub>3</sub>ATP (5–30 mCi/ $\mu$ mol; 1 Ci = 37 GBq) in a final volume of 30  $\mu$ l for 1 min and photolyzed with a hand-held UV lamp at 254 nm for 1 min. The reactions were quenched with protein solubilizing mixture [10% SDS/3.6 M urea/162 mM DTT/0.0025% Pyronin Y (Allied Chemical)/20 mM Tris, pH 8.0] and analyzed by SDS/6–12% PAGE. The gels were stained with Coomassie brilliant blue, destained, and dried by using a slab gel drier. <sup>32</sup>P incorporation was detected by autoradiography and quantified by either scanning on an optical image and acquisition analysis system (Ambis) or by cutting the appropriate band from the gel and counting the radioactivity by liquid scintillation counting.

**Saturation and Protection of Labeling.** Antibodies S1C5 (13) and 8019 (14), each 3  $\mu$ g, were incubated with increasing concentrations of [ $\gamma$ -<sup>32</sup>P]8-N<sub>3</sub>ATP for 1 min at 4°C and photolyzed and analyzed as described above. Protection of

*Abbreviations:* CDR, complementary determining region; FR, framework region; Fv, variable Ig fragment; Ig, immunoglobulin; 8-N<sub>3</sub>ATP, 8-azidoadenosine triphosphate; FACS, fluorescence-activated cell sorter.

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photolabeling of S1C5 and 8019 was performed by incubating 3  $\mu\text{g}$  of antibodies with increasing concentrations of ATP for 2 min followed by incubation with 200  $\mu\text{M}$  [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP for 1 min and photolyzed and analyzed as described above. Protection experiments using amino acids were performed by incubating 3  $\mu\text{g}$  of S1C5 antibody with increasing concentrations of amino acids for 2 min, followed by incubation with 200  $\mu\text{M}$  [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP for 1 min. Photolysis and analysis were performed as described above.

**Fluorescence-Activated Cell Sorter (FACS) Analysis.** Tumor cells (38C13 and LS174T,  $1 \times 10^6$ ) were incubated with biotinylated 8-azidoadenosine-labeled antibodies S1C5 or 8019 (unpublished data) for 60 min on ice. After washing with 5% fetal calf serum/phosphate-buffered saline, neutralite avidin-fluorescein isothiocyanate (Southern Biotechnology Associates) was added and incubated for 30 min, fixed, and subjected to flow cytometry. FACS data were generated from a minimum of 9900 cells per sample.

**Cloning and Sequencing of  $V_H$  and  $V_L$  Regions.** The genes coding for S1C5 and 8019 antibodies were cloned by using standard primers and sequenced as described (15, 16). S1C5 was cloned and sequenced by R. Streifer and 8019 by C. C. Meyers (personal communication).

**Tryptic Digestion and Peptide Purification.** Photolabeled peptides from antibodies S1C5 and 8019 were isolated as follows. Antibodies (2.5 mg) were incubated and photolabeled twice with 425  $\mu\text{M}$  of [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP. Photolabeled antibody was precipitated by the addition of 7%  $\text{HClO}_4$  and separated from the unbound probe by centrifugation. The pellet was suspended in a solution containing 2 M urea, 15.1 mM DTT, and  $\text{NH}_4\text{OH}$  (pH 8–9) and incubated at 50°C for 30 min. Iodoacetamide (235  $\mu\text{M}$  final concentration) was added and the solution was incubated at 25°C for 30 min followed by dialysis against 100 mM  $\text{NH}_4\text{CO}_3$  for 4 h. The pH of the solution was adjusted to 8–9 and the protein was digested with 60  $\mu\text{g}$  of L-tosylamido-2-phenylethyl chloromethyl ketone-trypsin (Promega) for 18 h. The photomodified peptides were purified by  $\text{Al}^{3+}$ -chelate affinity chromatography (17). The photomodified peptides eluted from the  $\text{Al}^{3+}$ -chelate column were further purified by reversed-phase HPLC by using a C8 column (Brownee Lab) on a LKB system equipped with a diode array detector. The mobile system consisted of a 0.1% trifluoroacetic acid solution (buffer A) and 0.1% trifluoroacetic acid/70% acetonitrile solution (buffer B). The gradient for HPLC was 0–30 min, 0% buffer A; 30–90 min, 0–75% buffer B; 90–95 min, 75–100% buffer B; 95–96 min, 100% buffer B. Radioactivity associated with the HPLC fractions were determined by using a liquid scintillation counter.

