

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/17002034>

Primary structure of papain-solubilized human histocompatibility antigen HLA-B40 (-Bw60). An outline of alloantigenic determinants

ARTICLE *in* BIOCHEMISTRY · SEPTEMBER 1983

Impact Factor: 3.02 · DOI: 10.1021/bi00285a036 · Source: PubMed

CITATIONS

37

READS

9

4 AUTHORS, INCLUDING:



José Antonio Lopez de Castro

Centro De Biología Molecular Severo Oc...

164 PUBLICATIONS 3,803 CITATIONS

SEE PROFILE



Douglas Michael Strong

University of Washington Seattle

276 PUBLICATIONS 5,539 CITATIONS

SEE PROFILE

- Moreland, R. B., & Dockter, M. E. (1980) *Anal. Biochem.* 103, 26-32.
- Phillips, D. R., & Morrison, M. (1971) *Biochemistry* 10, 1766-1771.
- Prochaska, L., Bisson, R., & Capaldi, R. A. (1980) *Biochemistry* 19, 3174-3179.
- Radda, G. K., & Vanderkooi, J. (1972) *Biochim. Biophys. Acta* 265, 509-549.
- Richards, F. M., & Brunner, J. (1980) *Ann. N.Y. Acad. Sci.* 346, 144-164.
- Rubin, M. S., & Tzagoloff, A. (1973) *J. Biol. Chem.* 248, 4275-4279.
- Schatz, G., & Mason, T. L. (1974) *Annu. Rev. Biochem.* 43, 51-87.
- Smith, P. A. S. (1970) in *Nitrenes* (Lwowski, W., Ed.) pp 99-162, Wiley-Interscience, New York.
- Tanaka, M., Haniu, M., Yasunobu, K. T., Yu, C.-A., Yu, L., Wei, Y.-H., & King, T. E. (1979) *J. Biol. Chem.* 254, 3879-3885.
- Thalenfeld, B. E., & Tzagoloff, A. (1980) *J. Biol. Chem.* 255, 6173-6180.
- Wu, C., & Stryer, L. (1972) *Proc. Natl. Acad. Sci. U.S.A.* 69, 1104-1108.

Primary Structure of Papain-Solubilized Human Histocompatibility Antigen HLA-B40 (-Bw60). An Outline of Alloantigenic Determinants[†]

José A. López de Castro, Rafael Bragado,* Douglas M. Strong, and Jack L. Strominger

ABSTRACT: The primary structure of papain-solubilized human histocompatibility antigen HLA-B40 (-Bw60) has been determined. Its comparison with that of the cross-reactive HLA-B7 antigen allows for the first time a direct sequence comparison between two HLA-B locus products and an outline of the location of their alloantigenic sites. Overall sequence homology between HLA-B40 and HLA-B7 is 93%. Of 19 detected differences, 18 are located in the two amino-terminal domains (residues 1-182). Half of them are clustered in two short segments spanning residues 63-74 and 147-156, re-

spectively, which suggests that they may encompass major sites of their alloantigenic determinants. The first of these segments is highly polymorphic in HLA and H-2 molecules. It is proposed that it may belong to a hypervariable region of class I histocompatibility antigens. The remaining substitutions are scattered through the N-terminal portions of the two external domains. Thus, in addition to the above-mentioned segments, other positions may contribute significantly to the antigenic polymorphism of these molecules.

Human class I histocompatibility antigens are a family of integral membrane glycoproteins encoded by the HLA-A, -B, and -C loci of the major histocompatibility complex (MHC).¹ They are ubiquitously distributed on the surface of the great majority of cells and appear to play a central role in mediating the recognition and killing of virus-infected or otherwise modified cells by specific cytotoxic T lymphocytes (McMichael et al., 1977; Dickmeiss et al., 1977). Serological analyses of class I HLA antigens have revealed an extraordinary degree of genetic polymorphism. This polymorphism is a consequence of the existence of three loci which encode for class I molecules, each locus containing one of a great number of alleles (Albert et al., 1980) which are codominantly expressed. The functional meaning of this diversity is obscure, although it has been proposed that it may be important in maximizing the repertoire of associative recognition of foreign antigens expressed on the

surface of target cells (Kimball & Coligan, 1983).

Biochemical characterization of HLA-A and -B antigens has established that the molecule consists of a polymorphic, MHC-encoded heavy chain of 44 000 daltons noncovalently bound to β_2 -microglobulin (Grey et al., 1973), an invariant 12 000-dalton polypeptide encoded outside the MHC (Goodfellow et al., 1975). The heavy chain consists of a large amino-terminal extracellular portion, a transmembrane hydrophobic segment, and a carboxy-terminal hydrophilic portion which is in contact with the cytoplasm (Springer & Strominger, 1976). Primary structural analysis suggests that the extracellular portion may be organized in 3 domains of approximately 90 residues each (Orr et al., 1979), which have been designated as $\alpha 1$ (residues 1-90), $\alpha 2$ (residues 91-182), and $\alpha 3$ (residues 183-275). Papain treatment of cell membranes solubilizes the extracellular portion of the molecule (HLA_{pap}), which includes the antigenic determinants (Sanderson & Batchelor, 1968; Springer et al., 1974). Papain-solubilized HLA antigens are suitable for large-scale purification (Parham et al., 1977; Trägård et al., 1979), and for this reason, they have been used for most studies dealing with detailed structural analyses of these molecules (Strominger et al., 1980). A major goal of these studies is to provide a

[†] From the Department of Immunology, Fundación Jiménez Díaz, Avenida Reyes Católicos, 2 Madrid-3, Spain (J.A.L.d.C. and R.B.), the Naval Medical Research Center, Bethesda, Maryland 20814 (D.M.S.), and the Department of Biochemistry and Molecular Biology, Harvard University, Cambridge, Massachusetts 02138 (J.L.S.). Received February 14, 1983. Supported by grants from the Spanish Ministerio de Educación y Ciencia (3448-79), from the Fondo de Investigaciones Sanitarias de la Seguridad Social (10/81 and 12/81), and from the National Institutes of Health (A1-10736). This work was also supported in part by the Naval Medical Research and Development Command (Work Unit M0095-PN.001-0045) and by Contract Y001CP00502 from the National Cancer Institute. R.B. was a fellow of the Spanish Fundación Jiménez Díaz.

¹ Abbreviations: MHC, major histocompatibility complex; HLA_{pap}, papain-solubilized HLA antigen; NaDodSO₄, sodium dodecyl sulfate; CNBr, cyanogen bromide; HPLC, high-pressure liquid chromatography; Pth, phenylthiohydantoin; CTL, cytotoxic T lymphocyte.

molecular understanding of HLA genetic polymorphism. Previous work on the chemical structure of various HLA and H-2 class I antigens has considerably advanced our appreciation of the nature and topographic location of polymorphic determinants (Kimball & Coligan, 1983). Particularly, the comparison of the amino acid sequence of two strongly cross-reactive antigens (HLA-A2 and HLA-A28) allowed a detailed mapping of alloantigenic sites (López de Castro et al., 1982). In this report, the primary structure of papain-solubilized HLA-B40 antigen is presented and related to that of HLA-B7 (Orr et al., 1979), providing the first comparison of two cross-reactive allelic products of the HLA-B locus at the molecular level.

Materials and Methods

Purification of Papain-Solubilized HLA-B40 Heavy Chain. The human lymphoblastoid cell line LB (HLA-A28,28; HLA-B40,40) was used as source of material. These cells were Bw60 (Dawkins & Houlston, 1980) as indicated by a recent typing with sera of the 8th Histocompatibility Workshop (J. McLean, personal communication). Preparation of membranes, papain solubilization, and purification of HLA-B40 protein were carried out as described for other HLA antigens (Parham et al., 1977). Preparations were judged to be homogeneous by polyacrylamide gel electrophoresis (Laemmli, 1970) in sodium dodecyl sulfate (NaDodSO₄).

