The class II histocompatibility molecule I-A\(^k\) was photoaffinity labeled by NH\(_2\)- and COOH-terminal photoactive conjugates of an immunogenic hen egg white lysozyme (HEL) peptide. The labeled \(\alpha\) and \(\beta\) chains were digested with protease V-8 (protease V-8) and/or trypsin, and the proteolytic fragments were separated by high performance liquid chromatography (HPLC) (peptide mapping). Reproducible peptide maps containing a major labeled component were obtained from the three conjugates reported here whose photoactive group was attached via short spacers of limited flexibility. The COOH-terminal conjugate N-acetyl HEL-(49-61)-iodo-4-azidosalicyloyl thioester (compound 1) labeled hydrophilic tryptic fragments on both chains of I-A\(^k\). The labeled digest fragments were homogeneous in reverse-phase and anion-exchange HPLC, indicating that the photoaffinity labeling was site-specific. Conversely, the NH\(_2\)-terminal conjugate iodo-4-azidosalicyloyl HEL-(46-61) (compound 2: IASA-(46-61)) labeled exceptionally hydrophobic sequences on both chains of I-A\(^k\). The labeling was also site-specific because reverse-phase HPLC of primary digests with protease V-8 and secondary digests with trypsin showed single major labeled components. The labeling of I-A\(^k\) by IASA-(46-61) was fully inhibitable by HEL-(46-61). In contrast, IASA attached to the smallest immunogenic peptide 52-61 (compound 3) labeled a distinctly different hydrophilic tryptic fragment.

The site of the I-A\(^k\) molecule that was photoaffinity labeled by IASA-(46-61) (compound 2) was determined. IASA-(46-61) labeled selectively at Pro-118 of a primary \(\alpha\) chain fragment most likely encompassing residues 115-134. It labeled Thr-121 of a primary \(\beta\) chain fragment most likely encompassing residues 109-138. We also obtained evidence that IASA-(46-61) occupied the antigen-specific site; the conjugate stimulated a T-cell hybridoma that recognizes the sequence 52-61 and also competed for the binding of this smaller peptide to I-A\(^k\). Thus, peptides that bind to the allele-specific binding site and are long enough to extend beyond it can interact with a hydrophilic area of class II molecules. This area is formed by sequences of the first halves of both \(\alpha\) and \(\beta\) chains.
MATERIALS AND METHODS

Syntheses of Photoactive Peptide Conjugates—The peptides (HEL)-46-61, (HEL)-49-61-Cys(SH) and (HEL)-Ac-49-61-Cys(SH) were synthesized on a Applied Biosystem peptide synthesizer following standard procedures. The deprotected peptides were purified by reverse-phase HPLC using a C-18 Vydac column and analyzed by amino acid composition. They were used to prepare the photoactive conjugates depicted in Fig. 1.

General Materials—Unless otherwise stated, all reagents and solvents were from Sigma (St. Louis, MO), unless otherwise stated. Acryloylmethyl ketone-trypsin (Worthington) at 12-h intervals. Digestion with protease V-8 was performed over 2 days by adding 4 aliquots (20 µg) of protease V-8 to the sample. The reaction mixture was dissolved in 800 µl of water and subjected to HPLC. The main product eluted from the HPLC solvent system or in PBS. All further purification procedures were performed under dimmed light.

Compound 1—(HEL)-Ac-49-61-Cys[4-azidosalicyloyl] Thioester—Compound 1 was obtained by acylation of Ac-49-61-Cys(SH) with 2-mercaptoethanol, and 5 µCi of either [35S]cysteine (Du Pont-New England Nuclear) or [3H]cysteine having average specific activity of 200 Ci/mmol (125) and 1000 (35S) Ci/mmol from Du Pont New England Nuclear. The conjugates and their intermediates were purified by HPLC using a Nova pak C-18 reverse phase column (ultrasphere ODS-IP, 5 µm, 4.6 X 250 mm). The column was eluted by the following linear gradients of acetonitrile in 0.1% trifluoroacetic acid in water; the second component. Both materials were indistinguishable in photoaffinity labeling.

As suggested, the main product probably is the 3-iodo-4-azidosalicyloyl derivative and the minor product the 5-iodo-4-azidosalicyloyl isomer (24). We also prepared the homologous (IASA)-52-61, using peptide 52-61 of HEL. The main eluted from the HPLC column at 29 min with a minor product at 30 min.