**Peptide Sequencing and Alignment.** HPLC fractions containing photolabeled peptides were sequenced on an Applied Biosystem model 477A protein sequencer with an on-line phenylthio-hydantoin identifications; sequenced tryptic peptides isolated from photolabeled S1C5 and 8019 antibodies were aligned with the  $V_L$  and  $V_H$  sequences.

**Computer Modeling.** The crystal structure of IgG Jel103 Fab (Protein Data Bank code 1MRC) was used to model an Fv. The coordinates in the Protein Data Bank (18) had a break in between H73 and H75. Missing residues were modeled as alanines using the lego-loop options in program o (19) and regularized. The coordinates of adenosine-5'-diphosphate were constructed from that of guanosine-5'-diphosphate as observed in the crystal structures of GDP-Jel103 (Protein Data Bank code 1MRE). The obtained coordinates of ADP were minimized by using X-PLOR (20). The program CHAIN was used to place the molecule of ADP in the Fv (21). Pictures were made by using the program SETOR (22). The model was not refined, and hence has a number of close contacts between ADP and Fv atoms. Some small rearrangement of the Fv will have to occur in order to relieve the close contacts with the ADP molecule.

## RESULTS

**Photoaffinity Labeling of Igs.** A variety of different monoclonal antibodies, affinity-isolated polyclonal antibodies, and  $\text{F}(\text{ab})_2$  fragments from different species were photolabeled with [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP and analyzed by SDS/PAGE and autoradiography. In Fig. 1, the autoradiogram of photolabeled antibodies shows labeled light and heavy chains with variable degrees of incorporation of the photolabel. Minor labeled bands represent degraded antibody fragments. Photolabeling of the two of S1C5 and 8019 antibodies with increasing concentrations of [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP showed saturation effects, which indicated that limited, specific sites were being photomodified (Fig. 2 A and B). For antibody S1C5, saturation of photolabeling was observed at  $\approx 150$ – $175$   $\mu\text{M}$ , with an apparent  $K_d$  of 75  $\mu\text{M}$ . For antibody 8019, saturation was at about 50  $\mu\text{M}$  with an apparent  $K_d$  of 25  $\mu\text{M}$ , which showed that these antibodies had affinity for 8- $\text{N}_3$ ATP. Both light and heavy chains were photolabeled, which indicated that the binding site was formed by both chains. Photolabeling could be prevented by using ATP for both antibodies (Fig. 2 C and D).

To analyze the specificity of the site for nucleotides, protection experiments were conducted with a variety of nucleotides and nucleosides. Although ATP was able to prevent photolabeling (Fig. 2 C and D), other nucleotides (1 mM) afforded about 50–60% protection against photolabeling with 200  $\mu\text{M}$  [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP. In addition, 8-azidoadenosine proved to have tighter binding than 8- $\text{N}_3$ ATP (data not shown). Protection experiments using a variety of amino acids showed that tryptophan produced the best protection (Fig. 3). These experiments demonstrated that this site had affinity for naturally occurring heterocyclic ring structures and suggested that the site was not a typical ATP binding site.

**Antigen Binding of Photoaffinity Labeled Antibodies.** To determine whether the modification of this site affects antigen recognition, the binding of photolabeled antibodies S1C5 (IgG2a,  $\kappa$ ) and 8019 (IgG1,  $\kappa$ ) to their respective antigens was tested. Antibody S1C5 is specific for a murine B-cell tumor idiotype 38C13 (13) and antibody 8019 recognizes the carcinoembryonic antigen (14) expressed by the LS17T4 tumor cell line. The binding of the  $\gamma$ - $^{32}\text{P}$ -labeled antibodies to their cellular targets was inhibited on an apparent 1:1 ratio by unlabeled antibodies (data not shown), which indicated that the integrity of the antibody was not disrupted. Also, these same antibodies were photomodified with biotinylated 8- $\text{N}_3$ -adenosine and used in FACS analysis. Both biotinylated antibodies recognized their specific tumor cell targets as assayed by flow cytometry (Fig. 4), while biotinylated control antibodies showed no binding, which indicated that Fc receptors were not involved. A comparison of the photoaffinity

