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The Influence of Sequence Microvariation Among HLA-DR3 Alleles on their Structure and Complex Formation with Presentation Peptides

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Abstract The three-dimensional structure of human leukocyte antigens HLA-DR*0301 and HLA-DR*0302 have been calculated using the homology modeling approach. General structural features of our models are similar to those of related HLA molecules. The typical layout of segments of the secondary structure is well preserved. However polypeptide chains are less tightly bound, which causes slightly broader opening of the binding groove. It also results in the modified layout of pockets in the binding groove. Amino acids defining the restricted sequence diversity of the proteins studied are easily available for interactions with ligands.

A set of docking simulations was performed using modeled structures of both HLA molecules and various specific peptide ligands. The control docking of influenza hemagglutinin peptide into the HLA-DR*0101 molecule gives a complex structure which is in good agreement with that from crystallographic studies. The extensive analysis of the structure of modeled complexes of HLA-DR*0301 and HLA-DR*0302 with various ligands indicates that sequence microvariation of both alleles does not directly control the binding specificity. Preferences for binding of specific ligands, as evaluated from interactions in modeled complexes, agree qualitatively with experimental observations. Thus, the computer aided docking simulations can be successfully used to calculate the three-dimensional structure of HLA-ligand complexes. However, a detailed explanation of binding specificity cannot be given using presently available modeling procedures.

Keywords Major histocompatibility compound, Human leukocyte antigen, Presentation peptide, Ligand docking

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Introduction

Major histocompatibility compounds (MHC) are proteins which form unusually stable complexes with peptide antigens, "presenting" them on the cell surface for recognition by T cells of the immune system. Human leukocyte antigens of the DR type (HLA-DR) are predominant alleles belonging to class II of MHC proteins. HLA-DR molecules are composed of two membrane-anchored chains (α and β) containing extracellular ($\alpha 1$ and $\beta 1$) and membrane embedded ($\alpha 2$ and $\beta 2$) domains, respectively. The binding site of HLA-DR molecules has the form of a groove between $\alpha 1$ and $\beta 1$ domains. HLA-DR alleles differ only in the amino acid sequence of $\beta 1$ domains but still preserve a high degree of the sequence homology. Many studies have been devoted to the structural aspects of complex formation [1] and ligand recognition [2] by HLA molecules. In spite of this effort the molecular basis of binding specificity as well as details of interactions with presentation peptides are still unclear.

Posch et al. [3,4] have performed extensive experimental studies on HLA-DR*0301 (DR31) and HLA-DR*0302 (DR32) alleles and their binding with several ligands. Wild type DR31 and DR32 proteins differ only in four amino acids (Scheme 1) and it has been shown that this sequence microvariation correlates well with the binding specificity towards several ligands - influenza hemagglutinin peptide, heat-shock protein, myelin basic protein and sperm whale myoglobin [4]. Thus, the restricted sequence diversity appears to be sufficient for expression of clear binding specificity. A simple explanation could be that such sequence microvariations are reflected in the three-dimensional structure of the binding groove and thus influence the binding properties of HLA-DR3 molecules. In order to prove the above reasoning, it is necessary to model the DR31 and DR32 molecules and perform a comparative analysis of their structures and interactions.

Several crystallographic structures of MHC class II proteins are available in the Protein Data Bank [5]. The structure of HLA-DR*0101 (PDB entry 1DLH) [6] was solved at high resolution and the amino acid sequence is highly homologous to the DR31 and DR32 molecules. Thus it is a suit-

| DR31 | Tyr26, Asp28, Phe47, Val86 |
| DR32 | Phe26, Glu28, Tyr47, Gly86 |

Scheme 1 *Microvariant amino acid residues in wild types of HLA-DR*0301 and HLA-DR*0302 molecules*

able template for molecular modeling studies. PDB entry 1DLH actually contains coordinates of HLA-DR*0101 complexed with influenza hemagglutinin peptide, which is one of ligands also used in studies by Posch et al. [4]. There is also a structure of HLA-DR*0301 complexed with CLIP peptide deposited recently in the PDB (entry 1A6A) [7]. However, this structure was solved by the molecular replacement method using HLA-DR*0101 as reference and could be biased towards the structure of the reference molecule. Thus it seems to be less suitable as a template for molecular modeling. Nevertheless, it can be used for comparison with modeled structures.

In this paper we present modeled structures of the $\alpha 1/\beta 1$ domains dimer for DR31, DR32 and DR*0101 proteins. Also models of docked molecular complexes formed by the above alleles with their specific peptide antigens are presented. Furthermore, analysis of the intermolecular and intramolecular interactions in the computed structures was performed to assess the quality of the models as well as to gain a deeper insight into complex formation between HLA-DR3 alleles and their ligands.

