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Both chains of HLA-DR bind to the membrane with a penultimate hydrophobic region and the heavy chain is phosphorylated at its hydrophilic carboxyl terminus*

(histocompatibility antigens/membrane proteins/phosphorylation/proteolysis)

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ABSTRACT **The HLA-DR antigen, a complex of two glycoproteins of 29,000 and 34,000 daltons, can be isolated from the membranes of human B-lymphoblastoid cell lines. Extensive proteolysis releases only 5–10% of the antigen, whereas detergent solubilizes all of it. Detergent solubilization after papain proteolysis of membranes produces antigen with chains cleaved near the carboxyl termini. Comparison of these three preparations demonstrates that each chain contains a carboxyl-terminal hydrophilic region that is sensitive to proteolytic degradation and a penultimate hydrophobic region, responsible for membrane binding, that is more resistant to papain. This two-step cleavage of each chain is also observed during the proteolysis of detergent-solubilized HLA-DR antigen. Both chains of HLA-DR in the membrane can be labeled with the lipophilic photoactivatable carbene reagent adamantane diazirine. This label is released from both chains during the second cleavage. The heavy chain can be reduced and alkylated under mild conditions, and this label is also lost during the second cleavage. The heavy chain is phosphorylated *in vivo*, and this label is lost upon the first cleavage. This observation suggests that the carboxyl terminus of the heavy chain is intracellular. Cumulatively, these data suggest that both chains of HLA-DR antigens are comprised of large extracellular NH_2-terminal regions, small penultimate intramembranous regions, and small carboxyl-terminal intracellular regions.**

The major histocompatibility complex is a genetic region originally defined as the major locus of tissue transplant rejection (2). It has since been demonstrated that this complex determines a number of molecules that play important roles in the immune system (3). The *HLA-D/DR* region in humans and the analogous *I* regions of mouse and guinea pig determine polymorphic cell-surface antigens, with a restricted tissue distribution, that are central to cell–cell communication within the immune system (3, 4). Thus, they have been shown to be important in such diverse phenomena as the mixed lymphocyte reaction, delayed type hypersensitivity, immune response gene effects, macrophage–T cell interaction (antigen presentation), T cell–B cell cooperation (help), and T-cell supression. All of these cellular interactions can be inhibited by various antisera directed against the relevant HLA-DR or Ia antigen. Some cell-derived factors with the ability to replace cellular subpopulations in immunological assays have been shown to carry Ia or HLA-DR determinants (2, 4).

Understanding the molecular basis of these cellular interactions requires a sophisticated level of knowledge concerning the structural features of HLA-DR and its analogues. The HLA-DR antigen is composed of two different glycoprotein chains associated in a tight, noncovalent complex (4, 5). The heavy chain appears to be invariant as assessed by isoelectric focusing and tryptic peptide mapping; the small chain is clearly variable and is likely to carry the alloantigenic determinant (refs. 1 and 6; unpublished data). The HLA-DR antigen is an integral membrane protein in which both chains may span the lipid bilayer (4, 7). Solubilization of the membrane with detergent yields a detergent-binding complex (p29,34) with chains of 29,000 and 34,000 daltons (4, 8). Other polypeptides may be loosely associated with this complex (unpublished data). Proteolysis of the membrane with papain produces a water-soluble complex (p23,30) with chains of 23,000 and 30,000 daltons. This fragment retains the carbohydrate and the alloantigenic activity of the HLA-DR antigen (9).

In the present paper, the papain cleavage of HLA-DR antigens is explored in detail and, in particular, some structural features of the carboxyl-terminal region are elucidated.

MATERIALS AND METHODS

Reagents. Papain was purchased from Worthington, Nonidet P40 (NP-40) from Particle Data (Elmhurst, IL), Brij 97 and 99 from Emulsion Engineering, (Elk Grove, IL), and lentil lectin from Vector (Burlingame, CA). Hydroxylapatite HTA and gel reagents including sodium dodecyl sulfate ($NaDodSO_4$) were purchased from Bio-Rad. Phosphate-buffered saline was from GIBCO, microgranular DEAE-cellulose from Whatman, and Sephadex G-150 and G-200 from Pharmacia. Film and Lanex screens were obtained from Kodak. Isotopes were purchased from New England Nuclear and were the highest specific activity available. All other chemicals were reagent grade. Anti-β_2-microglobulin beads were the kind gift of Richard Robb and tritiated adamantane diazirine was the kind gift of Hagan Bayley.

