Amino Acid Sequence of the Carboxyl-Terminal Hydrophilic Region of the H-2K^b MHC Alloantigen. Completion of the Entire Primary Structure of the H-2K^b Molecule†

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ABSTRACT: The amino acid sequence of the COOH-terminal hydrophilic region of the H-2K^b histocompatibility antigen was determined. The sequence was completed by analyses of four CNBr fragments obtained from the intact molecule as well as tryptic peptides. This region was composed of 39 amino acid residues with a cluster of basic residues at the NH_2 terminus and localized positions 308–346 of the H-2K^b molecule. These sequence data, together with those reported for the NH_2-terminal 284 residues [Martinko, J. M., Uehara, H., Ewenstein, B. M., Kindt, T. J., Coligan, J. E., & Nathenson, S. G. (1980) *Biochemistry 19*, 6188–6193] and for the intramembranous segment [Uehara, H., Coligan, J. E., & Nathenson, S. G. (1981) *Biochemistry* (preceding paper in this issue)], provided the complete primary structure of the H-2K^b molecule. This is the first histocompatibility antigen for which the entire primary structure is determined.

The regulation of the immune response depends on specific interactions between a variety of cells in the immune system. These cell–cell interaction events are mediated by a number of different cell surface molecules. The H-2 major histocompatibility complex (*MHC*)[1] of the mouse is a cluster of genes which control the expression of several sets of cell surface molecules involved in cooperation between lymphoid cells which leads to humoral and cell mediated immune responses [see reviews by Snell et al. (1976), Paul & Benacerraf (1977), Göetz (1977), Klein (1979), and Festenstein & Demant (1979)]. The class I H-2 histocompatibility antigens coded for by the H-2 K, D, and L genes in the murine MHC are a set of highly polymorphic, integral membrane glycoproteins [reviewed in Snell et al. (1976) and Nathenson et al. (1981)] involved in the immune recognition and killing of virally or chemically altered cells by cytotoxic T lymphocytes [reviewed in Shearer & Schmitt-Verhulst (1977) and Zinkernagel & Doherty (1979)]. Elucidation of the primary structure of the H-2 antigens is required in order to understand the structural basis of the function(s) of these molecules and to obtain knowledge of the molecular basis for the polymorphism.

Studies on the primary structure of H-2K^b MHC class I antigens from the H-2^b haplotype have been carried out by using radiochemical microsequencing techniques (Ewenstein et al., 1978; Coligan et al., 1978, 1979; Uehara et al., 1980a,b; Martinko et al., 1980). Data presented in the previous paper (Uehara et al., 1981) on the intramembranous hydrophobic segment together with the sequence determination presented here on the COOH-terminal hydrophilic region complete the determination of the primary structure of the H-2K^b glycoprotein.

Materials and Methods

Isolation of H-2K^b. Radiolabeled H-2K^b glycoproteins were isolated by indirect immunoprecipitation from glycoprotein fraction which was obtained by lentil lectin affinity chromatography of Nonidet P-40 cell extract (Brown et al., 1974). Sephadex G-75 gel filtration was utilized to separate noncovalently associated β_2-microglobulin (β_2m) (Ewenstein et al., 1978).

Isolation of CNBr Fragments. CNBr cleavage of the H-2K^b molecules and isolation of fragments on a Sephadex G-100 or Sephacryl S-200 column in 6 M guanidine hydrochloride were performed as described by Ewenstein et al. (1978). Fragments in CNBr pool III were separated by gel filtration on a column of Sephadex G-50 Superfine (1.5 cm × 220 cm) equilibrated with 1 M HCOOH, and fragments in CNBr pool V were separated on a column of Sephadex G-25 (0.9 × 210 cm) also equilibrated with 1 M HCOOH.

