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Major Histocompatibility Complex Class II Binding Characteristics of Peptoid–Peptide Hybrids

Ellen C. de Haan,[a] Marca H. M. Wauben,[b] Mayken C. Grosfeld-Stulemeyer,[b] John A. W. Kruijtzer,[a] Rob M. J. Liskamp[a] and Ed E. Moret[a,*]

[a]*Department of Medicinal Chemistry, Utrecht Institute for Pharmaceutical Sciences, Utrecht University, PO Box 80082, 3508 TB Utrecht, The Netherlands*

[b]*Immunology Division, Department of Infectious Diseases and Immunology, Utrecht University, PO Box 80165, 3508 TD Utrecht, The Netherlands*

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Abstract—The major histocompatibility complex (MHC) class II binding requirements for solvent-exposed peptide residues were systematically studied using amino acid and peptoid substitutions. In a peptoid residue, the side chain is present on the backbone nitrogen atom as opposed to the α-carbon atom in an amino acid residue. To investigate the effect of this side chain shifting on MHC binding, three amino acids in the central part of the peptide sticking out of the binding groove were replaced by corresponding peptoid residues. Two peptoid–peptide hybrids showed large affinity decreases in the MHC–peptide binding assay. To investigate this affinity loss, the individual contributions to MHC binding affinity of the side chain (position), the putative hydrogen bond, and the flexibility were dissected. We conclude that the side chain position as well as the backbone nitrogen atom hydrogen bonding features of solvent-exposed residues in the peptide can be important for MHC binding affinity.

Introduction

Major histocompatibility complex (MHC) class II proteins are cell surface glycoproteins that are present on professional antigen presenting cells. They bind peptides derived from merely extracellular proteins in an extended polyproline type II twist,[1] which is held by several conserved hydrogen bonds.[2,3] Such specific MHC–peptide complexes are recognized by CD4+ T cells via the T cell receptor and subsequent signaling results in activation of the T cell. In peptidomimetic compounds, amino acids are replaced by non-peptidic building blocks. Several reports have been published investigating MHC class I (e.g., refs 4 and 5) and MHC class II binding of certain types of peptidomimetics. These modifications can be divided into backbone modifications on the one hand, involving *N*-methylation,[6–8] amide bond reduction,[8,9] replacement of the amide bond by an ethylene bond[9] or a cyclic moiety,[6,8,10–12] and side chain modification on the other hand, introducing unnatural amino acids.[6,7,13]

Here, we describe the design, synthesis, and MHC class II binding characteristics of peptoid–peptide hybrid peptidomimetics. The synthesized hybrids consist of amino acids, in which only one amino acid is replaced by the corresponding peptoid residue. In peptoid residues, the position of the side chain is shifted from the α-carbon atom to the nitrogen atom.[14] The identity of the side chain is conserved, while the direction in three-dimensional space is changed. These compounds may be considered a combination of backbone and side chain modifications. Using stepwise replacement of three solvent-exposed amino acid residues for a peptoid residue, and an additional set of compounds, the structural features of peptoid–peptide hybrids were thoroughly investigated.

The MHC class II molecule RT1.B^L and peptide sequence 72–85 of guinea pig myelin basic protein (gpMBP) we studied, play a major role in the rat experimental autoimmune encephalomyelitis model (EAE, resembling human multiple sclerosis).[15] Starting from this peptide, peptoid–peptide hybrids were designed to investigate the influence on MHC binding of side chain shifting at solvent-exposed sites in the central part of the peptide.

*Corresponding author. Tel.: +31-30-253-6979; fax: +31-30-253-6655; e-mail: e.e.moret@pharm.uu.nl

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Results and Discussion

Design and synthesis of peptoid–peptide hybrids

For investigation of peptide-MHC class II binding requirements, a homology model of RT1.B^L complexed with peptide gpMBP72–85 was used,[16] since no crystal structure of this MHC molecule was available. The MHC–peptide model showed three amino acids in the peptide sticking out of the MHC binding groove: the lysine at relative position 2 (Lys2), Arg5, and Asp8 (Fig. 1). Replacements of these three amino acid residues into alanine and glycine (one at a time) did not reduce MHC binding affinity (Table 1),[16] indicating that the side chains of these three amino acids were not involved in MHC binding. The same three amino acids were changed into their corresponding peptoid residues (NLys, NArg, and NAsp, respectively) to investigate whether it was possible to shift solvent-exposed side chains, that are within the peptide binding core. The structures of the peptides and hybrids are shown in Figure 2 and Table 1. WT is the acetylated and amidated gpMBP72–85 peptide, and for example in hybrid Lys2NLys, Lys2 was replaced by the corresponding peptoid residue NLys.