Proteolysis of Membranes and Analysis of HLA Antigens. Membranes were prepared from the B-lymphoblastoid cell line JY (*HLA-A2,2; B7,7; DRw4,6*). Papain (13 units per mg) was activated with 1.3 mg of cysteine for 15 min at 37°C as described (10). Activated papain (0.01 ml, 6 units) was added to some aliquots of membrane from 0.1 g of cells (0.13 ml of 10 mM Tris·HCl, pH 8.0) in International A321 tubes and incubated at 37°C for 0, 5, 15, 30, 60, 90, and 120 min. Proteolysis was terminated by addition of 0.4 mg of iodoacetic acid in 0.02 ml of 35 mM Tris·HCl, pH 8.1/0.35 mM EDTA and incubation on ice. Other aliquots were simply solubilized with 0.1 ml of 10% (vol/vol) Brij 97,99 (2:1). Samples were centrifuged at 100,000 × *g* for 1 hr at 4°C. The resultant supernatant was removed and the pellet was solubilized with 0.1 ml of 10% (vol/vol) Brij 97,99 (2:1).

The radioimmunoassay used is a variant of that developed

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Abbreviations: NP-40, Nonidet P40; $NaDodSO_4$, sodium dodecyl sulfate.

* A preliminary report of this work has been published (1).

in our laboratory (A. Fuks and R. Pollock, personal communication). In brief, it involves preincubation of sample with anti-p29,34 [a B cell-specific xenoantiserum made in rabbits (5)] for 8 hr at 37°C followed by incubation with ^{125}I-labeled p23,30 (labeled with Bolton–Hunter reagent) for 18–48 hr at 4°C. Fixed *Staphylococcus aureus* Cowen I strain prepared according to Kessler (11) was used to precipitate the immune complexes which then were thoroughly washed. The assay was sensitive to the subnanogram range. Inhibition of antibody-dependent complement-mediated microcytotoxicity with JY cells as targets was performed as described (12).

Preparation of HLA-DR Antigens. The purification of papain-released HLA-DR antigen (p23,30) was a variation of the published methods (10) involving papain digestion of membranes, ultracentrifugation, DEAE-52 ion exchange, Sephadex G-150 gel filtration, and lentil lectin affinity chromatography. The membranes after papain treatment were pelleted through a sucrose cushion and solubilized with nonionic detergent. After ultracentrifugation, the papain-resistant detergent-solubilized HLA-DR antigens (p27,33) were purified from the detergent-solubilized supernatant by lentil lectin chromatography. The purification of detergent-solubilized HLA-DR (p29,34) is published (5), and the variation used here involves solubilization in nonionic detergent, ultracentrifugation, and chromatography on anti-β_2-microglobulin and lentil lectin affinity columns. Purification of the separated chains of HLA-DR antigens was accomplished either by preparative $NaDodSO_4$/polyacrylamide gel electrophoresis or by a new procedure involving Sephadex G-200 gel filtration and hydroxylapatite chromatography in $NaDodSO_4$. The details of all these procedures will be presented in another report.

Determination of Amino Acid Composition and NH_2-Terminal Sequence. Amino acid sequence determination was by automatic Edman degradation using an updated Beckman 890B Sequenator and a 0.1 M Quadrol program (13). Identifications were made by using thin-layer and gas/liquid chromatography. Dansyl end-group analysis was performed as described (14). Samples for amino acid composition were hydrolyzed *in vacuo* in 5.7 M HCl and 1% phenol for 24 hr at 110°C and were then analyzed on a Beckman 121M analyzer with a microbore single column assembly.

Modification of HLA-DR Antigen. Labeling with tritiated adamantane diazirine was carried out as described (15). Briefly, membranes from 1 g of cells were suspended in 0.1 ml of phosphate-buffered saline, and 1 mCi (1 Ci = 3.7×10^{10} becquerels) of tritiated adamantane diazirine in ethanol was added slowly. The membrane preparation was incubated on ice for 30 min and then photolyzed (with stirring) for 15 min at 10°C by using a Rayonet RPR 3500A UV lamp. The labeled membranes were washed twice with phosphate-buffered saline, extracted with 2% (vol/vol) NP-40, and applied to anti-β_2-microglobulin beads. The flow-through of such beads was applied to lentil lectin beads, and the modified HLA-DR antigen preparation was eluted with α-methylmannoside.

