Function-Related Regulation of the Stability of MHC Proteins

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ABSTRACT Proteins must be stable to accomplish their biological function and to avoid enzymatic degradation. Constitutive proteolysis, however, is the main source of free amino acids used for de novo protein synthesis. In this paper the delicate balance of protein stability and degradability is discussed in the context of function of major histocompatibility complex (MHC) encoded protein. Classical MHC proteins are single-use peptide transporters that carry proteolytic degradation products to the cell surface for presenting them to T cells. These proteins fulfill their function as long as they bind their dissociable ligand, the peptide. Ligand-free MHC molecules on the cell surface are practically useless for their primary biological function, but may acquire novel activity or become an important source of amino acids when they lose their compact stable structure, which resists proteolytic attacks. We show in this paper that one or more of the stabilization centers responsible for the stability of MHC-peptide complexes is composed of residues of both the protein and the peptide, therefore missing in the ligand-free protein. This arrangement of stabilization centers provides a simple means of regulation; it makes the useful form of the protein stable, whereas the useless form of the same protein is unstable and therefore degradable.

INTRODUCTION

Proteins must be stable to ensure their biological activity. However, protein stability must be limited, since degradation products of useless or inactive proteins are the main source of amino acid for de novo protein synthesis. A simple means of regulation, which makes a useful form of a protein stable and the useless form unstable, will be presented. It derives from the study of stabilization centers in an immunologically relevant peptide receptor and transporter.

Cell surface glycoproteins, encoded by genes of the major histocompatibility complex (MHC), begin and end their lifetime as empty molecules. At the cell surface however they are complexed with peptide ligands that are generated within the cell by limited proteolytic degradation and loaded intracellularly (reviewed in Germain and Margulies, 1993; Maenaka and Jones, 1999). A single MHC molecule can bind various, but not all, kinds of peptides (Rammensee, 1995; Rammensee et al., 1995). Peptide loading of MHC class I and class II molecules via the classical antigen presentation pathways occurs in distinct cellular compartments where various peptides compete for the available binding sites (reviewed in Harding and Geuze, 1993; Rajnavölgyi, 1994). The resulting complexes are then transported to the cell surface and presented to T cells (Garcia and Teyton, 1998). The interaction between newly synthesized MHC class I and class II molecules and their ligands is assisted by various chaperones, which support the peptide-accessible conformation of the molecule (Koopmann et al., 1997; Busch et al., 2000). Ligand-free MHC proteins usually do not reach the cell surface, since they are degraded rapidly inside the cell. MHC-peptide complexes expressed on the cell surface, however, are stable for several hours or even for days (Lanzavecchia et al., 1992; Hansen et al., 2000). Peptide ligands bind noncovalently to MHC molecules. Therefore, they dissociate from the heterodimers at a rate determined by the strength of interactions between the two molecules.

As a rule, ligand-free MHC molecules that happen to reach the cell surface are also degraded quickly, and only a few survive, either by reloading their ligand binding site with peptides present in the extracellular environment or by internalization in intact form into peptide-rich endosomes. Fluorescence measurements suggested the presence of a few percent of unliganded and still compact MHC I molecules on the cell surface (Matko et al., 1994; Edidin et al., 1997). Under in vitro conditions, ligand-free MHC class I proteins were demonstrated to exhibit a molten globule state, which resembled what was known as an intermediate in the denaturation process (Bouvier and Wiley, 1998). A molten globule is much less compact than a native protein; therefore, it is more sensitive to proteolytic attacks and it may interact differently with other proteins such as chaperones. Ligand-free MHC class II molecules are found on the surface of professional antigen-presenting cells, such as immature dendritic cells and B cells in association with chaperones (Santambrogio et al., 1999; Arndt et al., 2000).

