*Proc. Natl. Acad. Sci. USA*
Vol. 75, No. 7, pp. 3390–3394, July 1978
Immunology

# Primary structure of murine major histocompatibility complex alloantigens: Amino acid sequence studies of the cyanogen bromide fragments of the H-2K$^b$ glycoprotein*

(histocompatibility antigens/radiolabeling/immunoprecipitation)

J. E. COLIGAN†, T. J. KINDT†, B. M. EWENSTEIN‡, H. UEHARA‡, T. NISIZAWA‡§, AND S. G. NATHENSON‡

† Laboratory of Immunogenetics, Building 5, Room B2-31, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland 20014; and ‡ Departments of Microbiology and Immunology and Cell Biology, Albert Einstein College of Medicine, Bronx, New York 10461

*Communicated by Richard M. Krause, April 3, 1978*

ABSTRACT **Radiochemical microtechniques have been used in the amino acid sequence analysis of five major CNBr fragments of the glycoprotein specified by the murine major histocompatibility complex gene *H-2K*$^b$. These fragments have been tentatively aligned and represent the $NH_2$-terminal 80% of the intact molecule. All amino acids except Asp, Asn, and Gln have been assigned in 128 out of 149 possible positions in the $NH_2$-terminal portions of each of these fragments. These assignments, which represent approximately 50% of the total sequence from these fragments, are listed below in the order of their alignment in the intact H-2K$^b$ molecule: IIIn, -PHSLRYFVTAVSRP(G)L(G)(E)PRYM; IIIa, EVGYV--TEFVRF-S-AE(A)PRYEPR(A)--M; Ib, E-EGPEYWERET-KAK(G)-E-SFR--LRTLL(G)YY--TK; Ia, AALITK-KWE-AGEAERLRAYLEGTC-E-L; Ic, ELVETRPAG-GTF-KWAS-VVPLGKE-YY(T). The unassigned positions represented by dashes in the above sequences may be tentatively assigned as Asp, Asn, or Gln.**

**The $NH_2$-terminal sequence obtained for the H-2K$^b$ molecule was compared to the limited sequence information available for other major histocompatibility complex gene products. An 84% homology (16 of 19 residues) to the H-2K$^q$ and H-2K$^k$ molecules, which are identical to one another in the positions compared, was observed. A similar comparison with 28 of the 31 $NH_2$-terminal residues of HLA-B7 indicated 68% homology. Furthermore, significant homology was observed between H-K$^b$ and HLA-B7 in a region of glycosylation, which occurs between positions 85 and 100 in the two molecules.**

The genes in the mouse H-2 major histocompatibility complex (MHC), located on chromosome 17, determine a diversity of functions associated with immune recognition and reaction (1–5). The *H-2K* and *H-2D* genes, which map at the opposite extremes of the MHC, encode the major antigens involved in allograft rejection. The polymorphic nature of these genes and their products is one of the striking characteristics of this antigen system and is documented by the large number of different serologically detectable determinants on the *H-2* gene products expressed by a variety of mouse strains (1, 2).

Initial studies on the chemical composition and biochemical properties of the *H-2K* and *H-2D* gene products were done with an antigenically active fragment which was cleaved from the cell surface with papain. By using a lengthy purification scheme, only a few milligrams of this 37,000-dalton glycoprotein fragment was isolated from $10^4$ mouse spleens (6, 7). More recently, intact H-2 molecules (45,000 daltons), radiolabeled in their amino acid or monosaccharide components, have been obtained by immunoprecipitation from cell extracts solubilized with nonionic detergents (7, 8). These radiolabeled H-2 molecules have allowed extensive studies of the products from genetically different sources and have allowed characterization of materials isolated from single mouse spleens (5, 9, 10).

Analysis of the radiolabeled H-2K and H-2D products from a number of different mouse haplotypes by comparative peptide mapping techniques has indicated a remarkable diversity in chemical structure (9, 10). The radiochemical procedures have also permitted characterization of other MHC products, such as Ia (11–14) and TL (15, 16). Several of these studies have been extended to include partial amino acid sequence data for the amino-terminal portions of these molecules (5).

In order to relate structural differences among H-2 glycoproteins to changes in their biological activity and to examine various genetic and evolutionary relationships among the genes of the MHC, complete amino acid sequences of the H-2K and H-2D glycoproteins must be determined. Isolation and characterization of the CNBr fragments of the H-2K$^b$ glycoprotein were reported in a previous paper (17). We report the partial $NH_2$-terminal sequences for five major CNBr fragments isolated from the H-2K$^b$ glycoprotein; amino acids have been assigned to about 50% of the total positions in these fragments.