Compound 3: Iodo-4-azidosalicyloyl (HEL)-52-61—It was prepared as compound 2 but using the shorter peptide (HEL)-52-61.

Purification and Labeling—The purified I-A<sup>4</sup> A<sup>4</sup>, I-A<sup>E</sup>, and I-E<sup>P</sup> plus I-E<sup>P</sup> were purified from TAT cell membranes by affinity chromatography using Sepharose coupled with 10.36.2.6 (anti-I-A<sup>4</sup>), MKD6 (anti-I-A<sup>E</sup>), and 14.4.4 (anti-I-E<sup>P</sup> and I-E<sup>F</sup>) monoclonal antibodies, respectively. The photoactive conjugates (6 X 10<sup>-12</sup> mol) were exposed to purified class II molecules (20 µg) in PBS (60 µl) containing 20 mM MEGA 8 and 20 mM MEGA 9 detergents, in the absence or presence of the indicated molar-fold excesses of (HEL)-46-61, at room temperature for 2 days. The samples were irradiated with UV light (s-watt low pressure mercury lamp with parabolic reflector for 7 min at 4°C) in Pyrex glass tubes (which strongly absorb light below 270 nm) and then subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5%) under reducing conditions. In some experiments I-A<sup>4</sup> on cell membranes was photoaffinity labeled as described previously (12). The dried gels were exposed with an amplifying screen to Kodak XAR-5 x-ray film at -80°C. The labeled bands were cut out from the gels, rehydrated in 250 mM Tris buffer, pH 8.0, containing 8.3% glycerol and 0.5% SDS for 3 h. The labeled and unlabeled chains were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis using a disc electrophoresis apparatus with a constant current of 3 mA/disk overnight at 4°C. The anodic end of the disc was plugged with glass wool and connected to a closed dialysis membrane tubing (25-kDa cut-off). The eluted proteins were concentrated in a Centricron concentrator (10 kDa cut off, Amicon) to approximately 100 µl. Two ml of water were added, and the samples were concentrated. This procedure was repeated three times.

Labeling of I-A<sup>4</sup> with Amino Acids—CH-27 B-lymphoma cells (1 X 10<sup>9</sup>), in logarithmic growth phase, were washed three times and resuspended in 2 ml of RPMI 1640 medium deficient in the amino acid used for labeling, and complemented with either fetal calf serum or 10% dialyzed fetal calf serum. Cells were irradiated at 37°C in a humid chamber (100 mCi sodium iodide, 1 mCi [35S]cysteine, 35 Ci/mmol) and 10 µCi each of [3H]tryptophan or proline (Amersham Corp., 40-70 Ci/mmol). Cells were incubated at 37°C in a humidified atmosphere containing 4% CO<sub>2</sub> for 6 h, washed three times, and washed again at 0°C to 4°C in PBS containing 0.7% Triton X-100, and 20 µg iodoacetamide, 1 mM phenylmethylsulfonyl fluoride, and 10 µg/ml leupeptin. After 30 min incubation, the detergent-insoluble components were removed by centrifugation (100,000 x g, 1 h), and the supernatant was incubated with 1 to 2 ml of Sepharose 4B under agitation at 4°C for approximately 6 h. The Sepharose was washed by centrifugation, resuspended in medium (1 ml), and then loaded through 0.2-µm disposable membrane filters (Millipore, Watertown, MA). All further purification procedures of I-A<sup>4</sup> were as described using antibody-affinity chromatography. Approximately 1.2 X 10<sup>14</sup> dpm [35S]cysteine, 8 X 10<sup>13</sup> dpm [3H]tryptophan, and 1.5 X 10<sup>13</sup> dpm [3H]proline were incorporated in the purified I-A<sup>4</sup>, which was concentrated to a final volume of 60-80 µl. The biosynthetically labeled I-A<sup>4</sup> was incubated with 700 µCi of [35S] (IASA)-46-61 and 1 X 10<sup>-14</sup> mol of nonradioactive (IASA)-46-61 (approximately 300-fold molar excess) in 20 µl of PBS containing the same detergents.