MHC binding affinity of peptoid–peptide hybrids

In the concentration range tested, no MHC binding could be detected for two of the three hybrids with shifted side chains (Lys2NLys and Arg5NArg, Table 1), while Asp8NAsp displayed only a small affinity decrease ($IC_{50} = 16–32\,\mu M$) compared to the WT peptide ($IC_{50} \leq 8\,\mu M$). To investigate the differences between a peptoid and an amino acid residue systematically, we tried to dissect the overall change resulting from the modification of an amino acid to a peptoid residue into individual components. Therefore, the following consequences of the introduction of a peptoid residue were investigated, and possible reasons for the affinity loss were hypothesized:

Altered position of the side chain:
1a. The side chain of the amino acid is required at its original position.
1b. The side chain of the peptoid is in an unfavorable location.

Increased flexibility:
2. Increased loss of entropy occurs upon binding of the hybrid to the MHC.

Alkylation of the backbone nitrogen atom:
3. Potential hydrogen bond cannot be formed.

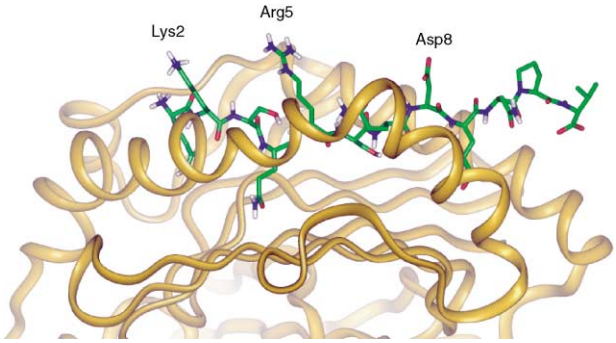


Figure 1. Schematic representation of the RT1.B^L-MBP72–85 model. Shown is the top part of the MHC (golden ribbon) and the peptide (colored by atom type, only polar hydrogens are shown) with Lys2, Arg5, and Asp8 projecting out of the MHC binding groove.

These effects were studied using sets of Ala (no functional side chain) and Gly (flexible) substituted peptides, and NAla (no NH) substituted peptoid–peptide hybrids (Table 1). MHC binding results of these compounds together with the flow charts shown in Figure 3, made it possible to confirm or reject the above described hypotheses.

Side chain binding of amino acids

The contribution of the amino acid side chains at the three positions to the binding affinity (hypothesis 1a) was studied by comparing the binding affinity of the WT peptide with that of the Ala substituted analogues. These peptides all showed high binding affinity (Table 1), which indicated that the side chain of the changed amino acid was not necessary for MHC binding. Hypothesis 1a was therefore rejected.

Influence of flexibility

The flexibility of the peptoid–peptide hybrid was expected to be larger than that of a peptide, since a peptoid residue can adopt more conformations than an amino acid.[14,17] To investigate whether increased flexibility and therefore increased loss of entropy upon binding to the MHC molecule affected the affinity (hypothesis 2), glycine substituted peptides were examined (Table 1). When compared to the alanine substituted peptides, the flexibility of those glycine substituted peptides has increased. As these glycine substituted peptides all showed high MHC binding affinity, we concluded that the expected increased flexibility of the peptoid–peptide hybrids was not deleterious to MHC binding.