## MATERIALS AND METHODS

**Antisera, Radiolabeling, and Preparation of Cell Extracts.** Antisera against the H-2K$^b$ glycoprotein were prepared and the radiolabeled amino acids or monosaccharides were incorporated into the E1.4.BU (H-2$^b$) cell line as described (17).

**Isolation of H-2K$^b$.** The H-2K$^b$ glycoprotein was isolated by immunoprecipitation of lentil-lectin-purified Nonidet P-40 extracts of cells, followed by Sephadex G-75 chromatography as described (17). The $\beta_2$-microglobulin was isolated as a by-product during this procedure; the $NH_2$-terminal sequence for approximately 30 steps was determined in order to establish relative levels of radioactivity for the incorporated amino acids. These data also indicated the extent of biosynthetic interconversion of radiolabeled amino acids. The CNBr fragments of the H-2K$^b$ molecule were obtained as described (17).

**Automated Amino Acid Sequence Analysis.** Automated Edman degradation of radiolabeled H-2K$^b$ peptides and $\beta_2$-microglobulin was performed with a Beckman Sequencer (model 890C) in the presence of several milligrams of carrier protein: bovine serum albumin and/or horse cytochrome *c* (Sigma Chemical Co., St. Louis, MO). One of two *N,N*-dimethyl-*N*-allylamine programs was used, either Beckman peptide program no. 102974 or the same program with additional dimethylallylamine delivery (5 sec) and coupling (400 sec) steps. In some cases, the sequences of smaller peptides were determined in the presence of Polybrene (Aldrich Chemical

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: MHC, major histocompatibility complex; PTH, phenylthiohydantoin.

* This is paper 2 in a series. Paper 1 is ref. 17.

§ Present address: National Institute of Health, Tokyo, Japan.

Table 1. Relative incorporation levels of different groups of radiolabeled amino acids

| Multilabel groups | Amino acid* | Specific activity, Ci/mmol | μCi added to culture | Relative radioactivity in $\beta_2$-microglobulin |
|---|---|---|---|---|
| I | Phe | 40.0 | 1.0 | 1.0 |
| | Leu | 60.0 | 1.5 | 0.80 |
| | Lys | 73.0 | 3.0 | 0.40 |
| | Cys | 61.7 | 1.0 | 0.17 |
| | Pro | 27.5 | 5.0 | 0.10 |
| | Ala | 16.1 | 5.0 | 0.06 |
| II | His | 11.3 | 2.0 | 1.0 |
| | Trp | 17.9 | 2.0 | 0.62 |
| | Thr | 2.9 | 2.0 | 0.50 |
| | Ile | 4.0 | 2.0 | 0.38 |
| | Val | 1.2 | 2.0 | 0.35 |
| | Ser | 2.8 | 2.0 | 0.14 |
| | Gly | 15.0 | 2.0 | 0.05 |
| III | Glu | 45 | 5.0 | 1.0 |
| | Pro | — | | 0.64 |
| | Arg | — | | 0.18 |
| IV | Ser | 2.8 | 5.0 | 1.0 |
| | Gly | | | 0.1 |
| V | Gly | 15.0 | 5.0 | 1.0 |
| | Ser | — | | 0.5 |

* All amino acids were $^3H$ labeled except Cys, which was $^{35}S$ labeled. In groups III, IV, and V, only a single radiolabeled amino acid, Glu, Ser, and Gly, respectively, was added to the EL-4 culture. The other radiolabeled amino acids present in these groups resulted from biosynthetic interconversions.

Co., Milwaukee, WI) as described by Klapper *et al.* (18). One-third or less of the butyl chloride extract was dried by a stream of $N_2$ in plastic MiniVials (Fisher Scientific Co,) and dissolved in 5.0 ml of Aquasol-2 (New England Nuclear Corp., Boston, MA) with 20 μl of glacial acetic acid; radioactivity was then measured.

**Determination of Amino Acid Sequence.** In many cases in which peptides were radiolabeled with only one amino acid, the radioactivity of the total butyl chloride extract was determined. Radioactivity significantly above background indicated the presence of the amino acid at that step. In most instances, assignments were verified either by conformity of radioactivity levels to expected repetitive yield values or by further analysis as described below.