The overall structures of the peptide-binding domains of MHC class I and class II molecules are rather similar. They are built up of a large eight-stranded β-sheet with two mostly α-helical regions on top of the β-sheets. The MHC class I helices are formed by the $\alpha_1$50-$\alpha_1$55 and $\alpha_1$58-$\alpha_1$84 residues on one side of the peptide binding groove and by the $\alpha_2$138-$\alpha_2$148 and $\alpha_2$151-$\alpha_2$180 amino acids on the other

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side. In MHC class II molecules, two long helices, α54-α76 and β52-β90, border the two sides of the ligand binding site (Brown et al., 1993; Stern et al., 1994; Madden, 1995). The major difference between the detailed structures of the ligand-binding sites is that they are closed by conserved amino acids at both the N- and C-termini in class I molecules (Madden et al., 1991), whereas they are open at both ends in class II molecules (Brown et al., 1993). Therefore, MHC class I molecules bind peptides of 8 to 10 residues in length, whereas MHC class II molecules can adopt much longer peptides that extend the binding groove (Rammensee, 1995; Rammensee et al., 1995; Rajnavölgyi et al., 1997; Gogolák et al., 2000).

Stabilization centers (SCs), defined as certain clusters of residues involved in cooperative long-range interactions, were described as being primarily responsible for keeping the three-dimensional structure of a protein intact (Dosztányi et al., 1997). Residues involved in SCs, i.e., elements of SCs, are defined by considering the contact maps of proteins of known structure. Residues are considered to be in contact if at least one of their heavy atom distances is less than the sum of the van der Waals radii of the two atoms plus 1.0 Å. Two residues are considered to be in long-range contact if they are at least 10 residues apart in the amino acid sequence or if they belong to separate polypeptide chains of the protein. To identify SC elements, we looked for supporting residues from the flanking tetrapeptides on both sides of the two residues that are involved in long-range interactions. There are $4^4 = 256$ ways to select these two pairs of supporting residues. One central residue and its two selected supporting residues (one on each side) are together called a residue triplet. If there is at least one of the 256 cases in which the two triplets form at least seven interresidue interactions (out of the nine theoretically possible), the central residues of both triplets are considered to be SC elements (Dosztányi et al., 1997). Properties of SCs are discussed in more detail elsewhere (Dosztányi et al., 1997; Dosztányi and Simon, 1999), and a public server is available at http://www.enzim.hu/scpred/scpred.html to identify SC elements of a given protein of known structure and to predict such elements when only the amino acid sequence of the protein is available.

According to our earlier study on a 600-member representative set of unrelated proteins of the Protein Data Bank (PDB), SCs were found in all proteins, and almost 25% of the residues were estimated to be SC elements. This percentage was rather variable in proteins that belonged to different secondary structure subclasses. For example, in all-β proteins SCs were abundant, whereas in the all-α subclass they were rather rare. This suggested that the stability of a certain structure requires a certain number of SC elements. Comparison of proteins of the same function in thermophilic microorganisms and in mammals showed that as few as one or two additional SC elements in a protein results in a denaturation temperature that is higher by 30–50°C. Apparently, protein stability can be adjusted significantly by introducing or removing a few SC elements (Dosztányi, Z., Szirtes, G., Magyar, Cs., Simon, I., manuscript in preparation).

In this work, the stabilization centers of peptide-complexed MHC class I and class II proteins were analyzed. Our studies revealed that residues of the bound peptide could be involved in SCs and thus may contribute to stability of the MHC molecule.