Preparation of Tryptic Peptides from H-2K^b. Radiolabeled H-2K^b glycoproteins were isolated by immunoprecipitation and separated from β_2m and high molecular weight materials by chromatography on a column of Bio-Gel A-0.5m (2.5 cm × 100 cm) in the presence of 0.1% $NaDodSO_4$ (Brown et al., 1974). The H-2K^b molecules were digested with TPCK-trypsin (Worthington Biochemicals, Freehold, NJ) as described by Brown et al. (1974). Peptides soluble in 20% CH_3COOH were fractionated by cation-exchange chromatography on a column of Spherix resin (type XX80-60-X) as described by Brown et al. (1974). In the case of one of the overlap peptides, the peak fraction was further purified by gel filtration on a column of Sephadex G-25 (0.9 cm × 200 cm) equilibrated with 1 M HCOOH.

Preparation of Tryptic Peptides from Citraconylated H-2K^b. Citraconylation of the H-2K^b molecule purified by Bio-Gel A-0.5m column chromatography was performed in the presence of 5 mg of bovine serum albumin (Martinko et al., 1980) by the modified method of Dixon & Perham (1968). The sample was digested by TPCK-trypsin, and peptides soluble in 20% CH_3COOH were applied to the column of Sephadex G-50 (Superfine) (0.9 cm × 170 cm) equilibrated with 1 M HCOOH. Pools were made based on the presence

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[1] Abbreviations used: MHC, major histocompatibility complex; β_2m, β-2-microglobulin; PTH, phenylthiohydantoin; LC, high-performance liquid chromatography; IgM, immunoglobulin M; TPCK, L-1-(tosylamido)-2-phenylethyl chloromethyl ketone; $NaDodSO_4$, sodium dodecyl sulfate.

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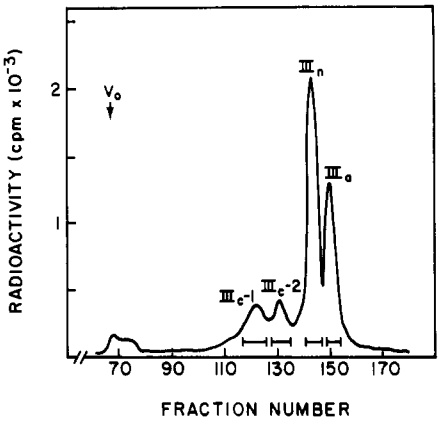

FIGURE 1: Gel filtration of pool III obtained from CNBr cleavage of intact molecule on a Sephadex G-50 column. A sample labeled with [^{3}H]FALKPYR was chromatographed on a Sephadex G-50 (Superfine) column (1.5 cm × 220 cm) equilibrated with 1 M HCOOH. The flow rate was 6.0 mL/h, and 2.0-mL fractions were collected. The fragments were detected by sampling each fraction for radioactivity; pools were made as indicated. V_0 indicates the void volume of the column.

of ^{35}S radioactivity and, after lyophilization, fractionated by cation-exchange chromatography as described by Brown et al. (1974).

Automated Amino Acid Sequence Analysis. Extensive details of these methods were described previously (Coligan et al., 1979; Uehara et al., 1980a). Briefly, a Beckman automated sequencer (Model 890-C) was utilized for Edman degradations with Beckman programs 102974, 121078, and 21980 in the presence of several milligrams of horse heart cytochrome *c*. The latter two programs were used after a cold trap was added to the sequencer. Polybrene (Aldrich Chemical Co., Milwaukee, WI) was added to the sequencer cup to prevent premature washout (Klapper et al., 1978). The detection of radioactivity, conversion of thiazolinone amino acids to phenylthiohydantoin (PTH) derivatives, and identification of PTH amino acids were performed as described before (Coligan et al., 1979; Uehara et al., 1980a).