Hydrogen bonding features

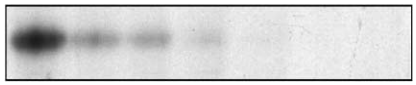
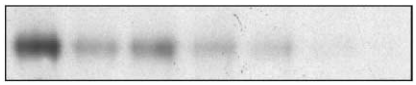
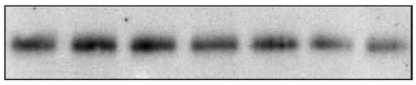
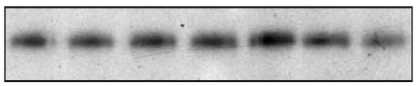
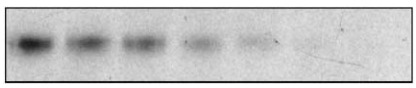
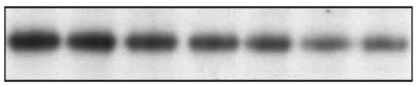
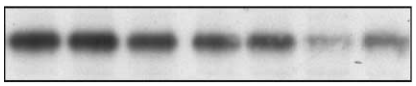
In the next step in the flow charts (Fig. 3) the contribution of a putative hydrogen bond between the backbone nitrogen atom in the peptide and the MHC molecule (hypothesis 3) was evaluated. After replacement by a peptoid residue no hydrogen bond involving the NH was possible. It has been shown that disruption of one hydrogen bond between the peptide backbone and the MHC can result in a decrease in binding affinity.[8,18] On the other hand, the contribution of hydrogen bonds to affinity is often overrated, especially at solvent-exposed positions.[19] The importance of the disappearance of the hydrogen bond donor was tested in peptoid–peptide hybrids with NAla (*N*-methyl glycine) inserted. Taking the step from a glycine substituted peptide to an NAla peptoid–peptide hybrid, the main difference was the disappearance of the hydrogen atom from the backbone nitrogen atom (Fig. 3). At position 2, methylation of the

backbone nitrogen atom (ccompare Lys2NAla to Lys2Gly, Fig. 3a) resulted in a large loss of affinity, which may point to the required presence of a hydrogen bond at that site. The same occurred at position 5 (Fig. 3b). At position 8, no reduction in binding affinity was detected. Apparently, no such hydrogen bond was present in the peptide at that position. The hydrogen bond between the backbone nitrogen atom at position 2 in the peptide and the MHC (Asnβ82) has been described in the literature as a conserved hydrogen bond.[2,3] Recently, McFarland et al. reported that this hydrogen bond contributes significantly to MHC binding affinity.[20] At positions 5 and 8, no conserved hydrogen bonds are known.

Side chain binding of peptoids

From the model of the peptide–MHC complex (Fig. 1), it could be deduced that the direction of the peptoid side chain in three-dimensional space could have been changed (about −30°), compared to the amino acid side chain. This is due to the polyproline type II twist, which is a general feature of MHC class II bound peptides.[1] The change in direction may have resulted in forcing the

Table 1. Structures and binding affinities of peptides and hybrids

Compound	Structure			MHC binding affinity	
	P2	P5	P8	Concentration of competitor (μM) 0 8 16 32 64 128 256[a]	IC_{50} (μM)[b]
WT	Lys	Arg	Asp		≤8
Lys2Ala	Ala	Arg	Asp		≤8
Arg5Ala	Lys	Ala	Asp		≤8
Asp8Ala	Lys	Arg	Ala		≤8
Lys2Gly	Gly	Arg	Asp		≤8
Arg5Gly	Lys	Gly	Asp		≤8
Asp8Gly	Lys	Arg	Gly		≤8
Lys2Nlys	NLys	Arg	Asp		≥227
Arg5Narg	Lys	NArg	Asp		≥256
Asp8Nasp	Lys	Arg	NAsp		16–32
Lys2Nala	NAla	Arg	Asp		64—128
Arg5Nala	Lys	NAla	Asp		64–256
Asp8NAla	Lys	Arg	NAla		≤8

[a]Inhibition of binding of biotinylated marker peptide to isolated $RT1.B^L$ molecules is shown in a dose range from 0 to 256 μM competitor peptide/hybrid.
[b]Relative binding affinity of the competitor is expressed as IC_{50}: concentration of competitor resulting in 50% inhibition of MHC binding of the marker peptide.

