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N-Terminal Amino Acid Sequences of the α and β Chains of HLA-DR1 and HLA-DR2 Antigens†

Leslie E. Walker,* Rodney Hewick, Michael W. Hunkapiller, Leroy E. Hood, William J. Dreyer, and Ralph A. Reisfeld

ABSTRACT: The N-terminal amino acid sequences of the α and β chains of HLA-DR1 and HLA-DR2 antigens were obtained with subnanomole quantities of material by using a gas–liquid solid-phase sequencer. A comparison of the N-terminal amino acid sequences of HLA-DR1 and HLA-DR2 α chains revealed no differences. However, in the first 35 N-terminal residues of the β chains from HLA-DR1 and HLA-DR2 antigens, two regions of variability are readily apparent, each comprising about six amino acids. Conceivably one or both of these variability regions may be responsible for the serologically defined polymorphism of HLA-DR alloantigens.

HLA-DR antigens constitute a set of highly polymorphic glycoproteins which are expressed mainly by B lymphocytes and have been implicated as functional components in graft rejection and cell–cell interactions between T and B cells necessary for immune responsiveness [for a recent review, see Amos & Kostyu (1980)]. These antigens are composed to two chains designated α and β with molecular weights of 34 000 and 29 000, respectively. Peptide maps of the β chains indicate that they express the majority of the detectable structural polymorphism whereas the α chains appear to be more conserved in structure (Silver and Ferrone, 1979; Walker et al., 1980). Recently, Kratzin et al. (1981) reported the amino acid sequence of the β chains of HLA-DR2 antigen and demonstrated the existence of a family of seven such chains whose structures were highly homologous except for one small region of five amino acids located between residues 65 and 69. In this report, we present and compare the amino acid sequences of the N-terminal 71 residues of the HLA-DR1 α chain, the N-terminal 40 residues of the HLA-DR2 α chain, and the N-terminal 35 amino acids of HLA-DR1 and HLA-DR2 β chains.

Materials and Methods

Cells. The homozygous B lymphoid cell lines LG-2 (HLA-DR1) and GM3107 (HLA-DR2) were cultured in RPMI 1640 media supplemented with 10% fetal calf serum and 25 μg/mL gentamycin.

Isolation of HLA-DR Antigens. The procedure used to isolate HLA-DR antigens has been described in detail in a separate report (Walker & Reisfeld, 1982). Briefly, 10 g of LG-2 or GM3107 cells was extracted with 20 mL of 0.01 M Tris buffer, pH 8.0, containing 1% Renex-30 (Accurate Chemical Co., Hicksville, NY) and 0.5 mM phenylmethanesulfonyl fluoride. Following centrifugation (30 min at 15000 *g*), the supernatant was applied to a 10-mL column of *Lens culinaris* lectin–Sepharose at a flow rate of 5 mL/h. After thorough washing of the column to remove unbound

† From the Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037 (L.E.W. and R.A.R.), and the Division of Biology, California Institute of Technology, Pasadena, California 91125 (R.H., M.W.H., L.E.H., and W.J.D.). *Received May 12, 1982.* This work was supported by Grant AI-10180 from the National Institutes of Health. This is Publication No. 2805 from Scripps Clinic and Research Foundation, La Jolla, CA 92037.

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proteins, glycoproteins were eluted by equilibration with buffer [0.1 M Tris–(pH 8.0)–0.15 M NaCl–0.1% Renex 30) containing 5% methyl α-mannoside. Eluted glycoproteins (10–12 mg) were concentrated by ultrafiltration on an Amicon YM-10 membrane to a concentration of 3 mg/mL, adjusted to 1% $NaDodSO_4$, and heated to 100 °C for ~2 min. The denatured glycoprotein solution was applied at a flow rate of 0.5 mL/min to a TSK3000SW HPLC gel filtration column equilibrated in 0.1 M sodium phosphate buffer, pH 7.0, containing 0.05% $NaDodSO_4$. Fractions containing α and β chains, as determined by $NaDodSO_4$–polyacrylamide gel electrophoresis, were pooled and applied to a 0.5 cm × 20 cm column of hydroxyapatite equilibrated at 37 °C with 0.01 M sodium phosphate buffer, pH 6.8, containing 0.1% $NaDodSO_4$ and 0.5 mM dithiothreitol (DTT), similar to the procedure described by Freed (1980). Bound proteins were eluted with a 300-mL linear gradient of 0.1 M sodium phosphate to 0.5 M sodium phosphate buffers, pH 6.8, each containing 0.1% $NaDodSO_4$ and 0.5 mM DTT. The fractions containing α or β chains were selected by $NaDodSO_4$–polyacrylamide gel electrophoresis and pooled. Following reduction with DTT and carboxamidomethylation with [^{14}C]iodoacetamide, final purification of the two polypeptide chains was by high-pressure liquid chromatography of each chain on the TSK3000SW column. The final yield of the sequencable HLA-DR1 and HLA-DR2 α and β chain was 4 nmol of each chain.