Complete reduction and ¹⁴C-carboxymethylation were performed essentially as described (Parham et al., 1977). Separation of HLA-B40 heavy chain and β_2 -microglobulin was done by gel filtration in a column (1.6 × 100 cm) of Sephadex G-75 superfine equilibrated with 1 M acetic acid. Fractions containing the heavy chain were pooled and lyophilized. Preparations were consistently pure as judged by NaDodSO₄ gel electrophoresis.

Preparation of Fragment by Mild Acid Hydrolysis. About 5 mg of reduced and ¹⁴C-carboxymethylated protein was dissolved in 2 mL of 70% formic acid containing 5 mM 2-mercaptoethanol, and nitrogen was bubbled through the solution. The mixture was incubated for 36 h at 37 °C, and then the sample was applied directly onto a column of Sephadex G-75 superfine (1.6 × 100 cm) equilibrated with 1 M acetic acid and 5 mM 2-mercaptoethanol. Fractions were monitored by reading the absorbance at 280 nm. Distribution of radioactivity was measured by liquid scintillation counting of aliquots in Aquasol (New England Nuclear). Fractions containing the purified fragments were pooled and lyophilized.

Preparation of CNBr Fragments. Cleavage by CNBr was performed as described (Gross, 1967). After 24 h, the reaction mixture was diluted in 10 volumes of water and lyophilized. Fragments were redissolved in 1 mL of 1 M acetic acid and were separated on a column (1.6 × 100 cm) of Sephadex G-75 superfine equilibrated with the same solution. Fractions were monitored as described above.

Peptide Purification. Tryptic and chymotryptic digestion of protein samples as well as peptide separation by microbore ion-exchange chromatography was carried out by following procedures detailed elsewhere (López de Castro et al., 1979). Peptide detection in the effluent fractions was performed by a fluorecamine assay (Nakai et al., 1974). ([¹⁴C]Carboxymethyl)cysteine-containing peptides were identified by liquid scintillation counting in Aquasol. A tryptic glycopeptide obtained from intact heavy chain was purified by affinity chromatography on a lentil-lectin column as described (Parham et al., 1977).

Amino Acid Analysis. Peptide samples were hydrolyzed at 110 °C for 24 h in constant-boiling 5.7 N HCl (Pierce)

containing 1% phenol. Amino acid analyses were performed on a Beckman 121 M analyzer.

Pth Determination. Conversion of the anilinothiazolinone fractions to the corresponding phenylthiohydantoins (Pth's) was performed with 0.1 N HCl at 80 °C for 10 min. Pth derivatives were identified by high-pressure liquid chromatography (HPLC) on a Hewlett-Packard 1081B instrument equipped with a Zorbax ODS C18 column (Du Pont). Pth's were separated at 62 °C with a solvent system of 0.01 M sodium acetate, pH 4.75, and acetonitrile. A linear gradient from 27% to 45% acetonitrile was used for 3 min followed by isocratic elution under final conditions for 5 additional min at a flow rate of 1.7 mL/min. After each run, the column was washed for 2 min with 90% acetonitrile. The column effluent was monitored at 266 nm with an automatic wavelength shift to 320 nm during the elution time of dehydro-Ser and dehydro-Thr derivatives, between 5.75 and 6.6 min. Pth-His and Pth-Arg derivatives were analyzed in a similar way but by using isocratic elution with 60% acetonitrile at a flow rate of 2 mL/min for 4 min. In some cases, a Waters instrument equipped with a Bondapak C18 radial compression column was used with chromatographic conditions as described elsewhere (López de Castro et al., 1982). Occasionally, gas-liquid chromatography was also employed as in a previous report (Orr et al., 1979).

Results

Chemical Cleavage. The strategy used for the sequence determination of the HLA-B40 heavy chain was very similar to that used for the sequencing of HLA-B7 (López de Castro et al., 1979) and was based on the isolation of large fragments generated by a combination of selective hydrolysis at the acid-labile Asp-Pro bond and of CNBr cleavage. The separation of fragments obtained after partial acid hydrolysis is shown in Figure 1A. The amino acid composition of HLA-B40_{pap} (Table I) was consistent with the presence of three methionine residues. Two of them were clustered near the amino terminus of the molecule as shown by NH₂-terminal sequence analysis of the intact heavy chain (see below). The third methionine was presumed to be at position 98 by homology with HLA-B7. Therefore, the ac-1 fragment (Figure 1A) was subjected to further cleavage with CNBr, and the resulting digest was fractionated by gel filtration (Figure 1B). The chromatographic pattern is analogous to that obtained with HLA-B7_{pap} (López de Castro et al., 1979). Amino-terminal sequencing of the material present in peaks ac-2 (Figure 1A), ac-1a, and ac-1b (Figure 1B) confirmed the identity of the chemical cleavage points with those of HLA-B7 (see below). Therefore, the fragments included in those peaks were designated as h3, h1, and h2, respectively (Figure 1D). A fragment (h23) extending from residue 99 to the carboxy terminus of the molecule was purified after CNBr cleavage of the whole HLA-B40_{pap} heavy chain by gel filtration of the resulting mixture (peak III, Figure 1C). This fragment was easily separated from aggregated material (peak I), undigested heavy chain (peak II), and heterogeneous breakdown products (peaks IV and V).

Purification and Characterization of Peptides. Fragments obtained by chemical cleavage of the polypeptide chain were digested by trypsin, and the resulting mixtures were fractionated by microbore ion-exchange chromatography (Figure 2). A set of 31 tryptic peptides was obtained which covered the whole extent of the heavy chain except the 14 N-terminal residues. Most peptides were obtained in a reasonably pure form after this single step such that they could be used directly for both compositional and sequence analysis. In those few

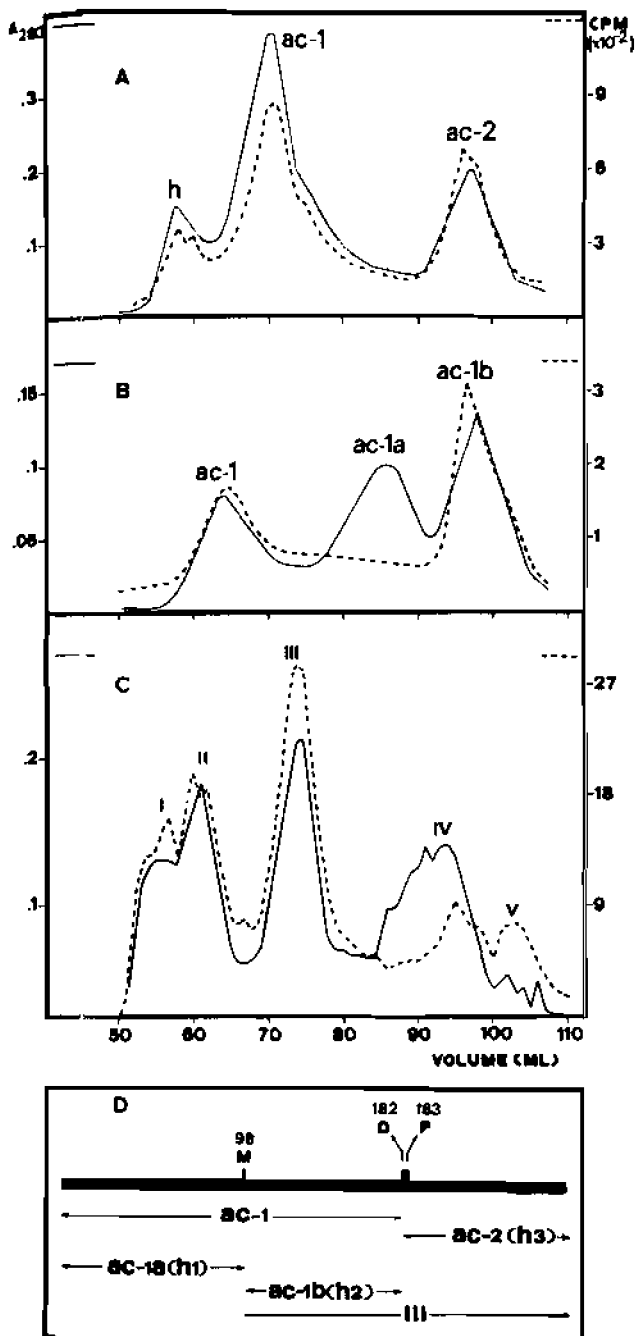


FIGURE 1: (A) Purification of fragments obtained after partial acid hydrolysis of reduced and ^{14}C -carboxymethylated HLA-B40_{pp} heavy chain. A superfine G-75 Sephadex column (1.6 \times 100 cm) equilibrated with 1 M acetic acid was used at a flow rate of 3 mL/h. Fractions of 1 mL were collected and monitored by reading the absorbance at 280 nm (A_{280}) and the radioactivity (^{14}C) in a liquid scintillation counter. (B) Fragment ac-1 was cleaved by CNBr and fractionated by gel filtration as described above. (C) Purification of fragments obtained after CNBr cleavage of reduced and ^{14}C -carboxymethylated HLA-B40_{pp} heavy chain. Chromatographic conditions were as described above. (D) Diagram of the location of chemically derived fragments in HLA-B40 heavy chain.