Mild reduction and alkylation were performed as described (16), except that the sample was freshly solubilized from membranes of 0.5 g of JY cells by using 1 ml of 5% NP-40/20 mM Tris·HCl, pH 8/2 mM dithiothreitol for 30 min on ice. The sample was centrifuged at 100,000 x *g* for 1 hr at 4°C, and 5 μl of 200 mM dithiothreitol and 0.1 ml of 3.5 M Tris·HCl, pH 8/35 mM EDTA was added to the supernatant. The sample was incubated for 15 min at 37°C and then 0.1 ml of aqueous iodo[^{14}C]acetic acid (1 mg) was added to make the sample about 10 mM. The sample was then incubated for 1 hr in the dark at room temperature, the reaction was stopped with 2-mercaptoethanol, and the sample then was partially purified by lentil lectin affinity chromatography.

Phosphorylation of cells was performed as described (17). In brief, 10^6 cells were incubated in 10 ml of phosphate-free buffer (150 mM NaCl/5 mM $MgCl_2$/5 mM KCl/2 mM glutamine/1.8 mM glucose/10 mM Tris acetate, pH 7.4) for 1 hr at 37°C. The cells were then resuspended in the same buffer containing 10 mCi of [^{32}P]orthophosphate and incubated for 1 hr at 37°C. The cells were washed three times with phosphate-buffered saline and then lysed with 2% NP-40/25 mM NaCl/5 mM $MgCl_2$/25 mM Tris·HCl, pH 7.4/1 mM phenylmethylsulfonyl fluoride on ice for 20 min. The detergent lysate was centrifuged at 17,000 rpm in a Sorvall SS34 rotor for 30 min. The supernatant was partially purified by lentil lectin chromatography.

Proteolysis of Detergent-Solubilized HLA-DR Antigen (p29,34). Papain (21.7 units/mg) was activated at 1 mg/ml in 1 mM dithiothreitol/0.1 mM EDTA/10 mM Tris·HCl, pH 8, for 5 min at 37°C and diluted to various concentrations with the same buffer. Proteolysis involved incubation of aliquots (0.02–0.05 ml) of partially purified detergent-solubilized modified HLA-DR preparations (about 25 mg of protein) with 0.025 ml of papain at various concentrations, times, and temperatures. In typical experiments, aliquots were incubated for 60 min at 37°C with 0.025 ml of papain at 1 mg/ml (papain/protein, 1:1), 0.5 mg/ml (1:2), 0.25 mg/ml (1:4), 0.125 mg/ml (1:8), or 0.0625 mg/ml (1:16), or with 0.0625 mg/ml (1:16) for 30 min at 37°C, for 15 min at 37°C, for 5 min at 21°C, or for 2 min on ice, or with 0.025 ml of buffer on ice. The proteolysis was stopped by addition of 0.01 ml of 25 mM iodoacetic acid/87.5 mM Tris·HCl, pH 8.1, on ice.

The proteolyzed samples were precipitated by addition of 5 vol of acetone and collected by centrifugation at 4000 rpm at room temperature in an IEC centrifuge. The precipitate was resuspended in Laemmli sample buffer containing 1.5% $NaDodSO_4$, boiled, and applied to a 7–15% polyacrylamide gradient gel prepared according to Laemmli (18). Gels were run overnight at 6–12 mA, stained with Vesterberg stain (19), and destained. Gels with ^{3}H were fluorographed (20) on Kodak SB-5 film, those with ^{14}C were autoradiographed on SB-5 film, and those with ^{32}P were autoradiographed by using a screen and Kodak XR-5 film, all at −70°C.

RESULTS AND DISCUSSION

Proteolysis of Membrane-Bound HLA-DR Antigen and Analysis of the Products. Papain proteolysis is a standard preparative procedure for solubilizing HLA-A, -B, and -C antigens from B-lymphoblastoid cell membranes (10). Under the standard conditions used (1 mg of papain for 2–3 mg of membrane protein at 37°C), HLA-A2 was totally released from JY membranes as a water-soluble fragment in 15 min, whereas only 10% of the HLA-DR antigen was released in 3 hr (Fig. 1). The remainder of the HLA-DR antigen was not destroyed but was found still in the membrane fraction. These conditions were nearly optimal because much greater amounts of papain released only slightly more HLA-DR antigen from the membrane and destroyed HLA-DR antigen in solution. Similar results were found for a number of HLA-DR antigens tested (unpublished data). In a previous report (9) of the preparation of papain-released HLA-DR antigen (p23,30), no yields were calculated.

