Structural diversity of human class II histocompatibility molecules induced by peptide ligands

Bertrand Georges[a,c,*], Estelle Loing[b], Raphaële Neveu[a], Oleg Melnyk[b], Helene Gras-Masse[b], Claude Auriault[a]

[a] *Laboratoire d'Immunopathologie Cellulaire des Maladies Infectieuses, CNRS UMR 8527, Lille, France*
[b] *Laboratoire de Chimie des Biomolécules CNRS UMR 8525, Lille, France*
[c] *Sedac-Therapeutics Inc., Institut de Biologie de Lille, 1bis rue du professeur Calmette, 59010 Lille Cedex, France*

Received 22 May 2000; revised 24 July 2000; accepted 17 August 2000

Edited by Masayuki Miyasaka

Abstract SDS–PAGE analyses of stable HLA-DR1 complexes indicate that the binding of T cell epitopes can lead to multiple conformational variants. Whereas short T epitopes (< 14-mer) induce complexes with apparent MW ranging from 47 to 57 kDa, longer peptides form generally high mobility complexes (44–45 kDa). The generation of HLA-DR1 conformational variants appears dependent on core peptide residues fitting inside the groove but can additionally be attributed to the presence of N- and C-terminal flanking residues (PFRs) acting as a complementary mechanism. These PFRs can jointly affect major histocompatibility complex class II conformation and stability, supporting the existence of alternative contacts at a distance from the classical binding site.

Key words: Soluble MHC class II; DRB1*0101; Stability; Conformation; Peptide binding

1. Introduction

The function of the major histocompatibility complex (MHC) class II glycoproteins is to present antigenic peptides derived from exogenous antigens to CD4 helper T cells [1]. Structural characterization of MHC class II heterodimers has identified a peptide binding groove composed of the N-terminal α1 and β1 domains whilst the membrane proximal α2 and β2 domains share an immunoglobulin-like conformation [2–5]. The length of naturally presented peptides comprises 12–25 residues [6,7] but MHC class II molecules recognize only a nine residue binding frame within the sequence of the peptide ligand. Pockets spaced within the overall MHC binding site accommodate side chains of the core peptide sequence and contribute to the peptide binding motif. In the structure of HLA-DR alleles, pockets are at P1, P4, P6 and P9 with a smaller one at P7. Additional residues at the amino- or carboxy-terminus extending out of the binding cleft have no effect on binding affinity but play a role in stabilizing (αβ) heterodimers [8–10].

Several studies have reported the existence of conformational variation in MHC class II folding states. Intermediate 'floppy' species, with reduced mobility in SDS–PAGE, were first described in response to low pH or elevated temperatures [11,12]. In addition, peptide binding to MHC class II molecules drives a slow conversion between two distinct states, one with unstable and looser conformation, the other with a long half-life and SDS stability [13–15].

Moreover, several works have demonstrated the additional role of peptides in the formation of mature MHC class II molecules with different conformations. Indeed, based on monoclonal antibody [16–18] and superantigen reactivities [19–21], but also on SDS–PAGE mobility [17,22], subsets of MHC class II expressed by presenting cells can be defined by the presence of particular peptides. However, molecular mechanisms that could be responsible for these changes remain unresolved. Here, we study the influence of peptide ligand residues on HLA-DR1 conformational changes.

2. Materials and methods

2.1. Peptide synthesis

Peptides were elaborated on a Rink Amide resin (France Biochem, Meudon, France) using the Fmoc/*tert*-butyl strategy [36] and HBTU/HOBT activation in an Applied Biosystems A431 peptide synthesizer (Foster City, CA, USA). All the peptides were purified by RP-HPLC using a water–acetonitrile linear gradient containing 0.05% trifluoroacetic acid and characterized by amino acid analysis and TOF-PDMS (Bio-Ion 20 Plasma Desorption Mass Spectrometer, Uppsala, Sweden). Sequences of all peptides are indicated in Table 1. The synthetic undecapeptide XYX_9 library was a kind gift of Dr. Burkhard Fleckenstein [23].