Amino Acid Sequence Analysis. Sequence analysis was performed as described (Hewick et al., 1981) in a gas–liquid solid-phase sequenator. Between 0.5 and 1 nmol of each HLA-DR polypeptide chain was used per analysis, and each sequence was confirmed by a duplicate analysis. Identification of PTH-amino acids was by high-pressure liquid chromatography as previously described (Hunkapiller & Hood, 1980).

Tryptophan Cleavage and Isolation of Tryptophan Peptides. The α chain (2 nmol) of HLA-DR1 antigen, containing a trace amount of [^{3}H]Leu-labeled α chain, was cleaved at its tryptophan residues by treatment with iodosobenzoic acid as previously described (Mahoney & Hermodson, 1979). The resulting peptides were purified by high-pressure liquid chromatography on a Bio-Rad ODS-5S column (4.6 mm × 250 mm) with a linear gradient of 0.1 M H_3PO_4–6 M urea to 0.02 M H_3PO_4 containing 1.2 M urea and 80% acetonitrile over a period of 90 min. Fractions were collected at 1-min intervals and positions of the peptides determined by counting aliquots of each fraction. Fractions containing the peptides were pooled and desalted by HPLC on a TSK3000SW gel exlusion column equilibrated with 0.05 M ammonium formate buffer, pH 6.8, containing 0.05% $NaDodSO_4$ and 0.5 mM DTT. The resulting eluate fractions containing a peak of radioactivity were lyophilized. Immediately prior to sequence analysis each peptide was dissolved in 88% formic acid.

Results

Isolation of HLA-DR α and β Chains. The purity of the isolated α and β chains of HLA-DR1 and HLA-DR2 antigens was determined by $NaDodSO_4$–polyacrylamide gel electrophoretic analysis and is shown in Figure 1. Both sets of α and β chains were practically homogeneous as to molecular weights. The isolated β chains of both HLA-DR1 and HLA-DR2 antigens contain a minor contaminant that migrates with an apparent molecular weight of 50 000 on $NaDodSO_4$–polyacrylamide gel electrophoresis. However, this component is probably a β-chain dimer since monoclonal antibodies that are known to react only with the β chain of HLA-DR antigen bind to this component and to monomer β chains by Western blotting techniques (data not shown).

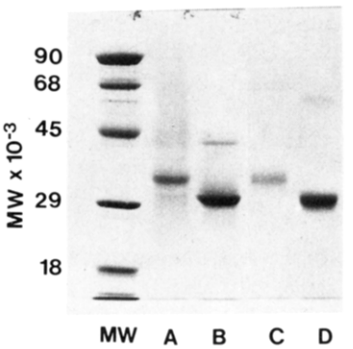

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoretic profile of purified HLA-DR1 aad HLA-DR2 α and β chains. The α and β chains of HLA-DR1 and HLA-DR2 antigens were purified as described under Materials and Methods and analyzed by $NaDodSO_4$–polyacrylamide gel electrophoresis on a 10% polyacrylamide slab gel. Proteins were visualized by Coomassie blue staining. The molecular weight standards are phosphorylase *b* (90 000), bovine serum albumin (68 000), ovalbumin (45 000), carbonic anhydrase (29 000), and myoglobulin (18 000). Lane A represents the α chain (5 μg) of HLA-DR1 antigen; lane B shows the β chain (5 μg) of HLA-DR1 antigen; lane C indicates the α chain (2.5 μg) of HLA-DR2 antigen; lane D depicts the β chain (5 μg) of HLA-DR2 antigen.