TABLE 1 MHC I-peptide complexes

No.	PDB code	MHC I allotype	Origin of peptide	Reference
1	1hhg	HLA-A*0201	HIV-1 gp120 (197–205)	Madden et al., 1993
2	1hhh	HLA-A*0201	Hep BV capsid (18–27)	as above
3	1hhi	HLA-A*0201	Flu-matrix (58–66)	as above
4	1hhj	HLA-A*0201	HIV-1 RT (476–484)	as above
5	1hhk	HLA-A*0201	HTLV-Tax	as above
6	2clr	HLA-A*0201	Calreticulin	Collins et al., 1994
7	1b0g	HLA-A*0201	HLA-A2 specific human peptide "P1049"	Zhao et al., 1999
8	1agd	HLA-B*0801	HIV-1 gag (24–31)	Reid et al., 1996
9	1aln	HLA-B*3501	HIV-1 Nef (74–81)	Smith et al., 1996a
10	1a9e	HLA-B*3501	EBNA3C derivative	Menssen et al., 1999
11	1alo	HLA-B*5301	P-falciparum liver antigen (1786–94)	Smith et al., 1996b
12	1alm	HLA-B*5301	HIV-2gag (182–190)	as above
13	1hoc	H-2D^b	Flu A Np (366–374)	Young et al., 1994
14	1ce6	H-2D^b	SV Np (324–332)	Glithero et al., 1999
15	1bz9	H-2D^b	Synthetic peptide "P1027"	Zhao et al., 1999
16	2vaa	H-2K^b	VSV Np (52–59)	Fremont et al., 1992
17	2vab	H-2K^b	SV Np (324–332)	as above
18	1vac	H-2K^b	Chicken OVA (258–265)	Fremont et al., 1995
19	1vad	H-2K^b	α-glucosidase (438–46)	as above
20	1ld9	H-2L^d	Endogenous peptide p29	Balendiran et al., 1997
21	1ddh	H-2D^d	HIV-1 gp160 V3 loop (P18–I10)	Li et al., 1998

TABLE 2 MHC II-peptide complexes

No.	PDB code	MHC II allotype	Origin of peptide	Reference
1	1dlh	HLA-DR1 (DRA*0101, DRB1*0101)	Flu HA (306–318)	Stern et al., 1994
2	1aqd	HLA-DR1 (DRA*0101, DRB1*0101)	Endogenous peptide A2 (104–117)	Murthy and Stern, 1997
3	2seb	HLA-DR4 (DRA*0101, DRB1*0401)	Human Collagen II (1168–1179)	Dessen et al., 1997
4	1bx2	HLA-DR2 (DRA*0101, DRB1*1501)	Human Myelin Basic Protein (85–98)	Smith et al., 1998
5	1a6a	HLA-DR3 (DRA*0101, DRB1*0301)	CLIP (87–101)	Ghosh et al., 1995
6	1iea	H-2E^k	Murine hemoglobin (64–76)	Fremont et al., 1996
7	1ieb	H-2E^k	Murine hsp 70 (236–248)	as above
8	1iao	H-2A^d	OVA (323–334) + signal residues	Scott et al., 1998
9	2iad	H-2A^d	Flu HA (126–138) + signal residue	as above
10	1iak	H-2A^k	Lysosyme HEL (50–62)	Fremont et al., 1998

DATABASE

Peptide binding domains of eight MHC class I and seven MHC class II proteins, complexed with one or several peptides, were studied. The PDB codes, the MHC allotype, and the origin of the bound peptides are listed in Tables 1 and 2.

RESULTS AND DISCUSSION

Stabilization centers that contain an SC element, located in the helices that form the sides of the peptide binding grooves, in all of the 31 complexes are listed in Tables 3–6. Representative examples, shown in Fig. 1, include the MHC class I molecules HLA-B*5301 complexed with the HIV-2 gag (182–190) peptide and HLA-B*0801 complexed with the HIV-1 gag (24–31) peptide, as well as the MHC class II molecule HLA-DR1 (composed of HLA-A*0101, HLA-B1*0101) complexed with the human influenza A virus hemagglutinin 306–318 peptide.

Fig. 1 clearly demonstrates that the eight-stranded β-sheets are stabilized by a large number of SCs; an average of 27 residues are involved in these interactions. On the other hand, the number of SCs connecting the helices to the eight-stranded β-sheet plateau is rather limited; these SC element pairs are listed in Tables 3 and 4. There are only a few cases where both helices are linked to the β-sheet by SCs. This finding is in good agreement with our previous results obtained on a large data set (Dosztányi and Simon, 1999), which indicated that in the α/β subclass of proteins, which include the MHC proteins, 46.5% of the SC elements connect one extended chain to another, whereas only 1.5% of the SC elements connect an extended chain to a helix.