cases in which two peptides coeluted, further purification was not attempted given the limited amount of material available. Instead, they were sequenced together, and assignments were done based either on independent sequencing of the corresponding portion of the molecule (see below) or on homology with the closely related HLA-B7 protein. The amino acid composition of each peptide of the coeluting pairs was calculated from the amino acid analysis of the mixture based on the proportion of each peptide as established by sequencing. The amino acid composition of tryptic peptides is presented

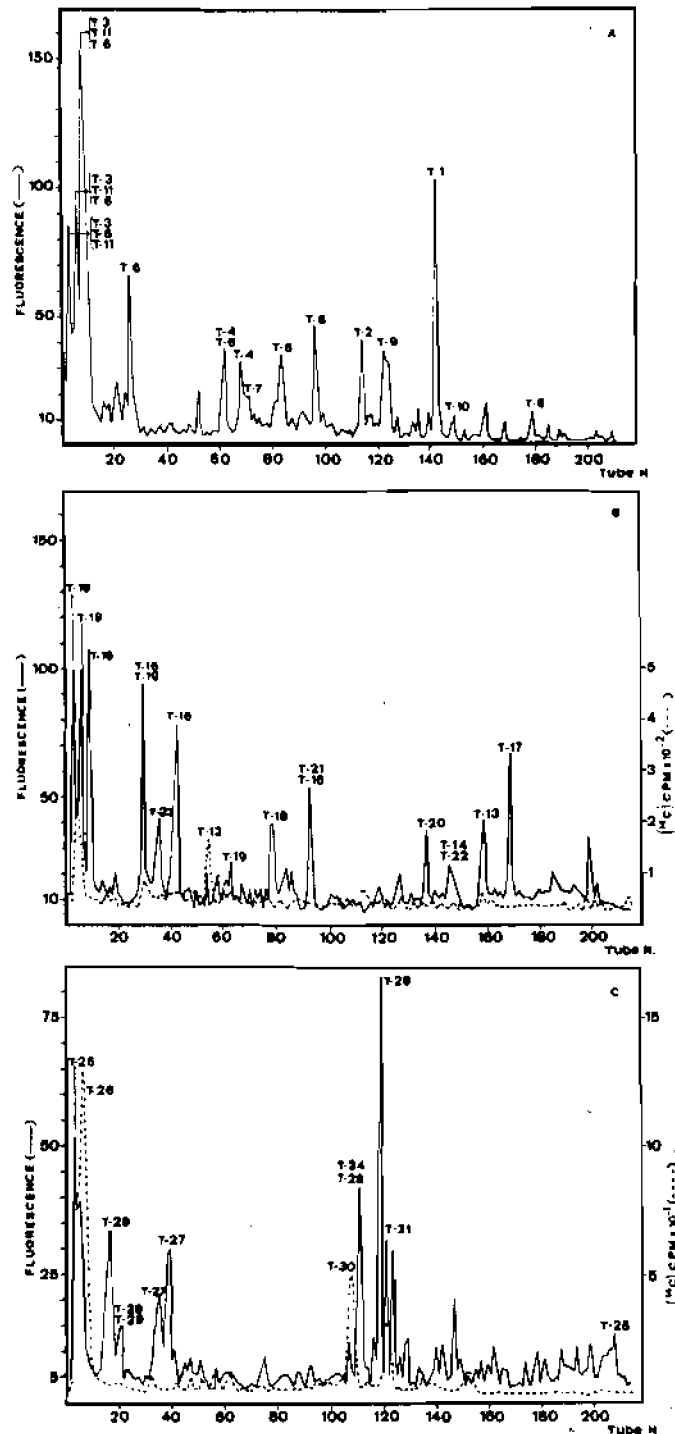


FIGURE 2: Purification of tryptic peptides. Ion-exchange chromatography of tryptic digests of fragments (A) ac-1a (h1), (B) ac-1b (h2), and (C) ac-2 (h3). A microbore column (0.2 \times 25 cm) was used following conditions described elsewhere (López de Castro et al., 1979). Fractions were monitored by fluorescamine reaction and ^{14}C radioactivity counting. Peptides identified in each peak are stated. Their numeration corresponds to the final alignment and correlates with that of Table I.

in Table I, except for T-4 and T-16. The latter was shown by sequence analysis to be contaminated with small amounts of T-3, T-6, and T-11 which obscured the interpretation of the amino acid analysis. (These peptides originated from a residual contamination of h2 by fragment h1 but were recovered in relatively high yield because they were excluded from the microbore column.) A tryptic glycopeptide, TG, was purified from a tryptic digest of the whole reduced and ^{14}C -carboxymethylated HLA-B40 heavy chain by lentil-lectin affinity chromatography. The pure peptide was eluted with

Table 1: Amino Acid Composition of HLA-B40_{pap} Heavy Chain (h) and Its Tryptic Peptides^a