(charged) functional group of the side chain of the peptoid residue into an unfavorable position (hypothesis 1b). The modification may also have caused dislocation of one of the adjacent MHC anchor residues, due to a distorted backbone.[11] In the last step in the flow charts, it was evaluated if the side chain on the backbone nitrogen atom could be elongated from NAla to the peptoid residue corresponding to the original amino acid at that position. At all three positions, a small (additional) affinity decrease was observed, indicating the position of the peptoid side chain was unfavorable.

Conclusion

It is intriguing that shifting the side chain from the C_α to the N atom at originally solvent-exposed peptide residues, had such an effect on MHC binding. Howard et al. have reported a retropeptoid mimicking a high-affinity binder.[21] No MHC binding could be detected for the retropeptoid, in which all amino acids were replaced by peptoid residues (in reversed order). A retropeptoid is expected to bind in the opposite direction in the MHC binding groove, as compared to a peptide or peptoid–peptide hybrid with one peptoid residue. As a result, the side chains of the peptoid residues can point in the same direction as the side chains in the original peptide, assuming that the backbone carbonyl groups reside in their original positions in the MHC. Therefore, the lack of MHC binding in this case may be attributed to the loss of all backbone hydrogen bond donors, or to the increased flexibility.

In this study, we investigated the differences between a peptoid residue and an amino acid residue at a solvent-exposed site in the peptide, in relation to MHC class II binding affinity. With the strategy that was described here, the contributions of the side chain, backbone, and overall structure to MHC binding were separated. We realize, however, that by adopting the above described deduction strategy, synergistic and counteractive effects of the separate contributions were not taken into