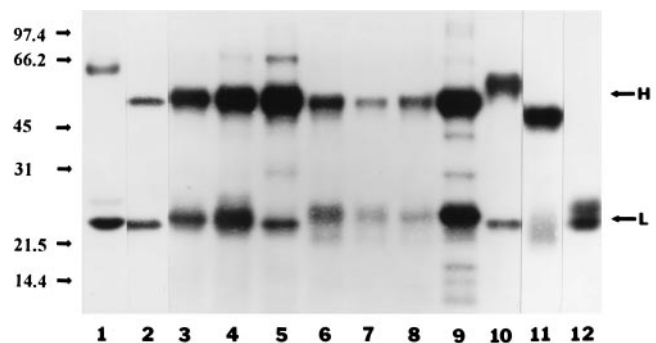


Fig. 1. Autoradiogram of various monoclonal and polyclonal antibodies photolabeled with [ $\gamma$ - $^{32}\text{P}$ ]8- $\text{N}_3$ ATP. Lanes: 1, human myeloma IgM,  $\lambda$ ; 2, S1C5, mouse IgG2a,  $\kappa$ ; 3, 8019, mouse IgG1,  $\kappa$ ; 4, 5D10, mouse IgG1,  $\kappa$ ; 5, OKT3, mouse IgG1,  $\kappa$ ; 6, GAHuIgM; 7, GAMIgM; 8, GAHukappa; 9, MOPC 21, mouse IgG1,  $\kappa$ ; 10, MOPC 315, IgA  $\kappa$ ; 11, rabbit IgG; 12, goat (Fab) $_2$  fragment.