Methods

The $\beta 1$ domain of HLA-DR*0301 (DR31) and HLA-DR*0302 (DR32) proteins, used in modeling studies, comprise fragment 1 - 90 of the β-chain sequence [8]. The three-dimensional structure of these domains were created using the homology modeling approach taking HLA-DR*0101 (PDB entry 1DLH) as the structure template. The aligned sequences are shown in Scheme 2.

The modeled $\beta 1$ domain was than combined with the $\alpha 1$ domain of HLA-DR*0101 so as to form the binding groove.

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            1         11         21         31         41         51

DRB*0101:  --TRPRFLWQ LKFECHFFNG TERVRLLERC IYNQEESVRF DSDVGEYRAV TELGRPDEAY

DRB*0301:  GDTRPRFLEY STSECHFFNG TERVRYLDRY FHNQEENVRF DSDVGEFRAV TELGRPDAEY

DRB*0302:  GDTRPRFLEY STSECHFFNG TERVRFLERY FHNQEENVRF DSDVGEYRAV TELGRPDAEY


            61        71         81

DRB*0101:  WNSQKDLLEQ RRAAVDTYCR HNYGVGESFT

DRB*0301:  WNSQKDLLEQ KRGRVDNYCR HNYGVVESFT

DRB*0302:  WNSQKDLLEQ KRGRVDNYCR HNYGVGESFT
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Scheme 2 *Alignement of the $\beta 1$-domains of HLA-DR*0101, HLA-DR*0301 and HLA-DR*0302 molecules*

Finally, α1/β1 dimers were optimized using molecular dynamics calculations. The crystallographic structures of HLA-DR*0101 (1DLH) and HLA-DR*0301 (1A6A) were also refined by molecular dynamics calculations.

Docking simulations were performed for 4 ligands: influenza hemagglutinin peptide (HA), heat-shock protein peptide (HSP), myelin basic protein peptide (MBP) and sperm whale myoglobin peptide (SWM) [8]. Amino acid sequences of respective fragments are given in Scheme 3.

Atomic coordinates of the peptides from influenza hemagglutinin and sperm whale myoglobin were cut out of the respective Protein Data Bank entries 1DLH and 1VXH and were only refined by molecular dynamics calculation. Three-dimensional structures of the binding peptides from heat-shock protein and myelin basic protein were built from the respective sequences and optimized by means of molecular dynamics.

All basic modeling tasks - sequence alignment, model building as well as model refinement by simulated annealing were done using the program MODELLER (Version 4) [9]. Structures obtained initially by homology modeling were subsequently optimized (molecular dynamics) to obtain the final models. This assures that all result analyses deal with structures obtained at the same level of modeling approximation. The molecular dynamics calculation procedure and parameterization were the same as defined in the respective macros supplied with the program packet.

Docking of presentation peptides in the groove of HLA-DR3 models was performed using the program AutoDock [10]. The HLA-DR3 molecules and peptide ligands were protonated and charged using the standard procedure PREPARE. Each docking simulation took 50 independent runs and each run consisted of 50 annealing cycles. A cycle terminated after reaching 25000 accepted or rejected conformers, whichever came first. Parameters for the annealing procedure were set to values suggested by Morris et al. [11].

Complexes with the lowest total energy were subsequently refined by molecular dynamics calculations and used for further structural analysis. Relatively time-consuming computations of model docking were done on a Sun ULTRA 2 server.

Interactions between peptides and HLA molecules were analyzed using the program CSU (Contacts of Structural Units) obtained by courtesy of Dr. Sobolev [12]. Identification of pockets in the binding grooves was performed on the CAST server (A server for Identification of Protein Pockets & Cavities) [13] located at the University of Minnesota. The modeled structures were uploaded for calculation into the server and results obtained *via* e-mail. The CAST server uses a solvent probe of 1.4 Å.

All molecular graphics were prepared using programs MOLMOL (Version 2.6) [14] and RasMol [15].

Results and discussion

Interactions in crystallographic structures

The crystallographic structure of the HLA-DR*0101 (1DLH) molecule indicates that α1 and β1 domains are in close contact with each other. There are 23 hydrogen bonds and 58 hydrophobic interactions between the two chains which stabilize their relative orientation required for the formation of the binding groove. Analysis of the secondary structure of the two domains with help of the program MOLMOL reveals the presence of 5 helical and 8 sheet segments, comprising 67.5% of all amino acids. Thus, the crystallographic structure of the α1/β1 dimer is highly structured and relatively compact. The detailed results are given in Table 1.