TABLE 3 Intramolecular stabilization center elements of MHC class I molecules

		Stabilization center elements					
PDB code	MHC I allotype	$\alpha_2157^\dagger$	$\alpha_2168^\dagger$	$\alpha_2169^\dagger$	$\alpha_2170^\dagger$	$\alpha_2171^\dagger$	$\alpha_2140^\dagger$
1hhg	HLA-A*0201	α_2130	α_2103				
1hhh		α_2130	α_2103				
1hhi		α_2130	α_2103	α_2107			
1hhj		α_2130	α_2103				
1hhk		α_2130					
2clr		α_2130	α_2103	α_2107		$\alpha_155^\dagger$	
1b0g		α_2130	α_2103				
1agd	HLA-B*0801	α_2130				$\alpha_155^\dagger$	
1aln	HLA-B*3501	α_2130					
1a9e			α_2103	α_2107			
1alo	HLA-B*5301		α_2103	α_2107		$\alpha_155^\dagger$	
1alm		α_2130	α_2103			$\alpha_155^\dagger$	
1hoc	H-2D^b	α_2130					
1ce6		$\alpha130$	α_2103	$\alpha_2103,107$		$\alpha_155^\dagger$	
1bz9		α_2130			$\alpha_155,59^\dagger$	$\alpha_155^\dagger$	
2vaa	H-2K^b					$\alpha_155^\dagger$	
2vab		α_2130				$\alpha_155^\dagger$	
1vac		α_2130				$\alpha_155^\dagger$	α_2123
1vad						$\alpha_155^\dagger$	
1ld9	H-2L^d						
1ddh	H-2D^d		α_2103				

Stabilization centers involving at least one residue from helices are documented.
†Residues of helical origin.

TABLE 4 Intramolecular stabilization center elements of MHC class II molecules

PDB code	MHC II allotype	Stabilization center elements				
		$\alpha 59^{\dagger}$	$\alpha 63^{\dagger}$	$\alpha 66^{\dagger}$	$\alpha 76^{\dagger}$	$\beta 80^{\dagger}$
1dlh	HLA-DR1 (DRA*0101, DRB1*0101)	$\alpha 36$			$\beta 54$	$\beta 21$
1aqd					$\beta 54$	$\beta 21$
2seb	HLA-DR4 (DRA*0101, DRB1*0401)	$\alpha 36$				$\beta 21$
1a6a	HLA-DR3 (DRA*0101, DRB1*0301)				$\beta 54$	$\beta 21$
1iea	H-2E^{k}	$\alpha 36$	$\alpha 12$	$\alpha 12$		$\beta 21$
1ieb			$\alpha 12$	$\alpha 12$		$\beta 21$
1iao	H-2A^{d}					
2iad						$\beta 21$
1iak	H-2A^{k}					$\beta 21$

Stabilization centers involving at least one residue from helices are documented.
†Residues of helical origin.

At least one residue of the bound peptide is involved in a SC in every one of the class II protein-peptide complexes analyzed, and also in most of the class I protein-peptide complexes studied (see Tables 5 and 6). In all these cases the complementary residues of the SC elements in the bound peptides were localized in the helices of the MHC molecule and never in the β-sheet. Because the accommodated peptides adopt an extended conformation in the MHC class I groove (Madden et al., 1991) and a polyproline II-like conformation in the MHC II groove (Jardetzky et al., 1996), the relatively large number of these helix-extended chain-associated SC connections suggests significant biological functions of these connections. Let us consider the two MHC classes separately.