Digestions of the Labeled Chains and Peptide Mapping—Aliquots (10-20 µl) of the above solutions were added to 180 µl of the following buffers: 10 mM Tris (100 µCi of [3H]proline); 10 µl of 100 mM Tris buffer, pH 7.8, for protease V-8; 100 mM ammonium carbonate, pH 8.65, for protease Lys-C; 100 mM phosphate buffer, pH 7.8, in 150 mM saline for clostripain and 100 mM phosphate buffer, pH 7.25, for mouse submaxillary endoprotease Arg-C. Digestions were performed at 37°C in the presence of 100 µg of human γ-globulin. Complete digestion with trypsin was achieved by adding 3 aliquots of 20 µg of L-1-tyosylamido-2-phenylamido chloromethyl ketone-trypsin (Worthington) at 12-h intervals. Digestion with protease V-8 was performed overnight by adding 4 aliquots (20 µg) of enzyme (Boehringer Mannheim) in 12-h intervals. Digestion of I-A<sup>4</sup> was performed for 37°C in the presence of 100 µg of human γ-globulin. Complete digestion with trypsin was achieved by adding 3 aliquots of 20 µg of L-1-tyosylamido-2-phenylamido chloromethyl ketone-trypsin (Worthington) at 12-h intervals. Digestion of I-A<sup>4</sup> was performed for 37°C in the presence of 100 µg of human γ-globulin. Complete digestion with trypsin was achieved by adding 3 aliquots of 20 µg of L-1-tyosylamido-2-phenylamido chloromethyl ketone-trypsin (Worthington) at 12-h intervals. Digestion of I-A<sup>4</sup> was performed for 37°C in the presence of 100 µg of human γ-globulin.
Peptide Binding by Histocompatibility Molecules

RESULTS

Peptide Mapping—We labeled the I-A<sup>α</sup> molecule with different photoreactive conjugates of (HEL)-46-61 and (HEL)-49-61-Cys and analyzed the labeled peptides after digestion of the separated α and β chains with trypsin or protease V-8. In this paper we will examine our initial results with the three compounds shown in Fig. 1 and in particular with compound 2 ((IASA)-46-61). The photoreactive IASA group was directly conjugated at the COOH-terminal cysteine of Ac-49-61-Cys (compound 1) or at the NH<sub>2</sub> terminus of either (HEL)-46-61 (compound 2) or (HEL)-52-61 (compound 3). With these three compounds, the tryptic peptide maps of the labeled α and β chain revealed resolved single major labeled components (Figs. 1 and 2). The fragment from the β chain labeled by compound 2 eluted considerably later (46 min) from the reverse-phase column than the one labeled by compound 1 (21 min) (Fig. 1), indicating that it was more hydrophobic. Indeed, peptide maps of unlabeled I-A<sup>α</sup> showed very few fragments eluting in the last third of tryptic peptide maps (see Refs. 19, 20). Furthermore, we failed to obtain a tryptic peptide map of the α chain labeled by compound 2, suggesting that an even more hydrophobic digest fragment was labeled which was no longer amenable to reverse-phase chromatography. In contrast to compound 2, a similar conjugate, but shorter, of the minimal immunogenic peptide from residues 52-61 (11) labeled a different, more hydrophilic, tryptic peptide fragment (Fig. 1C, dotted line).

The tryptic peptide map of the α chain labeled by compound 1 showed a major labeled component eluting at 22.0 min (Fig. 2A). To assess the homogeneity of this fragment, it was rechromatographed on anion-exchange (DEAE) HPLC. Again a single major labeled fraction was observed at 21 min (Fig. 2B). Likewise, rechromatography on DEAE HPLC of the major labeled β chain fragment (Fig. 2B) revealed a single major component eluting at 27 min (Fig. 2C). This later elution indicated that the labeled β chain fragment was more acidic than the labeled α chain fragment. The observation that the major labeled fragments of both chains were homo-

FIG. 1. Structures of the compounds 1, 2, and 3 (A). Reverse-phase HPLC (C-4) of the tryptic digests of I-A<sup>α</sup> β labeled by compound 1 (B) and compound 2 (C). The digest fragment labeled by the COOH-terminal conjugate eluted 24.7 min earlier than the one labeled by the NH<sub>2</sub> -terminal conjugate of (HEL)-46-61. The peptide fragment labeled by compound 3 ((IASA)-52-61) eluted at 30 min (C, dotted line).