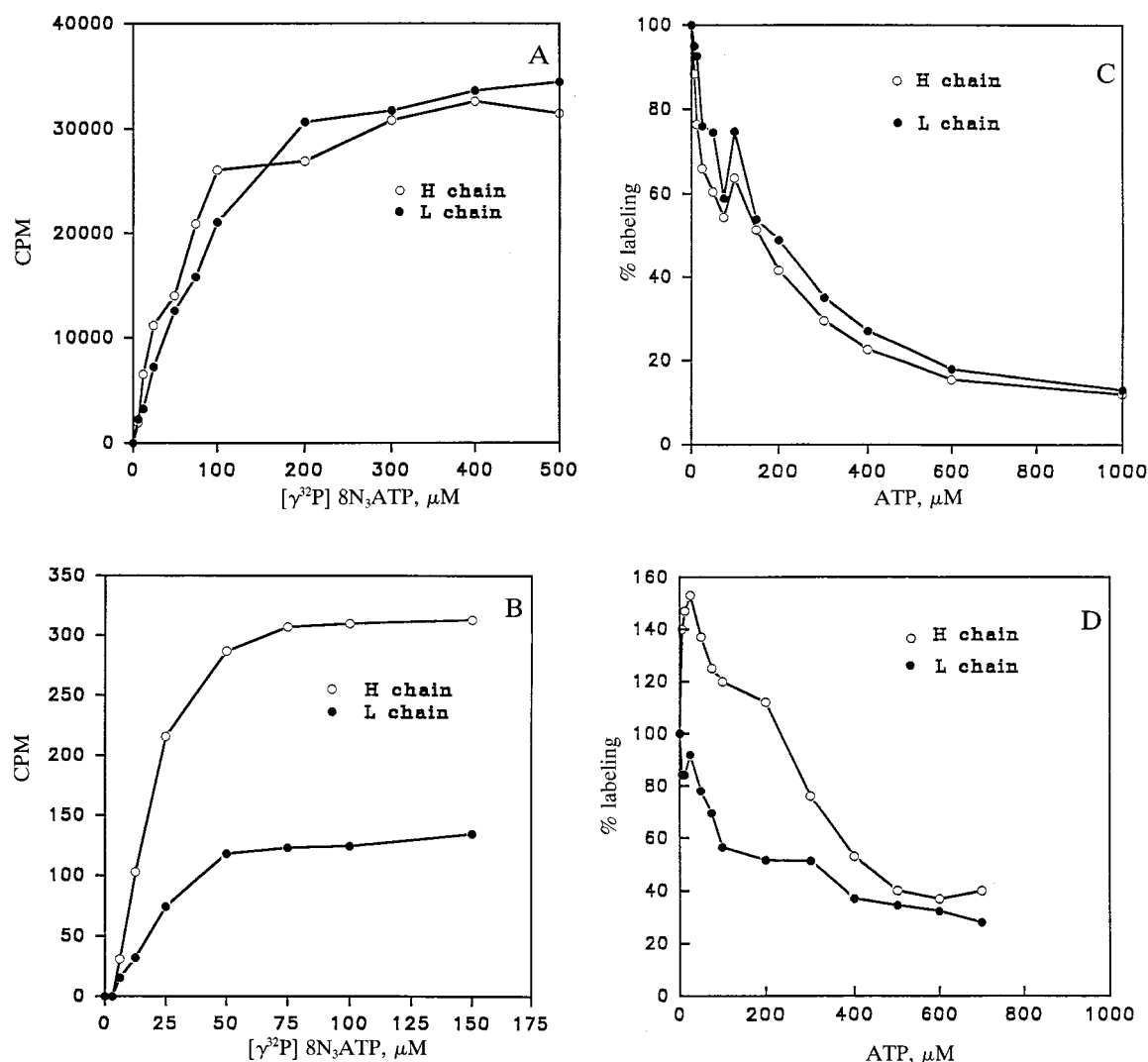


FIG. 2. Saturation and protection of photolabeling of antibodies S1C5 and 8019. (A) Saturation of photolabeling of antibody S1C5 with  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$ . (B) Saturation of photolabeling of antibody 8019 with  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$ . (C) Percent labeling of antibody S1C5 by using ATP as inhibitor. (D) Percent labeling of antibody 8019 by using ATP as inhibitor.

biotinylated antibodies with conventionally biotinylated antibodies in FACS analysis produced nearly identical staining (unpublished data). Furthermore, we have affinity-biotinylated human immune sera and performed ELISA and Western blot analyses with preparations stored for 3 months at  $4^\circ\text{C}$  without detectable loss of reactivity, which indicated that the biotin linkage was stable in serum.

**Sequence Alignment of Labeled Peptides.** To identify the peptide sequences involved in the formation of the site, antibodies S1C5 and 8019 were photolabeled with  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$  and digested with trypsin. The tryptic peptides from the photolabeled antibody were purified by a combination of  $\text{Al}^{3+}$ -chelate affinity chromatography and reversed phase HPLC (17). HPLC purification yielded two radioactive peaks with associated UV peaks at 214 nm (Fig. 5A and B). Fractions 64–65 for antibody S1C5 and fraction 71 for antibody 8019, which corresponded to the first peak, and fractions 70–71 for antibody S1C5 and 73 for antibody 8019, which corresponded to the second peak, were sequenced.

Amino acid sequence of antibodies S1C5 and 8019 were deduced from the cloned and sequenced V genes (Fig. 5C). The identified peptides from photoaffinity labeling corresponded to sequences located in the variable Ig domain. The peptides eluted first (T1) from each antibody are derived from the CDR1 domain of the variable light chain extending into

FR2. The second eluted peptides (T2) are from the CDR3–FR4 region of the variable heavy chain domain. It is striking that both peptides included invariant aromatic residues, Trp-H103 in the  $\text{V}_\text{H}$  domain and the highly conserved Tyr-L36 within the  $\text{V}_\text{L}$  domain. Triplicate experiments using quantitative immunoprecipitation (23) with  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$  labeled S1C5 showed an average number of  $1.9 \pm 0.2$  molecules of  $8\text{-N}_3\text{ATP/Ig}$  molecule, which is consistent with one  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$  incorporated into each Fab arm. The isolation of only two photoaffinity labeled peptides per Ig molecule together with the results of the saturation experiments suggested that insertion of photoprobe was directed into a single affinity site that is formed through the participation of both heavy and light chains within the variable domains of Igs.

**Computer Modeling of ATP Site.** The photoreactive site in tobacco Rubisco activase (24), which involved base-stacking interactions with tryptophans, had provided the concept for constructing a computer modeled insertion of nucleotide into a known Ig fragment structure. The isolated photolabeled peptides from both Ig chains contained the invariant Trp residue, which had the potential of base-stacking with purine rings.

The coordinates of Jel103 Fab were used to model the Fv (25). Fig. 6A shows the overall structure and the location of the nucleotide in relation to the antigen binding site. The phosphate groups are exposed to the solvent between the  $\text{V}_\text{L}$  and