Stabilization centers of MHC class II-peptide complexes

In MHC class II molecules, some of the SC elements of the peptides are identical to the so-called anchor residues, such as those located in relative positions 1 and 9 of the core sequence. These are characterized as strongly interacting peptide residues. When the anchors were not SC elements

TABLE 5 Stabilization center elements in MHCI-peptide complexes connecting the helices of the peptide binding groove to the peptide ligand

		Amino acids of the bound peptides Relative position*										Peptide-MHC stabilization centers†								
												α_1 helix					α_2 helix			
PDB code	MHC I allotype	1	2	3	4	5	6	7	8	9	10	$\alpha 63$	$\alpha 66$	$\alpha 70$	$\alpha 73$	$\alpha 77$	$\alpha 146$	$\alpha 147$	$\alpha 155$	$\alpha 156$
1hhg	HLA-A*0201	T	**L**	**T**	S	C	N	T	S	V			2,3							
1hhh		F	**L**	**P**	S	D	F	F	P	S	V		2,3							
1hhi		G	**I**	L	G	F	V	F	T	L			2							
1hhj		I	**L**	K	E	P	V	H	G	V			2							
1hhk		L	**L**	F	G	Y	P	V	Y	V			2							
2clr		M	**L**	L	S	V	P	L	L	L	G		2							
1b0g		A	**L**	**W**	**G**	F	F	P	V	L			2,3,4	3						
1agd	HLA-B*0801	G	G	K	K	K	Y	K	L											
1aln	HLA-B*3501	V	P	L	R	P	M	T	Y											
1a9e		L	P	P	L	D	I	T	P	Y										
1alo	HLA-B*5301	K	P	I	V	**Q**	Y	D	**N**	F						8			5	
1alm		T	**P**	**Y**	D	I	N	Q	**M**	L		2	3			8				
1hoc	H-2D^{b}	A	S	N	E	N	M	E	**T**	M						8	8	8		
1ce6		F	A	P	G	**N**	**Y**	P	**A**	L						8			5,6	
1bz9		F	A	P	G	V	F	P	**Y**	M						8				
2vaa	H-2K^{b}	R	G	Y	V	Y	Q	G	L											
2vab		F	A	P	G	N	Y	P	A	L										
1vac		S	**I**	**I**	N	F	E	K	L				2,3							
1vad		S	**R**	D	**H**	S	R	T	P	M			2	4						
1ld9	H-2L^{d}	Y	P	N	V	**N**	I	H	N	F									5	5
1ddh	H-2D^{d}	R	G	P	G	R	A	**F**	V	**T**	I				7		9	9		

*Amino acids of the bound peptide. Bold indicates residues that form stabilization centers with the MHC protein.
†Position of SC elements in the $\alpha 1$ and $\alpha 2$ helices and the relative position of the corresponding residues in the bound peptide (bold) are given as numbers.

TABLE 6 Stabilization center elements in MHCII-peptide complexes connecting the helices of the peptide binding groove to the peptide ligand

		Amino acids of the bound peptides Relative position*															Peptide-MHC stabilization centers#										
																	α_1 helix						$_1$ helix				
PDB code	MHC II allotype	−4	−3	−2	−1	1	2	3	4	5	6	7	8	9	10	11	α52§	α53§	α65	α68	α69	α72	β60	β71	β74	β82	β85
1dlh	HLA-DR1 (DRA*0101, DRB1*0101)			P	K	**Y**	V	K	Q	N	T	L	**K**	**L**	A	T					8	9	9			1	
1aqd			G	S	D	W	R	F	L	R	G	Y	H	**Q**	Y	A							9				
2seb	HLA-DR4 (DRA*0101, DRB1*0401)			Q	**Y**	M	R	A	D	Q	A	A	G	**G**	L			(−1)					9				
1bx2	HLA-DR2 (DRA*0101, DRB1*1502)	E	**N**	**P**	V	V	H	F	F	A	N	I	V	T	P		(−3),(−2)	(−2)									
1a6a	HLA-DR3 (DRA*0101, DRB1*0301)	P	V	S	K	M	R	M	A	**T**	P	L	**L**	**M**	Q	A	(−2)	(−2)			8	9		5	5		
1iea	H-2E^k	G	K	**K**	**V**	**I**	T	A	F	N	E	G	L	K			(−2)	(−1)								1	(−1)
1ieb		R	D	R	**M**	**V**	N	H	F	I	A	E	F	K											1	(−1)	
1iao	H-2A^d		R	G	I	S	Q	A	V	H	A	A	**H**	**A**	E	I				8	9						
2iad			G	H	A	**T**	Q	G	V	T	A	A	S	S	H	E										1	
1iak	H-2A^k			S	**T**	D	Y	G	I	L	Q	**I**	**N**	**S**	R	W		(−1)	7	8	9						