FIG. 2. Reverse-phase HPLC and DEAE HPLC of α and β chains labeled by compound 1. The tryptic digest of the I-A<sup>α</sup> α chain labeled by compound 1 and analyzed by reverse-phase HPLC (C-4) exhibited one major labeled component eluting at 22 min (A). This labeled species rechromatographed on DEAE HPLC eluted again as a major labeled component at 21 min (B). The labeled tryptic digest fragment of I-A<sup>α</sup> β chain that eluted at 21 min (shown in Fig. 1B) was likewise rechromatographed and shown to elute as a major labeled component at 27 min (C).
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FIG. 3. Reverse-phase HPLC of the α and β chains labeled by compound 2. The protease V-8 digest of the I-Aα chain labeled by compound 2 and analyzed by reverse-phase HPLC (C-4) exhibited one major labeled component eluting at 48 min (A). The same experiment for the I-Aβ chain showed one major component eluting at 41 min (B). These major components were digested with trypsin and the peptides chromatographed by reverse-phase HPLC. In the case of the α chain a derivative secondary labeled peptide product eluted at 52 min (C) and in the case of the β chain a derivative secondary peptide eluted at 47 min (D).

We assessed whether compound 2 labeled the membrane spanning domains of the I-Aα molecule. I-Aα was photoaffinity labeled on cells, and the separated chains were subjected to primary protease V-8 and secondary tryptic digestion. In contrast to purified I-Aα in detergent, in which both chains were labeled, I-Aα on cells was selectively labeled on the α chain (12). The peptide map of the I-Aα α chain labeled on the membrane was identical to that of I-Aα labeled in detergent (not shown). This result suggests that the I-Aα α chains on the membrane and in detergent were labeled at the same site, therefore excluding the transmembrane domain as the labeled site.

Identification of the Site Labeled by Compound 2 ([125I]IASA)-46-61 (compound 2) labeled protease V-8 digest products of the I-Aα α and β chains. A shows the proteolytic digestions of the α chain; top, primary protease V-8 product; bottom, secondary proteolytic digests with Lys-C, trypsin, or Arg-C (left to right). The arrow in the chromatograms of these secondary digests denotes the elution position of the primary protease V-8 product (fraction 48). B shows the proteolytic digests of the β chain; top, primary protease V-8 product; bottom, secondary proteolytic digests with Lys-C, trypsin, or Arg-C (left to right). The arrow in these secondary digests denotes the elution position of the primary protease V-8 product (fraction 41). For secondary tryptic digestion of both the α and β chains, a short digestion time (12 h) was used to produce incomplete proteolytic. All peptide maps were analyzed under identical conditions by reverse-phase (C-4) HPLC.

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acid residues (Fig. 4B). Secondary peptide maps with protease 
Lys-C and Arg-C showed two new labeled peptides, suggesting 
the presence of 2 lysines and arginines in the primary digest 
fragment (Fig. 4B). The relative amount of the secondary 
digest products in these peptide maps varied in different 
experiments. The earlier eluting fragment in the protease 
Arg-C peptide map (44 min) was the major peptide when clostripain rather than protease Arg-C from mouse submaxi-
illary gland was used.

We next examined whether the labeled protease V-8 digest 
fragments contained cysteine or tryptophan, infrequent 
residues 115-127 for the \( \alpha \) chain and 109-127 for the \( \beta \) chain.) 

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FIG. 6. Reverse-phase (C-4) HPLC of \([^{125}I]((\text{ASA})-46-61-
\text{labelled [}^{3}H\text{]}\text{Trp-I-A}^\alpha \text{ and \( \beta \) chains. I-A}^\alpha \text{ was intrinsically labeled with [}^{3}H\text{]}\text{tryptophan and photoaffinity labeled with [}^{125}I]\text{((ASA)}-46-
61. The isolated chains were subjected to protease V-8 peptide maps. 
The fractions of the \( \alpha \) chain map (A and B) and \( \beta \) chain map (C and 
D) were counted for [\(^{125}\)I] (A and C) and [\(^{3}\)H] (B and D). Peptide maps 
were performed on C-4 reverse-phase HPLC.

