Orr, H. T., Lopez de Castro, J. A., Lancet, D., & Strominger, J. L. (1979) *Biochemistry 18*, 5711.
Paul, W. E., & Benacerraf, B. (1977) *Science (Washington, D.C.) 195*, 1293.
Rask, L., Lindblom, J. B., & Peterson, P. A. (1974) *Nature (London) 249*, 833.
Schwartz, B. D., Kato, K., Cullen, S. E., & Nathenson, S. G. (1973) *Biochemistry 12*, 2157.
Seegers, W. H. (1967) in *Blood Clotting Enzymology*, Academic Press, New York.
Shearer, G. M., & Schmitt-Verhulst, A. (1977) *Adv. Immunol. 25*, 55.
Silver, J., & Hood, L. E. (1974) *Nature (London) 249*, 764.
Smyth, D. S., & Utsumi, S. (1967) *Nature (London) 216*, 332.
Snell, J. D., Dausset, J., & Nathenson, S. G. (1976) in *Histocompatibility*, Academic Press, New York.
Uehara, H., Ewenstein, B. M., Martinko, J. M., Nathenson, S. G., Coligan, J. E., & Kindt, T. J. (1980) *Biochemistry 19*, 306.
Vitetta, E. S., & Capra, J. D. (1978) *Adv. Immunol. 26*, 147.
Zaleska-Rutczynska, Z., & Klein, J. (1977) *J. Immunol. 119*, 1903.
Zinkernagel, R. M., & Doherty, P. C. (1979) *Adv. Immunol. 27*, 51.

Primary Structure of Murine Major Histocompatibility Complex Alloantigens: Completion of the Sequence of the Amino-Terminal 284 Residues of H-2K^{b}†

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ABSTRACT: The primary structure of the COOH-terminal cyanogen bromide (CNBr) cleavage fragment Ic (CN-Ic) of the extracellular portion of the murine histocompatibility antigen H-2K^{b} has been completed. CN-Ic contains a site of papain cleavage which has been utilized for solubilizing H-2K^{b} by cleaving off the membrane integrating portion of the molecule. The amino acid sequence of CN-Ic has been determined by using peptides recovered after trypsin digestion of CN-Ic before and after blockage of lysine groups with citraconic anhydride. Overlapping sequences for the tryptic fragments were obtained by amino-terminal sequence analysis. The sequence of fragment CN-Ic, which spans residues 229–284 in H-2K^{b}, is as follows: Glu-Leu-Val-Glu-Thr-Arg-Pro-Ala-Gly-Asp-Gly-Thr-Phe-Gln-Lys-Trp-Ala-Ser-Val-Val-Pro-Leu-Gly-Lys-Glu-Gln-Tyr-Tyr-Thr-Cys-His-Val-Tyr-Gln-Gln-Gly-Leu-Pro-Gln-Pro-Leu-Thr-Leu-Arg-Trp-Asp-Glu-Pro-Pro-Ser-Thr-Val-Ser-Asn-Met. This amino acid sequence determination completes the primary structure of the amino terminal 284 residues of H-2K^{b}, that portion of this histocompatibility antigen which is external to the cell membrane and which contains antigenic determinants. It was also possible to identify Val-281 as a papain cleavage site within CN-Ic. The completed structure was analyzed solely by radiochemical methods. The structure obtained for H-2K^{b} is 71% homologous to the reported structure of HLA-B7, a human homologue.

Radiochemical microsequencing techniques have been applied to the murine H-2 alloantigen H-2K^{b} [1] as an approach to determining the primary structure of proteins available in amounts too small for classical sequencing analysis. Determination of the sequence of the first 173 residues was accomplished in a series of studies on the CNBr fragments, CN-IIIn, CN-IIIa, and CN-Ib (Coligan et al., 1978, 1979; Uehara et al., 1980a). The sequence of CN-Ia (residues 139–228) is described in the preceding paper (Uehara et al., 1980b). The data for determination of CN-Ic (residues 229–284) are presented in the present paper, permitting assignment of the NH_2-terminal 284 amino acids (H-2K$^{b}_{284}$) which is approximately equivalent in size to the H-2K^{b} papain-solubilized fragment (H-2K$^{b}_{pap}$).