2.2. Expression of recombinant HLA-DRB1*0101 proteins

Recombinant baculovirus for both α and β chains was a generous gift from L.J. Stern and D.C. Wiley (Harvard University, Cambridge, MA, USA) [37]. The soluble human class II DR1 proteins were generated in Sf9 cells (*Spodoptera frugiperda*) or in BTI-Tn-5B1-4 cells (*Trichoplusia ni*) purchased from Invitrogen (The Netherlands). Cells were grown in X-press medium (Biowhittaker, Malkersville, MD, USA). Four days post-infection, soluble DR1 proteins were purified from medium and cell lysates on immunoaffinity columns with protein A-Sepharose beads CL-4B (Sigma) coupled with the conformation-sensitive monoclonal antibody L243 (ATCC). The elution step was performed with glycine buffer pH 11.5, immediately neutralized with glycine 2 M pH 3. After dialysis against phosphate-buffered saline, MHC heterodimers were concentrated up to 1 mg/ml on a Centricon 30 (Amicon, Beverly, MA, USA) and peptide binding capacity was confirmed by its ability to form SDS–PAGE stable complexes with peptide $HA_{(307-319)}$.

2.3. SDS–PAGE analysis

Acrylamide gel analyses were performed using SDS–PAGE with the following modifications. Samples were applied to the gel without mercaptoethanol and without being boiled. Sample buffer contained

*Corresponding author. Fax: (33)-3-20 87 12 33.
E-mail: bertrand.georges@ibl.fr

Abbreviations: PFR, peptide flanking residue; MHC, major histocompatibility complex

0014-5793/00/$20.00
PII: S0014-5793(00)01981-5

100 mM Tris, 0.4% SDS and 20% glycerol diluted at 1:4. Gels were run at low voltage to prevent temperatures exceeding 40°C. The separation gel used was 12.5% acrylamide/bisacrylamide (37.5/1). Acrylamide gels were stained with Coomassie brilliant blue R250 and were scanned on a Bio-Rad densitometer (quantitative analysis was performed using Molecular Analysis I software).

2.4. Binding assays

Biotinylated $HA_{(307-319)}$ was incubated at a final concentration of 0.1 μM with 10 nM of HLA-DR1 protein for 48 h at 37°C in the absence or presence of unlabeled peptide at final concentrations ranging from 1 nM to 100 μM. Samples were then added to ELISA plates coated with the monoclonal antibody L243. Bound biotinylated $HA_{(307-319)}$ was analyzed by streptavidin coupled to peroxidase enzyme.

2.5. Size exclusion chromatography

Gel filtration analyses were performed on a TSK3000PW0.75×30 column (TosoHaas, Montgomeryville, PA, USA). Separations were performed in phosphate-buffered saline at room temperature at a flow rate of 0.5 ml/min. Calibration was done with 50 μg of several standard modified proteins (Bio-Rad): γ-globulin (158 kDa), bovine serum albumin (82 kDa), ovalbumin (44 kDa), carbonic anhydrase (34 kDa) and myoglobin (17 kDa).

2.6. Circular dichroism (CD)

Peptide/MHC complexes were prepared by incubating MHC class II proteins at a concentration of 300 μg/ml with 50 μM of each peptide. After 96 h incubation at 37°C, free peptide was removed by dialysis with Spectra/por 25000 against 10 mM phosphate buffer. CD measurements were performed using a spectropolarimeter (Jobin-Yvon CD-6) equipped with a thermo-electric cell holder. Spectra were recorded in the far UV between 190 and 260 nm, using a 0.1 cm path length cell using a step size of 0.05 cm and a time constant of 5 s. Temperature was increased from 25 to 90°C with a step mode (5–10°C) with each temperature change followed by a 60 s equilibration time.

3. Results

3.1. Binding of different T cell epitopes induces multiple conformations of MHC class II HLA-DR1 complexes

Several peptide ligands were incubated with soluble DR1 molecules to generate stable (αβ) heterodimers before SDS–PAGE analysis under non-reducing, non-boiling conditions (Fig. 1). Short peptides (< 14-mer, Fig. 1A) induce stable complexes migrating with distinct mobility (MW 46–57 kDa). Interestingly, an undecapeptide library XYX_9 leads to the observation of a wide electrophoretic band compared to unique peptides (Fig. 1B), suggesting the existence of a family of peptide/DR1 isoforms. The presence of a tyrosine at the second position of the library was designed to ensure an optimal contact with the dominant P1 pocket of DR1 and to prevent peptide shifting on the binding cleft [23]. These results indicate that distinct MHC class II conformations can be induced by short peptides, depending on the residues fitting inside the binding groove. Interestingly, we did not observe any correlation between the apparent MW of DR1 complexed with short ligands and non-related binding parameters such as relative affinity and stability (see Table 2), indicating the existence of an unknown mechanism for the formation of MHC class II isoforms.