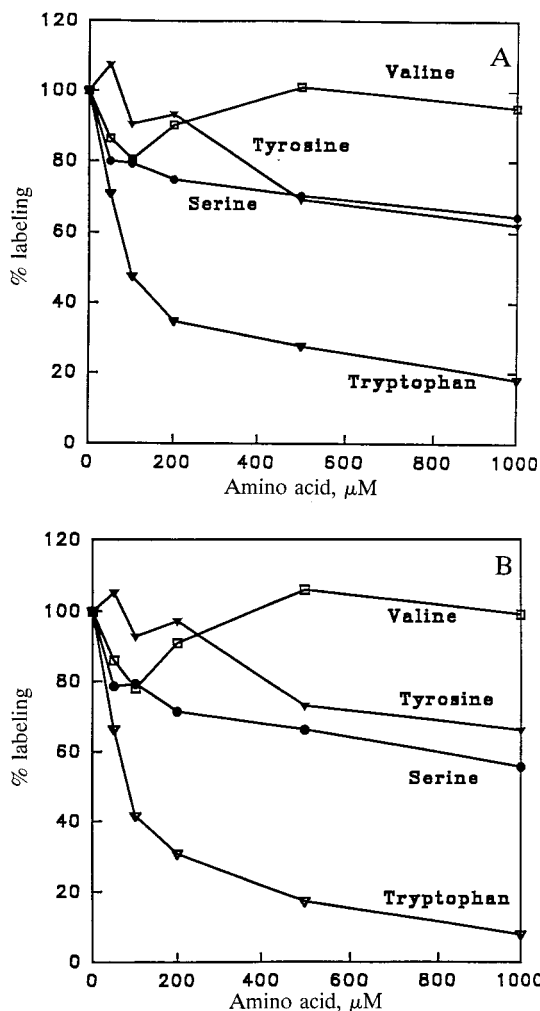


FIG. 3. Protection of photolabeling with  $[\gamma\text{-}^{32}\text{P}]\text{8-N}_3\text{ATP}$  by amino acids. (A) Percent labeling of antibody S1C5 in the presence of amino acids. (B) Percent labeling of antibody 8019 in the presence of amino acids.

$V_H$  domains and are visible in the model. In Fig. 6B, the predicted contacts of the purine ring with residues H101, H103, and L36 are indicated. The adenosine base is sandwiched between the rings of Trp-H103 and Pro-L44, providing non-specific stacking interactions. The stacking of the adenosine with the invariant Trp at H103 and the mostly invariant Pro-L44 allows sufficient flexibility to label residues from both chains. The azido group at C-8 of the purine ring is in proximity to the hydroxyl of Tyr-L36 so that it can form a covalent link upon photolysis. The azido group is also in a position to photoinject into residues of the CDR3 of heavy chains such as Asp-H101 in antibody S1C5 or Glu-H100 of antibody 8019. The model exemplifies how the  $8\text{-N}_3\text{ATP}$  probe binds into a hydrophobic pocket at the bottom of the antigen binding site. Because the actual chemical bonds of the reactive nitrene are formed with residues from the hypervariable regions, the CDR3 of the heavy chain, with the conserved Tyr-L36 and/or a residue from CDR1 of the light chain, each antibody is expected to be labeled at different residues within the site and with different efficiencies. Photolabeling studies using several antibodies did show differential labeling of the heavy and light chains (Fig. 1). Although we have identified the modified site from only two antibodies, it is very likely that most antibodies possess this site because it involves invariant or conserved residues and all antibodies tested, so far, could be photolabeled.

## DISCUSSION

In this report we show that antibodies can be modified with a photoreactive derivative of a biological molecule—i.e., ATP.