Materials and Methods

Preparation of Radiolabeled H-2K^{b} Antigen. The H-2K^{b} glycoprotein was isolated from EL-4.BU cells which had been cultured in the presence of various radiolabeled amino acids. After solubilization of the cell membrane in Nonidet P-40, the glycoprotein fraction was isolated by *Lens culinaris* hemagglutinin affinity chromatography, indirect immune precipitation, papain digestion (if indicated), and G-75 column chromatography as previously described (Ewenstein et al., 1978).

Preparation of CN-Ic. The disulfide-bonded complex of the major CNBr fragments of H-2K^{b}, which includes CN-Ia, CN-Ib, and CN-Ic, was purified by Sephadex G-100 chromatography. Reduction and alkylation of this material were done with 0.1 M dithiothreitol under N_2 at 37 °C for 2 h and 0.25 M iodoacetamide for 15 min at ambient temperature. CN-Ic was then isolated by Sephadex G-100 column chromatography. A more detailed description of these purification methods was previously published (Ewenstein et al., 1978).

Citraconylation of CN-Ic. Citraconylation of CN-Ic was accomplished by using a modification of previously described methods (Dixon & Perham, 1969). Radiolabeled CN-Ic and 2 mg of carrier bovine serum albumin were modified by re-

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[1] Abbreviations used: H-2K$^{b}_{284}$, H-2K^{b} glycopeptide comprising the NH_2-terminal 284 amino acid residues of intact molecule; MHC, major histocompatibility complex; HLA-B7$_{pap}$, HLA-B7 isolated after papain cleavage of membrane containing intact HLA-B7.

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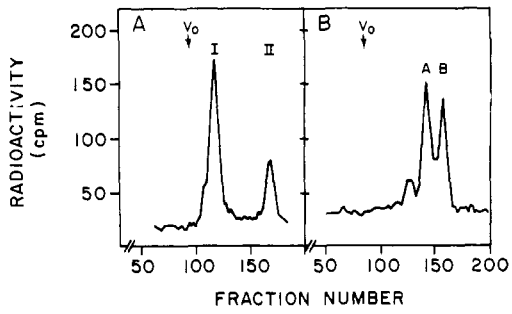

FIGURE 1: Chromatography of trypsin-digested CN-Ic labeled with [^{3}H]His, [^{3}H]Ile, [^{3}H]Thr, [^{3}H]Val, and [^{3}H]Trp on a Sephadex G-50 (Superfine) column (220 × 0.9 cm). The fraction size was 0.7 mL; the flow rate was 3.0 mL/h. (A) Citraconylated and trypsin-digested CN-Ic. Pool I includes residues 1–45. Pool II included residues 46-56. (B) Trypsin-digested pool I. Pool A includes residue 1–15 and 26–45. Pool B includes residues 16–25.

action with a 50 times molar excess of citraconic anhydride to carrier amino groups. In a typical experiment, the radiolabeled CN-Ic was dissolved in 1.0 mL of 2 M guanidine hydrochloride in 0.1 M Na_2HPO_4, pH 9.0. Two milligrams of bovine serum albumin (3×10^{-5} mmol of free amino groups) was added as the carrier, and 8 μL (9.0×10^{-2} mmol) of citraconic anhydride (Eastman Organic Chemicals, Rochester, NY) was added to the solution in two aliquots. The pH was maintained at ~9 with 10 N NaOH. After the pH stabilized, the reaction was allowed to proceed for 30 min, at which time the excess citraconic anhydride was removed by chromatography on a Sephadex G-15 column equilibrated in 0.5 M NH_4OH. Eluted fractions containing radioactive material were pooled and lyophilized.

Trypsin Digestion of Citraconylated CN-Ic. A stock solution of 5 mg of trypsin/mL (Trypsin-TPCK, Worthington Biochemicals Corp., Freehold, NJ) in 0.001 N HCl was added to the citraconylated CN-Ic dissolved in 0.1 M NH_4HCO_3, pH 8.55, to a final concentration of 1% trypsin/protein (w/w). The pH was adjusted to 8.0 with 1 N NaOH, and the solution was incubated at 37 °C for 1 h. The reaction mixture was adjusted to 7.8 M HCOOH with 88% HCOOH and allowed to incubate for 30 min at room temperature before applying to a Sephadex G-50 column.

G-50 Column Chromatography. The trypsin digested and citraconylated CN-Ic was applied directly on a Sephadex G-50 (Superfine) column (0.9 × 220 cm) and eluted at a flow rate of 3.0 mL/h with 1 M HCOOH. Exposure to acid conditions before and during chromatography was sufficient to remove citraconyl groups. Pool I material (see Figure 1A) was again digested with trypsin and chromatographed in the same manner.

