Antibody cross-reactivity and polyspecificity: interaction with peptide-homologous and non-homologous peptides

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Monoclonal antibodies usually show both cross-reactive and polyspecific behavior by reacting along with their original epitope both with epitope-homologous but also with non-epitope homologous structures. We investigate the peptide binding behavior of several murine monoclonal antibodies which recognize linear sequence epitope of the corresponding proteins used for immunization.

One of these antibodies is the anti-TGFα antibody tAb2. This antibody binds the N-terminal sequence of transforming growth factor α, VVSHFND. With the help of combinatorial peptide libraries it is possible to find homologous peptides that bind tAb2 with an affinity similar to that of the epitope. The conformational flexibility of short peptides can be constrained by cyclization in order to improve their affinity to the antibody and their stability towards proteolysis. Two cyclic peptides which are cross-reactive binders for tAb2 were selected earlier using combinatorial peptide libraries. One is cyclized by an amide bond between the N-alpha group and the side chain of the last residue (cyclo-SHFNEYE), and the other by a disulfide bridge (cyclo-CSHFNDYC). The complex structures of tAb2 with the linear epitope peptide VVSHFND and with cyclo-SHFNEYE were determined by X-ray structural analysis. Both peptides show a similar conformation and binding pattern in the complex [1], Fig.1. The linear peptide SHFNEYE does not bind tAb2, but cyclo-SHFNEYE is stabilized in a loop conformation suitable for binding. Hence the cyclization counteracts the exchange of aspartate in the epitope sequence to glutamate.

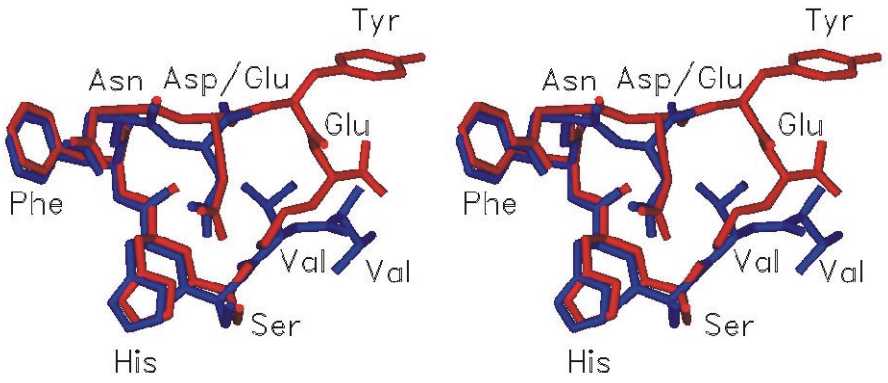

Fig.1. Comparison of the conformations of the linear peptide epitope and the cyclized epitope-homologous peptide in the state of complex with Fab tAb2 (taken from [1]).

Now we collected data at the X13 beamline with crystals from uncomplexed tAb2 Fab fragment to compare the structure of the native with the complexed form, especially with respect to changes in the domain arrangement and in the CDR loop conformations upon binding of the peptides to support or exclude an induced fit mechanism of binding.

The crystals were measured under cryo conditions and diffracted to 2.27 A resolution. A data set was obtained with 99% completeness in the highest resolution shell. The estimated unit cell constants in the space group $P2_12_12_1$ were a = 74.7; b = 86.6; c = 133.9 with $\alpha = \beta = \gamma = 90°$.

The structure is being solved now using the method of molecular replacement on the basis of the complex structures given above.

A second sample is the murine anti-p24(HIV-1) antibody CB4-1.

This antibody raised against p24 (HIV-1) recognizes a linear epitope of the HIV-1 capsid protein. Additionally, CB4-1 exhibits cross-reactive binding to epitope-homologous peptides and polyspecific reactions to epitope non-homologous peptides. Crystal structures solved earlier in our lab demonstrate that the epitope peptide and the non-homologous peptides adopt different conformations within the binding region of CB4-1 [2] (see Fig.2). It is also possible to select binding peptides forming a pathway of intermediates between the original epitope peptide sequence and that of epitope non-homologous sequences. We already solved the X-ray structure of a CB4-1 Fab in complex with one of those intermediates [3]. The conformation of the peptide backbone remains obviously constant during several steps of amino acid exchanges towards the unrelated peptide but switches suddenly to that of the unrelated peptide after accumulation of sufficiently deviating side chain interactions.
Now we are going to investigate the structure of more complexes of CB4-1 Fab fragments with hybrid peptides to trace the conformational prerequisites for this polyspecific binding behavior.
At the X13 beamline we measured several crystals of those Fab/peptide complexes which diffract usually to 2.6 or 2.8 A resolution. They belong to the space group $P6_122$ with unit cell constants a = b = 101.8; c = 295.2; with $\alpha = \beta = 90°$, $\gamma = 120°$.
The data sets were complete to 99% in the highest resolution shell.

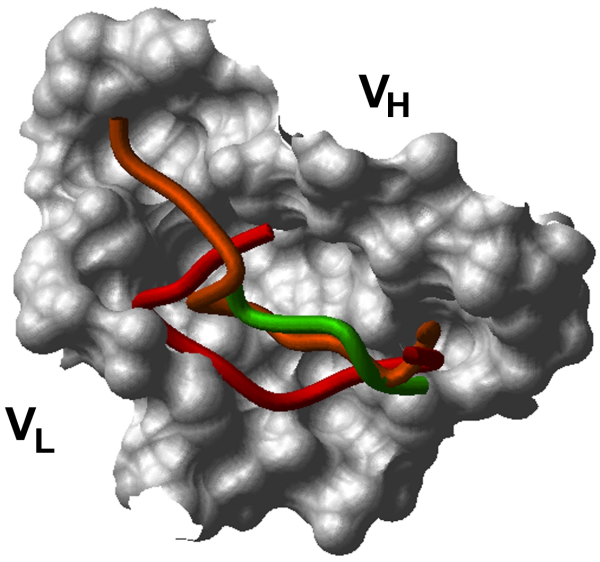

Fig.2. Comparison of the backbone conformation of the epitope peptide and two non-epitope homologous peptides (taken from [3])

References

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