For peptides containing multiple radiolabeled amino acids and for many peptides containing a single radiolabeled amino acid, the radioactivity of a portion of the butyl chloride extract was determined in order to determine which sequencer steps contained radioactive amino acids. The remainder of the butyl chloride extract from radioactive steps was then evaporated and the thiazolinones were converted to the phenylthiohydantoin (PTH) derivatives by heating at 80° in 1 M HCl for 10 min. Significant amounts of radioactivity remaining in the aqueous phase after conversion and ethyl acetate extraction indicated the presence of radiolabeled PTH-Arg or PTH-His. The ethyl acetate or aqueous phase was dried and unlabeled carrier PTH-amino acids were added to serve as standards for thin-layer chromatography or high-pressure liquid chromatography.

Thin-layer chromatography was performed as described by Summers *et al.* (19). Twenty to 30% of the sample remaining after conversion was chromatographed. The carrier PTH-amino acids were identified and individually cut out of the polyamide sheet; radioactivity was measured as described above. Conversion of the thiazolinone derivative of Thr to the PTH derivative yielded two products: one cochromatographed with standard PTH-Thr and the other with PTH-Met. The conversion product of Ser could not be identified by this method.

For liquid chromatography, 50 or 100% of the remaining sample was dissolved in 20 μl of methanol and analyzed with a Waters Associates (model 402) high-pressure liquid chromatograph that contained a μ-Bondapak C-18 column and an ultraviolet detector (254 nm). Isocratic chromatography was performed with 44% acetonitrile/56% sodium acetate buffer (pH 4.55, 0.22 M) at a flow rate of 1.2 ml/min. PTH-amino acid peaks were collected manually in MiniVials and dried with a stream of $N_2$ or air; then their radioactivity was determined. Of the 20 amino acids, only PTH-Met and PTH-Val could not be separated. The conversion product of Thr eluted partially as PTH-Thr and partially between PTH-Arg and PTH-Met. The conversion product of Ser could not be detected.

When both methods were used for identification, thin-layer chromatography was performed prior to liquid chromatography since larger amounts (40 compared to 200 nmol) of each carrier PTH-amino acid were used in the latter method.

Serine residues were assigned by determining the radioactivity of the total butyl chloride extract of preparations labeled mainly in Ser (group IV, Table 1). These assignments were confirmed by determining the sequence of fragments labeled in Gly and Ser (group V, Table 1). The presence of both radiolabeled Gly and Ser in these preparations presented no difficulties since their relative specific activities were known.

## RESULTS

**Preparation of Radiolabeled H-2K$^b$ Molecules for Sequence Studies.** Because the purpose of these studies was to determine the total amino acid sequence of the H-2K$^b$ molecule by radiochemical sequencing techniques, a prime issue was the strategy for preparing radiolabeled material. In order to do the extensive internal sequence studies reported here, high-specific-activity $^3H$-labeled amino acids were used. The problem was initially approached by preparing samples radiolabeled with groups containing six or seven amino acids (Table 1, groups I and II). The composition of each group of radiolabeled amino acids was determined, in part, by considering whether the given PTH-amino acids could be well separated by thin-layer and high-pressure liquid chromatography.

Amino acid sequence analysis of the $\beta_2$-microglobulin was used as a convenient means to estimate relative radioactivity of the amino acids. Since $\beta_2$-microglobulin is an early byproduct of H-2K$^b$ purification, this analysis was done prior to the isolation of individual peptides. The analysis indicated that, of the labeled amino acids in groups I and II (Table 1), only Ala and Gly were too low in radioactivity for convenient analysis.

Certain radioactive amino acids that either were not used or were poorly incorporated in the multilabel experiments were added individually to cell cultures and their incorporation was monitored as above. Multilabel groups III, IV, and V resulted from such cell cultures to which Glu, Ser, and Gly, respectively, had been added. For example, labeling with Glu gave incorporation into Pro and Arg in the ratios noted (1.0, 0.64, and 0.18). Labeling with Ser resulted in radioactivity being incorporated into Gly and, conversely, labeling with Gly gave incorporation into Ser. In addition to providing information concerning the location of Glu, Ser, and Gly in the CNBr peptides, the single label preparations that became multilabeled allowed confirmation of many previous assignments. Furthermore, the presence of previously assigned residues in the peptide sequences aided in analysis of sequencer function.