Aminex AG-50W-X2 Ion-Exchange Chromatography. Aminex AG-50W-X2 cation-exchange resin (Bio-Rad Laboratories, Richmond, CA) was equilibrated with 0.1 M pyridine acetate, pH 5.5. Radioactive peptides to be separated were dissolved in 0.1 M pyridine acetate, pH 5.5, and applied to a 1-mL column of the resin. The peptides were then eluted with 0.1 M pyridine acetate, pH 5.5, in ten fractions of 1-mL each. The buffer was then changed to 4 M pyridine acetate, pH 5.5, and the elution continued for ten more fractions of 1-mL each. Fractions containing radioactivity were pooled and lyophilized.

Automated Amino Acid Sequence Analysis. Automated Edman degradation of radiolabeled H-2K^b peptides and analysis of radioactive phenylthiohydantoin derivatives of amino acids were performed essentially as previously described (Coligan et al., 1979; Uehara et al., 1980a).

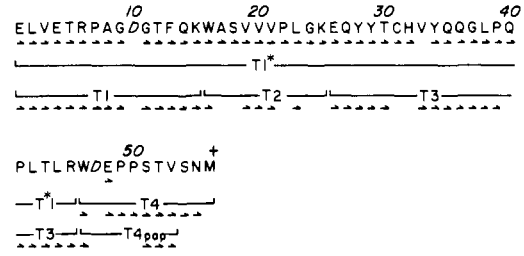

FIGURE 2: Amino acid sequence of CN-Ic showing the final ordering of the tryptic fragments obtained after citraconylation and trypsin digestion of NP-40 soluble material and papain-treated material. Numbering is given for CN-Ic (1–56). (+) The position of Met-56 was ascertained from [^{35}S]Met-labeled tryptic fragments of H-2K^b as further explained under Results (H. Uehara, unpublished experiments).

Scintillation Counting. Radioactivity was determined by dissolving samples in 4.5 mL of Bioflour liquid scintillation cocktail (New England Nuclear, Boston, MA).

Results

Citraconylation, Trypsin Digestion, and Fractionation of CN-Ic. Fractionation of citraconylated and trypsin-digested CN-Ic on Sephadex G-50 resulted in separation of two fractions (Figure 1A). After the removal of citraconyl groups, pool I was again digested with trypsin and chromatographed on a Sephadex G-50 column (Figure 1B), resulting in separation of two distinct fractions, pools A and B. Sequence analysis of pool A showed it contained a peptide spanning residues 1–15 (229–243) of CN-Ic and another peptide having a sequence starting at residue 26 (254). AG-50W-X2 ion-exchange chromatography of this mixture (pool A) led to the separation of the two peptides designated T1 and T3. Pool B contained peptide T2 (Figure 2). The amino acid sequence determined for pool II indicated that it was a pure peptide with a single sequence. It was designated T4 (Figures 2 and 3) and placed at the COOH terminus of CN-Ic because a tryptic peptide from H-2K^b labeled in successive experiments with [^{3}H]Trp and [^{35}S]Met, [^{3}H]Ser and [^{35}S]Met, and [^{3}H]Thr and [^{35}S]Met gave sequences which correspond to T4 with a Met at step 11 (H. Uehara, unpublished experiments).

Complete Sequence of CN-Ic. Table I summarizes the data documenting the sequence of 54 out of the 56 residues of CN-Ic. The order and designation of the tryptic peptides are shown in Figure 2. Figure 3 depicts the yields of radioactive residues at each step in the sequence except for steps 10 (238) and 47 (245). These positions have been indirectly assigned as aspartic acid residues since they were unassigned after definitive sequence determination for the other 19 amino acids had been completed [Asp is the only amino acid that has not been successfully incorporated into the H-2K^b molecule (Uehara et al., 1980a)].

The tryptic peptides of CN-Ic were aligned by NH_2-terminal sequences of CN-Ic labeled with [^{3}H]Glu, [^{3}H]Gln, and [^{3}H]Pro (see Figure 3A). The order and designation of the tryptic peptides are shown in Figure 2. All the amino acids identified, with the exception of Gln-268 and Cys-259, were identified in at least two separate sequence analyses and conformed to the rigid standards for positive determination of radiolabeled amino acid residues as previously described (Uehara et al., 1980a). The position of Cys-31 (259) has, however, been additionally established by other methods used for elucidating the position and relationship of the two intrachain disulfide bonds present in the H-2K^b molecules (J. Martinko, J. Adlersberg, R. Halpern, and S. G. Nathenson, unpublished experiments).