*Amino acids of the bound peptide. Bold indicates residues that form stabilization centers with the MHC protein.
#Position of SC elements in the α1 and β1 helices and the relative position of the corresponding residues in the bound peptide are given as numbers.
§N terminal cap of the helix.

FIGURE 1 Stabilization centers in peptide binding domains of MHC-peptide complexes. (*A*) MHC I-peptide complex, PDB code 1a1m. (*B*) MHC I-peptide complex, PDB code 1agd. (*C*) MHC II-peptide complex, PDB code 1dlh. Stabilization centers that involve residues from the bound peptide are marked in black. The rest of the stabilization centers are marked in dark grey. Sequence numbers of residues of the helices appearing as stabilization center element in any of the class I and class II complexes are shown in *A* and *C*, respectively.

themselves we often found them as supporting residues in the flanking tetrapeptides of the SC element. Some SC elements are located in the N-terminal flanking region of the peptide, which extends the binding groove of the protein. It is noteworthy that N-terminal elongation of peptide determinants beyond the first primary anchor was recently shown to improve peptide binding to MHC class II molecules (Bartnes et al., 1999).

The stability of MHC class II heterodimers as well as superdimers (Schafer et al., 1998) also found in the cell surface depends on the bound peptide (Germain and Margulies, 1993; Germain and Rinker, 1993), although apparently to a lesser extent than for class I molecules (Stern and Wiley, 1992; Reich et al., 1997). Peptides facilitate the assembly of the two chains in the endoplasmic reticulum, and determine their longevity in endosomal compartments and at the cell surface (Germain, 1995). Hydrogen bonds between conserved amino acids of MHC class II molecules and the backbone of the peptide make profound contributions to stabilization of the complex and to increasing enzyme susceptibility (Ceman et al., 1998). Stability of MHC class II-peptide complexes can be so delicately balanced that disruption of a single solvent-exposed hydrogen bond (such as substitution of histidine by asparagine at position β81) can lead to defects in complex assembly and speed up dissociation of the peptide, which is the triggering step in protein degradation (McFarland et al., 1999). It is noteworthy, that the conserved β81 histidine is located between residue β80 which forms an SC with another protein residue and β82 which forms an SC with peptide residues in many MHC class II proteins (see Tables 3 and 6). His β81 was found to be a supporting residue in all these cases. However, not all residues play the same role in stabilization, since, for example, loss of intramolecular interactions such as the salt bridge between α76 arginine and β57 aspartic acid in the resistant allotypes against insulin-dependent diabetes mellitus, can initiate local rearrangement of the peptide binding groove without altering global tertiary structure (Sato et al., 1999).

Empty MHC class II molecules were shown to have two peptide-free isomers; the active, peptide-receptive form is unstable and rapidly converts to an inactive one (Rabinowitz et al., 1998), which aggregates, becomes sensitive to proteases, and is degraded in endosomes (Germain and Rinker 1993). Under in vivo conditions, the folding, intracellular transport and continuous groove occupancy of MHC class II molecules is directed by the invariant chain (Ii), which acts as a chaperone for newly synthesized $\alpha\beta$ dimers, targets them to the endosomal compartments, and retards them from the cell surface in an inactive state. Removal of Ii is mediated by multistep proteolytic degradation, which results in the generation of class II associated invariant chain peptide (CLIP), peptides covering the 81–104 sequence of Ii, which bind to various MHC class II molecules and occupy the peptide binding groove until it is exchanged by a self- or an antigenic peptide. This process takes place in acidic MHC-rich compartments and only rarely on the cell surface, and it is catalyzed by the MHC-like HLA-DM/H-2M chaperone molecule (Koopmann et al., 1997). A large number of peptidic SC-elements were found in the CLIP peptide complexed with the HLA-DR3 molecule that is expressed on the surface of HLA-DM deficient cells (Table 6; Ghosh et al., 1995).