Results

Isolation of CNBr Fragments from the COOH-Terminal Hydrophilic Region of the Molecule. Primary structural studies of the H-2K^b molecule have employed CNBr cleavage as the initial step for generating peptides amenable to sequence analysis (Ewenstein et al., 1978). Among the nine CNBr fragments isolated from the intact H-2K^b molecule, four small fragments (<35 residues) (termed CN-IIIc, CN-Va, CN-Vb, and CN-Vc) were not present in the CNBr digest of the 37 000 molecular weight papain derived fragment which had lost the COOH terminal (about 60 residues) of the intact molecule (Ewenstein et al., 1978). This indicates that these four CNBr fragments were derived from the portion of the H-2K^b molecule carboxy terminal to the papain cleavage site at position 281 (Martinko et al., 1980). Previously, CN-IIIc could be resolved as a single peak separated from a mixture of CN-IIIn and IIIa by gel filtration on a column of Sephadex G-50 (Ewenstein et al., 1978). Improved resolution of CNBr pool III was gained in this study by using a larger column. CN-IIIn and CN-IIIa were resolved into separate peaks, and CN-IIIc was found to be heterogeneous in that it eluted as two peaks, CN-IIIc-1 and CN-IIIc-2 (Figure 1). CN-IIIc-1 and IIIc-2 have the same NH_2-terminal sequence and appear to differ only in length (see below). Fragments in pool V, CN-Va, CN-Vb, and CN-Vc are small fragments which were separated by gel filtration on a Sephadex G-25 column equilibrated with

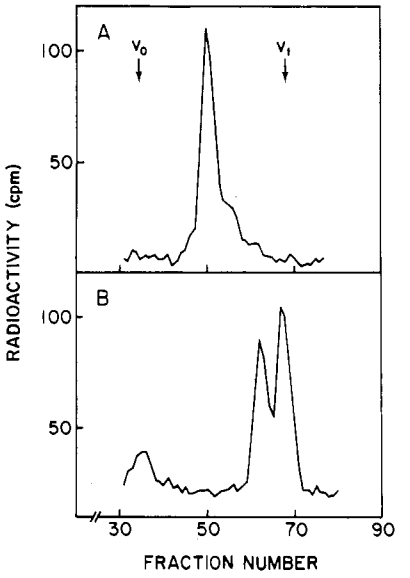

FIGURE 2: Gel filtration of pool V obtained from CNBr cleavage of intact molecular on a Sephadex G-25 (Superfine) column (0.9 cm × 110 cm) equilibrated with 1 M HCOOH. (A) Sample labeled with [^{3}H]SIGHWT. (B) Sample labeled with [^{3}H]Lys. The flow rate was 2.0 mL/h, and 0.7-mL fractions were collected. The void (V_0) and included (V_t) volumes of the column are indicated.

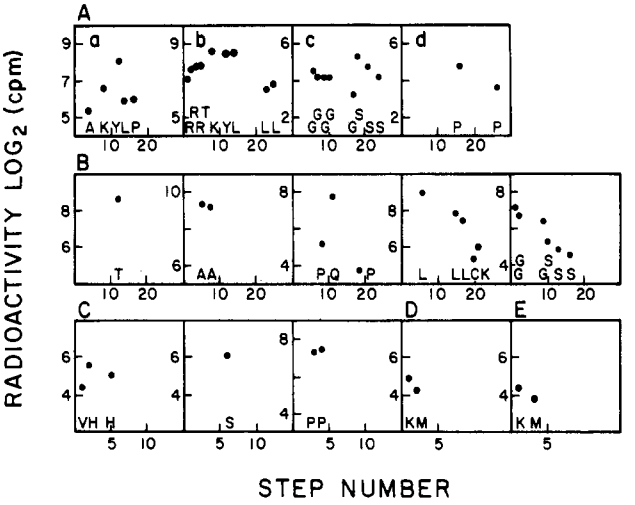

FIGURE 3: Radioactivity above background [as log$_2$ (cpm)] recovered in each step of the amino acid sequence analysis plotted against step number. The amino acid residue assigned at each position is indicated in single-letter amino acid code. (A) Sequence analysis of CN-IIIc: (a) CN-III$_c$-2, (b) CN-III$_c$-1, (c and d) mixture of CN-III$_c$-1 and CN-III$_c$-2. (B) Sequence analysis of TO4. (C) Sequence analysis of CN-Va. (D) Sequence analysis of CN-Vb. (E) Sequence analysis of CN-Vc.

1 M HCOOH (Figure 2). As shown in Figure 2, CN-Vb and -Vc were specifically labeled by [^{3}H]Lys whereas CN-Va was not.

Sequence Determination of the COOH-Terminal CNBr Fragments. The amino acid sequence analyses of the COOH-terminal hydrophilic fragments are shown in Figures 3 and 4. The method of identification of each residue (Table I-S) is provided as supplementary material.