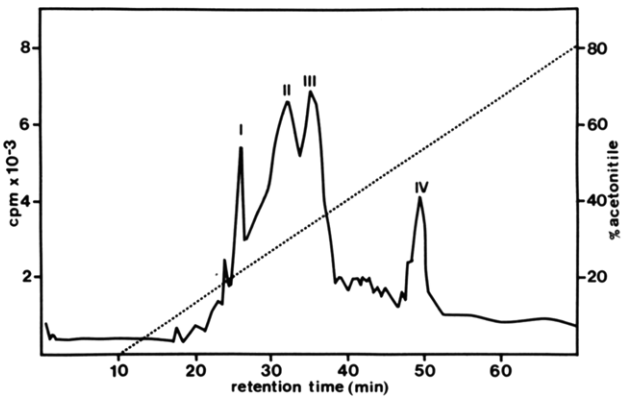

FIGURE 2: Separation of HLA-DR1 α chain peptides following digestion with iodosobenzoic acid. The peptides were subjected to high-pressure liquid chromatography on a Bio-Rad ODS column as described in the text. Aliquots (10 μL) of each fraction were counted to determine the positions of the peptides.

Furthermore, this component has too low a molecular weight to represent an intact HLA-DR chain complex of M_r ~60 000. The yield of α and β chains isolated from HLA-DR1 and HLA-DR2 antigens was 4 nmol/chain. The isolated α and β chains were found to be more than 90% homogeneous, as determined by $NaDodSO_4$–polyacrylamide gel electrophoresis and subsequent scanning of the gels.

Isolation of Peptides following Cleavage with Iodosobenzoic Acid. Shown in Figure 2 is the separation of the HLA-DR1 α chain tryptophan peptides. Four major [^{3}H]leucine-containing peaks were detected. Peak I consists most likely of undigested material since it has a molecular weight of about 34 000 as determined by gel filtration on a TSK3000SW HPLC gel exclusion column. Both peaks II and III have molecular weights of approximately 12 000–14 000 whereas peak IV is smaller than 5000. Peak II when subjected to amino acid sequence analysis was found to be a peptide whose N terminus begins at residue 43 of the HLA-DR1 α chain. Approximately 200 pmol of this peptide was recovered. Peaks III and IV were isolated in yields too low for any significant amino acid sequence determination.

N-Terminal Amino Acid Sequence of HLA-DR1 and HLA-DR2 α Chains. Figure 3 shows the N-terminal amino acid sequences of the α chains of HLA-DR1 and HLA-DR2

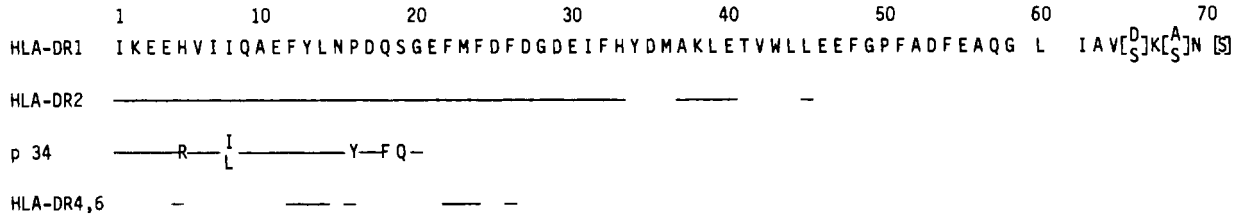

FIGURE 3: Comparison of the N-terminal amino acid sequences of HLA-DR1 and HLA-DR2 α chains. The HLA-DR1 α chain amino acid sequence was obtained with 700 pmol of protein and the HLA-DR2 α chain amino acid sequence from 800 pmol of protein. Included in the amino acid sequence of the HLA-DR1 α chain is the N-terminal sequence of peptide II obtained by tryptophan cleavage. Regions of homology are shown by a solid line. The p34 sequence is by Springer et al. (1978). The α chain of HLA-DR4,w6 antigen is by Allison et al. (1978). Brackets indicate some degree of uncertainty in the assignment of an amino acid residue.