Calculations performed on the CAST server have identified four pockets in the binding groove of HLA-DR*0101. The volume of the pockets amounts to 146(P1), 235(P2), 59(P3) and 342(P4) Å^3 respectively. All pockets have very large openings and they are smoothly interconnected. Inspection of the amino acids lining the wall of pockets does not allow a clear conclusion about the character of the individual pockets. Thus the individual pockets can accept charged as well as hydrophobic ligand residues. All four residues of the sequence microvariation set of DR3 molecules are also exposed in pockets of HLA-DR*0101. The layout of pockets in the binding groove deviates slightly from that postulated by Stern et al. [6]. This discrepancy can be due to the more rigorous method used in present studies for the pocket identification. It seems that some previous analyses of interactions in the binding groove must be revised considering the new pockets layout.

The crystallographic structure of the HLA-DR*0301 (1A6A) molecule has similar structural properties to those of HLA-DR*0101 (1DLH). The binding groove structure is stabilized by 44 interchain hydrogen bonds and 44 hydrophobic interchain interactions. Number and extent of secondary structure segments is identical in both molecules. The results are shown in Table 1 and Figure 1. CAST computation identifies 3 pockets in the binding groove with volumes of 145(P1), 461(P2) and 179(P3) Å^3 respectively (Figure 2). Most residues lining the wall of pockets are the same as in 1DLH. Residues of the sequence microvariation set are exposed in the binding groove, however Tyr26 is located in a small pocket and appears not to be easily accessible for interactions. The relatively small structural difference between the two molecules is rather surprising. Taking into account that out of 90 amino acids in the β1 domains of each allele, 18 are different (20%) and that 39% of the substituted amino acids have changed their character (hydrophobic into charged, etc.),

HA, fragment 306-318:	PKYVKQNTLKLAT	
HSP, fragment 3-15:	KTIAYDEEARR	
MBP, fragment 152-170:	KIFKLGGRDSRSGSPMARR	
SWM, fragment 132-151:	NKALELFRKDIAAKYKELGY	

Scheme 3 *Amino acid sequences of the presentation peptides used in this study. HA - influenza hemagglutinin, HSP - heat-shock protein, MBP - myelin basic protein, SWM - sperm whale myoglobin*

Table 1 *Regions of the secondary structures in some HLA-DR molecules assigned according to calculations using MOLMOL program. **DR11(1DLH)** - PDB entry code: 1DLH; **DR31(1A6A)** - PDB entry code: 1A6A; **DR11** - HLA-DR*0101; **DR31** - HLA-DR*0301; **DR32** - HLA-DR*0302*

Struct	DR11(1DLH) [a]	DR31(1A6A) [a]	DR11 [b]	DR31 [c]	DR32 [c]
			α-chain		
sheet	7-14	7-14	7-8		7-8
sheet			11-12		11-12
sheet	19-26	19-26	21-26	22-23	21-23
sheet					25-26
sheet	29-35	29-35	30-34	33-34	33-34
sheet	40-43	40-43	41-42	41-42	41-42
helix	46-50	46-51	47-50	46-50	46-50
helix	56-77	56-76	57-76	57-77	57-76
			β-chain		
sheet	9-18	9-18	9-12	11-12	11-13
sheet				16-17	16-17
sheet	23-32	23-32		24-25	24-25
sheet			28-32	27-31	27-31
sheet	35-41	35-41	39-40	36-41	36-41
sheet	47-49	47-49	47-48		47-48
helix	52-63	52-63	56-63	56-63	55-63
helix	65-77	65-72	65-77	66-72	65-72
helix		74-77		74-77	
helix	79-85	79-86	79-85	79-85	78-83

[a] crystallographic structure
[b] optimized structure
[c] modeled structure.

larger structural changes could be expected. This structural similarity can be an artifact of the crystallographic methodology and the structure of HLA-DR*0301 seems to be biased towards 1DLH, which was used as a reference for the molecular replacement method.

Interactions in modeled structures

The optimized structure of HLA-DR*0101 (DR11) shows a slightly larger separation of the α1 and β1 chains in comparison with the crystallographic structure. The structure of the binding groove is now stabilized by 14 hydrogen bonds
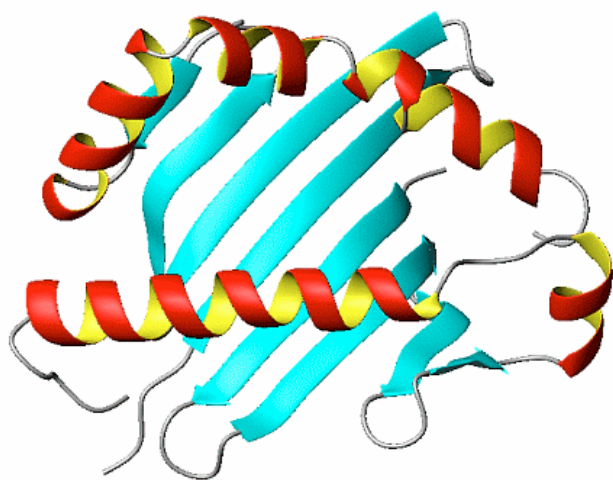