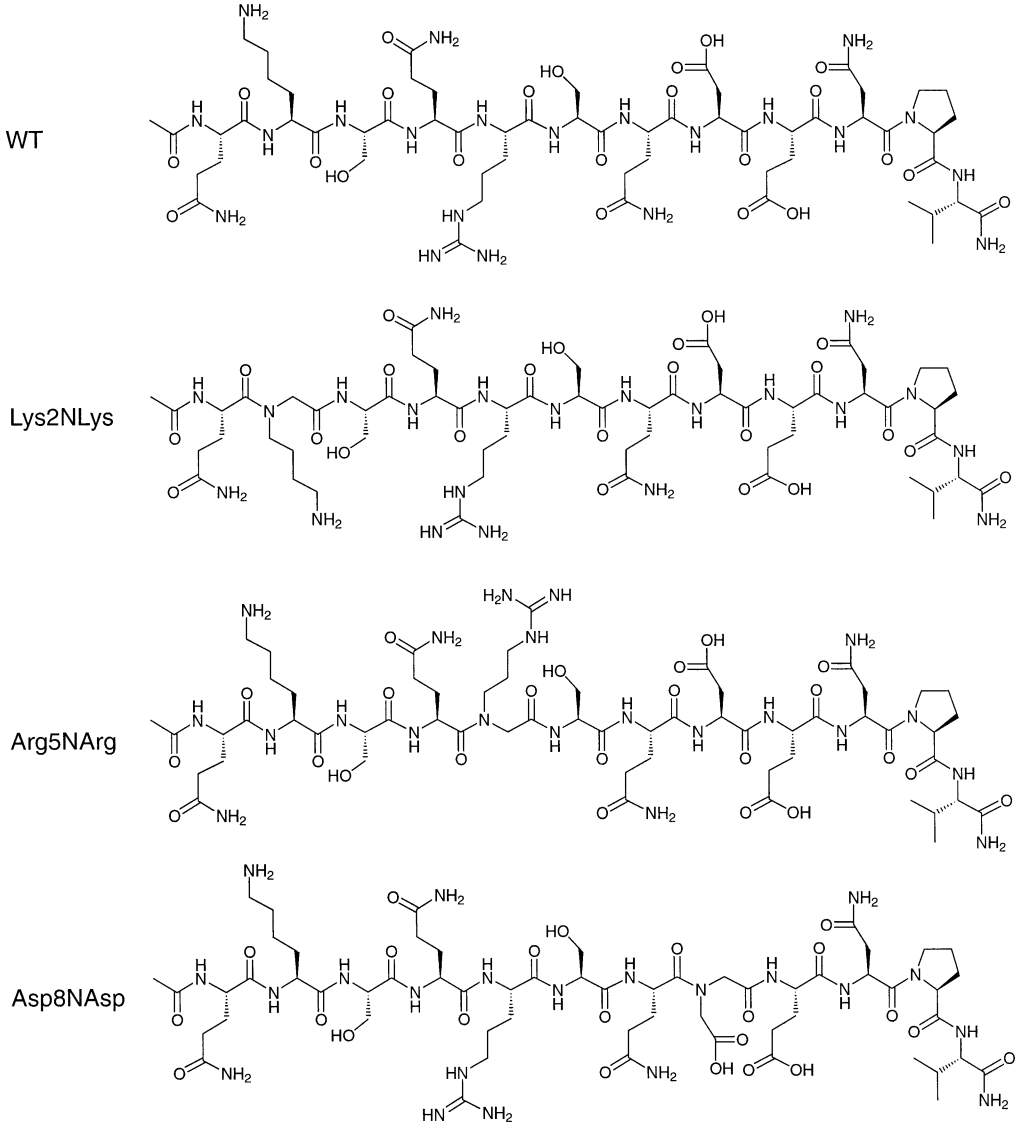


Figure 2. Structures of peptide WT and peptoid–peptide hybrids Lys2NLys, Arg5NArg, and Asp8NAsp, with amino acid residues at positions 2, 5, and 8, respectively, replaced by corresponding peptoid residues.