Table 2. Identification of amino acids in CNBr peptides

| SS* | AA† | Labeling group | LC | TLC |
|---|---|---|---|---|
| | | Peptide IIIn | | |
| 2 | P | S2; I; III | + | |
| 3 | H‡ | II | + | |
| 4 | S | IV; V | | |
| 5 | L | S3; I | + | + |
| 6 | R | S6 | | |
| 7 | Y | S2 | | |
| 8 | F | I | + | + |
| 9 | V | II | + | |
| 10 | T | II | + | |
| 11 | A | I | + | |
| 12 | V | II | + | |
| 13 | S | IV; V | | |
| 14 | R | S6 | | |
| 15 | P | S2; I; III | + | |
| 16 | (G) | V | | |
| 17 | L | S3; I | + | + |
| 18 | (G) | V | | |
| 19 | (E) | III | | |
| 20 | P | S2; I; III | + | |
| 21 | R | S6 | | |
| 22 | Y | S2 | | |
| 23 | M | S3 | | + |
| | | Peptide IIIa | | |
| 1 | E | III | | |
| 2 | V | II | + | + |
| 3 | G | IV; V | + | |
| 4 | Y | S2; I | + | + |
| 5 | V | II | + | + |
| 8 | T | II | + | + |
| 9 | E | III | | |
| 10 | F | I | + | + |
| 11 | V | II | + | + |
| 12 | R‡ | S3; III | | |
| 13 | F | I | + | + |
| 15 | S | IV; V | | |
| 17 | A | S; I | | |
| 18 | E | III | | |
| 19 | (A) | S | | |
| 20 | P | S2 | | |
| 21 | R‡ | S3 | | |
| 22 | Y | S2; I | | + |
| 23 | E | III | | |
| 24 | P | S2 | | |
| 25 | R‡ | S3 | | |
| 26 | (A) | S | | |
| 29 | M | S | | |

| SS* | AA† | Labeling group | LC | TLC |
|---|---|---|---|---|
| | | Peptide Ib | | |
| 1 | E | III | + | + |
| 3 | E | III | + | + |
| 4 | G | IV; V | + | |
| 5 | P | S; III | + | + |
| 6 | E | III | + | + |
| 7 | Y | S; I | | + |
| 8 | W | II | + | + |
| 9 | E | III | + | + |
| 10 | R‡ | S2; III | | |
| 11 | E | III | + | + |
| 12 | T | II | + | + |
| 14 | K | S; I | + | + |
| 15 | A | S | | |
| 16 | K | S; I | + | + |
| 17 | (G) | V | | |
| 19 | E | III | | |
| 21 | S | IV; V | | |
| 22 | F | I | + | + |
| 23 | R | S2 | | |
| 24 | V | I | + | |
| 26 | L | S; I | | |
| 27 | R | S2 | | |
| 28 | T | II | + | |
| 29 | L | S;I | + | |
| 30 | L | S;I | + | |
| 31 | (G) | V | | |
| 32 | Y | S; I | + | |
| 33 | Y | S; I | + | |
| 36 | T | II | + | |
| 37 | K | S; I | + | |

| SS* | AA† | Labeling group | LC | TLC |
|---|---|---|---|---|
| | | Peptide Ia | | |
| 1 | A | S; I | + | |
| 2 | A | S; I | + | |
| 3 | L | S; I | + | + |
| 4 | I | II | + | + |
| 5 | T | II | + | + |
| 6 | K | S; I | + | + |
| 8 | K | S; I | + | + |
| 9 | W | II | + | + |
| 10 | E | III | + | + |
| 12 | A | S | | |
| 13 | G | V | + | |
| 14 | E | III | + | + |
| 15 | A | S | | |
| 16 | E | III | + | + |
| 17 | R‡ | S2 | | |
| 18 | L | S: I | + | |
| 19 | R‡ | S2 | + | |
| 20 | A | S | | |
| 21 | Y | S | | |
| 22 | L | S; I | + | |
| 23 | E | III | | |
| 24 | G | V | | |
| 25 | T | II | + | |
| 26 | C | S2; I | | |
| 28 | E | III | | |
| 30 | L | S; II | + | |
| | | Peptide Ic | | |
| 1 | E | III | + | + |
| 2 | L | S; I | + | + |
| 3 | V | II | + | + |
| 4 | E | III | + | + |
| 5 | T | II | + | + |
| 6 | R‡ | S2; I; III | + | |
| 7 | P | S; I; III | + | + |
| 8 | A | I | + | + |
| 9 | G | V | | |
| 11 | G | V | | |
| 12 | T | II | + | + |
| 13 | F | I | + | + |
| 15 | K | S; I | + | + |
| 16 | W | II | + | |
| 17 | A | S; I | + | |
| 18 | S | IV; V | | |
| 20 | V | II | + | + |
| 21 | V | II | + | |
| 22 | P | S; III | + | |
| 23 | L | S; I | + | |
| 24 | G | V | | |
| 25 | K | S; I | + | |
| 26 | E | III | | |
| 28 | Y | S2 | | |
| 29 | Y | S2 | | + |
| 30 | (T) | II | | + |