In their immature differentiation state, dendritic cells, the most potent of the professional antigen presenting cells, express a large fraction of empty MHC class II molecules. These are in a peptide-receptive state, bind exogenous protein fragments, and present them for T cells (Santambrogio et al., 1999). Immature dendritic cells also express MHC-Ii complexes, which traffic to the cell surface before internalization into the endosomal peptide-loading compartment. Peptide uptake by these unusual MHC class II molecules is assisted by functional HLA-DM molecules expressed on the cell surface (Arndt et al., 2000). Thus they acquire a novel extracellular receptor function, which broadens the spectrum of peptides captured and saved by MHC class II molecules of dendritic cells, which in turn play a pivotal role in priming immune responses.

Stabilization centers of MHC class I-peptide complexes

In five out of the 21 MHC class I-peptide complexes, no peptide-protein SC could be identified. In these five cases, one of the helices was not stabilized by any SC. Binding experiments with various peptides indicated that terminal residues of the peptide, which interacted not only with residues of the helices but also with conserved amino acids, at the closed ends of the MHC class I groove, contributed significantly to the binding energy (Madden, 1995). These promising candidates could not be recognized as SC elements because of the special definition of SC that requires flanking segments to occur on both sides of an SC element (Dosztányi et al., 1997). Based on our earlier study of a large number of proteins, SC elements made 1.77 times more long range interatomic interactions than did those residues involved in long range interactions that were not SC elements (Dosztányi et al., 1997). Therefore, the extremely large number of inter-atomic interactions identifies SC element-like residues. In all 31 complexes studied, all 323 residues of the bound peptides had interactions with MHC-derived atoms; therefore, all of them were involved in long range interactions. Fifty-two were SC elements, and 42 were terminal residues of the bound peptides. A comparison of the numbers of long-range atomic contacts shows that the average number is larger for residues, at the termini of bound peptides than for the same type of SC residues and almost twice that of the rest of the residues (Table 7). This suggests that although the terminal residues in question can not be recognized as SC elements, their interactions with the

TABLE 7 Average number of stabilization centers and long range atomic contacts in globular proteins, in MHC molecules and of peptide termini in MHC I complexes

	LRI			SC	
AA	GPDB	MHC	TR	MHC	GPDB
A	13	30	53	36	27
C	22	6	—	—	32
D	18	34	—	—	31
E	17	25	—	—	36
F	40	54	103	16	62
G	12	16	37	13	24
H	26	37	—	40	49
I	26	25	74	72	45
K	19	54	60	26	39
L	23	53	77	78	41
M	23	43	79	40	41
N	20	29	—	46	34
P	18	32	—	49	30
Q	21	52	—	56	40
R	32	58	100	90	50
S	16	26	56	35	32
T	18	28	69	38	33
V	22	24	66	43	38
W	53	73	—	93	81
Y	40	47	100	72	58

AA, one letter code of amino acids; LRI, long-range interactions; TR, terminal residue; SC, stabilization center; GPDB, globular protein database.

−, lack of given residue in the respective positions in the database.

MHC class I protein are so pronounced that in fact they play the same role as do SC elements in structure stabilization, so they could be called quasi-SC elements.