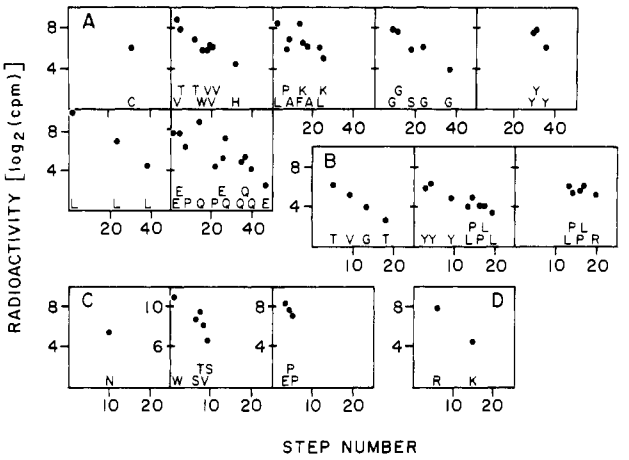

FIGURE 3: Repetitive yield of CN-Ic and tryptic fragments. (A) NH_2-terminal sequences of CN-Ic. (B) Sequences of T3. (C) Sequences of T4. (D) Sequences of T1.

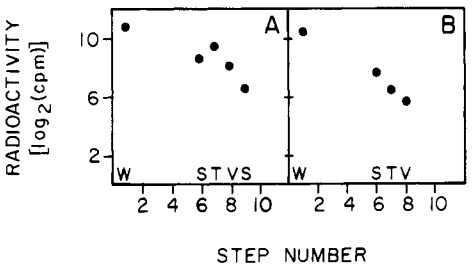

FIGURE 4: Automatic sequence analysis of the COOH-terminal peptide of CN-Ic from H-2K$^b_{pap}$ (A) and from intact H-2K^b (B) labeled with [^{3}H]Trp, [^{3}H]Ser, [^{3}H]Thr, and [^{3}H]Val.

Papain Cleavage Site. Figure 4A depicts the results of an amino acid sequence determination of the COOH-terminal peptide (T4) of CN-Ic obtained from intact H-2K^b labeled with [^{3}H]Trp, [^{3}H]Thr, [^{3}H]Val, and [^{3}H]Ser. The residue at step 9 was identified as Ser. Figure 4B shows the sequence information for the analogous peptide obtained from H-2K$^b_{pap}$ labeled with [^{3}H]Trp, [^{3}H]Thr, [^{3}H]Val, and [^{3}H]Ser. The sequence data clearly demonstrate the absence of Ser at step 9 and the presence of Ser, Thr, and Val at steps 6, 7, and 8, respectively, in T4 of Ic isolated from papain-cleaved H-2K^b (H-2K$^b_{pap}$). These comparative data indicate that a papain cleavage site exists after Val-281 in the H-2K^b molecule.

Discussion

The present communication presents the amino acid sequence of the most COOH-terminal of the five CNBr fragments which comprise the NH_2-terminal 284 residues of the murine major histocompatibility complex (MHC) product, H-2K^b (see Figure 5). These data complete our knowledge of the total amino acid sequence of the H-2K$^b_{pap}$ molecule. This portion of H-2K^b is external to the cell membrane and carries serologically reactive sites (Shimada & Nathenson, 1969) as well as histogenically reactive determinants (Graff & Nathenson, 1971). Sequence studies of the remaining positions of H-2K^b, which include a transmembrane piece and a hydrophilic COOH-terminal section (about 20% of the molecule), are in progress.

The determination of the amino acid sequence of H-2K$^b_{284}$ was a result of the successful adaptation of standard protein purification and sequence techniques to radiolabeled material (Ewenstein et al., 1976, 1978; Coligan et al., 1978, 1979; Uehara et al., 1980a,b). Therefore, this work demonstrates the feasibility of obtaining complete primary structure information for molecules not readily available in sufficient amounts for standard analysis. CNBr was utilized as the first step in the fragmentation of H-2K^b into peptides appropriate in length for automated sequence determination. CN-IIIn and CN-IIIa, 23 and 29 amino acids long, respectively, were sequenced as intact CNBr peptides. Enzymic methods employing trypsin, thrombin, and staphylococcal V8 protease were used to cleavage CN-Ia and CN-Ib into peptides of appropriate length for automated sequence analysis.