The sequences of CN-Vb and -Vc were determined as Lys-Met and Lys-( )-Met, respectively. The identification of a Met residue at the COOH terminus of the fragment agrees with the specificity of CNBr cleavage. On the other hand, the sequence of CN-Va was found to be Val-His-Pro-Pro-His-Ser. Sequence analyses of CN-IIIc-1 and CN-IIIc-2 revealed that they have identical NH_2-terminal sequences.

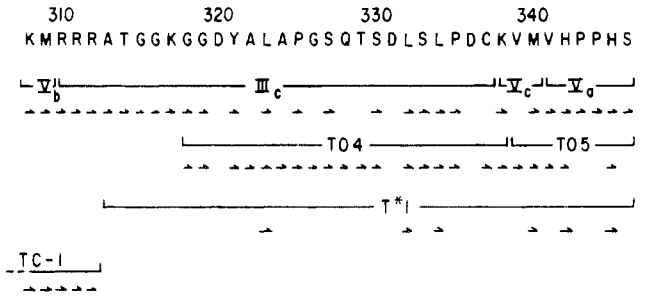

FIGURE 4: Summary of the amino acid sequence information for the COOH-terminal region of the H-2K^b molecule. The numbers above the sequence refer to residue positions in the intact molecule. The first line of arrows depicts residues identified by NH_2-terminal sequence analysis of each CNBr fragment. Other arrows depict the sequence analysis of tryptic peptides (TO, TC) and tryptic peptide obtained after citraconylation (T*). The assignment of Val in CN-V$_c$ is based on isolation and sequence analysis of the tryptic overlap peptide TO5. As described previously, Asp residues were assigned to positions where none of the other 19 amino acid residues could be detected (Uehara et al., 1980a).

This sequence is shown as IIIc in Figures 3 and 4. The difference between these two fragments is most likely due to a partial and inappropriate cleavage at Cys-337 of the H-2K^b molecule which results in the generation of peptides of different length (see below).

The NH_2-terminal sequence analyses of CN-IIIc-1 and CN-IIIc-2 were hampered by low yield of these peptides. Therefore, the sequence of the region from which CN-IIIc is derived was completed by analysis of the tryptic peptide TO4, a peptide isolated from the tryptic digest of the intact H-2K^b molecule by cation-exchange column chromatography (data not shown). The sequence of TO4 (positions 319–338) starts after the Lys-8 of CN-IIIc and ends with the Lys residue that initiates CN-Vc (Figures 3 and 4).

Alignment of the COOH-Terminal CNBr Fragments. The CN-IIIc, -Va, -Vb, and -Vc fragments were aligned by isolation and sequence analysis of the overlap peptides, TO4, TO5, T*1, and TC-1, as shown in Figure 4. TO5 (residues 339–346), which is the overlap peptide between CN-Vc and -Va, was isolated by ion-exchange column chromatography of the tryptic digest of [^{3}H]HITVW/[^{35}S]M-labeled H-2K^b molecule, followed by gel filtration on a Sephadex G-25 column (data not shown). The sequence of TO5 was determined to be Val-Met-Val-His-( )-( )-His-; thus the alignment of CN-Vc → CN-Va and the complete sequence of CN-Vc were established.

Peptide T*1 (residues 313–346) was obtained from a tryptic digest of the citraconylated H-2K^b molecule labeled with [^{3}H]His/[^{35}S]Met or [^{3}H]Leu/[^{35}S]Met. After the Lys residues were modifed by citraconic anhydride, the H-2K^b molecule was digested by trypsin, and peptides soluble in 20% CH_3COOH were applied on a column of Sephadex G-50 (Figure 5). The first eluted Met-containing peak was further purified by ion-exchange column chromatography, and the Met-containing peptide T*1 was isolated (Figure 6). The amino acid sequence of T*1 (the two peaks pooled as shown by bar gave identical sequence) was found to be Leu-11, Leu-20, Leu-22, Met-28, His-30, and His-33. The sequence of T*1 indicates that fragments CN-IIIc, CN-Vc, and CN-Va are aligned consecutively, as shown in Figure 4. These data further suggest that fragments CN-IIIc-2 and CN-Va arise from an inappropriate and partial CNBr cleavage after Cys-337. It is likely that CN-IIIc-1 is the appropriate CNBr cleavage product consisting of CN-IIIc-2 and CN-Va. The alignment of CN-Vb → CN-IIIc is based on the sequence of peptide TC-1. This peptide contains the transmembrane hydrophobic segment and is described in the preceding paper (Uehara et al., 1981).

