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Hydrogen Bond Formed between the DR1_{β81His} and the Peptide Main Chain Is the Primary Target for DM Induced Peptide/MHC Complex Dissociation

HLA-DR1 (DR1) is an allele of the Major Histocompatibility Complex Class II (MHC II), and presents peptides derived from endocytosed antigens to CD4+ T cells, thereby kickstarting the adaptive immune response.

Briefly, MHC II molecules are expressed by Antigen Presenting Cells (APCs) and are heterodimeric transmembrane proteins containing a peptide binding groove. APCs endocytose proteins and degrade them into peptides in their low pH endosomal / lysosomal compartments. Here the peptides meet DR and the MHC II homolog HLA-DM (DM). DM catalyses exchange of the default peptide, CLIP, with incoming peptides, and also mediates further rounds of peptide exchange depending on the ability of the bound peptide to fill Pocket 1 of DR1. DM thus plays a central role in selection of peptide to be presented by the APC to the T cell. However, little is known about the mechanism of DM action.

The disruption of Hydrogen bonds between peptide and the peptide binding groove of MHC II has been speculated to be a potential mechanism for DM assisted peptide exchange. To investigate this, we have introduced a single site directed mutation, βH81N, in wtDR1. Binding experiments demonstrate that the dissociation of DR1_{βH81N}/peptide complexes is severely affected and reduced to ~14 minutes *independent of peptide sequence*. Unexpectedly, complexes of DR1_{βH81N}/HA₃₀₆₋₃₁₈ are SDS stable, indicating that while stability of bound peptide to this mutant is severely debilitated, the structural integrity of the βH81N mutant is maintained.

Importantly, we show that DM does interact with DR1_{βH81N} molecules and is effective in enhancing peptide association, but cannot further enhance the dissociation rate of DR1_{βH81N}/peptide complexes. Thus, mutating this one residue does not abolish DM interaction with DR; it only affects the effector function of DM. We hypothesise that a DR1_{βH81N} molecule that fails to form Hydrogen bonds between β81His and peptide backbone might represent a “post DM effected” transitional state. We suggest that the distinct effects of DM on MHC class II, i.e., generation of peptide-receptive conformation and dissociation of floppy Peptide/MHC II complexes may be accomplished by a repetitive “hit-and-run” mechanism that involves breaking of the H bond between β81His and peptide.