The strategy for sequence determination of CN-Ic involved a different approach. After modification with citraconic anhydride, CN-Ic was digested with trypsin, which could then cleave only at arginine residues. The products of trypsin digestion were separated by molecular sieve chromatography. After removal of citraconyl groups, the larger molecular weight fragment was again digested with trypsin; the resulting peptides

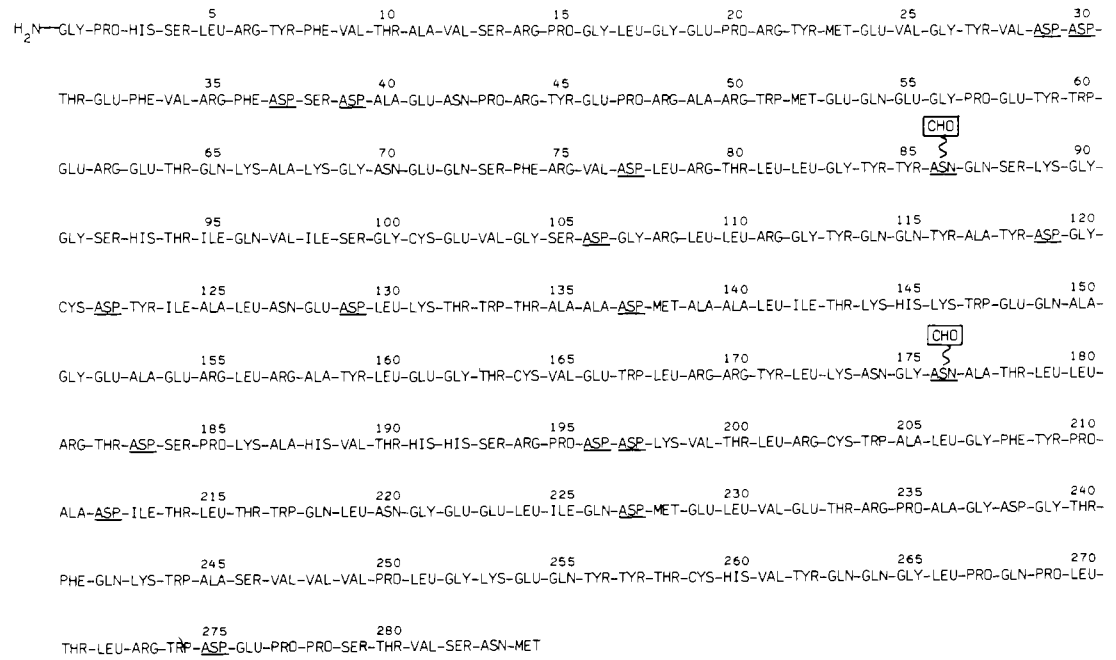

FIGURE 5: The complete amino acid sequence of the NH_2-terminal 284 residues of the H-2K^b glycoprotein. The aspartic acid residues which are underlined have been assigned by deductive methods previously described (Uehara et al., 1980).