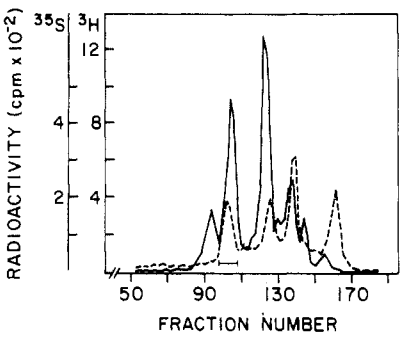

FIGURE 5: Gel filtration of tryptic digest of citraconylated H-2K^b on a column of Sephadex G-50 (Superfine) (0.9 cm × 170 cm) equilibrated with 1 M HCOOH. The tryptic peptides soluble in 20% CH_3COOH obtained from [^{3}H]His/[^{35}S]Met-labeled H-2K^b were applied to the column. The fraction size was 0.70 mL, and the flow rate was 2.2 mL/h. The pool indicated was made based on ^{35}S radioactivity and subjected to further purification (Figure 6). (—) ^{3}H; (---) ^{35}S.

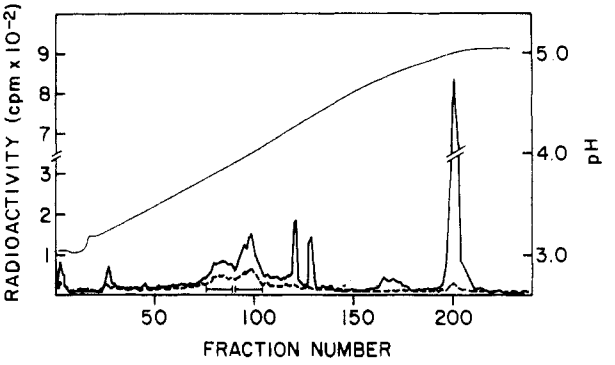

FIGURE 6: Spherix cation-exchange column chromatography of [^{3}H]His/[^{35}S]Met-labeled tryptic peptides. The peptides were obtained as indicated in Figure 5. Chromatographic conditions were described by Brown et al. (1974). Pools were made based on ^{35}S radioactivity. (—) ^{3}H; (---) ^{35}S.

Discussion

We have determined the amino acid sequence of the COOH-terminal region (positions 308–346) of the H-2K^b molecule. The sequence of this hydrophilic region (50% of the residues are polar) was determined by analyses of four CNBr fragments and two tryptic peptides. Tryptic peptides were also utilized to obtain overlapping sequences for the alignment of the CNBr fragments. The COOH-terminal region begins with a cluster of basic residues [Lys-(Met)-Arg-Arg-Arg-] immediately following the putative transmembrane segment [see Uehara et al. (1981)] and most likely ends at Ser-346. The COOH-terminal residue was assigned as Ser on the basis of the sequence analysis of CN-Va preparations labeled in all amino acids but Asp. Therefore, it is still possible that an Asp residue(s) is localized to the COOH terminus of Ser-346.