FIG. 7. Secondary tryptic peptide maps of the primary pro-

tese V-8-derived peptides of \((^{125}\text{I})((\text{ASA})-46-61\text{-labelled [}^{3}H\text{]} \text{Trp-I-A}^\alpha \text{ and \( \beta \) chains. Fractions 48 (Fig. 4A) of the primary 
protease V-8 digest of the \( \alpha \) chain was digested with trypsin and 
analyzed by reverse-phase (C-4) HPLC. A shows the [\(^{125}\)I] and D the 
[\(^{3}\)H] elution patterns. Fraction 41 (Fig. 4B) of the primary protease V-
8 digest of the \( \beta \) chain was digested with trypsin and the digest 
analyzed by reverse-phase (C-4) HPLC. C shows the [\(^{125}\)I] and D the 
[\(^{3}\)H] elution patterns. Note that in D the [\(^{3}\)H] radioactivity did not co-
elute with [\(^{125}\)I] radioactivity.
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FIG. 8. NH₂-terminal sequence analysis of [3H]Pro-I-Ak α and β chain fragments labeled with [125I]IASA-46-61. The [125I]-labeled α and β chains were obtained from a primary protease V-8 digestion after chromatography (as in Fig. 4, A and B). A and B show the results for the α chain. A indicates the [125I] cycle profile showing the label at position 4 of the peptide. B indicates the [3H] cycle showing proline at positions 4 and 5 of the peptide. C and D show the results for the β chain. C indicates the [125I] cycle profile showing the label at position 13 of the peptide. D indicates the [3H] cycle profile showing proline at position 17 of the peptide.

FIG. 9. Amino acid sequence of the β (A) and γ chain (B). The acidic amino acids residues D and E (protease V-8 cleavage sites) are circled, and the basic residues K and R (tryptic cleavage sites) are marked by boxes. The hydrophobic amino acid residues (A, F, I, L, M, V, W, and Y) are marked by a dot above the letter. The first two lines are the sequences of the first domains (α1, β1) and the following two lines of the second domain (α2, β2). The bottom lines are the sequences of the transmembrane and cytoplasmic domain (α TM-CY, β TM-CY). The rare cysteine (C) and tryptophan (W) residues are underlined by a solid line or a broken line.

DISCUSSION

We are in the process of determining the contact points of the HEL peptides 46-61 and 49-61 for the I-A^k MHC molecule by photoaffinity labeling. The identification of photoaffinity labeled sites requires that the photoaffinity labeling be site-specific and that the labeled proteins can be fragmented in a defined and reproducible manner, preserving the photocross-linked bond. The findings of this first study indicate that photoaffinity labeled sites on the I-A^k molecule can, indeed, be determined. Site-specific labeling that leads to resolved peptide maps, however, requires that the photoconjugate be conjugated to the peptide via short spacers of limited flexibility. Our other studies using photoconjugates containing cysteines resulted in unresolved peptide maps perhaps as a result of a longer and more flexible molecule. In this study we have also been able to identify the site of labeling of the α and β chains by an NH₂-terminal conjugate of HEL 46-61 (compound 2 in the text).

The photoconjugate IASA group conjugated directly at the NH₂-terminal amino group of HEL 46-61 (compound 2), and the COOH-terminal conjugate, compound 1, yielded resolved, labeled peptide maps (Figs. 1-3). However, compound 2 labeled exceptionally hydrophobic protease V-8 as well as tryptic fragments. The hydrophobicity of the labeled digest fragments was not accounted for by the photolabeling. (HEL)-46-61 itself is a tryptic digest fragment that contains two protease V-8 cleavage sites (Fig. 1); the protease V-8 digest fragment will therefore be labeled by a derivative of 4-amino-[125I]iodosalicyloyl Asn-Thr-Asp. This relatively hy-