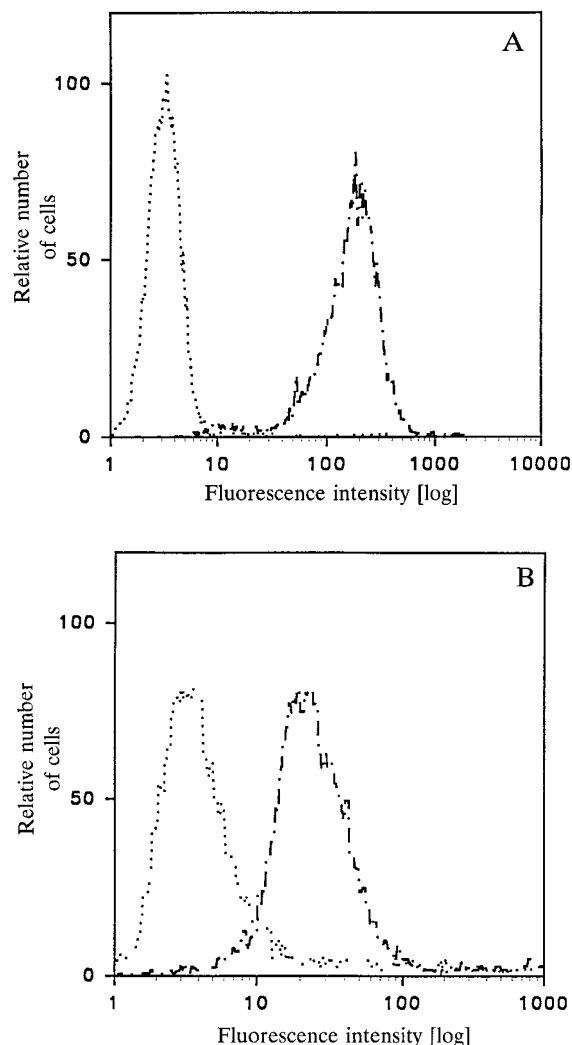
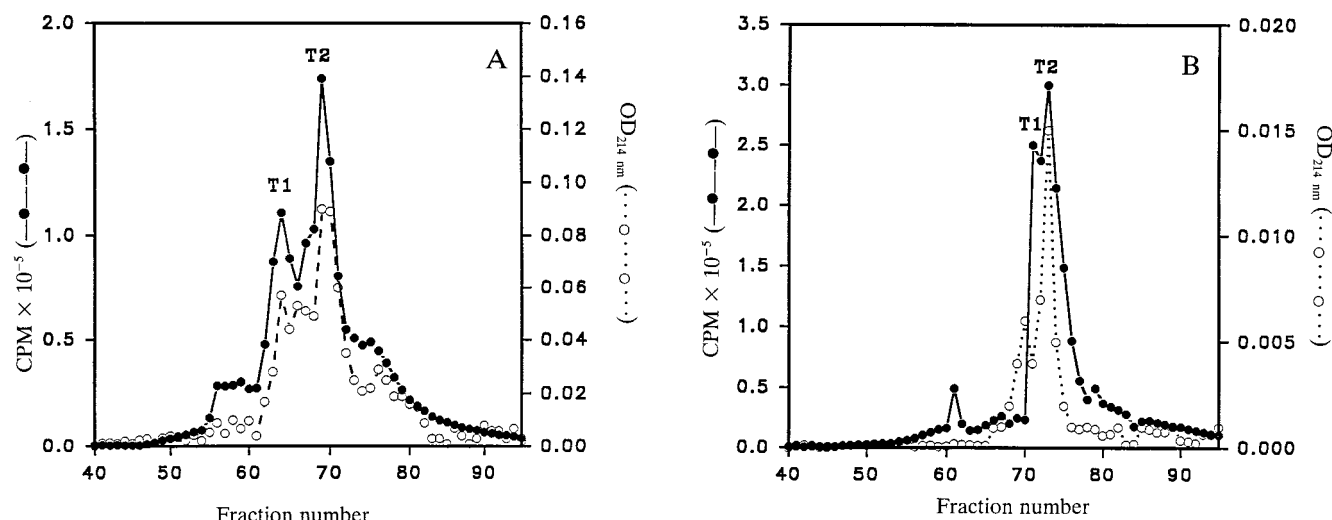


FIG. 4. FACS analysis of tumor cell lines using biotinylated antibodies. The first peak represents cells incubated with fluorescein isothiocyanate-streptavidin alone. (A) FACS analysis of 38C13 tumor cell line by using biotinylated antibody S1C5. (B) FACS analysis of LS174T tumor cell line by using biotinylated antibody 8019.

The labeling kinetics indicate the presence of unique affinity site for ATP with a  $K_d$  of 25–75  $\mu\text{M}$ . The site appears not to be a typical ATP binding site since other nucleotides and some aromatic amino acids are potent inhibitors of the photoinsertion. The presence of the affinity site was confirmed by the isolation of labeled peptides from a digestion of two complete antibodies. Interestingly, these peptides are derived from the Fv domains of heavy and light chains and from the same regions of both antibodies, thereby representing homologous peptides. The peptides from the light chain extend from FR1 over CDR1 to FR2, and the peptides from the heavy chain extend from FR3 over CDR3 into FR4. Because of the variability in this region, the presence of trypsin cleavage sites differs and therefore the length of the peptides is also different. However, the peptides include conserved framework structures, the tryptophan in position 103 of the heavy chain and a less conserved tyrosine in position 36 of the light chain.

Computer modeling of the ATP insertion into a Fv structure shows the close contact to these conserved residues with the probe, in particular a base stacking of the tryptophan with the purine ring of ATP. This model demonstrates several important features that helped us understand the interaction of the antibody site with the affinity probe. (i) The site is distant from