Longer peptides (> 14-mer) with residues extended outside the groove generally lead to complexes with higher mobility. Indeed, elongation of minimal T cell epitopes, such as $TT_{(830-850)}$ and $HA_{(301-331)}$, induce the formation of 44–45 kDa complexes (Fig. 1C, lanes 1 and 3). Curiously, $SM_{(24-43)}$, a 20-mer T cell epitope from *Schistosoma mansoni* [24], stabilizes two distinct conformational forms with respectively 45 and 78 kDa (Fig. 1C, lane 4). The nature of $SM_{(24-43)}$, containing a single putative anchor motif (see Table 1) and used in large excess to form the complexes, excludes the possible binding of two (αβ) heterodimers with a single peptide ligand.

Size exclusion chromatography was performed on these peptide/DR1 complexes to correlate the electrophoretic mobility with the hydrodynamic radius (Fig. 2). Complexes with 44–57 kDa apparent MW in SDS–PAGE have close elution profiles that were not considered significantly different. In this, we were not able to correlate the measurement of apparent molecular weight observed in SDS–PAGE and in gel filtration probably due to the different conditions used for the protein separation, notably the SDS. In turn, the $SM_{(24-43)}$/DR1 complexes revealed a biphasic elution profile. The first part of this profile is close to the retention time of a 82 kDa marker protein. This hydrodynamic alteration is in accordance with the formation of a 78 kDa isoform observed in SDS–PAGE (Fig. 1C).

3.2. Influence of peptide flanking regions (PFRs) on HLA-DR1 conformation and stability

Extensions of minimal peptide ligands seem to have a pro-

Table 1
Abbreviations and sequences of peptide ligands

Abbreviations	Origin	Length	Sequences, binding core and references (P1 … P9)	
HA	Influenza Virus Hemagglutinin (307-319)	13	P K Y V K Q N T L K L A T	[38]
MYO	Myoglobin (67-79)	13	T V L T A L G A I L K K K	*
NEF	HIV-1 nef protein (56-68)	13	A W L E A Q E A E E V G F	*
TT	Tetanus toxoid (830-843)	14	Q Y I K A N S K F I G I T E	[38]
TT_L	Tetanus toxoid (830-844)	15	Q Y I K A N S K F I G I T E L	*
TT_{LK}	Tetanus toxoid (830-845)	16	Q Y I K A N S K F I G I T E L K	*
TT_{LKK}	Tetanus toxoid (830-846)	17	Q Y I K A N S K F I G I T E L K K	*
$SM_{(24-43)}$	SM28GST protein from *Schistosoma*	20	L V A A G V D Y E D E R I S F Q D W P K	*
$CLIP_{(89-103)}$	Human invariant chain (89-103)	15	S K M R M A T P L L M Q A L P	[39]
$CLIP_{(81-103)}$	Human invariant chain (81-103)	23	L P K P P K P V S K M R M A T P L L M Q A L P	[39]
HA_{CLIP}	N-terminal part of CLIP associated to HA	21	L P K P P K P V P K Y V K Q N T L K L A T	*

Peptide sequences are aligned according to their HLA-DR1 motif. Peptide binding cores were determined by the presence of a HLA-DR1 sequence motif obtained by experimental studies [38,39] or by prediction (*) according to the published HLA-DR1 anchor motif [23]. Peptide binding cores covering nine residues (between P1 and P9) are represented in gray.