Table I: Identification of Amino Acids in CN-Ic

fragment no.	assigned residue[a]	NH_2-terminal label group[b]	NH_2-terminal ID	trypsin peptide	trypsin label group[b]	trypsin ID	trypsin peptide	trypsin label group[b]	trypsin ID
1	E	VI	LC	T1	VI	LC			
2	L	S, VII	LC		VII, I	LC			
3	V	IIa, II	TLC, LC						
4	E	VI	LC		VI	LC			
5	T	IIa	TLC						
6	R	S2			VIII				
7	P	VII	LC		VIII	LC			
8	A	VII, S	LC		IX	LC			
9	G	IV, V			IX				
10	(D)								
11	G	IV, V			IX				
12	T	IIa	TLC						
13	F	VII	LC		I	LC			
14	Q	VI	LC		VI	LC			
15	K	S, VII	LC		I	LC			
16	W	S, II	LC	T2	VIII, II	LC			
17	A	S			IX	LC			
18	S	IV, V			V				
19	V	II	LC		II	LC			
20	V	II, IIa	TLC, LC		II	LC			
21	V	II	LC		II	LC			
22	P	VII	LC						
23	L	S, VII	LC		VIII	LC			
24	G	IV, V							
25	K	S, VII	LC						
26	E	VI		T3	VI	LC			
27	Q	VI			VI	LC			
28	Y	S			I, Ia	LC			
29	Y	S	TLC		I, Ia	LC			
30	T				X	LC			
31	C	S, [^{35}S]							
32	H	IIa, II							
33	V				X	LC			
34	Y				I, Ia	LC			
35	Q	VI			VI				
36	Q	VI			VI				
37	G	IV			X	LC			
38	L	S, VII	LC		VII, I	LC			
39	P	S2			Ia, VIII	LC			
40	(Q)	VI							
41	P				Ia	LC			
42	L				I, Ia, VII	LC			
43	T				X, S	LC			
44	L				I, Ia, VIII	LC			
45	R				S2				
46	W			T4	II, IIa, X	LC	T4 (papain)	X, VIII	LC
47	(D)								
48	E	VI			IX	LC			
49	P				VIII	LC			
50	P				VIII	LC			
51	S				IIa, VIII, X	LC		X, VIII	
52	T				II, IIa, X	LC		X	
53	V				II, IIa, X	LC		X	LC
54	S				VIII, S, X	LC			
55	N				S2	LC			
56	M				S2,[c] [^{35}S]				

[a] The one letter amino acid code is taken from the IUPAC-IUB Commission on Biochemical Nomenclature (1968). [b] Label groups. All labels were ^{3}H unless otherwise noted. S, single-label preparation; I, FLKY; Ia, PLKY; II, HITVW; IIa, SIGHTVW, III, E,P; IIIa, R,E,P; IV, G,S; V, S,G; VI, Q,E,P; VII, FALKPC; VIII, WPLRS; IX, EAG; X,WTVS. [c] Sequence information from [^{35}S]M, [^{3}H]W labeled tryptic peptide of NP-40 soluble H-2.33 (H. Uehara, unpublished experiments).

were separated by molecular sieve and ion-exchange chromatography, and sequence analysis was performed.

Major structural features of the H-2K^b molecule, summarized schematically in Figure 6, are two carbohydrate chains linked to asparagine residues at positions 86 and 176 (Uehara et al., 1980a,b) and two intrachain disulfide bridges [Cys-101–Cys-164 and Cys-203–Cys-259 (J. Martinko, J. Aldersberg, R. Halpen, and S. G. Nathenson, unpublished experiments)]. With the exception of the second carbohydrate moiety at residue 176, these structural features correspond to those observed in the HLA-B7 and HLA-A2 molecules (Orr et al., 1979b).

A site of papain cleavage at the COOH terminus of Val-281 was demonstrated in H-2K^b by comparing CN-Ic fragments obtained from both H-2K^b and H-2K$^b_{pap}$. On the other hand, the sequence of the papain-derived HLA-B7$_{pap}$ is reported to end at residue 271 (Orr et al., 1979a). This difference in papain cleavage sites, however, may be due to the fact that H-2K$^b_{pap}$ is generated from immune precipitates whereas HLA-B7$_{pap}$ was derived from cell membranes. Traägärdh et

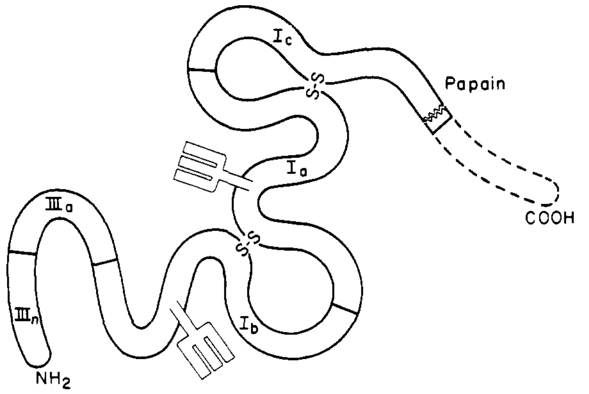

FIGURE 6: Schematic model of the H-2K^b glycoprotein. The five major cyanogen bromide peptides prepared by cleavage at Met residues are indicated as well as the relative positions of the disulfide bonds, carbohydrate moieties, and a papain cleavage site.

al. (1980) have reported a tentative papain cleavage site at residue 273 from papain-solubilized mixtures of HLA-A, -B, and -C, suggesting at least one more cleavage site for papain. They further suggest the existence of multiple papain cleavage sites in the heterogenous preparation which they examined. It remains to be established whether the differences observed in exact location of the papain cleavage site of the various preparations of histocompatibility antigens are the result of solubilization methods, intrinsic differences between the molecules investigated, or merely random differences attributable to the relatively nonspecific proteolytic action of papain.