The HLA-DR antigen that remains in the membrane during papain proteolysis can be solubilized with nonionic detergent and partially purified by lentil lectin chromatography. Such material was compared, by $NaDodSO_4$ gel electrophoresis, with partially purified preparations of HLA-DR antigen either solubilized from membranes with nonionic detergent (p29,34) or released from membranes with papain (p23,30) (Fig. 2*b*, lanes 11–13). The small amount of HLA-DR antigen released

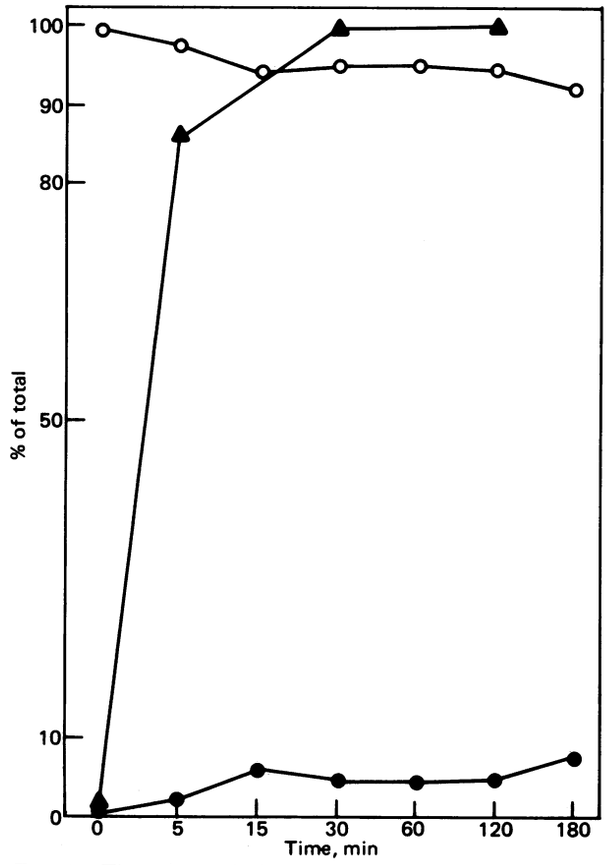

FIG. 1. Time course of release of HLA-DR and HLA-A2 antigens from JY cell membranes by papain. ●, Soluble HLA-DR; ○, HLA-DR remaining on membrane; ▲, soluble HLA-A2.