C

**T1**

S1C5 VK:	QIVLTQSPAI	NSASLGERVT	<u>MTCTASSSVS</u>	<u>SSYFHWYQK</u>	QGSSPKLWIY <sup>50</sup>		
8019 VK:	DIVLTQSPAS	LSASVGETVT	<u>ITCRASENIY</u>	<u>SYLLWYQK</u>	QGRKSPQLLVY		
S1C5 VK:	TTSNLAGSVP <sup>60</sup>	ARFSGSGSGT	SYSLTISSE	AEDAATYYCH	<u>OYHRSPLTFG</u>	AGTKLELKRA <sup>110</sup>	
8019 VK:	NAKTLAEGVP	SRFSGSGSGT	QFSLKINSLQ	PEDFGSYFCQ	<u>HHEGTPWTFG</u>	GGTSLLEIKRA	
S1C5 VH:	QVQLQQSDAI	LVKPGASVKI	SCKASGYTFT	<u>DHVLHWKQR</u>	PEQLGEWIGF	ISPGNGDIRY <sup>60</sup>	
8019 VH:	EVNLEESGGG	LVQPQGMKML	SCAASGFTES	<u>DAWMDWRQS</u>	PERGLEWVAE	IRTKVNNHAT	
S1C5 VH:	<u>NEKF</u>	<u>KDK</u> <sup>70</sup>	ATADKSSSTA	YMOLNSLTSE	<u>DSAVYFCKRS</u>	<u>FYYDDNYGD</u>	<u>YWGQGTTLTV</u> <sup>120</sup> SAAK
8019 VH:	<u>YYAESVKG</u> RFT	ISRDDSKSNV	YLQMSLRVE	<u>DTGIYYCTM</u>	<u>AYYE</u>	<u>A</u>	<u>YWGQGLTVT</u> SAAK

**T2**

FIG. 5. Separation and sequence alignment of radioactive tryptic peptides. (A) Isolation of labeled tryptic peptides on HPLC from antibody S1C5. (B) Isolation of labeled tryptic peptides on HPLC from antibody 8019. (C) Sequence alignment of photolabeled tryptic peptides into the deduced sequence of the V<sub>H</sub> and V<sub>L</sub> regions of antibodies S1C5 and 8019. T1 and T2 (boxed) are the isolated photolabeled tryptic peptides from antibodies S1C5 and 8019; CDRs are underlined.

the CDR loops that make contact with antigen; this explains why photolabeled antibodies still bind antigen. (ii) The purine ring penetrates the deepest into the site, while the ribose and the triphosphate are extruding from the molecule and are easily accessible from the molecule surface; this is in full agreement with the ability of the inserted adenosine or ATP probes to carry molecular substitutions with full accessibility, such as the biotin moiety, which can bind avidin. (iii) The penetrating purine structure engages in close contacts with conserved heterocyclic amino acid side chains, thereby predicting that most, if not all, antibodies carry this site.

While ATP is a molecule of great biological importance, it is not known whether nucleotides play a role in antibody functions. Possible functions involving energy-driven mechanisms are the surface movements of the B-cell receptor after crosslinking, the signal transduction of the B-cell receptor, or the folding of Ig chains and release from Ig binding protein during synthesis (26). Recently, we discovered an effect of photoaffinity modification on the catalytic activity of light chain dimers (27). Preliminary studies on photomodified catalytic light chain dimers (unpublished data) showed altered enzyme activity. This indicates that the catalytic site may be influenced by ligands for the novel site described here. Furthermore, the binding to the site by a ligand may have long-range

effects over the entire Ig molecule, modifying Fc-mediated functions like complement fixation and antibody-dependent cellular cytotoxicity (ADCC). These and other Ig effector mechanisms should be examined with respect to changes in activity by ligand binding to the nucleotide affinity site.

Antibodies have been considered as ideal vehicles to deliver biologically active and medically relevant molecules to selected targets such as tumor cells. However, their practical usefulness in clinical settings have been limited because of (i) incomplete tumor penetration, (ii) immune response against xenogeneic Igs, and (iii) biochemical and structural alteration caused by chemical coupling. Recently, significant progress has been made to generate recombinant smaller antibody fragments with improved tissue penetration and favorable pharmacological behavior; also, humanized or *de novo* human antibodies have been produced that do not induce xenograft immune responses in patients. Finally, gene fusion techniques and expression systems have allowed the production of recombinant fusion proteins with improved pharmacokinetics and biodistribution. Despite these advances in producing antibody or fragment conjugates for *in vivo* use, a simple general chemical conjugation method that does not affect the structural and biological integrity of the Ig molecule would be useful and would have practical advantages over more involved molecular engineering techniques.

