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Epitope Mapping Using H/D Exchange Combined with Proteolysis and Mass Spectrometry

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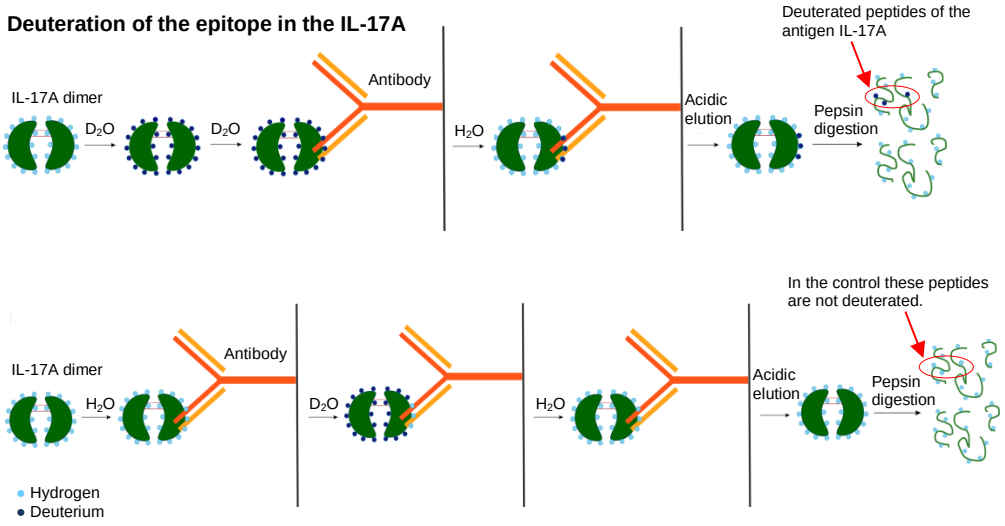
Introduction

Therapeutic monoclonal antibodies (mAbs) are playing an ever more important role in the treatment of previously insufficiently treatable diseases. Characterising the epitope on the antigen which is recognized by the mAb is essential for understanding the mode of action which is also requested by regulatory authorities. In this project, the epitopes on the homodimer antigen IL-17A were characterised for three different anti-IL17A mAbs.

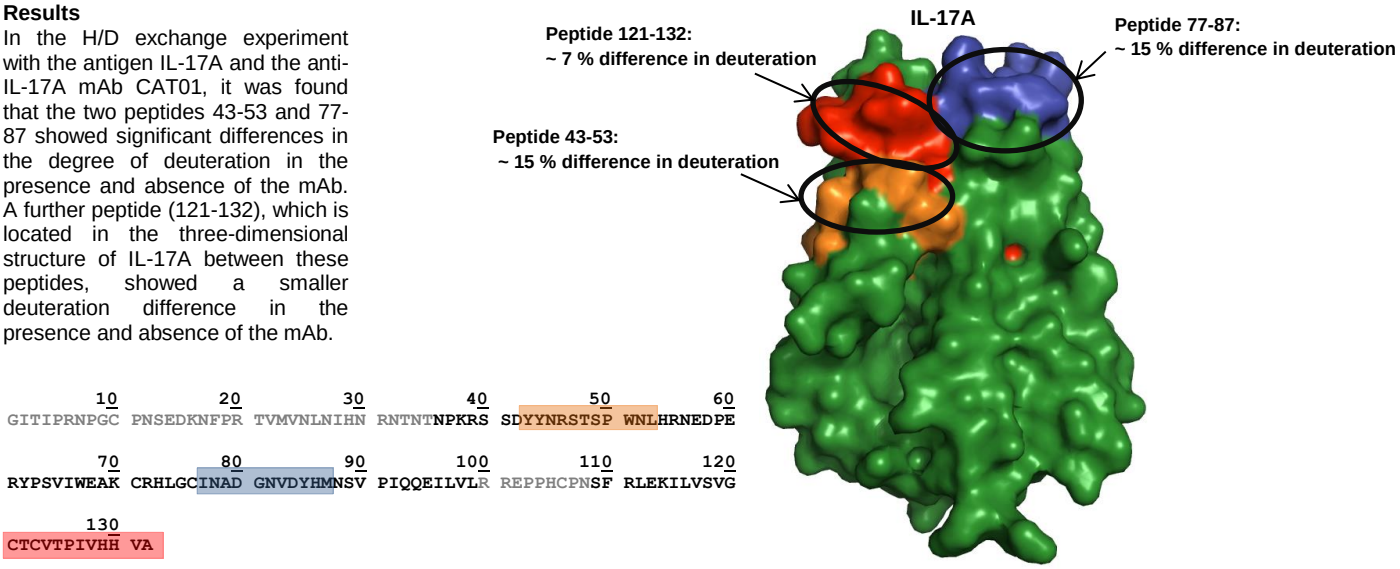
Method

Epitope mapping using H/D exchange, coupled with proteolysis and mass spectrometry, has proven to be a particularly effective method for characterising discontinuous epitopes. The principle of the H/D exchange method is based on the fact that the protonation of previously deuterated amino acids in the antigen is inhibited in the region of the epitope by the presence of an mAb. After H/D exchange, the antigen is enzymatically digested, and through exact determination of the masses of the peptides by mass spectrometry, differences in the degree of deuteration in the presence and absence of the mAb can be precisely localised (see the figure on the right).



Results

In the H/D exchange experiment with the antigen IL-17A and the anti-IL-17A mAb CAT01, it was found that the two peptides 43-53 and 77-87 showed significant differences in the degree of deuteration in the presence and absence of the mAb. A further peptide (121-132), which is located in the three-dimensional structure of IL-17A between these peptides, showed a smaller deuteration difference in the presence and absence of the mAb.



Three-dimensional structure of IL-17A (PDB number 2VXS) with orange, blue and red marked areas of the epitope (right). Grey marked amino acids in the sequence (left) are disordered segments and are therefore not defined in the three-dimensional structure. The colouring in the sequence also shows the peptides involved in the epitope.

Discussion

The resolution of epitopes defined by H/D exchange is in the range of ca. 10 amino acids. It might be possible to localise the epitopes still more precisely using MS/MS to identify deuterated amino acids.