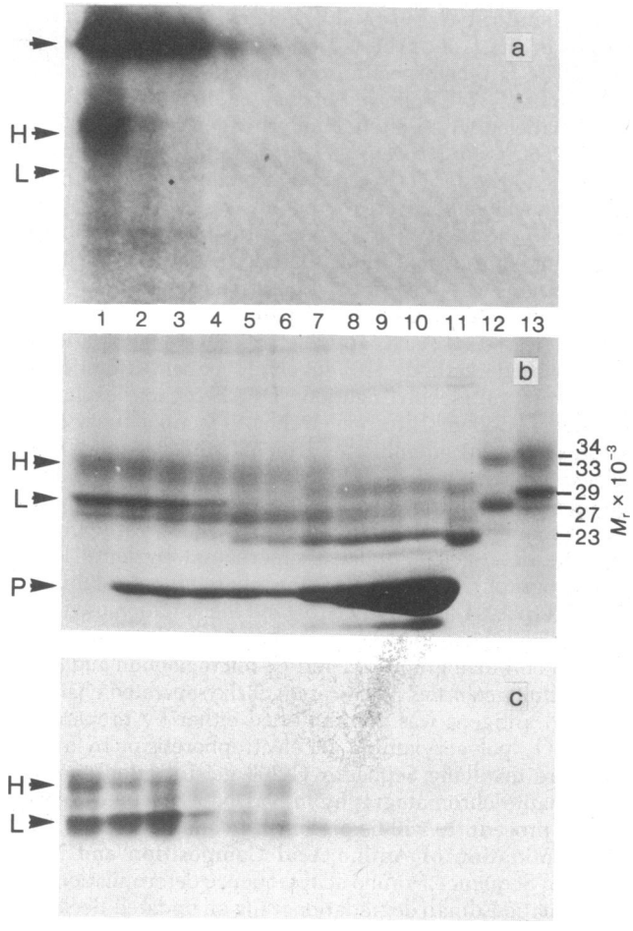

FIG. 2. Labeling of HLA-DR antigens with [^{32}P]phosphate and [^{3}H]adamantane diazirine and proteolysis by papain. A portion of $NaDodSO_4$/7–15% polyacrylamide gel is shown. (*a*) Fluorogram showing [^{32}P]phosphate labeled materials; (*b*) duplicate gel stained with Coomassie blue; (*c*) autoradiogram of duplicate gel showing [^{3}H]adamantane diazirine-labeled material. Lanes: 1–10, course of papain proteolysis of detergent solubilized HLA-DR; 11, papain-released HLA-DR (p23,30); 12, papain-resistant detergent-solubilized HLA-DR (p27,33); 13, detergent-solubilized HLA-DR (p29,34). H, heavy chain of HLA-DR; L, light chain; P, papain; unlabeled arrow, heavy chain of HLA-A and -B. HLA-A and -B are not present in (*b*) and (*c*).

by papain (p23,30 in lane 11) separated into three bands (23,000, 26,000, and 30,000 daltons) on these gradient gels. The material remaining in the membrane after papain treatment and subsequently solubilized by nonionic detergent appeared as two bands: a heavy chain of 33,000 daltons and a light chain of 27,000 daltons (lane 12). The detergent-solubilized HLA-DR antigen (p29,34 in lane 13) from membranes never exposed to papain also appeared as two major bands: a fuzzy doublet of heavy chain (34,000 daltons) and a sharp band of light chain (29,000 daltons with a minor proteolytic product of 27,000 daltons in this preparation). It should be stressed that these apparent molecular weights are only approximate, because different values were obtained from minor changes in gel buffers or procedures.

These three preparations could be further purified to give single homogeneous separated denatured chains either by preparative $NaDodSO_4$ gel electrophoresis or by a procedure (to be described elsewhere) involving Sephadex G-200 gel filtration and hydroxylapatite chromatography in $NaDodSO_4$. The heavy chains of all three preparations had an identical NH_2-terminal amino acid sequence (5–20 residues) which is in agreement with the published sequence of p34 from JY cells (21) and starts with isoleucine. The light chains had an identical NH_2-terminal amino acid sequence to that published for p29 of JY cells (21) and it begins with glycine. The middle band in the papain-released HLA-DR (p26) is a light chain by dansyl end-group analysis. These sequence data demonstrate that the proteolytic cleavages are at the carboxyl terminus of each chain.

Amino acid analyses of the separated chains were performed. The heavy chain of detergent-solubilized HLA-DR (p34) was assigned a molecular weight of 27,500 and the light chain (p29), a molecular weight of 26,000 based on relative gel mobilities of the nonglycosylated HLA-DR chains isolated from cells grown in the presence of tunicamycin (H. Ploegh, personal communication). The other chains were assigned molecular weights in accordance with those values. The normalized compositions of these chains are presented in Table 1. The approximate compositions of the peptides released by papain were determined by subtracting the compositions of the appropriate chains. The peptide released from each chain by the first cleavage is rather hydrophilic; the peptide released from each chain by the second cleavage is clearly hydrophobic. This assessment is confirmed by the values of polarity calculated from the inferred compositions of the released peptides, which also indicate that the peptide released from the light chain by the first cleavage is not as hydrophilic as the whole molecule.