Amino acids determined within the $NH_2$-terminal positions of CNBr peptides of H-2K$^b$ are presented. The labeling group indicates whether a given radiolabeled amino acid was incorporated singly, as denoted by S, or as one of several radiolabeled amino acids as denoted by I–V (see Table 1). Numbers following S indicate the number of replicate single-label runs. The radiolabeled amino acids for each multilabel group are as follows: I (FALKPC), II (SIGHTVW), III (EPR), IV (SG), and V (GS). Separately prepared, singly radiolabeled CNBr peptides of Y and R were added to group I peptides in some instances. All radiolabeled PTH-amino acids in multilabeled preparations and many in single radiolabeled preparations were identified by high-pressure liquid (LC) and/or thin-layer (TLC) chromatography, as indicated. The standard one-letter symbols (20) for amino acids are used.

* SS, sequencer step.

† AA, amino acid residue.

‡ The R or H was confirmed by determining the radioactivity of the aqueous phase after conversion and ethyl acetate extraction of PTH-amino acids.

Table 3. Comparison of $NH_2$-terminal sequences of murine H-2K and human HLA-B7 antigens*

| | 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| H-2K$^b$ | —— Pro His Ser Leu Arg Tyr Phe Val Thr Ala Val Ser Arg Pro (Gly) Leu (Gly) (Glu)† Pro Arg Tyr Met Glu Val Gly Tyr Val | | | | | Thr |
| H-2K$^k$; K$^q$ | (Gly)——— | His——— | — | Lys——— | Phe | — |
| HLA-B7 | Gly Ser——— | Met——— | Tyr—Ser——— | Arg——— | Pro Phe Ile Ala——— | Asp Gln—— |

* H2-K$^k$ and K$^q$ are from Capra and coworkers (personal communication). HLA-B7 is from Terhorst *et al.* (21).

† Position 19 of K$^b$ was determined as Lys by Capra and coworkers (personal communication) using material from mouse spleen (C57BL/6J). Absence of Lys from the fragment (IIIn) containing position 19 is supported by radiocompositional data (17).

**Amino Acid Sequence Studies.** Automated sequencing procedures were used to determine the $NH_2$-terminal sequence of the CNBr fragments, IIIn, IIIa, Ib, Ia, and Ic, which were radiolabeled with either a single or several radioactive amino acids (Table 1). Table 2 gives the amino acid established for each step and the bases for these identifications. Assignments have been made for all amino acids except Asp, Asn, and Gln, which have not as yet been successfully incorporated into the H-2K$^b$ glycoprotein. These three amino acids comprise approximately 15% of the molecule, as estimated by previous compositions on papain-solubilized materials (6). Considering that 128 assignments have been made for the 149 positions examined (86%), it is likely that positions in which no amino acid has been identified contain Asp, Asn, or Gln. In multiple sequencer runs, which averaged between six and eight determinations per fragment, no instance was noted where two amino acids were assigned to a single position.

A summary of the presently available information on the primary structure of the H-2K$^b$ molecule is shown in Fig. 1. The position numbers beyond the first 52 amino acids are estimates based on the molecular weight determination of the fragments and preliminary alignment data (17), as well as on homology to human histocompatibility antigens (21). Carbohydrate moieties of approximately 3300 daltons are present in fragments Ib and Ia (17). The carbohydrate linkage site for fragment Ib has been estimated to be at position 86 (see *Discussion*). The position of the papain cleavage site in fragment Ic was ascertained by comparing the size of the Ic fragment isolated from the CNBr digests of molecules treated with papain to the size of the Ic fragment obtained from CNBr digests of molecules obtained by detergent (Nonidet P-40) solubilization.