amino acid	h	T-1	T-2	T-3	T-4 ^f	T-5	T-6	T-7	T-8	T-9	T-10
Asp ^b	23.6 (24)			2.2 (2)	(2)		0.8 (1)		1.0 (1)		0.9 (1)
Thr	19.3 (22/23) ^g			1.7 (2)	(1)		0.7 (0)	1.0 (1)	2.8 (3)		
Ser	12.7 (12/13) ^g			0.4 (0)	(2)		0.8 (0)	0.9 (1)		0.7 (1)	
Glu ^b	38.9 (39)	0.4 (0)	0.9 (1)	0.7 (0)		1.1 (1)	2.5 (4)	2.4 (2)	1.1 (1)	1.1 (1)	
Pro	15.0 (15)	1.0 (1)	0.8 (1)		(1)	1.1 (1)	1.6 (2)				
Gly ^c	19.0 (19)	1.1 (1)	0.8 (1)	1.2 (1)			1.8 (1)				
Ala	21.9 (21)			0.3 (0)	(1)		0.8 (1)				
Cys ^d	nd ^e (4)										
Val	11.9 (12)			2.2 (3)							
Met	2.9 (3)										
Ile	7.4 (7)			0.7 (1)			1.0 (1)	1.0 (1)			
Leu	20.0 (20)			1.0 (1)			0.5 (0)			1.0 (1)	1.0 (1)
Tyr	14.0 (14)			1.1 (1)			1.0 (1)		1.2 (1)		
Phe	6.7 (6)			1.5 (2)	(1)						
Lys	9.8 (9)					0.7 (1)		1.0 (1)			
His	10.2 (10)										
Trp	nd ^e (7)						nd ^e (2)				
Arg	26.1 (26)	1.0 (1)	1.0 (1)	1.0 (1)	(1)	1.0 (1)	1.3 (1)		1.0 (1)	0.8 (1)	1.0 (1)
res no.	1-271	15-17	18-21	22-35	36-44	45-48	49-62	63-68	69-75	76-79	80-82
amino acid	T-11	TG	T-12	T-13	T-14	T-15	T-16 ^f	T-17	T-18	T-19	T-20
Asp ^b	1.7 (1)	1.3 (1)	2.6 (2)		1.9 (2)	2.4 (3)	(1)	0.4 (0)		0.8 (0)	
Thr	1.1 (1)	1.0 (1)					(2/3) ^g			0.3 (0)	
Ser	1.7 (2)	1.5 (2)	0.4 (0)			0.6 (0)	(1/2) ^g	0.3 (0)		0.4 (0)	
Glu ^b	2.7 (3)	3.0 (3)			1.2 (1)	1.4 (1)	(2)	1.4 (1)	1.9 (2)	2.2 (3)	
Pro	0.5 (0)		1.3 (1)			0.4 (0)					
Gly ^c	1.9 (2)	2.3 (2)	2.8 (3)		2.0 (2)	1.3 (0)		1.2 (0)		1.1 (1)	0.4 (0)
Ala	1.1 (1)	1.1 (1)	0.6 (0)		1.0 (1)	1.1 (1)	(4)	1.9 (2)	1.1 (1)	1.0 (1)	
Cys ^d			nd ^e (1)							nd ^e (1)	
Val	0.5 (0)		0.9 (1)			0.3 (0)			1.0 (1)	0.7 (1)	
Met											
Ile						0.6 (1)	(1)			0.3 (0)	
Leu	1.0 (1)	1.1 (1)	0.3 (0)	1.9 (2)		1.8 (2)		1.2 (1)	1.1 (1)	1.3 (2)	
Tyr	1.3 (2)	2.0 (2)	0.7 (1)		1.9 (2)	0.6 (1)				0.9 (1)	
Phe											
Lys					0.8 (1)			0.8 (1)			
His	0.6 (1)	0.9 (1)									
Trp							(1)			nd ^e (1)	
Arg	0.5 (1)	0.8 (1)	1.0 (1)	1.0 (1)		1.0 (1)	(1)	0.7 (1)	0.8 (1)	1.0 (1)	1.0 (1)
res no.	83-97	83-97	99-108	109-111	112-121	122-131	132-145	146-151	152-157	158-169	170
amino acid	T-21	T-22	T-23	T-24	T-25	T-26	T-27	T-28	T-29	T-30	T-31
Asp ^b	0.7 (1)	0.9 (1)	1.1 (1)		0.9 (1)		3.8 (4)		0.3 (0)	0.3 (0)	
Thr					2.1 (3)	1.5 (2)	2.6 (3)	0.8 (1)	0.4 (0)	0.7 (1)	1.5 (2)
Ser				0.3 (0)	0.9 (1)	0.4 (0)			0.8 (1)		
Glu ^b	1.2 (1)	1.2 (1)		0.3 (0)	1.0 (1)	2.7 (2)	5.4 (5)	1.2 (1)	3.2 (3)	1.8 (2)	1.9 (2)
Pro				2.1 (2)	0.9 (1)	0.8 (1)	0.7 (1)	0.4 (0)	0.7 (1)	1.8 (2)	2.0 (2)
Gly ^c	1.7 (1)		1.7 (0)	0.4 (0)		1.2 (1)	1.9 (2)		1.3 (1)	1.3 (1)	0.9 (1)
Ala			1.0 (1)		0.9 (1)	2.0 (2)	1.2 (1)		2.2 (2)		
Cys ^d						nd ^e (1)				nd ^e (1)	nd ^e (1)
Val					0.9 (1)		1.1 (1)		3.2 (3)	0.9 (1)	0.9 (1)
Met											
Ile					0.9 (1)	0.8 (1)					
Leu	1.0 (1)	1.2 (1)			1.0 (1)	1.9 (2)	1.2 (1)			1.9 (2)	2.0 (2)
Tyr	0.7 (1)					1.2 (1)				0.8 (1)	0.9 (1)
Phe						0.7 (1)		1.0 (1)			
Lys	0.7 (1)	0.8 (1)		1.0 (1)				1.0 (1)		1.0 (1)	1.1 (1)
His					3.5 (4)					1.9 (2)	1.8 (2)
Trp						nd ^e (2)			nd ^e (1)		
Arg		1.0 (1)			1.0 (1)	0.9 (1)	2.1 (2)	0.4 (0)	0.7 (1)	0.4 (0)	
res no.	171-176	177-181	182-183	184-186	187-202	203-219	220-239	240-243	244-256	257-270	257-271

^a Values are given as residues per mole. In parentheses are the values determined by sequencing. ^b Aspartic and glutamic acid values include those of Asn and Gln, respectively. ^c Increased Gly values were found occasionally. It is assumed that they have originated from external contamination since no additional Gly was found by sequence analysis. ^d Determined as S-(carboxymethyl)cysteine. ^e nd, not quantitatively determined. ^f A satisfactory amino acid analysis was not obtained due to the presence of low-amount contaminants which precluded an interpretable quantitation of peptide composition (see text). ^g Indetermination of the values of Thr and Ser derives from the lack of assignment at position 143 (see text).

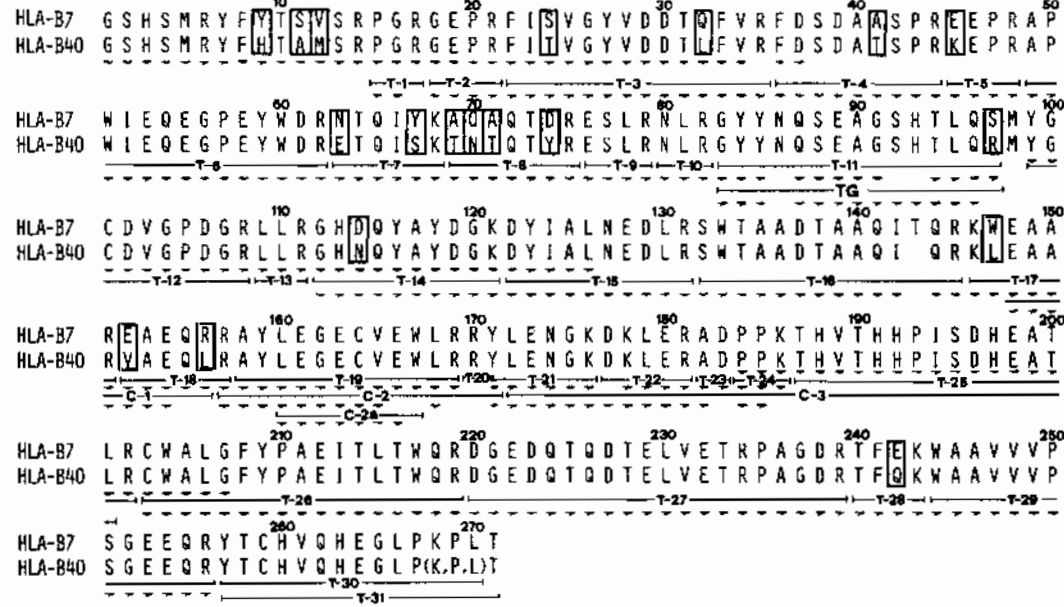


FIGURE 3. Amino acid sequence of HLA-B40_{pap} heavy chain aligned with that of HLA-B7. Tryptic (T) and chymotryptic (C) peptides used to determine the sequence are indicated. Arrows indicate residues directly identified after Edman degradation. Differences between both molecules are boxed.

α -methyl-D-mannoside and did not contain methionine or any significant amount of radioactivity. The amino acid composition of this peptide was identical with that of T-11 (Table I). Peptides T-30 and T-31 were identical except for the presence of an additional Thr residue in peptide T-31. These peptides arose from the C-terminal end of the heavy chain, and their compositions probably reflect the heterogeneity of the papain cleavage as described for HLA-B7 (López de Castro et al., 1979).

Chymotryptic peptides covering the portion of the molecule spanning residues 148–201 were purified after digestion of h23 fragment by microbore ion-exchange chromatography (data not shown). Characterization of these peptides was undertaken to provide an overlap between h2 and h3 fragments as well as to reinforce the sequence determination in the second half of h2 (residues 148–183). The area is covered by three peptides designated as C-1, C-2, and C-3 whose amino acid composition is given in Table II. Peptide C-2 (residues 158–171) was a result of incomplete chymotryptic cleavage. A fourth peptide, C-2a, spanning residues 160–167, was also purified and sequenced (see below) although its composition is not included in Table II.