Figure 1 *Secondary structure of the α1/β1-domains of HLA-DR*0301 (crystal structure, PDB entry 1A6A). Red/yellow ribbon - α-helix; turquoise stripe - β-sheet*
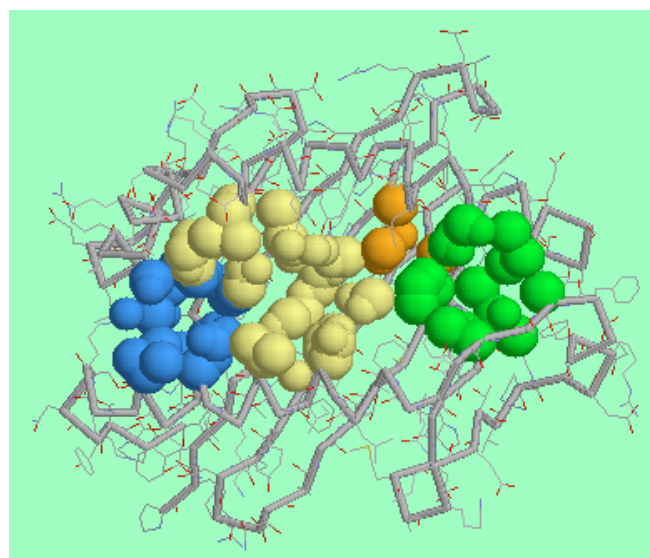

Figure 2 *Pockets in the binding groove of HLA-DR*0301 (crystal structure, PDB entry 1A6A). Different CPK colors indicate distinct pockets*
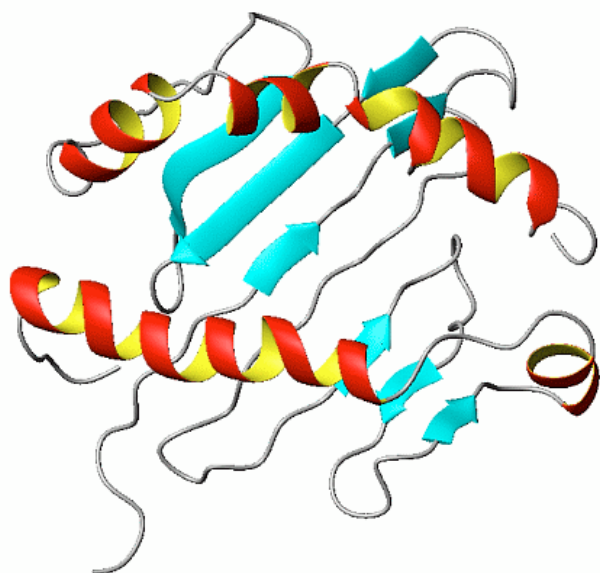
Figure 3 *Secondary structure of the α1/β1-domains in the computed model of HLA-DR*0301. Red/yellow ribbon - α-helix; turquoise stripe - β-sheet*
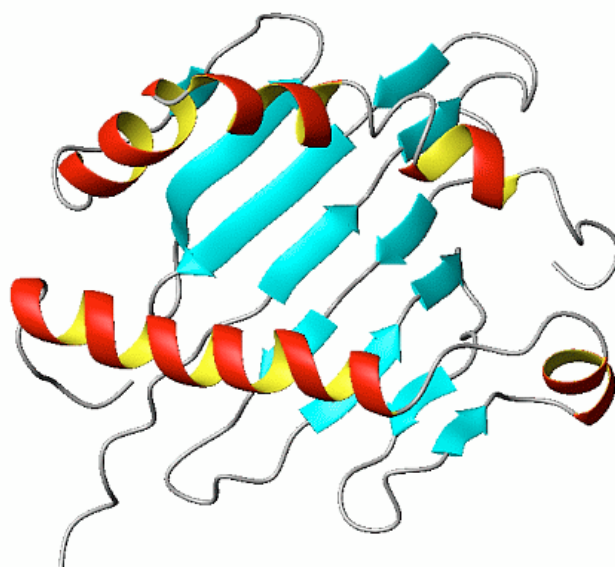

Figure 4 *Secondary structure of the α1/β1-domains in the computed model of HLA-DR*0302. Red/yellow ribbon - α-helix; turquoise stripe - β-sheet*

and 48 hydrophobic interactions. The secondary structure segments identified by means of the program MOLMOL are similar to those found in the the crystallographic structure but all segments of secondary structures are shorter. The details are given in the Table 1. The total number of amino acids involved in structural segments reduces to 48% of all amino acids in the two domains. Optimized structures of the α1/β1 dimers are more relaxed than crystallographic but the

general layout is still preserved. Such a relaxed structure is likely for the native protein contrary to the more compact crystallographic structure.