Taken together, these data suggest that both chains of membrane-bound HLA-DR antigen have a carboxyl-terminal region that is susceptible to papain proteolysis and a penultimate hydrophobic region responsible for membrane binding that is relatively resistant to removal by papain. The light chain ap-

Table 1. Amino acid compositions* of the heavy and light chains of HLA-DR antigens and the products of its proteolysis by papain

Amino acid	Light chain					Heavy chain				
	p29	p27	p23	p29 − p27	p27 − p23	p34	p33	p30	p34 − p33	p33 − p30
Cys	3.10	3.01	2.01	0	1	3.00	2.99	2.09	0	1
Asx	19.29	16.13	16.01	3	0	23.53	23.20	23.14	0	0
Thr	15.53	15.60	12.52	0	3	15.53	16.03	16.28	0	0
Ser	15.06	13.88	11.03	1	3	15.94	10.83	10.84	5	0
Glx	29.88	29.98	26.51	0	3–4	29.50	29.60	26.42	0	3
Pro	12.24	11.33	10.11	2	1	14.11	15.59	15.41	0	0
Gly	19.76	17.68	14.44	2	3	19.76	16.51	14.96	3	2–3
Ala	12.94	10.39	9.27	2–3	1	17.16	14.37	13.64	3	0–1
Val	20.94	20.74	17.02	0	4	19.28	16.30	15.84	3	0–1
Met	3.76	3.17	2.11	0–1	1	4.05	4.10	2.64	0	1–2
Ile	7.76	7.12	5.07	0–1	2	12.49	11.97	8.36	1–2	4
Leu	23.53	21.13	13.11	2–3	8	25.28	24.12	16.72	1	7–8
Tyr	9.88	7.44	8.53	2	0	7.46	4.71	4.92	3	0
Phe	12.00	12.05	7.20	0	5	13.74	13.43	11.88	0	3
His	6.59	6.26	6.11	0	0	7.59	6.93	6.60	1–2	0
Lys	11.53	7.28	6.07	4	1	13.92	9.68	10.12	4	0
Arg	15.59	15.42	14.25	0	1	10.75	10.77	5.72	0	5
Polarity†	48.0	47.9	51.0	39.0	30.6	46.7	46.5	48.7	46.0	27.1

* Values listed are residues per molecule.
† Polarity was determined as described (22).

pears to be more sensitive to both steps of papain proteolysis than the heavy chain. The heterogeneity responsible for the fuzzy band of the heavy chain may be located near the carboxyl terminus because the first cleavage product is a much better defined band (Fig. 2*b*, lane 12). Also, the release of two light chains by papain could well be a consequence of the presence of two alloantigenic specificities in JY cells.

Proteolysis of Detergent-Solubilized HLA-DR Antigen and Various Modification Products. The results of papain proteolysis of partially purified detergent-solubilized HLA-DR antigen support the notion that each chain is cleaved by papain in two steps (Figs. 2*b* and 3*a*). The first cleavage required relatively mild conditions (Fig. 2*b*, lanes 1–5; Fig. 3*a*, lanes 1–7). It generated a defined intermediate of the light chain—with roughly the same mobility as the light chain of papain-resistant detergent-solubilized HLA-DR—and a series of intermediates of the heavy chain. The second cleavage required much harsher conditions of proteolysis and generated products with mobilities roughly comparable to those found in papain-released HLA-DR (Fig. 2*b*, lanes 6–10; Fig. 3*a*, lanes 8–11). Under the conditions used, the amount of HLA-DR measured by radioimmunoassay did not decrease with proteolysis (data not shown). The pattern of papain proteolysis of detergent-solubilized HLA-DR and membrane-bound HLA-DR appear to be similar although not identical. The proteolysis of detergent-solubilized material was used to localize several interesting modification sites along the molecule.

Labeling the Hydrophobic Region. JY membranes were labeled with the tritiated photoactivatable lipophilic carbene reagent admantane diazirine (kind gift of Hagan Bayley). Detergent-solubilized HLA-DR was purified, digested with papain, and analyzed by gel electrophoresis and fluorography (Fig. 2*c*). Both the heavy and light chains were labeled, and the label was retained in both chains after the first cleavage but lost from both chains after the second cleavage. This suggests that the second cleavage of each chain removes a hydrophobic region buried in the lipid bilayer.