NH₂-Terminal Sequence of HLA-B40 Heavy Chain and the Sequence of h1. This portion encompasses residues 1–98. Its sequence was determined by NH₂-terminal sequence analysis of intact heavy chain and by isolating and sequencing 11 tryptic peptides derived from h1 fragment and a tryptic glycopeptide obtained from a digest of the whole HLA-B40_{pap} heavy chain. Automatic Edman degradation of the intact chain was carried out for 37 cycles with a repetitive yield of 94% (Table III). Two methionine residues were identified at positions 5 and 12. This clustering resulted in heterogeneous cleavage by CNBr at the N terminus as determined by sequence analysis of the h1 fragment (data not shown). Thus, tryptic peptides purified from h1 (Figure 2A) covered the portion of the molecule from residue 14 to residue 97. The sequence of these peptides is presented in Table IV. Peptides T-1 to T-4 provided an overlap with the N-terminal run of the heavy chain. Peptides T-5 to T-11 were aligned by homology with HLA-B7 (Figure 3), thus extending the sequence up to residue 97. Positions 92 and 93 were assigned by sequencing

Table II: Amino Acid Composition of HLA-B40_{pap} Heavy-Chain Chymotryptic Peptides^a

amino acid	C-1	C-2	C-3
Asp ^b			3.8 (4)
Thr			3.0 (3)
Ser			1.6 (1)
Glu ^b	2.9 (3)	3.0 (3)	3.4 (3)
Pro			2.4 (3)
Gly ^c		1.4 (1)	1.9 (1)
Ala	2.8 (3)	1.4 (1)	2.4 (2)
Cys ^d		nd ^e (1)	
Val	1.0 (1)	1.1 (1)	1.1 (1)
Met			
Ile			1.1 (1)
Leu	1.2 (1)	1.6 (2)	2.5 (3)
Tyr		1.7 (2)	
Phe		0.3 (0)	
Lys			2.2 (3)
His			4.0 (4)
Trp			
Arg	1.1 (1)	2.6 (3)	1.3 (1)
res no.	148–156	157–171	172–201

^a Only those peptides used for sequencing are included. Values are given as residues per mole. In parentheses are the values determined by sequence analysis. ^{b–e} See footnotes in Table I.

the tryptic glycopeptide TG (Table IV). The Asn residue at position 86 was not directly identified and was assigned on the basis of the following evidence: (a) Both T-11 and TG were glycopeptides. They were identical by amino acid composition and sequence analysis (Tables I and IV). (b) They contained a single Asx residue that was not detected by sequencing. (c) Position 86 was obtained as blank in the Pth analysis of the corresponding organic phase, a result which is consistent with the retention of the glyco moiety in the aqueous phase during Pth extraction. (d) Position 86 is known to be a glycosylated Asn residue in all HLA and H-2 antigens investigated so far (Nathenson et al., 1981; López de Castro et al., 1982). The presence of a Ser residue at position 88 provides the necessary sequence (Asn-X-Ser/Thr) which is common among N-linked glycosylation sites (Hubbard & Ivatt, 1981).

Position 98 was assigned as methionine on the basis of the cleavage specificity of CNBr and on the N-terminal sequence

HLA-B40 heavy chain	1 1.8	2 b	3 1.8	4 b	5 4.5	6 2.5	7 4.0	8 5.3	9 b	10 3.3	11 3.0	12 b	13 2.0	14 1.8	15 2.8	16 1.3	17 1.0	18 b	19 1.3	20 1.3	21 1.8	22 1.8	23 b	24 2.0	25 0.8	26 1.3	27 2.0	28 2.3
	Gly-Ser-His-Ser-Met-Arg-Tyr-Phe-His-Thr-Ala-Met-Ser-Arg-Pro-Gly-Arg-Gly-Glu-Pro-Arg-Phe-Ile-Thr-Val-Gly-Tyr-Val-Asp...																											
	Asp-Thr-Leu-Phe-Val-X-Phe-Asp...																											
h23	99 0.9	100 1.0	101 2.4	102 2.3	103 1.0	104 0.8	105 0.7	106 1.8	107 0.9	108 0.9	109 1.8	110 1.1	111 0.7	112 0.7	113 0.5	114 0.3	115 0.9	116 0.5	117 1.0	118 0.8	119 0.9	120 0.2	121 0.3	122 1.1	123 0.9	124 0.2	125 0.9	126 1.3
h3	184 4	185 3	186 10	187 b	188 4	189 10	190 b	191 6	192 2	193 8	194 b	195 8	196 3	197 b	198 2	199 b	200 3	201 6	202 4	203 2	204 6	205 3	206 1	207 1	208 1	209 1	210 1	211 1
TG	83 0.9	84 0.7	85 0.6	86 0.1	87 b	88 b	89 0.1	90 0.04	91 b	92 b	93 b	94 0.06	95 0.07	96 0.07	97 0.07	98 0.07	99 0.07	100 0.07	101 0.07	102 0.07	103 0.07	104 0.07	105 0.07	106 0.07	107 0.07	108 0.07	109 0.07	110 0.07

^a Numbers under residues are yields in nanomoles of the Pth derivatives. Numbers above residues indicate their position in the HLA-B40 heavy chain sequence. Peptides sequenced to the carboxyl terminus end with -COOH. Peptides and fragments which were not sequenced completely end with (...). ^b Identified as the Pth derivative by HPLC but not quantitated.

Table IV: Sequence of Tryptic Peptides from the h1 Fragment of HLA-B40 Heavy Chain^a

T-1	¹⁵ 0.5	¹⁶ 0.5	Pro-Gly...															
T-2	¹⁸ 1	¹⁹ b	²⁰ 1	²¹ b	Gly-Glu-Pro-Arg-COOH													
T-3	²² 2	²³ 2	²⁴ b	²⁵ 1	²⁶ 0.5	²⁷ 1	²⁸ 1	²⁹ 0.5	³⁰ 0.5	³¹ b	³² b	³³ 0.2	³⁴ 0.2	Phe-Ile-Thr-Val-Gly-Tyr-Val-Asp-Asp-Thr-Leu-Phe-Val-Arg-COOH				
	³⁵ b	Arg-COOH																
T-4	³⁶ 3	³⁷ 4	³⁸ b	³⁹ b	⁴⁰ 1.5	⁴¹ b	⁴² b	⁴³ 0.3	⁴⁴ 0.2	Phe-Asp-Ser-Asp-Ala-Thr-Ser-Pro-Arg-COOH								
T-5	⁴⁵ 8	⁴⁶ b	⁴⁷ 2	⁴⁸ 1	Lys-Glu-Pro-Arg-COOH													
T-6	⁴⁹ 0.5	⁵⁰ 0.2	⁵¹ 0.2	⁵² 0.2	⁵³ b	⁵⁴ 0.3	⁵⁵ b	⁵⁶ 0.3	⁵⁷ b	⁵⁸ b	⁵⁹ 0.1	⁶⁰ b	⁶¹ b	Ala-Pro-Trp-Ile-Glu-Gln-Glu-Gly-Pro-Glu-Tyr-Trp-Asp-Arg-COOH				
	⁶² 0.05	Arg-COOH																
T-7	⁶³ b	⁶⁴ b	⁶⁵ 3	⁶⁶ 6	⁶⁷ b	⁶⁸ 3	Glu-Thr-Gln-Ile-Ser-Lys-COOH											
T-8	⁶⁹ b	⁷⁰ 2	⁷¹ b	⁷² 1	⁷³ b	⁷⁴ 2	⁷⁵ 0.6	Thr-Asn-Thr-Gln-Thr-Tyr-Arg-COOH										
T-9	⁷⁶ b	⁷⁷ b	⁷⁸ 13	⁷⁹ 7	Glu-Ser-Leu-Arg-COOH													
T-10	⁸⁰ 5	⁸¹ 9	⁸² 1	Asn-Leu-Arg-COOH														
T-11	⁸³ 2.5	⁸⁴ 6	⁸⁵ 6	⁸⁶ 1	⁸⁷ b	⁸⁸ b	⁸⁹ 3	⁹⁰ 1	⁹¹ b	⁹² 6	⁹³ 0.4	Gly-Tyr-Tyr-X-Gln-Ser-Glu-Ala-Gly-X-X-Thr-Leu-Gln-Arg-COOH						
	⁹⁷	Arg-COOH																

^{a, b} See footnotes in Table III.

of h2 fragment which indicated that this fragment starts at position 99 (Table IV). No formal overlap was obtained between h1 and h2. However, a clustering of Met residues in this area that could generate a small peptide upon CNBr treatment which would go undetected may be excluded because the amino acid composition of HLA-B40_{pap} heavy chain (Table I) shows the existence of only three Met residues in the molecule, which are accounted for by those in positions 5, 12, and 98.