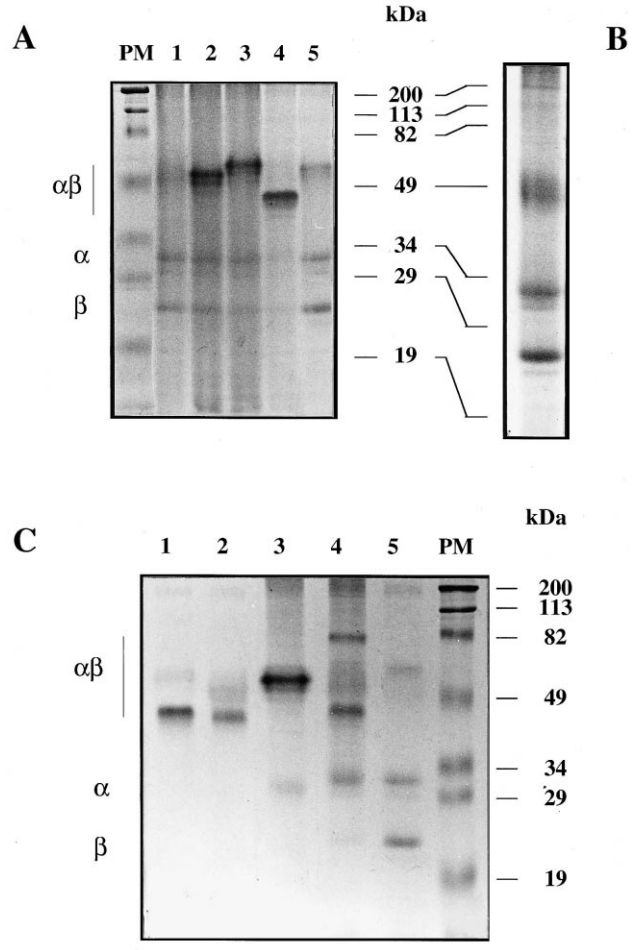

Fig. 1. Peptide binding-induced DR1 complexes with different mobilities in SDS–PAGE. DR1 was incubated with a large excess of peptide ligands. Stable MHC complexes were assessed in 12.5% acrylamide SDS–PAGE analysis under non-reducing non-boiling conditions. A: Influence of short T helper epitopes on HLA-DR1 electrophoretic mobility: empty HLA-DR1 (lane 1), TT/DR1 (lane 2), HA/DR1 (lane 3), NEF/DR1 (lane 4), MYO/DR1 (lane 5). B: Influence of a undecapeptide library XYX_9 on HLA-DR1 mobility. C: Longer T helper epitopes associated with HLA-DR1: $TT_{(830-850)}$ (lane 1), $HA_{(301-331)}$ (lane 2), HA (lane 3), $SM_{(24-43)}$ (lane 4) and empty HLA-DR1 (lane 5).

found influence on DR1 conformation. To determine more specifically the importance of PFRs in determining HLA-DR1 structure, N- and C-terminal elongations of different minimal binding sequences were investigated.

Fig. 3A shows that binding of $CLIP_{(81-103)}$ generates SDS-resistant DR1 complexes with lower apparent MW relative to the complexes obtained with $CLIP_{(89-103)}$. This has been previously noticed but its relevance has been disputed considering the difference of mobility as an electrophoretic artefact induced by the presence of the N-terminal part of $CLIP_{(81-103)}$ [25]. In contrast, when the N-terminal part of CLIP was linked to the HA epitope (HA_{CLIP}, see Table 2), no difference in electrophoretic mobility was observed compared to HA/DR1 complexes (Fig. 3B). In this, the $CLIP_{(81-89)}$ residues dictate HLA-DR1 conformation but this effect is not necessarily transferable to another peptide ligand. These data suggest a possible interaction of $CLIP_{(81-89)}$ residues with MHC residues distant from the N-terminal binding groove. Critical MHC residues may be engaged in this binding and cause the whole molecule to undergo a structural transition. This hypothesis was first suggested by Kropshofer et al. [26,27] who identified N-terminal residues of CLIP (lysine 83, lysine 86, proline 87) responsible for the reduction of MHC class II stability. In contrast, we observed rather a conformational change and maintenance of HLA-DR1 stability (see Table 2) in the presence of $CLIP_{(81-89)}$ suggesting the existence of multiple distinct molecular mechanisms associated with the binding of the CLIP peptide.

On the other hand, C-terminal elongation of the minimal $TT_{(830-843)}$ peptide with leucine (TT_L), leucyl-lysine (TT_{LK}), or leucyl-lysyl-lysine (TT_{LKK}) increases the electrophoretic mobility of the corresponding complexes, from 55 to 44 kDa (Fig. 3C). The more pronounced effect was observed with the addition of lysine 845 which increases the apparent MW to 8 kDa. C-terminal elongation of TT is also accompanied by an increase in the thermostability of peptide/DR1 complexes (56°C for TT compared to 63°C for TT_{LKK}, see Table 2), a phenomenon independent of relative binding affinity. Together, these results indicate the role of C-terminal residues extending outside the binding site in determining jointly MHC class II conformation and stability.

3.3. CD analysis of stable peptide/HLA-DR1 complexes

Far-UV CD spectroscopy was previously used to compare the secondary structure of empty and peptide-filled MHC class II [28]. The spectra present a broad absorption band from 210 to 230 nm, consistent with a protein containing a substantial amount of β-sheet and a portion of α-helical structure.

