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Do antigenic peptides have a unique sense of direction inside the MHC binding groove?

A molecular modelling study

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In the models suggested recently for antigenic peptides binding in the α_1, α_2 groove of MHC class I molecules, the orientation of the peptide has been shown uniquely as: the N→C vector of the peptide being parallel to the N→C vector of the α_1 helix of MHC. Here, we demonstrate that the reverse orientation of the peptide is equally probable. This hypothesis is supported by molecular modelling calculations and computer graphic analyses on a murine class I MHC molecule H-2K^d and its complexes with a restricted peptide RYLENGKETLQ. Analysis of the complementary interactions between the peptide residues and the amino acid side chains lining the MHC groove shows that the binding orientation of the peptide may be allele-specific and could depend on the sequence and structure of the antigenic epitope.

Major histocompatibility complex, class I; Antigen presentation; Molecular modelling; Binding orientation; Allele-specific

1. INTRODUCTION

T cells recognise viral protein fragments presented on the surface of an infected cell by a class I major histocompatibility complex glycoprotein (MHC) [1]. It is now well established that the association with peptide fragments, either endogenously processed or exogenously added, enhances the structural stability of the MHC class I proteins [2–5]. X-Ray crystallographic analysis of the three-dimensional structures of human MHC proteins, HLA-A2 [6,7]. HLA-Aw68 [8] and HLA-B27 [9] have revealed the presence of additional electron density corresponding to one or more peptides in the binding groove formed by the α_1, α_2 helices and the eight-stranded β-sheet of the MHC heavy chain.

Whereas the rules regarding the putative sequence motif characteristic of naturally processed self-peptides by MHC are being understood [10], relatively little has been known so far about the structure and/or conformation as well as the interactions of these peptides inside the MHC groove. The length and sequence of the characteristic motifs were found to be allele-specific [11,12]; similarly, one would expect differences in the manner of presentation and the conformation of the peptides by different MHC alleles. However, in the putative models recently proposed for peptide–MHC complexes [9,13,14], the orientation of the peptide inside the MHC groove is shown as: the N-terminal to the C-terminal direction (N→C vector) of the peptide is parallel to the N→C vector of the α_1 helix of MHC (Figs. 1 and 2A) (this complex will henceforth be referred to as complex I). Since binding of the peptide in the reverse orientation, where the N→C vector of the peptide is antiparallel to the N→C vector of the MHC α_1 helix (Figs. 1 and 2B) (henceforth referred to as complex II), is also possible, we undertook to study the peptide-binding in the MHC groove in both orientations. In this paper we mainly address the following questions: (i) in which orientation does the peptide in question interact better? (ii) why is one orientation more favourable than the other? and (iii) can general rules for MHC-peptide interactions be formulated? We use here the example of the HLA-A2.*170–180* peptide restricted to the mouse MHC H-2K^d. In previous studies, three agretopic (*Y171, T178, L179*) and two epitopic (*E173, E177*) have been identified based on cytolytic assays with CTL clones restricted to H-2K^d [15,16].

Abbreviations: MHC, major histocompatibility complex coded glycoprotein; NMR, nuclear magnetic resonance.

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2. MATERIALS AND METHODS

The molecular modelling calculations were performed with the murine MHC, H-2K^d and one of its restricted peptide epitopes *RYLENGKETLQ* derived from HLA-A2 and correspond to the region