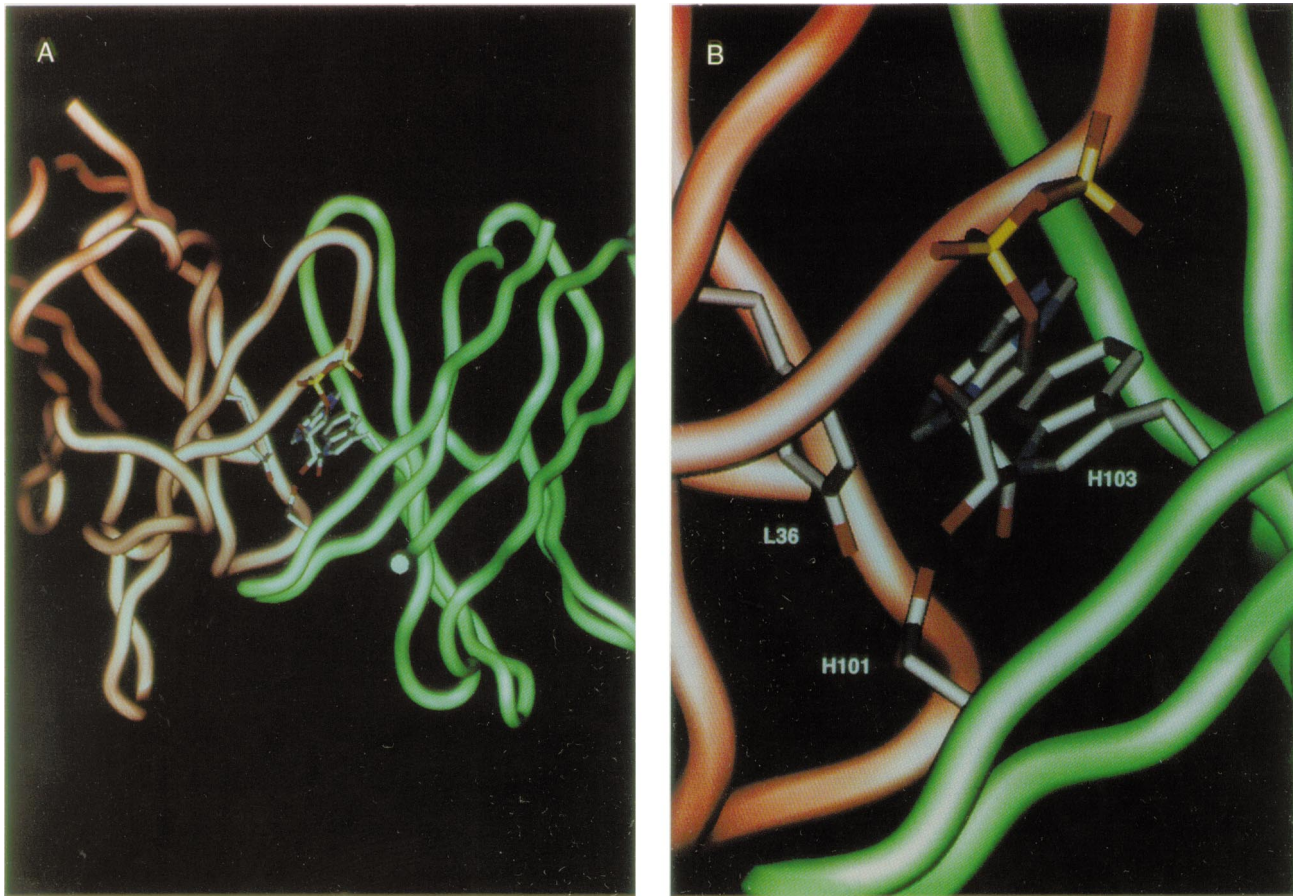


FIG. 6. Computer generated model of Fv with inserted ADP based on coordinates from ref. 17. (A) Fold of the backbone of the Fv model with site of nucleotide insertion. The light chain is in red, and the heavy chain in green. The antigen binding site with CDR loops is facing the top. (B) Close-up view showing the inserted ADP and the close contacts with side chains of Trp-H103, Tyr-L36, and Asp-H101.

In summary, a novel conserved site has been described in the Fv domains of the Ig molecule with affinity for ATP. This site has been used to attach reporter molecules to antibodies without impairing antigen binding, demonstrating the general utility for using affinity photolinker chemistry to attach drugs, metal chelates, antisense oligonucleotides, or biologically active peptides to selected antibodies for target-specific delivery.

K.R. and G.P. contributed equally in this work. We thank Dr. M. Chatterjee for providing antibody 8019 and the LS17T4 cell line, Dr. Ashok J. Chavan for providing biotinylated 8-azidoadenosine, and George Johnson for help with the computer modeling. The work was supported by grants from the Council of Tobacco Research, the National Institutes of Health, and the U.S. Department of Energy, Office of Health and Environmental Research, under Contract W-31-109-ENG-38.

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