Conformational variations between HA/DR1 and TT_{LKK}/

Table 2
Influence of specific HLA-DR1 peptides on apparent MW, thermal stability and relative affinity

Peptide	Apparent MW[a]	T_m^b (°)	Relative affinity[c]
HA	57	70	0.1
MYO	54	42	10
NEF	47	63	nt
TT	55	56	0.8
TT_L	53	57	0.8
TT_{LK}	45	62	0.85
TT_{LKK}	44	63	0.9
$SM_{(24-43)}$	45/79	55	25
$CLIP_{(89-103)}$	56	62	1
$CLIP_{(81-103)}$	44	59.5	0.6
HA_{CLIP}	57	nt	0.1

nt: not tested.
[a]Apparent molecular weights are representative of the electrophoretic mobility of stable peptide/HLA-DR1 complexes in SDS–PAGE under non-boiling, non-reducing conditions. Values are expressed in kDa.
[b]Melting temperature (T_m) corresponds to the temperature at which 50% of stable complexes are dissociated. Each sample containing stable peptide/HLA-DR1 complexes was heated for 10 min at temperatures ranging from 40 to 85°C before loading on non-reducing, non-boiling SDS–PAGE. Densitometric analysis was performed to calculate the ratio of stable (αβ) heterodimers versus dissociated chains and the values for the midpoint transition (T_m) were calculated.
[c]Relative affinity (IC_{50}) is measured in a heterologous competition assay. Soluble HLA-DR1 molecules are incubated with different concentrations of the peptide in the presence of biotinylated $HA_{(307-319)}$ at 0.1 μM. After 48 h incubation at 37°C individual samples were analyzed by ELISA. Results are expressed in μM as a mean of at least two independent experiments.

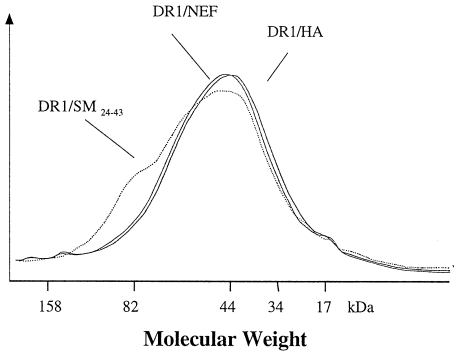

Fig. 2. Size exclusion chromatography of HLA-DR1/peptide complexes. The elution profile of different HLA-DR1/peptide complexes were analyzed relative to different molecular markers as references. Three complexes with different SDS–PAGE mobilities are presented: HLA-DR1/HA, a 57 kDa isoform, HLA-DR1/NEF, a 47 kDa isoform, and HLA-DR1/SM leading to two different isoforms with respectively 45 and 78 kDa.

DR1 complexes were monitored by far-UV CD (Fig. 4). These two stable complexes possess very distinct SDS–PAGE mobility and high thermostability (see Table 2). The contribution of peptide ligands to the ellipticity was found to be negligible and the absence of aggregate complexes was checked by gel filtration (data not shown). At neutral pH, the spectra of both complexes are very similar to each other with a broad minimum centered at 216–218 nm characteristic of proteins with a portion of α-helical structure and a substantial amount of β-sheet. The analysis was then performed in the presence of SDS, an optically neutral surfactant. We used the detergent at a concentration of 0.1% at which both complexes (αβ+peptide) are resistant to dissociation and lack of the peptide at 25°C (data not shown). The addition of 0.1% SDS in each sample induces a change in secondary structure for both complexes, but this change was less pronounced for DR1/TT_{LKK} complexes even at elevated temperatures (Fig. 4). In this, the presence of SDS revealed structural differences between stable peptide/HLA-DR1 complexes and suggested the importance of a hydrophobic site on these conformational changes.

4. Discussion

Several studies based on monoclonal antibody or superantigen reactivities have demonstrated the role of peptide sequences in determining MHC class II structural changes on antigen presenting cell surface [16,18,21]. Recently Chervonsky et al. [17] showed that structural transitions imposed by peptides upon murine MHC class II molecules could affect T cell recognition. However, molecular mechanisms that could account for this phenomenon remain unresolved. Our study demonstrates the role of peptide ligands in determining conformational changes on MHC class II molecules. The alternative forms of HLA-DR1 seem to be different from the previously described MHC floppy conformation. Floppy isoforms were obtained after exposure of MHC class II molecules to acidic pH or elevated temperature [29] and were associated with an unfolded intermediate state that could promote peptide loading in the endosome. Here, conformational changes of DR1/peptide complexes are spontaneously driven by the peptide, independent of parameters such as temperature, incubation time or pH. The results obtained with short T epitopes (< 14-mer) including the undecapeptide library indicate that the contact between peptide ligands and MHC class II residues within the binding groove may be responsible for changes observed in electrophoretic mobility of the DR1 complexes. Peptide ligands are known to accommodate in the binding groove of MHC class II with small rearrangement of MHC residues. Indeed, conformational alterations between two crystal structures of HLA-DR1 complexed with $HA_{(307–319)}$ or $A2_{(103–117)}$ were observed in a flexible 'kink', located in the α-helix of the β-chain, a region that may vary according to the peptide side chain near the P7 pocket [5,30]. This subtle molecular mechanism could explain the difference in electrophoretic mobility of MHC class II complexes formed with minimal peptide epitopes and may be related to the altered antibody reactivities or T cell recognition observed in other studies [16,17].