Determination of the complete amino acid sequence of H-2K$^b{}_{284}$ makes possible for the first time an extensive comparison between MHC products of different species. Thus, as shown in Figure 7, when the first 271 residues of H-2K^b and HLA-B7 (Orr et al., 1979b) are compared, a 71% homology is seen. Of 80 differences in the comparable residues, 64 amino acid changes can be attributed to a minumum of one nucleotide base-pair change, while only 16 require two or three base-pair changes.

In general, the sequence differences noted in Figure 7 are distributed more or less randomly throughout the 271 residues. However, two clusters of accumulated differences are apparent. The region spanning positions 61–82 exhibits 13 differences out of 22 residues (41% homology). Five of these differences require a minimum of a two-nucleotide base-pair substitution. Comparisons of the MHC products of man and mouse, or between loci in man, have revealed a large number of differences at positions 61–82 (Coligan et al., 1979; Orr et al., 1979b; Uehara et al., 1980a). It remains to be seen, however, if this portion of the MHC molecule also exhibits the same diversity within the mouse when products of multiple alleles at the *H-2K*, *H-2D*, and *H-2L* loci are compared. In any event, current findings suggest that this region may be a site of diversity.

The region spanning positions 173–199 also exhibits a high degree of diversity between H-2K$^b{}_{284}$ and HLA-B7$_{pap}$. For these 27 residues, 14 differences were found (48% homology), but only one would require more than a one-nucleotide base-pair change. Position 176 is a glycosylation site in H-2K^b (Uehara et al., 1980b) and probably for other H-2 molecules (Coligan et al., 1980; Kimball et al., 1980; Nairn et al., 1980a) but not in HLA-B7 (Orr et al., 1979b) or, it appears, in any other HLA molecule (Trägårdh et al., 1980). HLA-B7 and H-2K^b have identical sequences at residues 86–88 which correspond to known sequence signals for glycosylation sites on other eukaryotic glycoproteins (Marshall, 1972), and both HLA-B7 and H-2K^b are glycosylated at position 86. However, comparison at residues 176–178 shows no homology, and HLA does not have a carbohydrate moiety attached at position 176. This may represent a species-specific difference.

The successful use of microsequence analysis to determine the complete primary structure of the H-2K$^b{}_{284}$ molecule encourages the belief that structural analysis of a series of H-2K, H-2D, and H-2L gene products will be possible by using these same techniques. Indeed, data are already available for H-2D^d (Nairn et al., 1980a) and H-2L^d (Coligan et al., 1980).

The specific analysis of the H-2K^b molecule is also of particular importance in view of the availability of a series of over 16 mouse strains carrying mutations in their H-2K^b gene (Kohn et al., 1978; R. Melvold, unpublished experiments). These strains show strong reciprocal histogenic reactivity among members of the mutant series as well as with the parent standard strain. The H-2K^b molecules from a number of the mutants have been analyzed at the levels of peptide mapping and partial amino acid sequencing (B.M. Ewenstein, S.G. Natheson, T. Nisizawa, H. Uehara, and K. Yamaga, unpub-

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                      15             30             45             60
H-2Kb   GPHSLRYFVTAVSRPGLGEPRYMEVGYVDDTEFVRFDSDAENPRYEPRARWMEQEGPEYWERETQKAKGNEQSFR
HLA-B7  -S--M---Y-S-----R----FIS-------Q--------AS--E----P-I--------D-N--IY-AQA-TD-
                *       *      *                                      *   *  *   *

                 CHO
                      90             105            120            135
H-2Kb   VDLRTLLGYYNQSKGGSHTIQVISGCEVGSDGRLLRGYQQYAYDGCDYIALNEDLKTWTAADMAALITKHKWEQA
HLA-B7  ES--N-R------EA----L-SMY--D--P-------HD------K---------RS-----T--Q--QR---A-
        *                    *                *      *                           *