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170–180, (peptide residues are in *italics* to avoid confusion with the residues of MHC) following the work of Bouillot et al. [17] and Martinon et al. [15]. Since the three-dimensional structure of H-2K^d is not known, it was derived from the known refined crystal structure coordinates of HLA-A2 [7] (Brookhaven Protein Data Bank entry 3HLA [18]), on the basis of extensive sequence homology (73% identity in the α_1 and α_2 domains) between the two MHC proteins. The first step consisted of incorporating the appropriate amino acid changes corresponding to H-2K^d sequence, and then to remove any resultant van der Waals clashes. The final, working three-dimensional structure was obtained by energy minimisation of the modified protein using the molecular mechanics program AMBER [19]. An 11 residue peptide was chosen for complexation instead of the predicted 10-mer for HLA-derived epitopes [12] as it is known that longer peptides can also bind to MHC [15]. The peptide epitope in its conformation derived from the crystallographic coordinates of HLA-A2 [6] followed by modelling based on ^{1}H NMR data (B. Gopalakrishnan, F. Cornille, F. Martinon, N. Morellet, M.C. Founié-Zaluski and B.P. Roques, unpublished) was positioned in the α_1, α_2 groove of H-2K^d. In this orientation the delineated anchor residues *Y171* (2nd residue), *T178* and *L179* (9th and 10th residues) [11,12], which were well oriented on one side of the peptide backbone, contact the MHC residues on the β-sheet floor of the groove. Van der Waals clashes between the peptide atoms and the MHC atoms were manually removed as much as possible before energy minimisation of the complexes (both I and II) with AMBER. To facilitate the peptide backbone to adapt itself to the environment of the H-2K^d groove and to avoid getting stuck in a local minimum on the interaction surface, a systematic and stepwise approach was followed: successive minimisations of the complex of H-2K^d with (i) *AYAAAGAATLA*, (ii) *AYLEAGAETLA* and (iii) the actual epitope *RYLENGKETLQ* were carried out. Alanine was chosen for replacement as it is the smallest representative chiral amino acid and does not disturb the local secondary structure of the polypeptide chain [20]. The total space available for the peptide inside the MHC groove was sampled by rigid-body translations and rotations of the peptide; the best representative complexes were chosen for analysis on the basis of the total energy of interaction. All the calculations (AMBER) were carried out at the CNRS-CIRCE computer center at Orsay (France) and graphic visualisations (Biostructure Inc., Strasbourg) were performed on a Silicon Graphics IRIS 4D/35 workstation.

3. RESULTS AND DISCUSSION

The HLA-A2.*170–180* peptide interacts favourably within the binding groove of H-2K^d in both the parallel and antiparallel orientations. Fig. 1 shows a detailed scheme of nearest neighbour interactions and hydrogen bonds between the peptide and various residues of the binding groove of H-2K^d. The salient features of the two complexes are as follows: the total energy of complex II was more favourable compared to that of complex I by about 20 kcal/mole. The peptide bound slightly