Observations made with longer peptides (> 14-mer) suggest that PFRs extending outside the groove play an additional role in determining MHC class II structure. The C-terminal extension of TT peptides reduce the apparent molecular

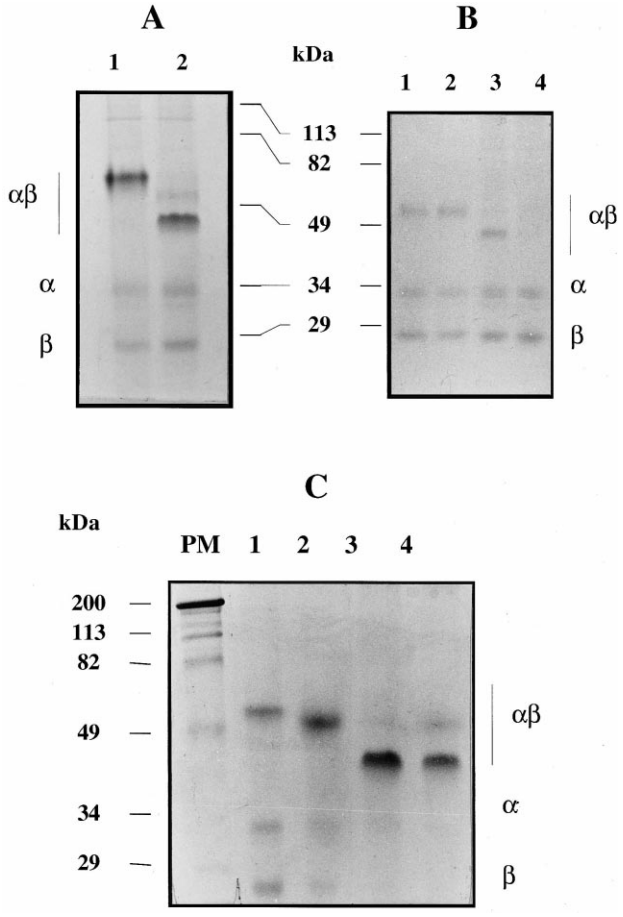

Fig. 3. N- and C-terminal extension of peptide ligands influence the HLA-DR1 electrophoretic mobility. 5 μg of soluble DR1 was incubated for 4 days at 37°C in the presence of 100 μM of peptides. The peptide/DR1 complexes were analyzed on SDS–PAGE under non-reducing, non-boiling conditions. A: Relative migration of HLA-DR1 complexes associated with $CLIP_{(89–103)}$ (lane 1) or $CLIP_{(81–103)}$ (lane 2). B: Relative migration of HLA-DR1 complexed with HA_{CLIP} (lane 1), HA (lane 2) and $CLIP_{(81–103)}$ (lane 3). Empty HLA-DR1 is presented lane 4. C: Relative migration of HLA-DR1 complexes formed with TT (lane 1) peptide, TT_L (lane 2), TT_{LK} (lane 3) and TT_{LKK} (lane 4).

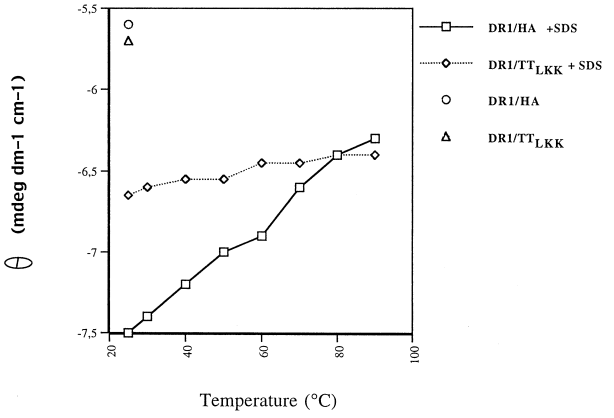

Fig. 4. Far-UV CD spectra of peptide/DR1 complexes. Stable HLA-DR1 complexes performed with HA or TT_{LKK} were analyzed in the absence or presence of 0.1% SDS. Data are expressed in units of mean residue ellipticity at 216 nm.

weight of complexes and concurrently increase thermostability, results that strongly suggest the existence of some contact outside the binding site. These potential contacts have been previously proposed to explain the positive or negative influence of N- and C-terminal extension of peptide ligands on MHC class II stability [9,26]. Moreover, direct evidence using photoaffinity labeling has suggested the existence of a contact site beyond the peptide binding site to a nearby area in the α2 and β2 domains [31]. In this, one epitope could be presented by different MHC class II isoforms depending on the nature of the N- or C-terminal part of the ligand extending outside the classical binding site. However, the influence of exopeptidases that participate in antigen processing and in the trimming of peptides associated with class II molecules may naturally limit the presence of PFRs and the interaction with effector binding sites distant from the classical groove.