3 I. F. Luescher, D. L. Crimmins, B. D. Schwartz, and E. R. Unanue, unpublished data.
tions of p& tide or conjugates were added to a constant number of
leukin-2-dependent CTLL cells. The
fixed CH-27 cells and 3A9 cells. The supernatants were assayed after
residue (Fig. 4A). In the case of the p chain, the same exper-
4). In the case of the cy chain, digestion of the V-8 fragment
fragments, indicating the presence of an arginine and a lysine
elution of the labeled fragments.
fig. dimple) was expected to give only a small delay in the
elution of the labeled fragments.
Reverse-phase peptide maps of I-A\(^k\) labeled by (IASA)-46-
61 indicated that the labeled primary protease V-8 digest
fragments of both chains contained tryptic cleavage sites (Fig. 4).
In the case of the \(\alpha\) chain, digestion of the V-8 fragment
with endoprotease Arg-C and Lys-C showed new labeled
fragments, indicating the presence of an arginine and a lysine residue (Fig. 4A).
In the case of the \(\beta\) chain, the same experi-
ment showed two new digest fragments in both peptide maps.
Because the \(^1\)H label was found at only one position during
sequencing, these results suggest the presence of 2 lysine and
2 arginine residues in the primary protease V-8 digest frag-
ment (Fig. 4B). Assuming that protease V-8 cleaved at all
acidic residues, the following generated peptides are compat-
ible with these findings: I-A\(^k\) α:76-89 and -115-134 and I-A\(^k\)
β:123-138 and -195-238 (Fig. 9).
In the case of the \(\alpha\) chain, the former peptide contains three and
the latter eight hydropho-
bic amino acids. In view of the late elution of the labeled
protease V-8 peptide (Fig. 4A), the latter sequence seems to be
more likely to contain the labeled site(s). This hypothesis
was further supported by the failure to obtain a primary tryptic
peptide map of the labeled \(\alpha\) chain. The corresponding
peptide that encompasses residues 99-127 contains 15
hydrophobic amino acids and may indeed not be amenable to
reverse-phase HPLC (Fig. 9).  
Peptide maps on \(^{35}S\) cysteine intrinsically labeled I-A\(^k\)
showed that only the labeled peptide derived from the \(\beta\) chain
contained cysteine (Fig. 5). In contradiction to these find-
ings, however, the predicted labeled \(\beta\) chain peptide, including
residues 123-138, did not contain cysteine (Fig. 9). It is,
however, conceivable that protease V-8 failed to cleave at
Asp-122, resulting in the primary labeled protease V-8 peptide
being 109-138. Protease V-8 has been reported to cleave
sluggishly when aspartic acid residues are followed by bulky
hydrophobic residues, such as is found in the present sequence
(Asp-Phe-Tyr) (26). Furthermore, a primary V-8 peptide
spanning residues 109-138 better accounts for the secondary
tryptic peptide maps. The secondary digest \(\beta\) chain peptide
derived from this larger peptide spans residues 109-127 and
contains eight hydrophobic amino acids. It, rather than the
alternative hydrophobic tryptic peptide spanning residues 126-127,
would be expected to elute at 47 min (Figs. 4B and 7C).
Primary protease V-8 peptide maps of I-A\(^k\) labeled intrin-
sically with tryptophan showed that the labeled digest prod-
ucts of both chains contained tryptophan (Fig. 6). This finding
is compatible with the primary labeled protease V-8 fragments
being I-A\(^k\) \(\alpha\):115-134 and I-A\(^k\) \(\beta\):109-138. More importantly,
the secondary tryptic digest product of the \(\alpha\) chain, but not
the \(\beta\) chain, contained tryptophan (Fig. 7). Tryptophan in the
predicted primary \(\beta\) chain fragment is located between tryptic
cleavage sites and is therefore expected to be absent in the
secondary peptide. Conversely, in the case of the \(\alpha\) chain,
tryptophan is located in the hydrophobic sequence NH-
terminal of the tryptic cleavage sites and is therefore expected
to be present in the secondary peptide. In fact, there are no
other protease V-8 peptides that contain tryptophan and
tryptic cleavage sites in a manner compatible with these
peptide maps.
The predicted labeled protease V-8 \(\alpha\) chain peptide 110-
134 contains proline at positions 4 and 5 (Pro-118 and -119)
and the \(\beta\) chain fragment 109-138 at position 17 (Pro-125).
Sequencing of V-8 protease peptides prepared from \(^3H\)pro-
line intrinsically labeled I-A\(^k\) molecules localized proline at
the predicted sites (Fig. 8). These sequencing experiments
further showed \(^125I\) at the 4th residue of the \(\alpha\) chain and at the
13rd residue of the \(\beta\) chain, indicating that the photo-
affinity labeled residues were I-A\(^k\) Pro-118 and I-A\(^k\)
Thr-121.
Our interpretation is that (IASA)-46-61 folds into the
allele-specific peptide-binding site at the critical stretch from
residues 52 to 61 and that the peptide from residues 46 to 61
extends beyond the peptide-binding site to a nearby area rich
in hydrophobic residues in the \(\alpha\) and \(\beta\) domains. Several
observations support this interpretation. First, the finding
that (IASA)-46-61 stimulated a T-cell hybridoma similarly to
the unmodified peptide (Fig. 1) argues that the 52-61 residues
of the peptide must be specifically bound to the allele-com-
bining site. This was also confirmed by showing that it com-
peted for the binding of labeled 52-61 or an unrelated lyso-
zyme peptide. We had previously established that (HEL)-46-
61 can be NH\(_2\)-terminally truncated to the minimal immu-
nogenic structure 52-61 (27). The residues that contact I-A\(^k\)
are Asp-52, Ile-58, and Arg-61. The photoactive group
is spaced by 7 residues from Asp-52. Second, a shorter compound
such as compound 3, (IASA)-52-61, failed to label these
hydrophobic sites on I-A\(^\lambda\), indicating that their labeling
requires a spacer. Third, a less hydrophobic conjugate ABA-
Met-46-61 only partially labeled these hydrophobic sequences
but also labeled more hydrophilic ones (data not reported).
Fourth, the ability of (IASA)-46-61 or (IASA)-52-61 to label
other alleles of class II molecules correlated with the labeling
of this hydrophobic site, suggesting allele nonspecific hydro-
phobic interactions with the photoactive groups (12). The
potential of the conjugated photoactive groups to undergo
hydrophobic interactions was indeed observed by reverse-
phase HPLC; ABA-Met-46-61 eluted 4 min later from a
C-18 column than (HEL)-46-61, and (IASA)-46-61 was even
10 min later.
The presence of a nonpolymorphic, hydrophobic domain
located in the vicinity of the allele-specific antigen binding
site of class II molecules constitutes a major difference from
class I molecules. In class I molecules, the beginning of the
constant (third) domain forms a "loop" at the outer side of
the molecule, connecting the helical part of the second domain
with \(\beta\)-pleated sheet structures in the constant domain (5).
This class I constant domain is hydrophilic, containing mainly
charged amino acids. In contrast, the sequences of the corre-
sponding constant (second) domains of class II molecules
show a considerably different hydrophobicity pattern,
containing mainly uncharged, hydrophobic amino acids. Further-
more, in contrast to class I, class II molecules have two such
sequences which, according to the model, exit the antigen
binding site on opposite sites (4). It is tempting to speculate