## DISCUSSION

The present report documents studies aimed at establishing radiochemical methods for complete primary structural analysis of membrane proteins and the application of these methods to the study of H-2 antigens. The previous paper (17) reported the isolation and biochemical characteristics of five large CNBr fragments (IIIn, IIIa, Ib, Ia, and Ic) from the H-2K$^b$ glycoprotein. These fragments together contain approximately 265 residues and account for the $NH_2$-terminal 80% of the H-2K$^b$ molecule. Of 149 $NH_2$-terminal positions in these fragments, a total of 128 (86%) have been positively identified (Table 2); the remaining 14% are most likely Asn, Asp, or Gln. The amino-terminal sequence for 50% of the positions in these fragments is known (Fig. 1).

While the present study emphasized the use of multilabeled molecules, certain radioactive amino acids were incorporated singly for various reasons. For example, preliminary studies

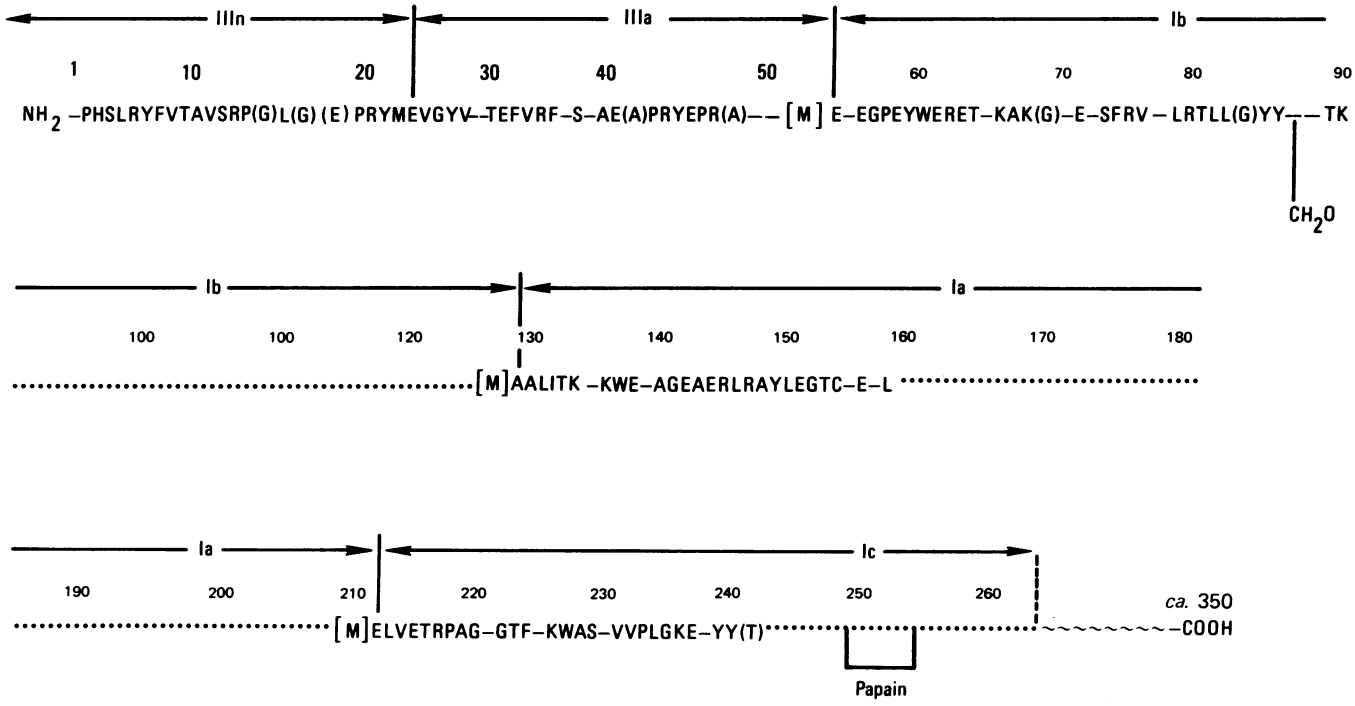


FIG. 1. Schematic representation of the H-2K$^b$ molecule depicting the approximate regions of known sequence. Positions of the Met residues in brackets are based on molecular weight estimates of the CNBr fragments obtained by Sephadex column chromatography and sodium dodecyl sulfate/polyacrylamide gel electrophoresis (21). Hence, the numbering of positions after residue 50 is approximate. The region of papain cleavage was estimated by similar analysis of the difference in Ic fragments obtained by detergent solubilization compared to papain digestion. Single dashes represent an unidentified residue, which more than likely is an Asp, Asn, or Gln. The position of glycosylation in fragment Ib, as indicated by $CH_2O$, was estimated as described in the *Discussion*.