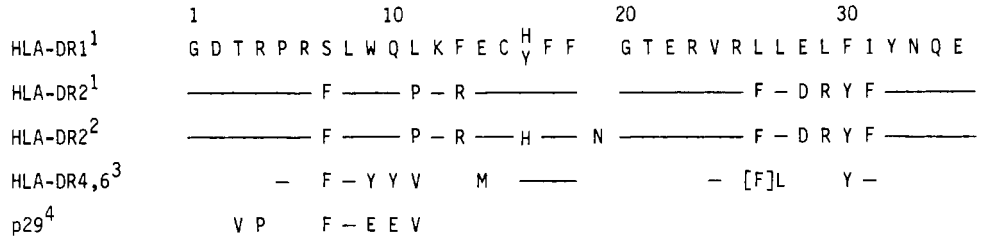

FIGURE 4: Comparison of the N-terminal amino acid sequences of HLA-DR1 and HLA-DR2 β chains. The HLA-DR1 β chain amino acid sequence was obtained from 500 pmol of protein and the HLA-DR2 β chain sequence from 1 nmol of protein. The sequences shown are the following: (1) from this study, (2) from the sequence of a HLA-DR2 antigen isolated from a homogeneous cell (HLA-A3,3, -B7,7, -DR2,2) reported by Kratzin et al. (1981); (3) the β-chain amino acid sequence of HLA-DR4,w6 antigen isolated from the cell line Victor (Allison et all, 1978), and (4) p29 antigen (β chain) isolated from the cell line JY (HLA-DR4,6) (Springer et al., 1978). Brackets indicate some degree of uncertainty in the assignment of an amino acid residue.

antigens and compares them with the published sequences of the α chain (p34 antigen) of Springer et al. (1978) and with the HLA-DR4,6 antigen α chain (Allison et al., 1978). The HLA-DR1 α chain sequence was extended from residue 47 by analysis of tryptophan cleavage peptide II. The α chains of HLA-DR1, HLA-DR2, and HLA-DR4,6 antigens are all homologous at each residue that can be compared. However, p34 antigen, the α chain of human Ia antigen isolated from the heterozygous JY cell line (HLA-DR4,w6), contains four distinct differences when compared to the amino acid sequences of the other three HLA-DR α chains.

N-Terminal Amino Acid Sequence of HLA-DR1 and HLA-DR2 β Chains. The N-terminal amino acid sequences of the β chains from HLA-DR1 and HLA-DR2 antigens are depicted in Figure 4. Included in this figure for comparison are the N-terminal sequences of a HLA-DR2 β chain isolated from a different cell line (Kratzin et al., 1981), the β chain from HLA-DR4,6 antigen (Allison et al., 1978), and the β chain isolated from JY cells, i.e., the p29 antigen described by Springer et al. (1978). In contrast to the α-chain sequences, a comparison of the sequences of the β chains clearly indicates a high degree of structural polymorphism. The first 35 amino acids contain two regions of high variability comprised of residues 7–13 and 26–31. It is noteworthy that residue 16 of the HLA-DR1, HLA-DR2, and HLA-DR4,6 β chain contains equimolar amounts of histidine and tyrosine. Position 19 in the HLA-DR1 and HLA-DR2 β chains could not be identified. This residue is most likely asparagine and the attachment site of a carbohydrate moiety analogous to the HLA-DR2 β chain position determined by Kratzin et al. (1981).

Discussion

The N-terminal amino acid sequences determined by us were all obtained by sequence analysis in a gas–liquid solid-phase sequenator using very limited quantities (less than 1 nmol) of protein. This sequenator is unique in that extended amino acid sequence analysis can be performed on subnanomole quantities of polypeptides with high repetitive yields and without covalent attachment of the sample to the support phase. The sensitivity of the sequenator is demonstrated by the fact that in a typical analysis of 700 pmol of HLA-DR2 α chain and 500 pmol of β chain, 49 and 35 amino acid residues were identified, respectively.

The N-terminal amino acid sequences of the α chains of HLA-DR1, HLA-DR2, and HLA-DR4,w6 antigens were completely homologous at comparable positions. The amino acid sequence of the p34 molecule, however, differs from the sequences of the other three α chains at positions 5, 16, 18, 19, and possibly 8, which was identified as either isoleucine or leucine. It is difficult to find a ready explanation for this lack of homology, especially since the cell line JY, from which p34 was isolated, expresses the same phenotype as cell line Victor, i.e., HLA-DR4,w6. One possible explanation for this discrepancy is that the amino acid sequence differences observed are attributable to products of different genetic loci being sequenced. In this regard, human Ia antigens were found to be encoded for by the DR locus as well as by an additional locus, DC1, which has a population association with HLA-DR1, -2, and -w6 specificities (Tosi et al., 1978). Coincidentally, all the α chains compared by sequence analysis in this study were isolated from cell lines which typed for these specificitites, and consequently either p34 (HLA-DR4,w6) or the three other α chains (HLA-DR4,w6, HLA-DR1, or HLA-DR2) may contain significant amounts of material encoded by the DC1 locus.