![Fig. 10. Dose-response curves of HEL 46-61 (C) and IASA-
46-61 (Δ) on 3A9 T-cell hybridomas. The indicated concentra-
tions of peptide or conjugate were added to a constant number
of fixed CH-27 cells and 3A9 cells. The supernatants were assayed after
24 h for interleukin-2 by [\(^3H\)h]thymidine incorporation by the inter-
leukin-2-dependent CTLL cells. The bars indicate standard deviation
of the triplicate culture.](http://www.jbc.org)
that these hydrophobic areas would associate to minimize their contact with the hydrophilic medium, rather than assume conformations at the surface of the molecule, as their counterparts do in class I molecules (5).

The configuration in which these hydrophobic areas interact may be expected to depend on the symmetry of class II molecules, in particular on the angle between the floor of the antigen binding site and the center axis of the constant domain. If this angle is close to 90°, these sequences would "coil together" under the antigen binding site, approximately in the center of the molecule. In this case, the photoreactive groups conjugated NH2-terminally to HEL-46-61 would need to access the labeled sites through a sufficiently wide intramolecular opening. Conversely, if this angle is considerably less than 90°, the labeled sites would be located closer to an end of the antigen binding site. In this case, this hydrophobic domain could be accessible for macromolecules from the aqueous milieu. It is possible that allele nonspecific interactions of class II molecules involve hydrophobic interactions with this site. Examples include the interaction of class II molecules with the CD4 structure (28), the invariant chain (29), and "super antigens" such as staphylococcal enterotoxin B or Mls molecule (30).

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