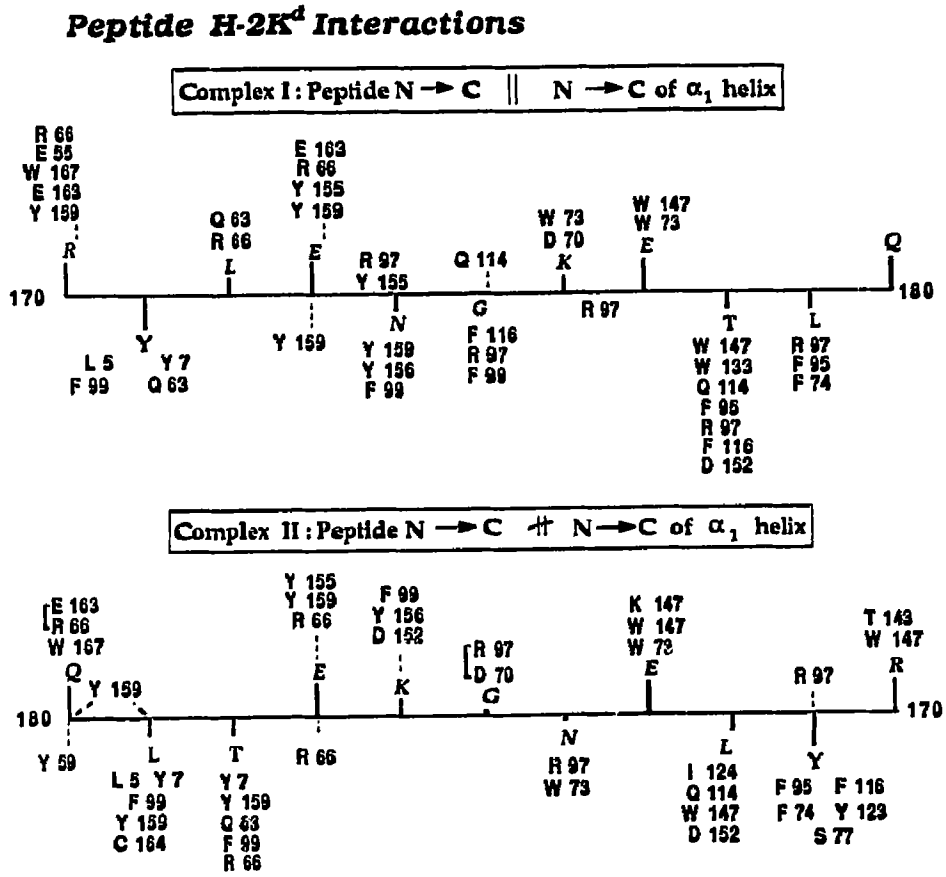

Fig. 1. The nearest neighbour interaction scheme between the residues of HLA-A2.*170–180* peptide and the residues of H-2K^d binding groove in the complexes I and II. Horizontal line represents the peptide chain and the vertical lines indicate residues pointing into (below) the pocket or out (above) of the pocket. No vertical lines mean that the residues are partially into and out of the pocket. Inter-molecular hydrogen bonds are indicated by dashed lines. Salt-bridges in H-2K^d are indicated by '|'.

deeper in complex II than in complex I. The delineated anchor residues of the antigenic peptide *Y171*, *T178* and *L179* are surrounded by hydrophobic residues of the H-2K^d groove in both complexes, though less efficiently in complex I. Thus in complex II the phenolic ring of the principal anchor residue *Y171* of the peptide stacks ideally with F95; *Y171* is flanked by F116 as well and seems to be participating in the middle of a festoon of aromatic residues consisting of F74, F95, F116 and Y123 (Fig. 3). In complex I *Y171* of the peptide is situated between Y7 and 1.5 of H-2K^d but stacks poorly with Y7 (Fig. 2A). The other two anchor residues *T178* and *L179* are also surrounded by hydrophobic residues in both complexes (Fig. 1). Moreover, the greater stabilisation of complex II when compared with complex I also comes from additional intermolecular hydrogen bonds between peptide and H-2K^d molecules. There are eight inter-molecular hydrogen bonds in complex II compared to only four in complex I (Fig. 1).

While possessing several general features common to both complexes, the peptide in complex II has a more well-defined conformation inside the binding groove of H-2K^d. A salt-bridge interaction between the side chains of *R170* and *E173* and four intra-peptide backbone hydrogen bonds: *L172* >C=O --- HN< of *G175* ($i \rightarrow i+3$) and *K176* ($i \rightarrow i+4$), *R170* >C=O --- HN< *N174* ($i \rightarrow i+4$) and *K176* >C=O --- HN< *T178* ($i \rightarrow i+2$), suggesting a helicoidal conformation of the peptide, were found in both complexes. However, the lengths of the hydrogen bonds are longer and hence became weaker in complex I. Residues *L172–E173–N174–G175* formed an ideal type III hydrogen bonded β-turn ($\phi_2, \Psi_2 = -51°, -31°$; $\phi_3, \Psi_3 = -45°, -53°$) in the complex II. This turn has changed its shape slightly in complex I ($\phi_2, \Psi_2 = -45°, -48°$; $\phi_3, \Psi_3 = -45°, -40°$) resulting in the loss of the internal hydrogen bond. In addition, complex II has a γ-turn comprising residues *K176–E177–T178*, adjacent to the β-turn which may be imparting structural stability to the peptide.

The superposition of the two complexes is shown in Fig. 4. The peptide in complex I is slightly pushed towards the α_2 helix, whereas it is positioned more or less in the middle of the groove in complex II as observed in the crystal structure of HLA–B27 complex [9]. The orientation of the peptide in complex I, although comparable to that proposed by Maryanski et al. [14] with respect to its proximity to the α_2 helix, the latter presents a different interaction scheme with the *Y171* of the peptide in close proximity to Y155, Y156 and Y159 on the α_2 helix. Two arginines, R66 on the α_1 helix and R97 situated in the middle of the β-sheet floor seem to largely guide the positioning of the peptide in the groove. These arginines are involved in salt-bridges with their nearest acidic amino acid side chains (E163 and D70, respectively) in complex II. The R66–E163 salt-bridge has been disrupted by *R170* of the peptide in complex I, while the R97–D70 salt-bridge was retained. In both complexes, the peptide residues implicated in T cell receptor recognition, namely, *E173* and *E177*, project outside the groove in a similar fashion ruling out possible distinction between the two orientations by T cell receptors. Similar results have been obtained with the *Plasmodium berghei* PB-CS 253-260 peptide binding to H-2K^d (to be published).