The amino acid sequences of the β chains were found to contain a very high degree of structural polymorphism as was reported previously (Silver & Ferrone, 1979; Walker et al., 1980; Kaufman et al., 1981). One residue, 16, in the β-chain sequences of HLA-DR1, HLA-DR2, and HLA-DR4,w6 antigens proves of considerable interest since histidine and tyrosine were detected in equimolar amounts. The mixed sequence of HLA-DR4,w6 antigens could be explained by the presence of polyeptide chains that bear either the HLA-DR4 or HLA-DRw6 specificity possessing either a tyrosine or a histidine at residue 16. However, this explanation is negated by the fact that the β chains of HLA-DR1 and HLA-DR2 antigens also contain equimolar amounts of histidine and

tyrosine at residue 16. Consequently, the most likely explanation for this observation is the coisolation of the β chains of HLA-DR antigens with that of a structurally very similar human Ia antigen that is phenotypically distinct from HLA-DR.

The two regions of high polymorphism in the β chains, residues 7–13 and 26–31, are of adequate length to represent distinct antigenic sites since it has been reported that a hexapeptide is approximately the size of an antigenic determinant (Atassi, 1975). We are currently synthesizing these two regions of the molecule and are attempting to produce both polyclonal and monoclonal antibodies reactive with them to determine whether these two regions of amino acid sequence variability are important for the recognition of allospecific sites. The seemingly high degree of structural polymorphism that exists among the β chains of HLA-DR1 and HLA-DR2 antigens is somewhat surprising in light of a report by Kratzin et al. (1981), who showed that an antigen preparation isolated from a homozygous B lymphoid cell line (HLA-DR2,2) is actually comprised of a pool of seven β chains which are highly variable in only one five amino acid region (residues 65–69) of each molecule. If, as suggested by Kratzin et al. (1981), these β chains are indeed the product of a cluster of different genes, then the structural difference between two HLA-DR allospecificities (HLA-DR1 and HLA-DR2) are more numerous than those detected between HLA-DR and other human Ia gene products.

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Carbohydrates of Influenza Virus Hemagglutinin: Structures of the Whole Neutral Sugar Chains†

Akira Matsumoto, Hideo Yoshima, and Akira Kobata*

ABSTRACT: The carbohydrates of BHA, a solubilized hemagglutinin of influenza virus by bromelain digestion, were quantitatively released as oligosaccharides by hydrazinolysis. The oligosaccharide mixture was separated into a neutral and two acidic fractions by paper electrophoresis. Both acidic fractions were resistant to sialidase digestion but were slowly converted to the neutral fraction by incubation with sulfatases. The neutral fraction which comprised about 80% in molar ratio of total oligosaccharides was separated into 13 oligosaccharides by paper chromatography and by Con A–Sepharose column chromatography. Structural studies of these oligosaccharides by sequential exoglycosidase digestion and by methylation analysis revealed that BHA contains a series of high mannose type and bi-, tri-, and tetraantennary complex type sugar chains. Occurrence of Galβ1→3GlcNAc outer chain in two and bisectional *N*-acetylglucosamine in one of the biantennary sugar chains is an interesting characteristic of the sugar chains of BHA.

Among the proteins coded by influenza virus, the hemagglutinin and the neuraminidase are integral membrane glycoproteins which construct spikes on the viral envelope. When hemagglutinin (HA) binds specifically to the sialated components of host cell plasma membranes, HA adsorbs virus particles to the cell surface and induces penetration of viral RNA into the cell by fusing the viral envelope with the cellular membrane. The hemagglutinin is also the major surface antigen of the virus, and variations in its antigenic structure accompany the recurrences of influenza epidemics in man.

The HA of A2/Hong Kong/1968 virus is a trimer of molecular weight of about 220000 (Wiley & Skehel, 1977), and each subunit of the glycoprotein is composed of two di-

† From the Department of Biochemistry, Kobe University School of Medicine, Chuo-ku, Kobe, Japan. *Received August 3, 1982.* This work has been supported in part by Grant-in-Aids for Scientific Research, the Ministry of Education, Science and Culture of Japan, and by a research grant from Yamanouchi Foundation for Research on Metabolic Disorders.

* Address correspondence to this author at the Department of Biochemistry, Institute of Medical Science, Tokyo University, 4-6-1 Shirokanedai, Minato-ku, Tokyo, Japan.

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