Comparison of the amino acid sequence of the COOH-terminal hydrophilic regions of H-2K^b to the corresponding regions of HLA-B7 and HLA-A2 (Robb et al., 1978) as well as to amino acid sequences deduced from cDNA clones (obtained from H-2^d haplotypes) (Steinmetz et al., 1981) reveals remarkable sequence differences (Figure 7). Thus, the amino acid sequence homology of HLA-B7 and A2 to H-2K^b is only 50% and 44%, respectively, whereas these two HLA molecules are 88% homologous to each other. Furthermore, gaps at two different sites, one in the human molecule and one in H-2K^b,

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                310          320          330          340
H-2K^b   KMRRRATGGKGGDYALAPGS***QTSDLSLPDCKVMVHPPHS
pH-2 II  -*--N----------***-S-EM--R--GDTLGSDWGGAMWT
pH-2 I       N----------***-S--M----------
HLA-B7   C*--KSS-------S-SQ-AC-DSA-G--V--TA
HLA-A2   W*--KSSDR----S-SQ-AS-DSAZG--V--TA
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FIGURE 7: Amino acid sequence comparison of the COOH-terminal hydrophilic region of the H-2K^b, pH-2I, pH-2II, and HLA molecules. The numbers above the sequences refer to residue positions in the intact H-2K^b molecule. The sequences of other molecules were aligned on the basis of a cluster of basic residue (Arg-Arg-) at the NH_2 terminus. Identical residues are identified. (*) Gaps required to achieve maximum homologies. The data for HLA-B7 and A2 were taken from Robb et al. (1978). The sequences of pH-2I and pH-2II are from Steinmetz et al. (1981).

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1                                                    45
GPHSLRYFVTAVSRPGLGEPRYMEVGYVDDTEFVRFDSDAENPRY

46                                                   90
EPRARWMEQEGPEYWERETQKAKGNEQSFRVDLRTLLGYYNQSKG
                                                  CHO

91                                                  135
GSHTIQVISGCEVGSDGRLLRGYQQYAYDGCDYIALNEDLKTWTA
                 └──────S──S──────┐
136                                                 180
ADMAALITKHKWEQAGEAERLRAYLEGTCVEWLRRYLKNGNATLL
                                                  CHO

181                                                 225
RTDSPKAHVTHHSRPDDKVTLRCWALGFYPADITLTWQLNGEELI
                              └──S──S──┐
226                                                 270
QDMELVETRPAGDGTFQKWASVVVPLGKEQYYTCHVYQQGLPQPL

271          Papain                                 315
TLRWDEPPSTV↓SNMATVAVLVVLGAAIVTGAVVAFVMKMRRRATG
                       |  Transmembrane  |
316
GKGGDYALAPGSQTSDLSLPDCKVMVHPPHS
                                                   346
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FIGURE 8: Amino acid sequence of the H-2K^b molecule. (CHO) carbohydrate moiety.

are required to maximize the interspecies homology to these levels. These levels of homology between the murine and human molecules are similar to what was observed for the intramembranous region (Uehara et al., 1981) but are markedly different from the high homology (about 70%) noted for the extracellular portion of the molecule (positions 1–284) (Martinko et al., 1980). Also, the two HLA molecules are eight amino acid residues shorter than the H-2K^b molecule. The amino acid sequences of two cDNA clones also reveal differences in length from H-2K^b in their COOH terminus. Furthermore, for one of the cDNA clones, pH-2II, there was only a 64% sequence homology to H-2K^b. In contrast, the amino acid sequence comparison of the NH_2-terminal 100 residues of H-2K^b, D^b, K^d, and D^d yields 75–88% homologies without introducing any gaps [reviewed in Nathenson et al. (1981)].

These extensive sequence differences found in the COOH-terminal regions both within and between species products might be related to the possible difference of function(s) of these regions from those of the NH_2-terminal portion of the molecule. The COOH-terminal portion of the molecule might be involved in transmittance of signal(s) from outside of the cell to cytoplasmic component(s) in the recognition of the immune system (Edelman, 1976). It is possible that different evolutionary pressures, reflecting different functions of these regions, have generated the features observed. The questions

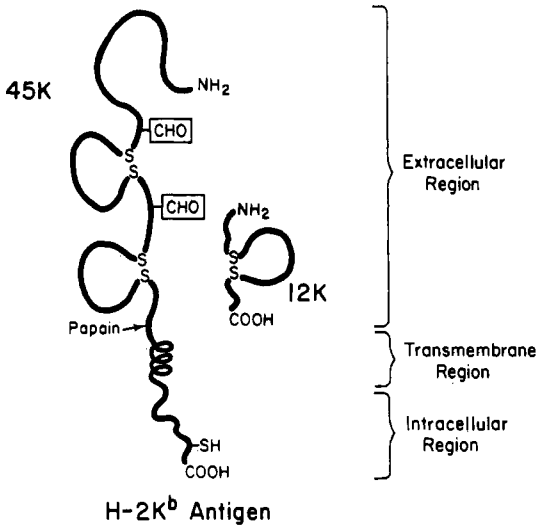

FIGURE 9: Schematic presentation of the H-2K^b molecule.

remain as to how the remarkable diversities are related to the possible function(s) of this region.