CAST calculation identifies 1 large pocket, with a volume of 1626 Å^3, which is due to the larger separation of the α1 and β1 chains. Nevertheless, the pocket wall lining amino acids are mostly the same as found in the crystallographic structure. The satisfactory agreement between crystallo-
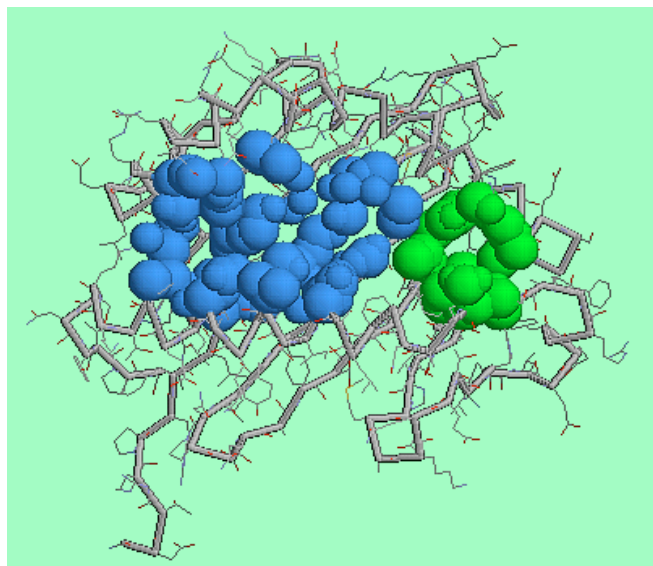

Figure 5 *Pockets in the binding groove in the computed model of HLA-DR*0301. Different CPK colors indicate distinct pockets*
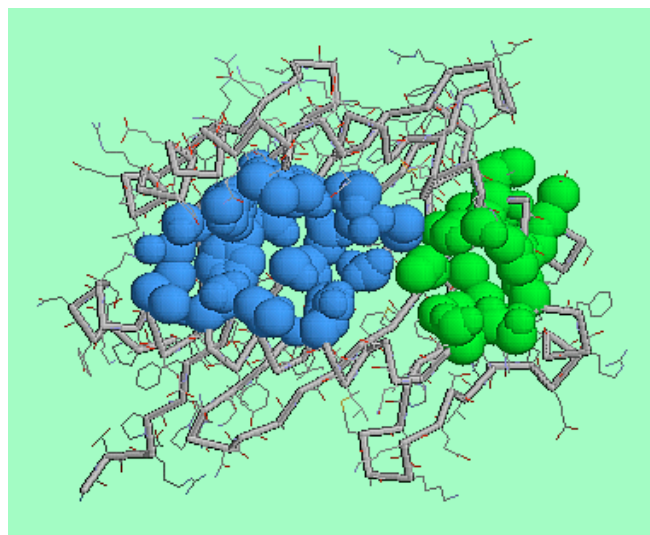

Figure 6 *Pockets in the binding groove in the computed model of HLA-DR*0302. Different CPK colors indicate distinct pockets*

Table 2 *Total number of intermolecular interactions in some human leukocyte antigens complexes with various peptide ligands.* **1DLH** *- HLA-DR*0101;* **1A6A** *- HLA-DR*0301;* **DR11** *- HLA-DR*0101;* **DR31** *- HLA-DR*0301;* **DR32** *- HLA-DR*0302;* **HA** *- influenza hemagglutinin peptide;* **HSP** *- heat-shock protein peptide;* **MBP** *- myelin basic protein peptide;* **SWM** *- sperm whale myoglobin peptide.*

Complex	hydrogen bonds	aromatic--aromatic	hydrophobic--hydrophobic	destabilizing
1DLH + HA [a]	20	4	32	47
1A6A + CLIP [a]	23	0	34	49
DR11 + HA	12	1	14	24
DR31 + HA	10	0	14	27
DR31 + HSP	11	0	14	22
DR31 + MBP	5	0	16	24
DR31 + SWM	6	4	23	34
DR32 + HA	13	0	12	21
DR32 + HSP	11	0	13	30
DR32 + MBP	8	1	12	25
DR32 + SWM	4	1	21	26

[a] crystallographic structures

graphic and modeled structures justifies application of our modeling approach for the calculation of yet unknown three-dimensional structures of HLA alleles.