Concerning the question of whether an antigenic peptide has a preferred orientation/direction of binding inside the MHC groove, several examples need to be analysed experimentally and theoretically before a definitive answer could be given. For the peptide in the present study (HLA-A2.*170–180*) binding in the groove of H-2K^d, there is a preference for the orientation as in complex II. The results of Latron et al. [21] for the influenza matrix peptide *57–68* (*KGILGFVFTLTV*) binding in the groove of HLA-A2 support the parallel orientation (complex I). However, the orientational/directional preference may be allele-specific, implying that various antigenic peptides possessing an allele-specific sequence motif interact in a particular orientation. The directional binding mode may be governed by several factors such as, the amino acid sequence and the conformational characteristics of the restricted peptide epitope as well as the polymorphism of MHC molecules which determines the landscape (surface undulation) of the binding groove. Since the length of most peptide epitopes has now been determined to be only 8–10 residues [12], there is enough space for the peptide to move inside the groove, and polymorphic residues on the floor of the groove would guide the optimal positioning of the pep-

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Fig. 2. Stereoviews of the two binding orientations of an antigenic peptide (*RYLENGKETLQ*) inside the MHC groove: (A) complex I and (B) complex II. Only the terminal amino acids are labelled for clarity. The MHC backbone (C^α) is shown in red and the side chains in cyan. The antigenic peptide is shown in yellow.

Fig. 3. Computer graphic picture of the strong hydrophobic stacking interactions of the principal anchor residue in the peptide antigen *Y171* with residues F95, F116, Y123 and F74 of H-2K^d in complex II. Van der Waals dot surface highlights this festoon of closely interacting aromatic residues. Strong hydrogen bonds between the backbone carbonyl of *Y171* (peptide) and the guanidyl group of R97 (H-2K^d) side chain are also shown.

Fig. 4. Stereoview showing the relative positions of the peptides (cyan = complex I; yellow = complex II) inside the H-2K^d binding groove from the two energy-minimised complexes. The α_1 and α_2 helices forming the edges of the binding groove are enveloped in van der Waals dot surface.

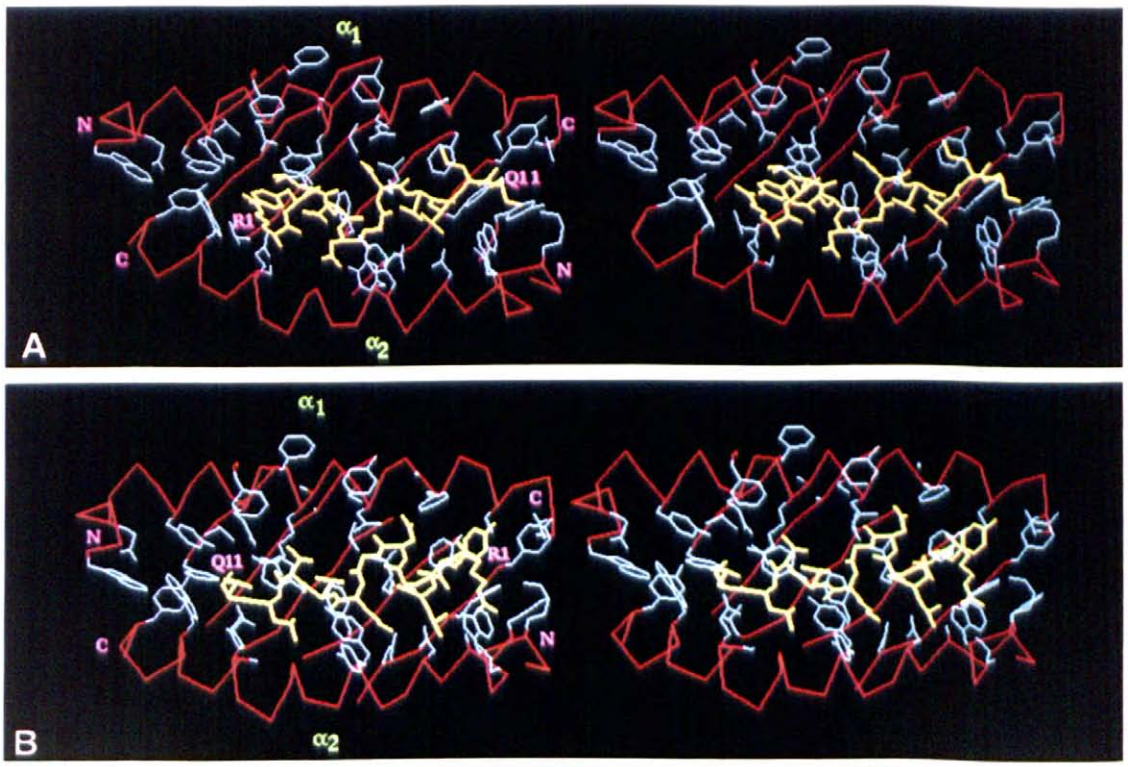

Fig. 2.