The amino acid sequence reported in this paper (residues 308–346) along with that reported previously for the extracellular portion (residues 1–284) (Martinko et al., 1980) and the membrane-associated portion (residues 285–307) (Uehara

et al., 1981) provides the complete primary structure of the H-2K^b molecule (Figure 8). The intramolecular organization of the molecule as well as postulated transmembrane orientations, which are suggested from the amino acid sequence information, is schematically shown in Figure 9. The H-2K^b molecule consists of 346 amino acid residues and two carbohydrate chains attached via Asn linkages at positions 86 and 176 (Uehara et al., 1980a,b). The molecular weight of the molecule is calculated to be 45 517 assuming the molecular weight of the carbohydrate moieties to be approximately 6600 (Muramatsu & Nathenson, 1971). Two linearly arranged intrachain disulfide loops are present in the extracellular portion of the molecule, between Cys-101 and Cys-164 and between Cys-203 and Cys-259 (J. M. Martinko, R. Halpern, J. Adlersberg, and S. G. Nathenson, unpublished results). A highly hydrophobic transmembrane segment (residues 284–307) occurs between the extracellular region (residues 1–283) and intracellular region (residues 308–346). The presence of a cluster of basic residues in the COOH terminus of the transmembrane segment (residues 308–312) has also been reported in a number of membrane-binding proteins, including glycophorin A (Tomita et al., 1978), membrane-bound IgM (Rogers et al., 1980), and the homologous HLA-B7 and A2 molecules (Robb et al., 1978). The function of these positively charged residues may be to anchor the molecule by interacting with negatively charged phospholipid groups in the membrane. Whether the phosphate group suggested to be present in this portion (Rothbard et al., 1980) is involved in interactions with cellular components is an important point which remains to be pursued.

The complete primary structure of the molecule was determined solely by radiochemical techniques; this demonstrates the feasibility of using such methodology for the structural studies of large proteins. As described previously (Coligan et al., 1978; Uehara et al., 1980a; Martinko et al., 1980), except for those modifications necessitated by the use of radioactivity for identification, the overall strategy employed for this study has been similar to that used for structural studies of proteins available in milligram amounts. The advantage of the approach is that the primary structure of proteins available in minute amounts can be determined as long as they can be radiolabeled biosynthetically by an appropriate cell population.

The primary structural studies on H-2K^b have provided a prototype both for methodology for structural studies on other H-2 molecules and for amino acid sequence comparisons to such molecules. In this regard, structural studies on several other H-2 molecules, H-2D^b (Maloy et al., 1981), H-2K^d (Kimball et al., 1981), H-2D^d (Nairn et al., 1981), and H-2K^k (Rothbard et al., 1980) are at various stages of completion. For H-2K^b, D^b, K^d, and D^d, where sequence information is complete for the NH_2-terminal 100 residues, sequence homologies ranging from 75% (K^b vs. K^d) to 88% (K^b vs. D^d) were observed (Nathenson et al., 1981; Coligan et al., 1981). The sequence differences between H-2 molecules are not distributed randomly, but in the main occur in several discrete regions such as positions 61–83 and positions 95–99. It is tempting to speculate that regions of diversity, which are observed between various H-2 molecules, are involved in recognition by alloantibodies and by T-lymphocytes. However, biochemical studies on H-2 mutant molecules indicate that only a small number of amino acid differences are sufficient for T-cell recognition. H-2 molecules isolated from K^b mutants, which reveal histogenic reactivity with parental strain, have only one or two amino acid differences from the parent H-2K^b molecule (Ewenstein et al., 1980; Nisizawa et al., 1981; reviewed by Nairn et al. (1980)]. Thus, because of the large number of differences between standard H-2 allelic glycoproteins, it is difficult to define specific structure–function relationships for the H-2 molecules. Therefore it is clear that a great deal more information, including more actual sequence data as well as studies on three-dimensional structure, is needed to develop a precise understanding of structural "sites" recognized by immune T cells and by antibodies. The attainment of the first total sequence of an H-2 molecule is but the first essential step toward gaining this level of understanding.