Modeled structures of DR31 and DR32 molecules are similarly relaxed as that of DR11. The relative orientation of the α1 and β1 domains in the DR31 molecule is stabilized by 17 hydrogen bonds and 51 interchain hydrophobic interactions. Patterns of the secondary structure are less pronounced than in the crystallographic structure or the DR11 molecule (see Table 1 and Figures 3 and 4). The secondary structure in the DR31 dimer comprises 38% of all amino acids (Table 1) but the general, usually observed, layout of the backbone is not distorted. Two large pockets in the binding groove with volumes of 866(P1) and 198(P2) Å^3 have 3 large openings (Figure 5). The P1 pocket of DR31 is equivalent to pockets P1 and P2 identified in DR11. Thus, the general structural layout of the binding groove is not substantially changed. However these minor changes can suffice to control the specificity
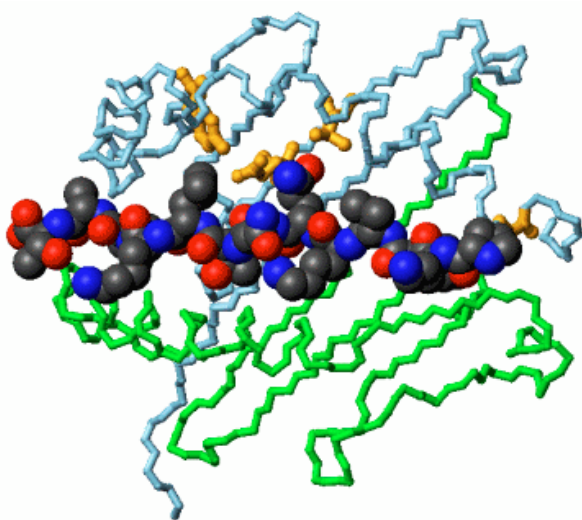

Figure 7 *Complex of the HLA-DR*0101 molecule with influenza hemagglutinin peptide (crystal structure, PDB entry 1DLH). Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of the ligand atoms*

Figure 8 *Computed complex of the HLA-DR*0101 molecule with influenza hemagglutinin peptide. Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of the ligand atoms*
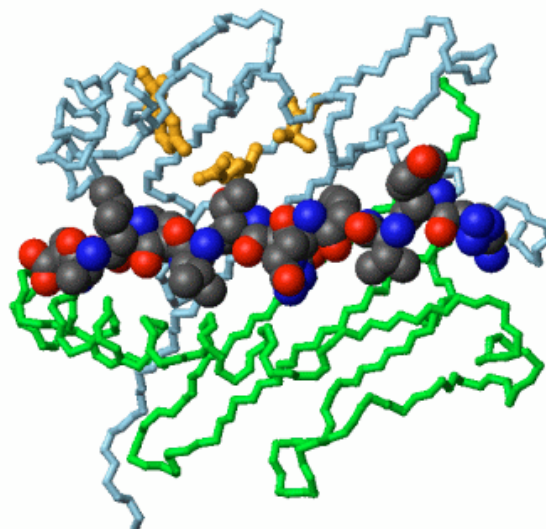
Table 3 *Amino acids of some human leukocyte antigens involved in hydrogen bond formation with respective ligands.* **1DLH** *- HLA-DR*0101;* **1A6A** *- HLA-DR*0301;* **DR11** *- HLA-DR*0101;* **DR31** *- HLA-DR*0301;* **DR32** *- HLA-DR*0302;* **HA** *- influenza hemagglutinin peptide;* **HSP** *- heat-shock protein peptide;* **MBP** *- myelin basic protein peptide;* **SWM** *- sperm whale myoglobin peptide*

| 1DLH [a] | 1A6A [a] | DR11 [b] | DR31 [b] | | | | DR32 [b] | | | |
HA	CLIP	HA	HA	HSP	MBP	SWM	HA	HSP	MBP	SWM
					α-chain					
Q9	Q9	Q9	S53	S53	F51	E55	S53	S53	S53	E55
S53	F51	S53	E55	E55	S53	N62	E55	E55	E55	R76
N62	S53	E55	A61	N62	N69	N69	N62	N62	N69	
N69	E55	N69	N62	R76		N69				
R76	N62		N69			R76				
	N69		R76							
	R76									
					β-chain					
D57	Y30	Y47	P56	P56	R74		P56	R74	T12	P56
Y60	P56	D57	H81	R74			D57	H81	R74	L67
W61	D57	Y60	V85	V85			Y60	V85	H81	
Q70	E59	Q70					Q70			
R71	W61	R71					R74			
T77	K71	T77					V85			
H81	R74	H81								
N82	N77									
	H81									
	N82									

[a] crystallographic structures
[b] modeled structures.

of the two molecules e.g. by restricting access of some ligands to the binding groove. All residues of the sequence microvariation set are easily available in the binding groove and can also contribute to differences in the binding specificity.