Sequence of h2 and h3. Fragment h23 was subjected to automatic Edman degradation for 28 cycles with a repetitive yield of 97% (Table III). Twelve tryptic peptides, T-12-T-23, were purified from a digest of h2 fragment (Figure 2b) that

Table V: Sequence of Tryptic Peptides from h2 and h3 Fragments of HLA-B40 Heavy Chain^a

T-14

112

117

121

Gly-His-Asn-Gln-Tyr-Ala-Tyr-Asp-Gly-Lys-COOH

1

b

1

1

1

1

1

0.5

0.5

T-15

122

127

131

Asp-Tyr-Ile-Ala-Leu-Asn-Glu-Asp-Leu-Arg-COOH

4

4

2

2

3

1

b

2

1.5

0.5

T-16

132

137

Ser-Trp-Thr-Ala-Ala-Asp-Thr-Ala-Ala-Gln-Ile-X-Gln-Arg-COOH

b

b

b

0.4

0.3

0.2

b

0.04

0.1

0.1

0.1

0.05

T-17

146

151

Lys-Leu-Glu-Ala-Ala-Arg-COOH

3

4

b

2.5

2

1

T-18

152

157

Val-Ala-Glu-Gln-Leu-Arg-COOH

6

5

b

5

4

b

T-19

158

163

169

Ala-Tyr-Leu-Glu-Gly-Glu-Cys-Val-Glu-Trp-Leu-Arg-COOH

3

1

1

b

0.2

b

0.3

0.5

b

0.3

0.2

0.1

T-21

171

176

Tyr-Leu-Glu-Asn-Gly-Lys-COOH

1

1

b

b

0.2

0.1

T-22

177

181

X-Lys-Leu-Glu-Arg-COOH

1.5

2

b

0.4

T-23

182

183

Ala-Asp-COOH

9

4

T-24

184

186

Pro-Pro-Lys-COOH

9

2.5

2

T-26

203

208

213

Cys-Trp-Ala-Leu-Gly-Phe-Tyr-Pro-Ala-Glu-Ile-Thr-Leu-Thr-Trp-Gln-Arg-COOH

b

1

3

1

b

2

2

1

b

1

b

1

T-27

220

225

230

Asp-Gly-Glu-Asp-Gln-Thr-Gln-Asp-Thr-Glu-Leu-Val-Glu-Thr-Arg-Pro-Ala-Gly-Asp-Arg-COOH

12

2

b

5

1

b

1

3

b

b

1

1

b

T-28

240

243

Thr-Phe-Gln-Lys-COOH

b

14

4

3

T-29

244

249

Trp-Ala-Ala-Val-Val-Val-Pro-Ser-Gly-Glu-Glu-Gln-Arg-COOH

13

16

16

9

9

7.5

3

b

2

b

b

1

T-31

257

262

Tyr-Thr-Cys-His-Val-Gln-His-Glu-Gly-Leu-Pro...

6

b

b

1

0.5

0.3

0.6

b

0.1

0.1

0.1

^{a, b} See footnotes in Table III.

covered the area of the heavy chain spanning residues 99-183 (Table I). Peptides T-12 and T-13 corresponded to the N-

Table VI: Sequence of Chymotryptic Peptides from the h23 Fragment of HLA-B40 Heavy Chain^a

C-1	¹⁴⁸ Glu-Ala-Ala-Arg-Val-Ala-Glu... _{b 1 1.5 0.5 0.6 0.3 b}
C-2	¹⁵⁷ Arg-Ala-Tyr-Leu-Glu-Gly-Glu-Cys-Val-X-X-Leu-X-X- _{b 0.3 0.3 0.2 b 0.1 b b 0.2 0.1} ¹⁷¹ Tyr-COOH _{0.04}
C2a	¹⁶⁰ Leu-Glu-Gly-Glu-Cys-Val-Glu... _{1.5 b 0.6 b b 0.1 b}
C-3	¹⁷² Leu-Glu-Asn-Gly-Lys-Asp-Lys-Leu-Glu-Arg-Ala-X- _{0.4 b 0.1 0.2 0.2 0.2 0.1 0.1 b 0.1 0.1} ¹⁸⁵ Pro-Pro... _{0.05 0.05}

^{a,b} See footnotes in Table III.

terminal part of h2 and were not sequenced. The amino acid sequence of peptides T-14-T-23 is shown in Table V. Alignment of peptides T-12-T-15 was done by overlapping with the N-terminal sequencing of h23 and allowed extension of the sequence up to residue 131. T-16 was aligned by homology with HLA-B7 (Figure 3). This peptide was sequenced to the end, but at cycle 12 (which corresponds to position 143), a blank was consistently obtained when the corresponding Pth was analyzed in both the organic and the aqueous phases. This fact probably reflects technical difficulties in detecting Pth-Thr or Pth-Ser when small amounts of peptide material are sequenced. Thus, this position was not assigned. However, in all other HLA and H-2 antigens which have been sequenced, it is a Thr residue. T-16 extended the sequence up to residue 145. Peptides T-16-T-23 were formally aligned by isolating and sequencing three overlapping chymotryptic peptides from a digest of h23 fragment (Table VI and Figure 3), which completed the sequence up to residue 183. Position 170 was assigned based on the identification of T-20 as free arginine (Table I) and on the amino acid composition of C-2 (Table II). This is the only region of h2 (and of the whole molecule) with an Arg-Arg sequence.

Automatic Edman degradation of fragment h3 was carried out for 24 cycles with a repetitive yield of 96% (Table III). As expected from the generation of h23 fragment by selective acid hydrolysis, proline was the N-terminal residue. Formal overlap between h2 and h3 fragments was obtained through amino-terminal sequencing of peptide C-3 (Table VI). Completion of the sequence up to the papain cleavage point was accomplished by isolating eight tryptic peptides from h3 fragment (Figure 2C). The sequence of these peptides is presented in Table V, except for T-25 which was shown by amino acid composition (Table II) to correspond to an area sequenced from the N terminus of h3 (Figure 3). Peptides T-24-T-26 were aligned by overlap with the N-terminal sequence of h3 fragment. Peptides T-27-T-31 were aligned by homology with HLA-B7 (Figure 3). As mentioned above, T-30 and T-31 were identical in their amino acid compositions except for the presence of an extra Thr residue in T-31. This fact reflects heterogeneity at the papain cleavage point, as in HLA-B7 (López de Castro et al., 1979), and establishes the extra Thr residue as the C-terminal residue of HLA-B40_{pap} heavy chain, at position 271.

Figure 3 shows the proposed amino acid sequence of papain-solubilized HLA-B40 heavy chain along with the peptides used in its determination. The sequence of HLA-B7, that was used for alignment of several HLA-B40 peptides, is also

presented. The number of residues, the position of the single glycosylation site, and the disposition of the disulfide bonds are the same in both molecules.