                                CHO
                      165            180            195            210
H-2Kb   GEAERLRAYLEGTCVEWLRRYLKNGNATLLRTDSPKAHVTHHSRPDDKVTLRCWALGFYPADITLTWQLNGEELI
HLA-B7  R---QR------E---------E--KDK-E-A-P--T-----PIS-HEA------------E-----RD---DQT
                    *            △   *

                      240            255            270
H-2Kb   QDMELVETRPAGDGTFQKWASVVVPLGKEQYYTCHVYQQGLPQPLTLRWDEPPSTVSNM
HLA-B7  --T----------R--E---A----S-E--R-----QHE---K---
                                      *     *
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FIGURE 7: Amino acid sequence homology comparing H-2K^b and HLA-B7. Straight lines indicate homology. Only the differences are shown for HLA-B7. HLA-B7 data were obtained from Orr et al. (1979a). Asterisks indicate that more than one nucleotide base-pair change is necessary for the observed amino acid substitution. Triangles indicate a change in glycosylation.

lished experiments; Nairn et al., 1980b). Because of our knowledge of the primary structure of the standard H-2K^b molecule, we have been able to establish that the mutant-standard strain differences are localized in discrete regions of the molecule and appear to consist of one or, at most, only a few amino acid changes. Such structural alterations have a correlation with biological specificity [see Nairn et al. (1980a,b)] and provide insight into the nature of the T-cell recognition determinants on the H-2K^b molecule. Further analysis of such mutant strains, combined with comparative analyses of MHC molecules of existing standard haplotypes, should provide clues to the structure–function relationships of gene products of the murine MHC and may suggest a mechanism for the generation of polymorphism within the MHC.

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References

Coligan, J. E., Kindt, T. J., Ewenstein, B. M., Uehara, H., Nisizawa, T., & Nathenson, S. G. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 3390.

Coligan, J. E., Kindt, T. J., Ewenstein, B. M., Uehara, H., Martinko, J. M., & Nathenson, S. G. (1979) *Mol. Immunol. 16*, 3.

Coligan, J. E., Kindt, T. J., Nairn, R., Nathenson, S. G., Sachs, D., & Hansen, T. H. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1134.

Dixon, H. B. F., & Perham, R. N. (1969) *Biochem. J. 109*, 312.

Ewenstein, B. M., Freed, J. H., Mole, L. E., & Nathenson, S. G. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 915.

Ewenstein, B. M., Nisizawa, T., Uehara, H., Nathenson, S. G., Coligan, J. E., & Kindt, T. J. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 2909.

Graff, R. J., & Nathenson, S. G. (1971) *Transplant. Proc. 3*, 249.

IUPAC–IUB Commission on Biochemical Nomenclature (1968) *J. Biol. Chem. 243*, 3557.

Kimball, E. S., Maloy, W. L., Martinko, J. M., Nathenson, S. G., & Coligan, J. E. (1980) *Mol. Immunol. 17*, 1283.

Kohn, H. I., Klein, J., Melvold, R. W., Nathenson, S. G., Pious, D., & Schreffler, D. C. (1978) *Immunogenetics (N.Y.) 7*, 279.

Marshall, R. D. (1972) *Annu. Rev. Biochem. 41*, 673.

Nairn, R., Nathenson, S. G., & Coligan, J. E. (1980a) *Eur. J. Immunol. 10*, 495.

Nairn, R., Yamaga, K., & Nathenson, S. G. (1980b) *Annu. Rev. Genet. 14* (in press).

Orr, H. T., Lopez de Gastro, J. A., Lancet, D., & Strominger, J. L. (1979a) *Biochemistry 18*, 5711.

Orr, H. T., Lopez de Castro, J. A., Parham, P., Ploegh, H. L., & Strominger, J. L. (1979b) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4395.

Shimada, A., & Nathenson, S. G. (1969) *Biochemistry 8*, 4048.

Trägärdh, L., Rask, L., Wiman, K., Kohlman, J., & Peterson, P. A. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 1129.

Uehara, H., Ewenstein, B. M., Martinko, J. M., Nathenson, S. G., Coligan, J. E., & Kindt, T. J. (1980a) *Biochemistry 19*, 306.

Uehara, H., Ewenstein, B. M., Martinko, J. M., Nathenson, S. G., Kindt, T. J., & Coligan, J. E. (1980b) *Biochemistry* (preceding paper in this issue).