In the case of DR32, there are 15 hydrogen bonds and 50 hydrophobic interactions stabilizing the α1/β1 dimer. The secondary structure in the DR32 dimer comprise 41% of all amino acids and the general layout of the backbone, typical for HLA molecules, is again well preserved (Table 1 and Figure 3). Similarly, 2 large pockets can be found in the binding groove of DR32 with volumes of 750(P1) and 409(P2) Å^3 (Figure 6). Thus, the binding groove has a larger total volume and is more open in comparison with DR31, allowing for accommodation of larger ligands. Residues of the sequence microvariation set are also in this case easily available in the binding groove and can control the binding specificity.

Docking of specific peptides into the binding groove

A control docking of HA peptide into modeled DR11 was performed in order to assess the applicability of the docking procedure for HLA-ligand complexes. The structure of the docked complex resembles closely that known from crystallographic studies (Figure 8 and 7 respectively). The RMSD value calculated for docked versus crystallographic atom coordinates of HA peptide in the binding groove is 5 Å. The total number of interactions between ligands and the binding groove of respective HLA molecules is summarized in Table 2. Detailed analysis of hydrogen bonds between HA peptide and DR11 models shows slightly different set of amino acids involved in the binding than found in the complex determined crystallographically (1DLH). The results are given in the Table 3. It is obvious that the quantitative agreement between our model complex and crystallographic structure complex is not possible within the approach used here. The structure of the target molecule is more relaxed and the binding groove is slightly more open. Thus, the docking peptide chooses another position in the binding groove which is energetically favorable. Nevertheless, good qualitative agreement between docked and crystallographic complex validates the application of the docking approach for exploration of unknown structures of HLA-ligand complexes.

Structures of modeled complexes of DR31+HA, DR31+HSP, DR31+MBP and DR31+SWM are shown in Figures 9 - 12, respectively. The full set of Figures for all studied complexes is available in the Supplementary Material.
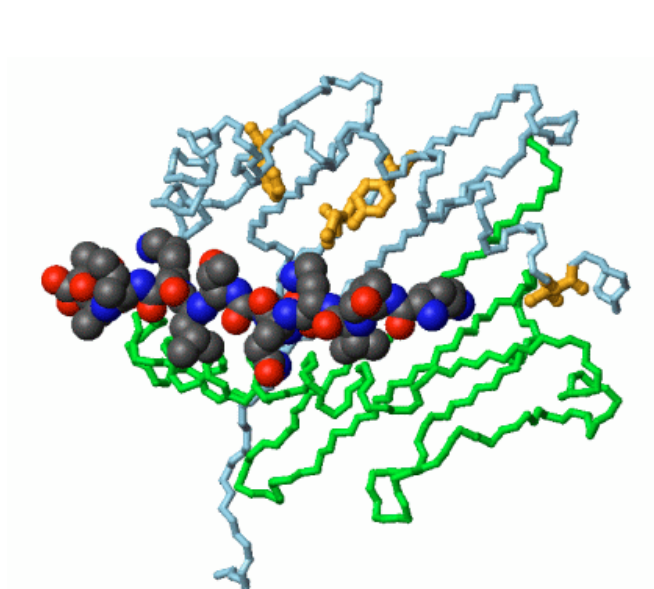
Figure 9 *Computed complex of the HLA-DR*0301 molecule with influenza hemagglutinin peptide. Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of the ligand atoms*
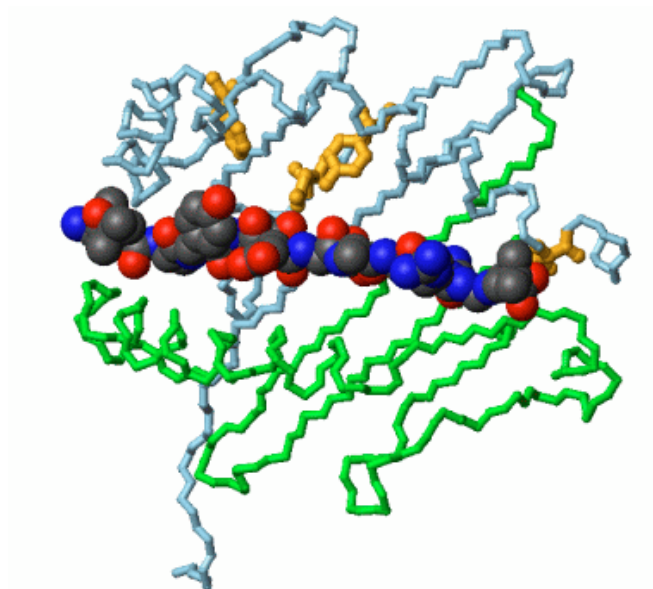
Figure 10 *Computed complex of the HLA-DR*0301 molecule with heat-shock protein peptide. Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of the ligand atoms*
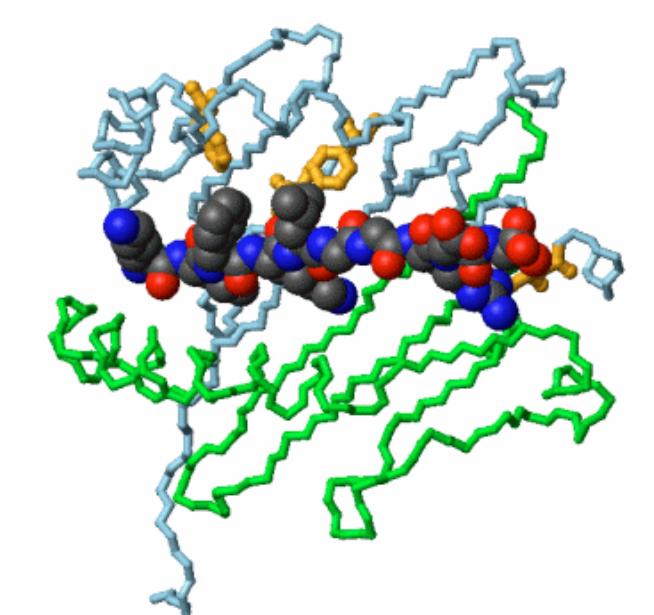
Figure 11 *Computed complex of the HLA-DR*0301 molecule with myelin basic protein peptide. Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of ligand atoms*
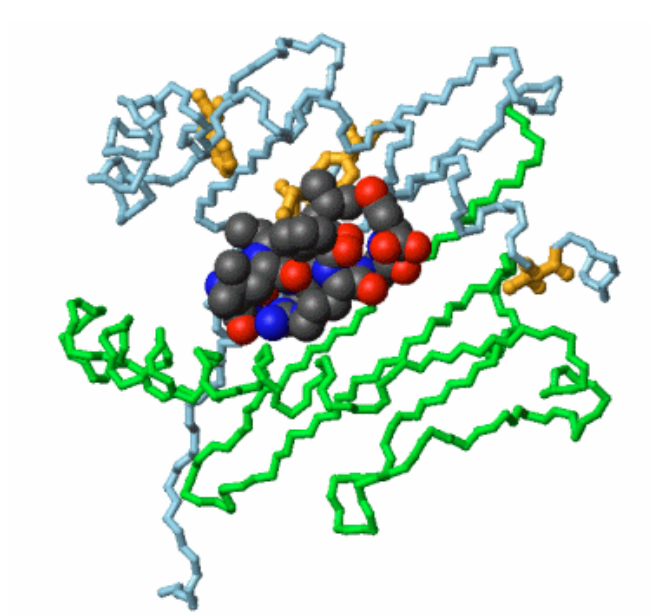
Figure 12 *Computed complex of the HLA-DR*0301 molecule with sperm whale myoglobin peptide. Color scheme: green - α-chain, blue - β-chain, orange - amino acids of the sequential microvariation set, standard CPK coloring of the ligand atoms*