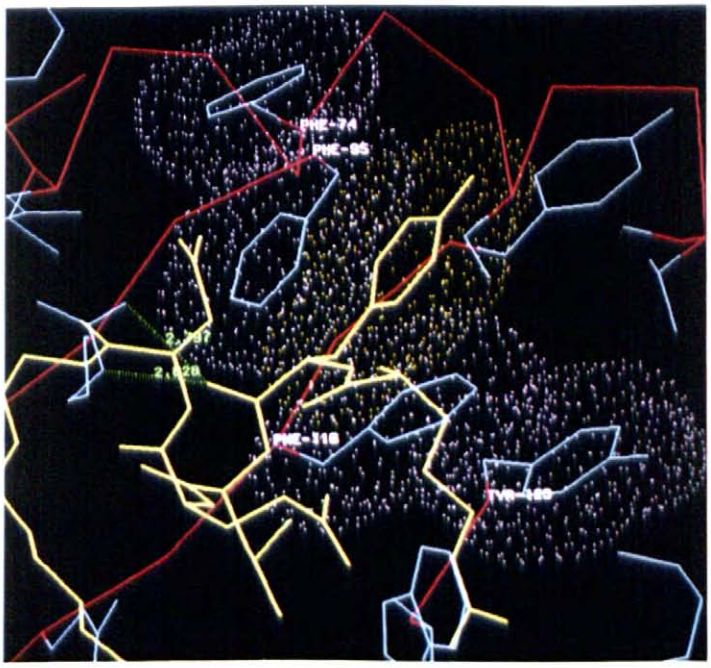

Fig. 3.

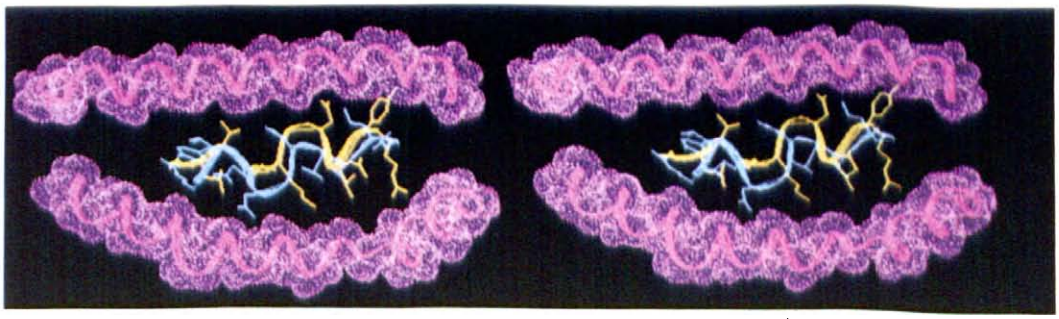

Fig. 4.

tide inside it. However, a unique binding orientation of the antigenic peptides vis-a-vis the helix dipoles [22] of the α_1 and α_2 helices could be imposed by a hypothetical MHC-dependent protease [23] for processing longer peptides bound to MHC.

4. CONCLUSIONS

In summary, the peptide epitope HLA-A2.*170–180* presented by the mouse MHC H-2K^d interacts better when the peptide is orientated with its amino-terminus at the righthand end of the MHC groove, guided by favourable hydrophobic and electrostatic interactions. An understanding of the general interaction pattern of several antigenic peptides in the binding grooves of their restriction MHC molecules could well help to design potent blocking peptide or peptidomimetic antigens. Also, the orientation of antigen presentation by MHC molecules could be allele-specific and perhaps governed by their sequence and structure. More detailed theoretical and experimental analysis on a variety of MHC–peptide complexes is required for confirmation of the models and such studies are in progress in our laboratory.

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