Table 4. Comparison of sequences in the region of glycosylation of murine H-2K$^b$ and human HLA-B7 and HLA-A2

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 35 | | | | ↓ | |
| H-2K$^b$ CNBr fragment Ib | Gly- | Tyr- | Tyr- | * | -?- | Thr- | Lys | | | | |
| ↓ | | | | | | | | | | | |
| HLA-B7 glycopeptide | Gly- | Tyr- | Tyr- | Asn† | - | Glx- | Thr- | Glx- | | | |
| ↓ | | | | | | | | | | | |
| HLA-A2 glycopeptide | Gly- | Tyr- | Tyr- | Asn† | - | Glx- | Ser- | Glx- | | | |

The sequences for HLA-B7 and HLA-A2 are from Parham *et al.* (22). Number shown refers to the H-2K$^b$ CNBr fragment Ib. ↓, Site of trypsin cleavage.

* Presumptive site of glycosylation in H-2K$^b$; most likely to be Asn (24).

† Site of glycosylation in HLA glycoprotein.

were conducted using tritiated Arg or Lys in order to facilitate future work on tryptic peptides. Serine was labeled separately because of the problems that have been encountered in analyzing the PTH-Ser derivatives by either thin-layer or high-pressure liquid chromatography. Finally, certain other amino acids, such as Gly, Ala, and Pro, were also labeled singly because of their relatively poor incorporation in multilabeled studies.

The 31 amino-terminal residues of H-2K$^b$ are compared with certain published sequences for murine and human histocompatibility antigens in Table 3. Addition of new assignments at positions 16, 18, 19, 23, 24, 25, 26, 28, and 31 to the H-2K$^b$ sequence confirms the high degree of homology among these molecules. A comparison of H-2K$^b$ with 20 positions of H-2K$^q$ and H-2K$^k$, which were determined by Capra and his coworkers (personal communication), shows only three differences, giving an 85% homology among these molecules which are encoded by allelic genes. Similar degrees of homology exist between the H-2K$^b$ molecule and the H-2D$^b$ and H-2D$^d$ molecules (5). Comparison of H-2K$^b$ with HLA-B7 (21) shows 68% homology for the 28 $NH_2$-terminal residues available for comparision. It is tempting to speculate that the unassigned positions 29 and 30 of H-2K$^b$ are also homologous to the HLA molecule. These residues, which are proximate to the amino-terminal of fragment IIIa, are most likely occupied by Asp, Asn, or Gln residues, because no other assignment could be made in our study. It is significant that all amino acid differences between the H-2 and HLA molecules in the $NH_2$ terminus can be explained in terms of single base changes in the genetic code, with the exception of the Val-His interchange at position 9.

An additional homology to human transplantation antigens was noted between a region in the Ib CNBr fragment of H-2K$^b$ and the glycopeptide from the region around position 100 of HLA-B7 and A2 (22) (Table 4). That this homology involves a glycopeptide of H-2K$^b$ was verified by the isolation of a tryptic glycopeptide encompasing residues 28–37 of the Ib CNBr fragment (H. Uehara, unpublished observation). Of the five residues that have been assigned within positions 30–37 of fragment Ib, four are identical to those present in the HLA-B7 glycopeptide. Because glycosylation is presumed to occur at the Asn residue of the recognition sequence Asn-X-Thr/Ser (23), and because it was previously shown that the carbohydrate moieties of the H-2K$^b$ molecule are covalently attached to Asn (24), the position of carbohydrate attachment can be tentatively assigned to residue 34 of fragment Ib.