Discussion

The nature of HLA polymorphism has attracted the attention of immunologists since it was realized that histocompatibility antigens play a role as markers of self and as essential units in MHC-restricted immune recognition. The need for a molecular understanding of this polymorphism stems from the assumption that it may enlighten the mechanisms of associative recognition of foreign antigens by T-cell receptors (Zinkernagel & Doherty, 1979). Thus, much effort is being devoted to defining the number and arrangement of polymorphic sites in HLA and H-2 molecules. An approach to this problem is by means of comparison of the amino acid sequences of cross-reactive antigens. Figure 3 shows an alignment of the HLA-B40 sequence with that of HLA-B7. It provides the first comparison of two HLA-B locus products. There are 19 differences between both molecules, which means an overall homology of 93%. This number of differences is somewhat high, considering the degree of antigenic cross-reactivity of HLA-B40 and HLA-B7. By comparison, 10 differences were found between the cross-reactive HLA-A2 and -A28 antigens (96% homology) López de Castro et al., 1982). Thus, conventional allosera used in tissue typing may not generally differentiate between antigens with much higher homology. Therefore, the actual polymorphism of class I antigens may be significantly greater than that established by serological analysis. That this may, indeed, be the case is also suggested by analysis of the reactivity of specific, HLA-A2-restricted immune CTL's against a panel of influenza virus infected HLA-A2 target cells as well as of allogenic HLA-A2-directed CTL's (Biddison et al., 1980, 1982). In these studies, some -A2 cells are killed much less efficiently, suggesting the existence of structural differences between various HLA-A2 antigens. Comparative biochemical analyses of some of these variants have shown that they may differ by as little as two positions in their amino acid sequence (Kraegel et al., 1982, 1983).

The distribution of differences between HLA-B40 and HLA-B7 follows a characteristic pattern (Figure 3). As many as 13 differences (68%) occur in $\alpha 1$. There are five differences in $\alpha 2$ (26%) and only one difference in $\alpha 3$ (5%). This pattern of distribution is analogous to that observed when HLA-A2 and -A28 sequences are compared, where 60% of the substitutions are located in $\alpha 1$ and 30% in $\alpha 2$ (López de Castro et al., 1982). Whether or not this feature reflects intrinsic differences in the diversification rate of $\alpha 1$ and $\alpha 2$ domains must await further structural information. Thus, polymorphism is essentially restricted to the amino-terminal two-thirds of the extracellular portion of the heavy chain as established for other HLA (López de Castro et al., 1982) and H-2 (Kimball & Coligan, 1983) class I molecules. Within this region, the most conspicuous clustering of variability involves the accumulation of six differences between residues 66 and 74, where homology drops to 50%. Previous structural comparisons suggest that this area may constitute the segment of greatest diversity in class I antigens (Kimball & Coligan, 1983), and it is probably an essential portion of the alloantigenic determinants of HLA-A2 and HLA-A28 (López de Castro et al., 1982). Serological analyses with allospecific monoclonal antibodies also suggest that this region (or a closely related one) may constitute an alloantigenic epitope of H-2K^b antigen (Hämmerling et al., 1982). Although the relatively high number of differences between HLA-B40 and -B7 makes

directly responsible for their respective allospecificities, it seems likely that this segment may be an integral part of some HLA-B40 and -B7 allodeterminants.

Three of five differences in the $\alpha 2$ region correspond to positions 147, 152, and 156. These positions lie within an area of variability which was loosely outlined on the basis of sequence comparisons between HLA-B7, -A2, and -A28 (López de Castro et al., 1982). Interestingly, they may be included in the only α -helical segment of the molecule, spanning residues around 146–160, as estimated by prediction analyses, so that at least positions 152 and 156 would be brought into close proximity by the helical turn (M. A. Vega and J. A. López de Castro, unpublished results). Prominent variability in this area exists also in H-2 antigens (Kimball & Coligan, 1983). Some recent findings suggest that it may be a significant functional site. For instance, an H-2K^b mutant, bm1, detected by its capacity of eliciting graft rejection in H-2K^b recipients (Nairn et al., 1980), has been shown to differ from normal H-2K^b molecules in two substitutions at positions 155 and 156, which suggests that these positions may be important for CTL recognition. Mutations at these two positions also produce a drastic effect on the binding capacity of some allospecific anti-H-2K^b monoclonal antibodies, which has been interpreted as an indication that these positions contribute to an H-2K^b allospecific determinant (Hämmerling et al., 1982). At least two HLA-A2 variants which are distinguishable by alloimmune cytotoxic T lymphocytes have been shown to differ from the known HLA-A2 sequence in several positions within the segment 147–157, suggesting that residues in it may play an essential role in forming a recognition site for immune CTL's (Kraegel et al., 1982, 1983). The chemical nature of residues at positions 152 and 156 strongly suggests that they may play a significant role in determining the antigenic differences between HLA-B7 and HLA-B40, since Glu-152 and Arg-156 in HLA-B7 are changed to valine and leucine, respectively, in HLA-B40. These substitutions induce a drastic increase in the local hydrophobicity of the polypeptide chain in this area. Such a change is all the more remarkable since the remaining differences do not significantly alter the relative local hydrophobicity of both molecules (M. A. Vega and J. A. López de Castro, unpublished results). Binding inhibition experiments with a set of anti-H-2K^b monoclonal antibodies indicate that this region of the molecule forms an antigenic epitope which is spatially separated from the polymorphic segment including residues 60–80 (Hämmerling et al., 1982). Thus, it seems likely that HLA-B7 and HLA-B40 antigens may differ in at least two antigenic determinants located in separate sites of the three-dimensional molecule. In addition, 7 substitutions exist between both molecules distributed through the amino-terminal 45 residues (Figure 3). In the absence of a three-dimensional model, their spatial relation to the above-mentioned polymorphic sites cannot be established nor may it be unambiguously decided if they contribute to the alloantigenic sites or instead are responsible for some of the cross-reactive ("public") determinants which characterize the antigenic reactivity of histocompatibility antigens. That this may be the case, at least for some of the substitutions, is suggested, however, by a consideration of the structural relationship of HLA-B7 and -B40 with other HLA class I antigens in the vicinity of these difference. For instance, there are 3 differences between HLA-B7 and HLA-B40 at positions 9, 11, and 12, but the sequence of HLA-B7 is identical with that of HLA-A28 at the N-terminal 23 residues (López de Castro et al., 1982). Thus, putative antibodies distinguishing

between -B7 and -B40 on the basis of these substitutions would not probably differentiate between -B7 and -A28. Differences at positions 24, 41, and 45 (Figure 3) lie within short polymorphic segments described in H-2 molecules (Nathenson et al., 1981). Position 24 appears to be highly polymorphic in murine class I antigens, and it might integrate a recognition site as suggested by the nature of the H-2K^b mutant bm8 which differs from the parental molecule by a substitution at position 23. Still, bm8 and normal H-2K^b individuals vigorously reject exchanged grafts (Nairn et al., 1980).

In conclusion, the data discussed above show that a molecular analysis of HLA polymorphism by means of sequence comparisons of cross-reactive antigens may provide valuable information concerning the nature and topography of polymorphic sites and may be helpful in interpreting the complexities of HLA serology. Structural research, in conjunction with a number of other experimental approaches, including serological studies with monoclonal antibodies, biochemical characterization of mutants, and genetic analysis, will be instrumental in the identification of functional regions and in clarifying the molecular mechanisms of HLA recognition by T lymphocytes.

Acknowledgments

The excellent technical assistance of Bill Lane, Anastasia Khaykova, and Marylou Coe is gratefully acknowledged. We also thank Dr. F. Ortiz for critical comments and Dr. D. Andrews for assistance.

Registry No. Antigen HLA-B40, 86025-68-5.