The number of interactions and residues forming hydrogen bonds is compiled in Tables 2 and 3, respectively.

HA, HSP and MBP peptides adopt preferably the extended conformation and they can be well accommodated in the binding groove. HA peptide forms more interactions with DR32 than with DR31 and it can be predicted that the HA-DR32 complex will be energetically preferred. This is in agreement with results of experimental studies [4]. In the case of HSP and MBP molecules such clear preferences for specific binding cannot be predicted but experimental studies indicate that both HSP and MBP bind preferentially (stronger) to DR31. SWM peptide adopts a total α-helix conformation and thus because of its bigger radial size does not fit well into the binding groove. There are less interactions between SWM and DR31 or DR32 than in other models, but it can be predicted that SWM will bind stronger to the DR31 molecule, again in agreement with experimental studies [4]. However, in this case further docking simulations with the option of dynamic opening of the binding groove are necessary. Microvariation residues are exposed in pockets of the binding groove in all modeled complexes and they could interact with ligands but results indicate that they are not involved directly in interactions with the ligand. Thus, the specificity of studied HLA-DR3 alleles cannot be explained easily by the microvariation of the amino acid sequence. It can be postulated that these amino acids modify the local environment allowing another amino acids to make strong interactions with the ligand. Thus, a more advanced analysis of the properties of the pockets is required in order to elucidate the nature of ligands binding to HLA molecules.

Supplementary materials available The full set of Figures for all studied HLA proteins and all docked complexes of HLA proteins with their specific ligands as well as set of files for dynamic visualization and manipulation of computed structures (1 archived file).

The Figures can be viewed using an HTML browser with installed Chime plug-in (MDL Information Systems, Inc.). The entry file - index.htm - contains link to the Chime download site.

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