Interest in the structure of the H-2K molecule derives not only from the fact that it is a membrane-integrated glycoprotein, but also from consideration of the biological significance of genetic polymorphism. Of particular relevance are recent studies which revealed a series of mutants in H-2K$^b$ that lead to intrastrain skin-graft rejection and positive *in vitro* reactivity, but do not give rise to serologically detectable differences in their protein products (10). In the *H-2K* gene product of one of these mutants (B6-H-2$^{bd}$, M505), two structural differences were localized by peptide mapping studies to the Ib CNBr fragment. In the *H-2K* gene product of another mutant (B6.C-H-2$^{ba}$, Hz1), a primary structural difference has been detected near the $NH_2$ terminus of CNBr fragment Ia. A logical extension of the present study involves determination of the precise chemical nature of the structural changes that give rise to drastic changes in biological reactivity in these mutants. Such studies have the potential for probing the relationships between the structure and function of these cell-surface glycoproteins.

We gratefully acknowledge the expert technical assistance of Ms. S. Bernstein, Ms. D. McGovern, Ms. S. Schumacher, and Mr. S. Harrison and thank Dr. Rod Nairn for assistance in developing methods for incorporating radiolabeled amino acids into cells. We thank Ms. V. Frye, Ms. C. Whelan, and Ms. N. Goldberg for secretarial assistance. This work was supported partially by U.S. Public Health Service Grants AI-07289, AI-10702, 5T5-GM-1674, and ACS-IM-77.

1. Klein, J. (1975) *Biology of the Mouse Histocompatibility Complex* (Springer-Verlag, New York).
2. Snell, G. D., Dausett, J. & Nathenson, S. G. (1976) *Histocompatibility* (Academic, New York).
3. Schreffler, D. C. & David, C. F. (1975) *Adv. Immunol.* **20,** 125–195.
4. Paul, W. E. & Benacerraf, B. (1977) *Science* **195,** 1293–1300.
5. Vitetta, E. S. & Capra, J. D. (1978) *Adv. Immunol.* **26,** 147–193.
6. Shimada, A. & Nathenson, S. G. (1969) *Biochemistry* **8,** 4048–4062.
7. Schwartz, B. D., Kato, K., Cullen, S. E. & Nathenson, S. G. (1973) *Biochemistry* **12,** 2157–2164.
8. Schwartz, B. D. & Nathenson, S. G. (1971) *J. Immunol.* **107,** 1363–1367.
9. Brown, J. L., Kato, K., Silver, J. & Nathenson, S. G. (1974) *Biochemistry* **13,** 3174–3178.
10. Nathenson, S. G., Brown, J. L., Ewenstein, B. M., Nisizawa, T., Sears, D. W. & Freed, J. H. (1976) *Cold Spring Harbor Symp. Quant. Biol.* **41,** 343–349.
11. Cullen, S. E., Freed, J. H. & Nathenson, S. G. (1976) *Transplant. Rev.* **30,** 236–270.
12. Silver, J., Russel, W. A., Reis, B. L. & Frelinger, J. A. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5131–5134.
13. McMillan, M., Cecka, J. M., Murphy, D. B., McDevitt, H. O. & Hood, L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5135–5139.
14. Cook, R., Vitetta, E. S., Capra, J. D. & Uhr, J. W. (1977) *Immunogenetics* **5,** 437–443.
15. Vitetta, D. S., Uhr, J. W. & Boyse, E. (1972) *Cell Immunol.* **4,** 187–191.
16. Ostberg, L., Rask, L., Wigzell, H. & Peterson, P. A. (1975) *Nature* **253,** 735–737.
17. Ewenstein, B. M., Uehara, H., Nisizawa, T., Nathenson, S. G., Coligan, J. E. & Kindt, T. J. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 2909–2913.
18. Klapper, D., Wilde, C. W. & Capra, J. D. (1978) *Anal. Biochem.* **85,** 126–131.
19. Summers, M. R., Smythers, G. W. & Oroszlan, S. (1973) *Anal. Biochem.* **53,** 624–628.
20. IUPAC-IUB Commission on Biochemical Nomenclature (1968) *J. Biol. Chem* **243,** 3557–3559.
21. Terhorst, C., Robb, R., Jones, C. & Strominger, J. L. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 4002–4006.
22. Parham, P., Alpert, B. N., Orr, H. T. & Strominger, J. L. (1977) *J. Biol. Chem.* **252,** 7555–7567.
23. Hughes, R. C. (1973) *Prog. Biophys. Mol. Biol.* **26,** 141–268.
24. Nathenson, S. & Muramatsu, T. (1971) in *Glycoproteins of Blood Cells,* eds. Jameson, G. A. & Greenwalt, T. J. (Lippincott, Philadelphia, PA), pp. 245–262.