References

- Albert, E., Amos, D. B., Bodmer, W. F., Ceppellini, R., Dausset, J., Kissmeyer-Nielsen, F., Mayr, W., Pyne, R., van Rood, J. J., Terasaki, P. I., & Walford, R. L. (1980) *Bull. W. H. O.* 58, 945–948.
- Biddison, W. E., Ward, F. E., Shearer, G. M., & Shaw, S. (1980) *J. Immunol.* 124, 548–552.
- Biddison, W. E., Kostyu, D. D., Strominger, J. L., & Kraegel, M. S. (1982) *J. Immunol.* 129, 730–734.
- Dawkins, R. L., & Houlston, J. B. (1980) in *Histocompatibility Testing 1980* (Terasaki, P. I., Ed.) pp 456–457, UCLA Tissue Typing Lab., Los Angeles, CA.
- Dickmeiss, E., Soeborg, B., & Svejgaard, A. (1977) *Nature (London)* 270, 526–528.
- Goodfellow, P. N., Jones, E. A., van Heyningen, V., Solomon, E., Bobrow, M., Miggiano, V., & Bodmer, W. F. (1975) *Nature (London)* 254, 267–269.
- Grey, M. M., Kubo, R. T., Colon, S. M., Poulik, M. D., Creswell, P., Springer, T. A., Turner, M., & Strominger, J. L. (1973) *J. Exp. Med.* 138, 1608–1612.
- Gross, E. (1967) *Methods Enzymol.* 11, 238–255.
- Hämmerling, G. J., Rüsche, E., Tada, N., Kimura, S., & Hämmerling, U. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 4737–4741.
- Hubbard, S. C., & Ivatt, R. J. (1981) *Annu. Rev. Biochem.* 50, 555–583.
- Kimball, E. S., & Coligan, J. E. (1983) *Contemp. Top. Mol. Immunol.* (in press).
- Kraegel, M. S., Taketani, S., Biddison, W. E., Strong, D. M., & Strominger, J. L. (1982) *Biochemistry* 21, 6313–6321.
- Kraegel, M. S., Biddison, W. E., & Strominger, J. L. (1983) *J. Immunol.* 130, 1856–1862.
- Laemmli, U. K. (1970) *Nature (London)* 227, 680–685.
- López de Castro, J. A., Orr, H. T., Robb, R. J., Kostyk, T. G., Mann, D. L., & Strominger, J. L. (1979) *Biochemistry* 18, 5704–5711.

- López de Castro, J. A., Strominger, J. L., Strong, D. M., & Orr, H. T. (1982) *Proc. Natl. Acad. Sci. U.S.A.* 79, 3813-3817.
- McMichael, A. J., Ting, A., Zweerink, H. J., & Askonas, B. A. (1977) *Nature (London)* 270, 524-526.
- Nairn, R., Yamaga, K., & Nathenson, S. G. (1980) *Annu. Rev. Genet.* 14, 241-277.
- Nakai, N., Lay, C. Y., & Horecker, B. L. (1974) *Anal. Biochem.* 58, 563-570.
- Nathenson, S. G., Uehara, H., Ewenstein, B. M., Kindt, T. J., & Coligan, J. E. (1981) *Annu. Rev. Biochem.* 50, 1025-1052.
- Orr, H. T., López de Castro, J. A., Lancet, D., & Strominger, J. L. (1979) *Biochemistry* 18, 5711-5720.
- Parham, P., Alpert, B. N., Orr, H. T., & Strominger, J. L. (1977) *J. Biol. Chem.* 252, 7555-7567.
- Sanderson, A., & Batchelor, J. R. (1968) *Nature (London)* 219, 184-186.
- Springer, T. A., & Strominger, J. L. (1976) *Proc. Natl. Acad. Sci. U.S.A.* 73, 2481-2485.
- Springer, T. A., Strominger, J. L., & Mann, D. L. (1974) *Proc. Natl. Acad. Sci. U.S.A.* 71, 1539-1543.
- Strominger, J. L., Engelhard, V. H., Fucks, A., Guild, B. C., Hyafil, F., Kaufman, J. F., Korman, A. J., Kostyk, T. G., Krangel, M. S., Lancet, D., López de Castro, J. A., Mann, D. L., Orr, H. T., Parham, P. R., Parker, K. C., Ploegh, H. L., Pober, J. S., Robb, R. J., & Shackelford, D. A. (1980) in *The Role of the Major Histocompatibility Complex in Immunobiology* (Dorf, M. E., Ed.) pp 115-171, Garland, New York.
- Trägård, L., Curman, B., Wiman, K., Rask, L., & Peterson, P. A. (1979) *Biochemistry* 18, 2218-2226.
- Zinkernagel, R., & Doherty, P. C. (1979) *Adv. Immunol.* 27, 52-142.

Lectin Receptors on the Plasma Membrane of Soybean Cells. Binding and Lateral Diffusion of Lectins[†]

Thomas N. Metcalf, III, John L. Wang,* Karel R. Schubert, and Melvin Schindler

ABSTRACT: Protoplasts prepared from suspension cultures of root cells of *Glycine max* (SB-1 cell line) bound soybean agglutinin (SBA), concanavalin A (Con A), and wheat germ agglutinin (WGA). Binding studies carried out with ¹²⁵I-labeled SBA, Con A, and WGA showed that these interactions were saturable and specific. Fluorescence microscopy demonstrated uniform membrane labeling. The mobility of the lectin-receptor complexes was measured by fluorescence redistribution after photobleaching. The diffusion constants (*D*)

for SBA and Con A were 5×10^{-11} and 7×10^{-11} cm²/s, respectively. In contrast, WGA yielded a diffusion constant of 3×10^{-10} cm²/s. Pretreatment of the protoplasts with either SBA or Con A resulted in a 6-fold reduction in the mobility of WGA ($D \approx 5 \times 10^{-11}$ cm²/s). The results suggest that the binding of SBA or Con A may lead to alterations of the soybean plasma membrane which, in turn, may restrict the mobility of other receptors.

The measurement of the dynamics of plasma membrane receptors has catalyzed research into the role of lateral mobility as an important component of transmembrane signaling mechanisms (Cherry, 1979; Edidin, 1981; Peters, 1981). From these investigations, two different schemes have emerged for the motion of membrane components: (a) lateral mobility as a consequence of Brownian movement (Frye & Edidin, 1970; Saffman & Delbruck, 1975; Schlessinger et al., 1976) and (b) directional flow on the cell surface (Taylor et al., 1971; Koppel et al., 1982). Lateral mobility based on diffusional fluxes in a two-dimensional continuum has been characterized for a great number of membrane proteins (Peters, 1981), and to a lesser extent for a few varieties of lipid (Peters, 1981) and glycolipid (Wolf et al., 1977; Schindler et al., 1980b). In general, these measurements have been performed by using the technique of fluorescence redistribution after photobleaching (FRAP; Koppel, 1979; Peters, 1981) and have

yielded values of from 10^{-8} - 10^{-12} cm²/s for membrane proteins to 10^{-8} - 10^{-9} cm²/s for phospholipids and glycolipids in the same membranes. Of particular interest was the observation that a particular diffusing species of protein could have an immobile component (Peters, 1981). A number of theories have been presented to explain this type of protein mobility in the context of intraplasma membrane and cytoskeletal interactions with the diffusing molecule (Edelman, 1976; Schindler et al., 1980b; Koppel et al., 1981).

The other type of movement for cell membrane components observed was a directional flow ultimately leading to cap formation on lymphoid cells (Taylor et al., 1971) and the movement of a concanavalin A (Con A)-receptor complex during late anaphase or telophase to the developing cleavage furrow in J7742 mouse macrophages (Koppel et al., 1982). This type of protein mobility has been ascribed to cross-linking of membrane receptors that normally occurs only when exogenously added ligands bind to the receptors. One receptor movement mechanism need not exclude the other, since receptors cross-linked by specific ligands and undergoing directed

[†] From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48824. Received January 12, 1983. This work was supported by Grant PCM-8011736 from the National Science Foundation, by Grant GM-30158 from the National Institutes of Health, and by the Michigan Agricultural Experiment Station (Project No. 1276H). J.L.W. was supported by Faculty Research Award FRA-221 from the American Cancer Society. Publication No. 10849 from the Michigan Agricultural Experiment Station.

¹ Abbreviations: SBA, soybean agglutinin; Con A, concanavalin A; WGA, wheat germ agglutinin; FRAP, fluorescence redistribution after photobleaching; MGB, modified Gamborg buffer; MGB-N₂-BSA, modified Gamborg buffer containing 0.01 M NaN₃ and 0.2% (w/v) bovine serum albumin (BSA).