Differences in serology and thermal lability indicated that MHC class I molecules lacking peptide have a structure significantly different from those complexed with peptide ligands. Empty MHC class I molecules possess partially folded α_1 and α_2 domains and many of their physical properties are typical of molten globules (Bouvier et al., 1998). A small proportion (3–5%) of native α-chains was detected on the surface of T cells (Edidin et al., 1997), but empty MHC class I molecules can be transported to the cell surface only at sub-physiological temperatures (Ljunggren et al., 1990). These results demonstrate that the MHC class I ligand binding site is inherently unstable in the absence of peptide. To select peptide under in vivo conditions, the highly polymorphic MHC class I α-chains interact with β2-microglobulin. Analyzing SC elements in the peptide binding domains we found that the polymorphic α76 residue and the tryptophan at position 60 in β_2-microglobulin form an SC in almost all studied MHC-peptide complexes except one of the HLA-A2 complexes (Table 1, no.2).

Intracellular loading of MHC class I molecules is also assisted by chaperones, which maintain the fragile peptide-binding sites in a peptide-receptive conformation, and retain the class I molecules in the endoplasmic reticulum until peptide binding occurs. MHC class I molecules, transported to the cell surface, retain their folded peptide binding site and are stable for hours or even days (Micheletti et al., 1999). However, some MHC class I molecules, such as the murine H-2L^d protein, exhibit weak interaction with peptides and with β2-microglobulin (Balendiran et al., 1997). As a consequence, their peptide loading within the cell is insufficient and results in low level cell surface expression; the L^d molecules have an unusually short half-life and are able to exchange their peptides for exogenous ligands (Hansen et al., 2000). In accord with these data, our analysis failed to detect SCs in the α-helices of the H-2L^d molecule (Table 3), but SC elements were detected in the peptide ligand (Table 5). Thus, MHC class I molecules, which display quantitative differences in their interactions with peptides and other molecules involved in intracellular peptide loading, may also acquire novel functions that provide alternative pathways for antigen presentation (Jondal et al., 1996; Khare et al., 1996; Hansen et al., 2000).

Our survey suggests that SCs and quasi-SCs composed of residues derived from both the protein and the complexed peptide can be vital for ensuring the compact structure of MHC proteins by fixing their α-helices. These highly ordered structures play a pivotal role in stabilizing the molecular surface which contacts the T cell antigen receptor and also support a stable MHC conformation resistant to proteolytic attack (Willcox et al., 1999).

CONCLUSION

We suggest a new view of function-related regulation of the stability of MHC proteins by SCs, which were analyzed in 31 MHC-peptide complexes of known three-dimensional structure. The primary biological function of MHC proteins is to bind intracellular peptides, generated by limited proteolysis and loaded onto nascent or recycling MHC class I or class II proteins inside the cell, to transport them to the cell surface and hold them there until they are recognized by T cells. To accomplish this job MHC molecules must acquire a stable conformation. Although MHC molecules can bind a wide array of peptides, almost every one of them carries only one ligand to the cell surface in its lifetime; only a few molecules lose and exchange their ligands at the cell surface or after their recycling to endosomes. The peptide binds to the protein noncovalently; without the dissociable peptide ligand, the MHC molecule loses its stable structure very rapidly and becomes sensitive to proteolytic degradation.

We suggest in this paper that regulation of protein stability is based on the formation of SCs composed of residues from both the peptide binding groove of the MHC protein and the small dissociable ligand. These SCs stabilize the helices bordering the peptide binding groove and the remainder of the MHC protein. Thus, these interactions may also be essential for capturing the peptide and so generating a novel molecular surface, recognizable by the T cell recep-

tor (Willcox et al., 1999). Because these SCs are missing in the ligand-free protein, the empty MHC molecules are fragile. The lifetime of MHC class I-peptide complexes at the cell surface determines the efficiency of cytotoxic T lymphocytes, which play a pivotal role in virus- and tumor-specific responses. Stable MHC class II-peptide complexes trigger helper T cell responses, which regulate immune responses against foreign and self-antigens and may cause autoimmune diseases. Ligand-free MHC molecules are immunologically incompetent and have a short half life, but they may acquire novel biological functions or become a source of amino acids for de novo protein synthesis.

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