We postulate that the conformational changes induced by N- or C-terminal extension of minimal T cell epitopes may preferentially affect the structure of the α2 and β2 distal domains as previously suggested by photoaffinity labeling experiments [31]. This hypothesis is also in agreement with the CD analysis of two distinct peptide/HLA-DR1 complexes. At 25°C and in the presence of SDS, the conformation of both species is different while the peptide ligand still remains tightly bound to the α1-β1 peptide binding domain. Interactions between domains of the (αβ) heterodimer are known to be modulated by large pockets under the β-sheet from the peptide binding domain. These interactions consist in hydrophobic patches [30] that could be responsible for MHC conformational variations observed in SDS–PAGE and CD analysis due to SDS binding. The α2 and β2 domains were also previously implicated in a mechanism proposed for $I\text{-}E^k$ conformational changes occurring at mildly acidic pH [32]. In any case, it is clear that the conformational variations observed with soluble DR1 molecule lacking transmembrane and intracellular parts are much more important compared to complete (αβ) heterodimers [33]. In this, the absence of structural constraints at the proximal part of the protein may increase the amplitude of molecular changes observed in our studies.

The existence of multiple configurations of MHC class II molecules may have an impact on T cell reactivity as previously demonstrated [17]. Peptide ligands presented by MHC class II molecules represent a small part of the contact area with the T cell receptor and if we consider the ability of a single immunogenic peptide to be presented in different topological configurations induced by adjustment of MHC residues, an expanded spectrum of T cell reactivities could be elicited. However, MHC class II conformational changes induced by peptide ligand may have other implications. Indeed, during intracellular trafficking, MHC class II molecules interact with chaperone molecules (such as HLA-DM), a mechanism that has previously been demonstrated to be MHC class II conformation-dependent [34]. Moreover, the conformation of MHC class II has recently been demonstrated to influence the selectivity of transport to the cell surface [35], a mechanism that can now be imputable to the presence of different peptide ligands on the MHC class II binding site. These hypotheses are now the subject of current investigations.

References

[1] Germain, R.N. and Margulies, D.H. (1993) Annu. Rev. Immunol. 11, 403–450.
[2] Brown, J.H., Jardetzky, T.S., Gorga, J.C., Stern, L.J., Urban, R.G., Strominger, J.L. and Wiley, D.C. (1993) Nature 364, 33–39.
[3] Dessen, A., Lawrence, C.M., Cupo, S., Zaller, D.M. and Wiley, D.C. (1997) Immunity 7, 473–481.
[4] Ghosh, P., Amaya, M., Mellins, E. and Wiley, D.C. (1995) Nature 378, 457–462.
[5] Stern, L.J., Brown, J.H., Jardetzky, T.S., Gorga, J.C., Urban, R.G., Strominger, J.L. and Wiley, D.C. (1994) Nature 368, 215–221.
[6] Rudensky, A., Preston-Hurlburt, P., Hong, S.C., Barlow, A. and Janeway Jr., C.A. (1991) Nature 353, 622–627.
[7] Chicz, R.M., Urban, R.G., Lane, W.S., Gorga, J.C., Stern, L.J., Vignali, D.A. and Strominger, J.L. (1992) Nature 358, 764–768.
[8] Nelson, C.A., Petzold, S.J. and Unanue, E.R. (1993) Proc. Natl. Acad. Sci. USA 90, 1227–1231.
[9] Siklodi, B., Vogt, A.B., Kropshofer, H., Falcioni, F., Molina, M., Bolin, D.R., Campbell, R., Hammerling, G.J. and Nagy, Z.A. (1998) Hum. Immunol. 59, 463–471.
[10] Verreck, F.A., Vermeulen, C., Poel, A.V., Jorritsma, P., Amons, R., Coligan, J.E., Drijfhout, J.W. and Koning, F. (1996) Int. Immunol. 8, 397–404.
[11] Dornmair, K., Rothenhausler, B. and McConnell, H.M. (1989) Cold Spring Harbor Symp. Quant. Biol. 54, 409–416.
[12] Sadegh-Nasseri, S. and Germain, R.N. (1991) Nature 353, 167–170.
[13] Sadegh-Nasseri, S., Stern, L.J., Wiley, D.C. and Germain, R.N. (1994) Nature 370, 647–650.
[14] Witt, S.N. and McConnell, H.M. (1994) Biochemistry 33, 1861–1868.
[15] Beeson, C., Anderson, T.G., Lee, C. and McConnell, H.M. (1996) J. Am. Chem. Soc. 118, 977–980.
[16] Fling, S.P., Arp, B. and Pious, D. (1994) Nature 368, 554–558.
[17] Chervonsky, A.V., Medzhitov, R.M., Denzin, L.K., Barlow, A.K., Rudensky, A.Y. and Janeway Jr., C.A. (1998) Proc. Natl. Acad. Sci. USA 95, 10094–10099.
[18] Rath, S., Lin, R.H., Rudensky, A. and Janeway Jr., C.A. (1992) Eur. J. Immunol. 22, 2121–2127.
[19] Thibodeau, J., Cloutier, I., Lavoie, P.M., Labrecque, N., Mourad, W., Jardetzky, T. and Sekaly, R.P. (1994) Science 266, 1874–1878.
[20] Albert, L.J., Denzin, L.K., Ghumman, B., Bangia, N., Cresswell, P. and Watts, T.H. (1998) Cell. Immunol. 183, 42–51.
[21] Lavoie, P.M., Thibodeau, J., Cloutier, I., Busch, R. and Sekaly, R.P. (1997) Proc. Natl. Acad. Sci. USA 94, 6892–6897.
[22] Srinivasan, M., Marsh, E.W. and Pierce, S.K. (1991) Proc. Natl. Acad. Sci. USA 88, 7928–7932.
[23] Fleckenstein, B., Kalbacher, H., Muller, C.P., Stoll, D., Halder, T., Jung, G. and Wiesmuller, K.H. (1996) Eur. J. Biochem. 240, 71–77.
[24] Auriault, C., Wolowczuk, I., Gras-Masse, H., Marguerite, M.,