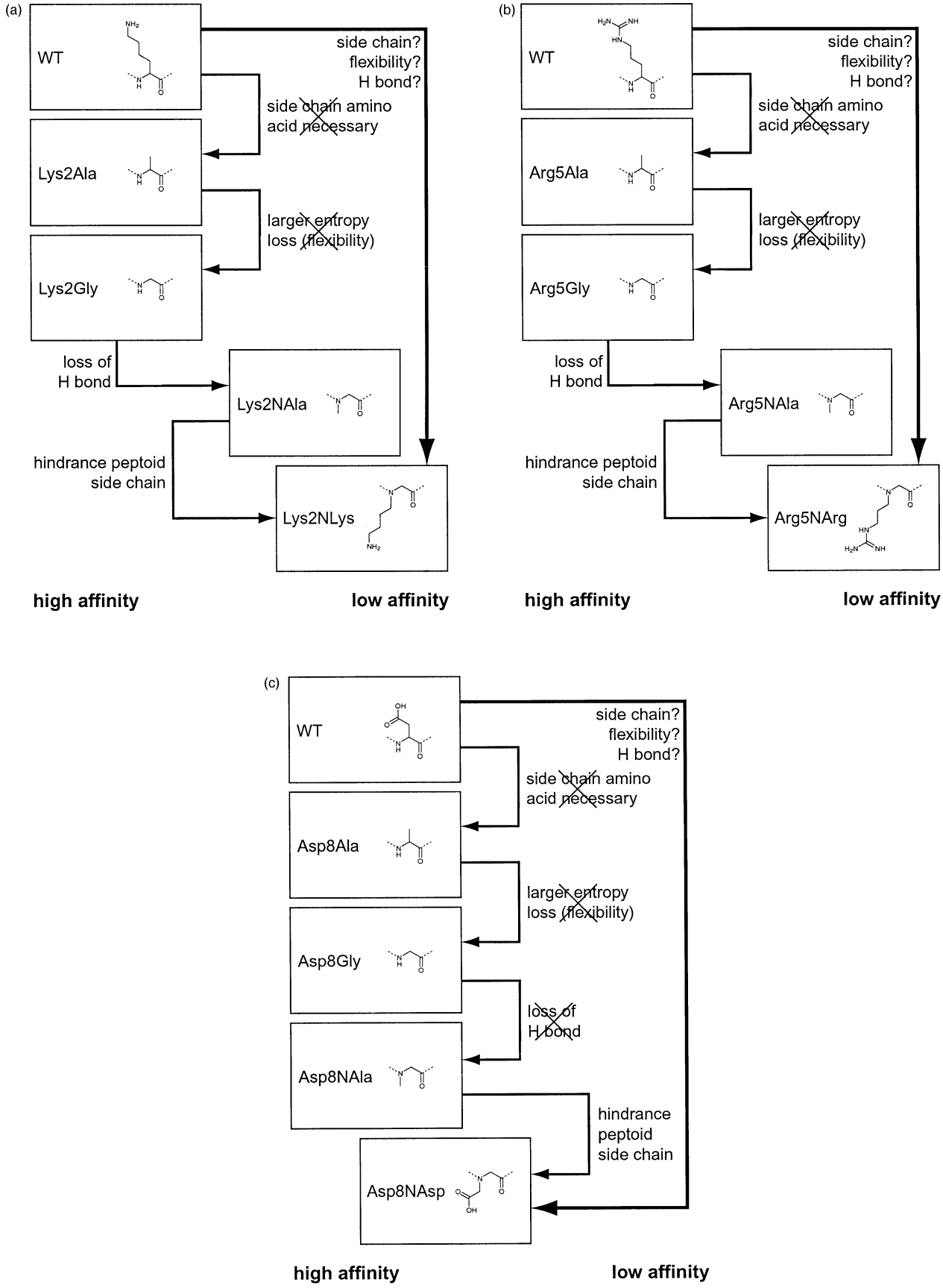


Figure 3. Flow charts describing the structural changes resulting from the replacement of an amino acid residue by a peptoid residue. Shown are parts of structures and their contributions to binding affinities (left: high affinity; right: low affinity) of compounds altered at (a) position 2, (b) position 5, and (c) position 8.

account. It was shown that the side chains of the three selected solvent-exposed amino acid residues were not involved in MHC binding, whereas the side chains of the peptoid residues were in unfavorable locations. Presumably increased flexibility of the peptoid–peptide hybrids did not hamper MHC binding. A large affinity loss was seen when amino acid residues at positions 2 or 5 were altered into their corresponding peptoid residues, and this could be mainly attributed to the loss of a putative hydrogen bond of the backbone nitrogen atom with the MHC. Apparently, no such hydrogen bond was formed at position 8. In conclusion, these results indicate that the side chain position, as well as the backbone nitrogen atom hydrogen bonding features of solvent-exposed residues of the peptide could be important for MHC binding.

Experimental

Synthesis of peptides and peptoid–peptide hybrids

Peptoid monomers NLys (peptoid residue corresponding to Lys), NArg, and NAsp were synthesized according to general procedures as described earlier.[22] Peptide WT (gpMBP72–85, sequence QKSQRSQDENPV) was synthesized on an automated peptide synthesizer (model 433A, Applied Biosystems, USA), as described previously.[22] ArgoGel® Rink-NH-Fmoc resin (Argonaut Technologies Inc., USA) was used to obtain C-terminal amides. The N-terminus of the peptide was acetylated with acetic anhydride capping solution. The peptoid–peptide hybrids were synthesized analogously, with minor variations: coupling of the peptoid monomer to the preceding amino acid was carried out with *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) and 1-hydroxy-7-azabenzotriazole (HOAt) instead of *O*-benzotriazole-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxybenzotriazole (HOBt). Subsequent coupling of the next amino acid to the peptoid monomer was performed as a double coupling, also using HATU/HOAt, after which a capping step was introduced. The peptides with Ala and Gly substitutions[16] were synthesized by automated simultaneous multiple peptide synthesis (SMPS).[23] The peptides and hybrids were purified by reversed phase HPLC (C18) when necessary, and checked by MS analysis.

MHC–peptide binding assay

The MHC binding capacity of the compounds was determined using a direct MHC–peptide binding assay, as has been described before.[24] Briefly, a concentration range (0–256 μM) of competitor (peptide or hybrid) was incubated together with biotinylated marker peptide (gpMBP72–85) and detergent-solubilized MHC class II molecules (RT1.B^L) for 40 h at room temperature and pH 5, in the presence of a protease inhibitor mix. Non-reducing SDS-PAGE and Western blotting were used for analysis of the MHC-peptide mixtures. Marker peptide–MHC binding was visualized by enhanced chemoluminescence. The larger the decrease of signal of the marker peptide, the higher the affinity of the competitor peptide (expressed as IC_{50}: concentration of competitor resulting in 50% inhibition of binding of the marker peptide).

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