Labeling the Easily Reduced Cysteines. JY membranes were detergent-solubilized and the membrane residue was removed by ultracentrifugation. The supernatant was reduced and alkylated with iodo[^{14}C]acetic acid under mild conditions and then the HLA-DR and HLA-A, -B, and -C antigens were partially purified by lentil lectin chromatography. This preparation was digested with papain and analyzed by gel electrophoresis followed by staining with Coomassie blue (Fig. 3*a*) and autoradiography (Fig. 3*b*). As reported (16), HLA-A, -B, and -C heavy chains were digested by papain in two steps and the label was substantially lost on the first step. Only the heavy chain of HLA-DR was labeled by this procedure: the label was retained after the first cleavage and lost in the second cleavage. In contrast, when old preparations of HLA-DR, which had been

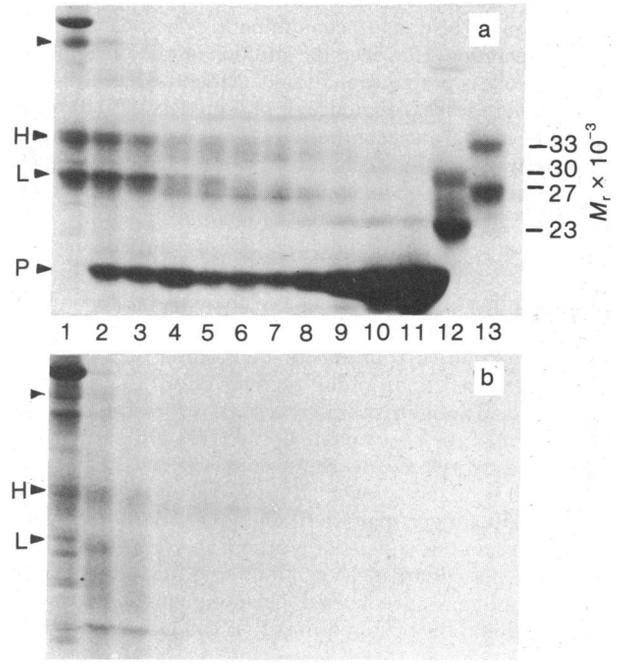

FIG. 3. Labeling of HLA-DR antigens with iodo[^{14}C]acetic acid under conditions of mild reduction and alkylation and proteolysis by papain. (*a*) Coomassie blue-stained gel; (*b*) autoradiogram of same gel. Lanes: 1–11, course of papain proteolysis; 12, papain-released HLA-DR (p23,30); 13, papain-resistant detergent-solubilized HLA-DR (p27,33). Symbols as in Fig. 2.

previously frozen and thawed, were reduced and alkylated under the same conditions, label was sometimes found in both chains, in agreement with previous findings (8). This label was substantially removed from both chains in the first cleavage (data not shown). The subtractive amino acid compositions (Table 1) indicate that there is a cysteine in the region removed from the heavy chain by the second cleavage. This cysteine need not be within the hydrophobic region but may lie at the edge of the membrane, as does a cysteine in HLA-B7 (23). The reactivity of the light chain may result from partial denaturation during storage.

Labeling with Phosphate. JY cells were incubated in the presence of [^{32}P]orthophosphate and then detergent-solubilized. The membrane residue was removed by ultracentifugation and the HLA-DR and HLA-A, -B, and -C antigens were partially purified by lentil lectin chromatography. This preparation was digested with papain and analyzed by gel electrophoresis and autoradiography (Fig. 2*a*). The heavy chains of HLA-A and -B were labeled and the label was removed in the first cleavage (17). Under these conditions, only a band in the position of the heavy chain of HLA-DR was labeled and only to approximately 20% of the specific activity of HLA-A and -B. The label was lost during the first cleavage but more rapidly than the shift in the Coomassie-stained band (Fig. 2*b*). A number of possible explanations could account for this result. The phosphate could be very near the carboxyl terminus of the heavy chain and the papain could be digesting that portion away without affecting the mobility of the band significantly. The fact that the heavy chain appears to be digested through a number of intermediates in the first cleavage supports this hypothesis. Alternately, only a subpopulation of the HLA-DR antigens could be phosphorylated, and this material may be digested more rapidly than the major population of the HLA-DR antigens (e.g., phosphorylation could alter the conformation of the carboxyl-terminal region and thus affect the rate of proteolysis). Two other possibilities are deemed less likely. Removal of the phosphate might be due to a contaminating phosphatase in the papain preparation. However, the addition of phosphate buffer to the proteolysis preparation did not affect the pattern of release and there was no evidence of phosphatase activity upon the HLA-A and -B antigens. Lastly, the labeled band might be due to an impurity that fortuitously migrates in the same position as the heavy chain, but this band is specifically immuno-precipitated by anti-p29, 34 (data not shown).