Acknowledgments

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Supplementary Material Available

A table outlining the method of identification for each of the residues in the peptides (2 pages). Ordering information is given on any current masthead page.

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Characterization of Short-Chain Alkyl Ether Lecithin Analogues: ^{13}C NMR and Phospholipase Studies†

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ABSTRACT: Several short-chain ether-linked lecithin analogues (*rac*-1,2-dihexyl-, *rac*-1,2-diheptyl-, and *rac*-1,2-dioctyl-*sn*-glycero-3-phosphocholine and L-1,2-diheptyl-*sn*-glycero-3-phosphocholine) have been synthesized and characterized. When dispersed in aqueous solution, these synthetic phospholipids form micelles (not bilayers) and can be used to investigate phospholipase action. Critical micellar concentrations are 1.5- to 2-fold lower than those of the comparable chain length diacyllecithins. This critical micelle concentration difference corresponds to the methylene ether being approximately 200 cal/mol more "hydrophobic" than an ester moiety. This value is compatible with the solvent free energy transfer potentials for ester/ether substitution in model compounds. ^{13}C NMR has been used to characterize the conformation and mobility of short-chain lecithins as monomers and micelles [Burns, R. A., Jr., & Roberts, M. F. (1980) *Biochemistry 19*, 3100]. ^{13}C T_1 relaxation times, chemical shift differences generated in the monomer/micelle transition, and interchain magnetic shift nonequivalence generated by micellization are similar at corresponding carbon positions in ester and ether lecithins. However, T_1 relaxation times do indicate greater fluidity near the terminal methyl end of ether lecithin chains. These data suggest that the carbonyl groups make little contribution to overall lipid conformation and mobility. Ester and ether lecithins form a useful assay system for the phospholipases because substrates and inhibitors can be comixed with minimal change in the characteristics of the interface. Racemic and chiral diheptylphosphatidylcholines are equivalent inhibitors of phospholipase A_2 from *Naja naja naja*. If Michaelis–Menten kinetics are assumed, $K_I \sim 0.2K_m$ of the corresponding diester lecithin. The *sn*-2 ester carbonyl, although the site of hydrolysis, does not dominate the enzyme–lecithin binding interaction. Racemic and chiral diheptyllecithins are extremely poor substrates and/or inhibitors of phospholipase C from *Bacillus cereus*. Enzymatic activity against the ether lecithins could not be detected in the assay system used, nor do the ether lecithins affect the rate of hydrolysis of diheptanoylphosphatidylcholine in a mixed micellar system. This kinetic result suggests a requirement of *B. cereus* phospholipase C for fatty acyl carbonyl groups for efficient substrate binding.

Lecithins are the major lipids in a variety of membranes. Many physical studies have elucidated the conformation, structure, and packing of 1,2-diacyl-*sn*-glycero-3-phosphocholines and -ethanolamines (Seelig & Niederberger, 1974; Yeagle, 1978; Seelig & Browning, 1978; Buldt et al., 1978; Elder et al., 1977; Burns & Roberts, 1980). Yet, the extent to which phospholipid structural features affect chain conformation or aggregation is not well established. Modified lecithins occur in several membranes and may have distinct roles in altering membrane structure. In particular, phospholipids where one or both acyl groups are replaced by alkyl ether moieties are found in a variety of systems (Mangold, 1979). In diacyllecithins the two fatty acyl chains are conformationally nonequivalent: the *sn*-2 carbonyl and first few carbons of the chain are oriented parallel to the interface, while the *sn*-1 chain is perpendicular to the surface. This packing

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