Peptide Revolution

Genomics, Proteomics & Therapeutics

Proceedings of the Eighteenth American Peptide Symposium

Edited by
Michael Chorev
and
Tomi K. Sawyer

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Dimeric Analogs of the Immunosuppressory Fragment of HLA-DR

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Introduction

Our previous studies revealed that the nonapeptide fragment of human histocompatibility antigens (HLA) class DR, located in the β 164-172 loop with the VPRSGEVYT sequence, suppresses the immune response [1]. The sequence is located on the loop of the molecule exposed toward the solvent and, therefore, may be involved in interactions with other proteins. We suggest that the loop may serve as a functional epitope on the HLA class II surface for intermolecular binding. The possible mechanism of biological action of the synthesized peptides is connected to specific interference of adhesion between HLA class II molecules and their coreceptors [2,3]. It has been postulated that oligomerization of the coreceptors is required for stable binding to class II HLA [4]. On the basis of the crystal dimeric structure of HLA-DR [5], we designed and synthesized molecules able to induce the putative coreceptors dimerization, which consist of two VPRSGEVYT sequences linked through their C-termini by spacers of different length (Figure 1).

I. (H-VPRSGEVYT***GGGG)$_2$K-NH$_2$*** (31)
II. (H-VPRSGEVYT***GGGGG)$_2$K-NH$_2$*** (37)
III. (H-VPRSGEVYT***GGGGGG)$_2$K-NH$_2$*** (43)
IV. H-VPRSGEVYT***GGGGGG-NH$_2$***

*Fig. 1. Synthetic dimeric analogs of the immunomodulatory fragment of HLA-DR molecules **(I-III)** and their monomeric counterpart containing six glycine residues at C-terminus **(IV)**. The linker structure is given in bold; numbers of atom in linker are in brackets-counting only the atoms contributing to the length of the linker.*

Results and Discussion

The bivalent analog should consist of two monomers covalently linked by a spacer with sufficient length to allow for positioning in the same orientation as in the superdimers. Therefore, we began the design by examining the three-dimensional structure of the superdimers [5] (Figure 2A). To quickly determine the optimal linker length, we decided to synthesize a series of dimeric analogs containing linkers of various lengths. Whereas compound **I** contains a 31-atom linker, which is too short to orient the suppressors in the same position as in the crystal structure, its analogs **II** and **III** possess sufficient length of the linkers (Figure 2B and 2C). The 43-atom linker in compound **III** seems even longer than necessary, although flexible enough to orient the peptides more precisely, and without any distortion.

Peptides were prepared by manual solid-phase techniques. The peptide was assembled on the solid support, using standard Fmoc procedure. The first amino acid attached to the MBHA-Rink Amide resin (0.55mmol/g) was di-Fmoc-Lys in case of dimeric compounds. Successive amino acids were coupled simultaneously to the α and ε-amino groups of the lysine residue.

The synthesized peptides were investigated for their activities in the humoral and cellular immune responses. Their potencies were compared to that of cyclosporine

(CSA). Dimeric peptides **II** and **III** exhibited higher potencies than their linear counterpart **IV**. The immunosuppressory activity of the dimeric peptides depended on their linker length. Both in humoral and cellular immune response, the shorter linker dimeric analog **I** exhibited the weakest potency in the series, although slightly higher than that of its monomeric analog **IV.** The longer linker analogs **(II** and **III)** were very active immunosuppressants, more effective than CSA. CD spectra of compounds **I–IV** were similar to each other, suggesting that dimerization has little effect on stabilization of their structure in water solution. The unstructured conformation of the linker may enable the orientation the immunosuppressory peptides in a desired position.

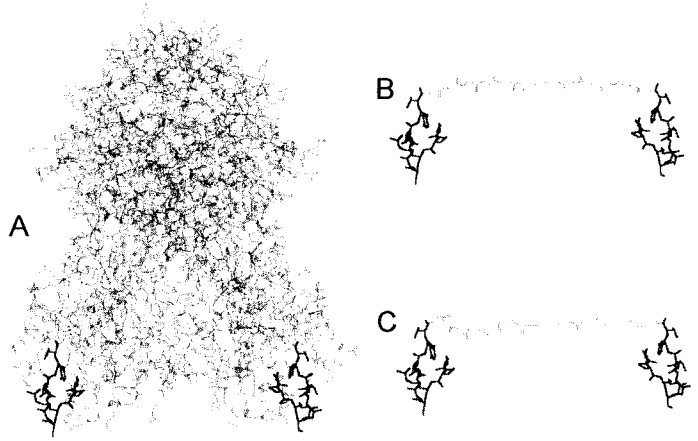

Fig. 2. ***A.*** *View of the superdimer of human class II histocompatibility antigen HLA-DR. The β164-172 loops with the immunosuppressive sequences (VPRSGEVYT) are in bold.* ***B.*** *Peptide contains a 37-atom linker that is able to span the distance between the carbonyl groups of* $Thr^{\beta 172}$ *as in the superdimer structure.* ***C.*** *43-atom linker is longer than necessary and may have conformational flexibility to orient the VPRSGEVYT loops in the same position as in the crystal structure.*

Our results demonstrate that the immunosuppressive activity of β 164-172 fragment of HLA-DR is enhanced by its dimerization. The linker length affects the immunosuppressory effects.

Acknowledgments

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