In summary, these experiments establish that both chains of the HLA-DR antigen are inserted into the membrane in a manner similar to that of the heavy chain of HLA-A and -B antigens or glycophorin (24). They contain a penultimate hydrophobic region responsible for membrane binding and a carboxyl-terminal hydrophilic region. As in the case of the HLA-A and -B antigens (16, 17) the carboxyl-terminal hydrophilic region of the heavy chain of the HLA-DR antigen contains an easily reduced cysteine and appears to be phosphorylated. It has been reported by Walsh and Crumpton (7) that HLA-DR antigen spans the membrane. These investigators prepared inside-out vesicles of lymphocytes, labeled with ^{125}I by the lactoperoxidase method, and found that both chains of HLA-DR antigens were labeled. Unambiguous demonstration of orientation, however, requires that the labeled region be localized, and thus their experiments do not establish which portion of the HLA-DR antigen is intracellular. The demonstration of phosphorylation localized to the carboxyl terminus of the heavy chain presented here strongly suggests that it is this region that is located inside the cell. An interaction between the hydrophilic regions of the two chains of the HLA-DR antigens or of them with cytoskeletal elements could be regulated by phosphorylation of the heavy chain or by oxidation of the cysteine residues.

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1. Kaufman, J., Shackelford, D., Ploegh, H., Engelhard, V. & Strominger, J. (1979) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **38,** 1280.
2. Klein, J. (1975) *The Biology of the Mouse Histocompatibility 2 Complex* (Springer, New York).
3. Klein J. (1979) *Science* **203,** 516–521.
4. Ferrone, S., Allison, J. P. & Pellegrino, M. A. (1978) *Contemp. Top. Mol. Immunol.* **7,** 239–281.
5. Springer, T. A., Kaufman, J. F., Siddoway, L. A., Mann, D. L. & Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 6201–6207.
6. Silver, J. & Ferrone, S. (1979) *Nature (London)* **279,** 436–437.
7. Walsh, F. S. & Crumpton, M. J. (1977) *Nature (London)* **269,** 307–311.
8. Springer, T. A., Kaufman, J. F., Terhorst, C. & Strominger, J. L. (1977) *Nature (London)* **268,** 213–217.
9. Humphreys, R. E., McCune, J. M., Chess, L., Hermann, H. C., Malenka, D. L., Mann, D. L., Parham, P., Schlossman, S. F. & Strominger, J. L. (1976) *J. Exp. Med.* **144,** 98–112.
10. Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 7555–7567.
11. Kessler, S. W. (1976) *J. Immunol.* **117,** 1482–1490.
12. Springer, T. A., Mann, D. L., DeFranco, A. L. & Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 4682–4693.
13. Orr, H. T., Lopez de Castro, J. A., Parham, P., Ploegh, H. L. & Strominger, J. L. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 4395–4399.
14. Woods, K. R. & Wang, K.-T. (1967) *Biochim. Biophys. Acta* **133,** 369–370.
15. Goldman, D. W., Pober, J. S., White, J. & Bayley, H. (1979) *Nature (London)* **280,** 841–843.
16. Springer, T. A. & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. USA* **73,** 2481–2485.
17. Pober, J. S., Guild, B. C. & Strominger, J. L. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 6002–6006.
18. Laemmli, U.K. (1970) *Nature (London)* **227,** 680–685.
19. Vesterberg, O. & Hansen, L. (1977) in *Electrofocusing and Isotachophoresis,* eds. Radola, B. J. & Graesslin, D. (Gruyter, Berlin, West Germany), pp. 123–129.
20. Bonner, W. M. & Laskey, R. A. (1974) *Eur. J. Biochem.* **46,** 83–88.
21. Kaufman, J. F., Fuks, A., Giphart, M., Solheim, B. & Strominger, J. L. (1978) in *Ir Genes and Ia Antigens,* ed. McDevitt, H. O. (Academic, New York), pp. 235–242.
22. Capaldi, R. A. & Vanderkooi, G. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 930–932.
23. Robb, R. J., Terhorst, C. & Strominger, J. L. (1978) *J. Biol. Chem.* **253,** 5319–5324.
24. Marchesi, V. T., Furthmayr, H. & Tomita, M. (1976) *Annu. Rev. Biochem.* **45,** 667–698.