Boulanger, D., Capron, A. and Tartar, A. (1991) Peptide Res. 4, 6–11.
[25] Natarajan, S.K., Assadi, M. and Sadegh-Nasseri, S. (1999) J. Immunol. 162, 4030–4036.
[26] Kropshofer, H., Vogt, A.B., Stern, L.J. and Hammerling, G.J. (1995) Science 270, 1357–1359.
[27] Kropshofer, H., Vogt, A.B. and Hammerling, G.J. (1995) Proc. Natl. Acad. Sci. USA 92, 8313–8317.
[28] Reich, Z., Altman, J.D., Boniface, J.J., Lyons, D.S., Kozono, H., Ogg, G., Morgan, C. and Davis, M.M. (1997) Proc. Natl. Acad. Sci. USA 94, 2495–2500.
[29] Dornmair, K., Rothenhausler, B. and McConnell, H.M. (1989) Cold Spring Harbor Symp. Quant. Biol. 54, 409–416.
[30] Murthy, V.L. and Stern, L.J. (1997) Structure 5, 1385–1396.
[31] Luescher, I.F., Crimmins, D.L., Schwartz, B.D. and Unanue, E.R. (1990) J. Biol. Chem. 265, 11177–11184.
[32] Boniface, J.J., Lyons, D.S., Wettstein, D.A., Allbritton, N.L. and Davis, M.M. (1996) J. Exp. Med. 183, 119–126.
[33] Stumptner, P. and Benaroch, P. (1997) EMBO J. 16, 5807–5818.
[34] Ullrich, H.J., Doring, K., Gruneberg, U., Jahnig, F., Trowsdale, J. and van Ham, S.M. (1997) Proc. Natl. Acad. Sci. USA 94, 13163–13168.
[35] Thery, C., Brachet, V., Regnault, A., Rescigno, M., Ricciardi-Castagnoli, P., Bonnerot, C. and Amigorena, S. (1998) J. Immunol. 161, 2106–2113.
[36] Fields, G.B. and Noble, R.L. (1990) Int. J. Peptide Protein Res. 35, 161–214.
[37] Stern, L.J. and Wiley, D.C. (1992) Cell 68, 465–477.
[38] O'Sullivan, D., Arrhenius, T., Sidney, J., Del Guercio, M.F., Albertson, M., Wall, M., Oseroff, C., Southwood, S., Colon, S.M., Gaeta, F.C. and Sette, A. (1991) J. Immunol. 147, 2663–2669.
[39] Malcherek, G., Gnau, V., Jung, G., Rammensee, H.G. and Melms, A. (1995) J. Exp. Med. 181, 527–536.