Semisynthetic antibody-antigen complexes

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General discussion and summary

The subject of this thesis is size reduction of antibody and antigen molecules to small peptide molecules. In case of synthetic antigen-binding antibody fragments (synthetic mini-antibodies) these peptides should be able to mimic the antibody molecule in specific antigen-binding capacity, and in case of synthetic linear epitopes these peptides should be able to mimic the antigen molecule in antibody-recognition capacity. The demand on synthetic antibody fragments to maintain antigen-binding capacity is much greater than the demand on synthetic epitopes to maintain antibody-recognition capacity, because not all amino acid residues of the antibody involved in antigen binding are available for interaction with antigens. This in contrast to the interaction of antibodies with synthetic linear epitopes.

Two approaches were used in the design of synthetic antibody fragments, which are able to bind protein antigen molecules. The first approach was based on three-dimensional structure information of the antibody-antigen complex of antibody D1.3 and the protein antigen lysozyme. Surface Plasmon Resonance (SPR) measurements showed that none of the tested peptides was able to bind lysozyme in the same manner as monoclonal antibody D1.3. However, a 36-amino acid residue peptide which contained two Complementarity Determining Regions (CDRs) of the heavy chain of D1.3 was able to retard lysozyme in an immunoaffinity chromatography study. This points to a weak interaction of this peptide with lysozyme.

The second approach was a general procedure to develop synthetic antibody fragments without prior knowledge about the three-dimensional structure of the antibody-antigen complex. In this approach, all six CDRs were synthesized as peptides and were tested for binding the antigen. The target antigen was glycoprotein D of herpes simplex virus type 1. CDR-derived peptides of monoclonal antibody A16 were synthesized and tested for binding glycoprotein D by SPR measurements and by an immunoassay (ELISA). Peptides containing two CDRs of the heavy chain of antibody A16 and antibody Fd138-80 were also tested for binding glycoprotein D. It was not possible to synthesize CDR-derived peptides which could bind the antigen glycoprotein D.

These results strongly suggest, that there is no general way to obtain synthetic mini-antibodies. Possible explanations for this are that the conformation of a CDR-derived peptide is different from that in the antibody, or that the affinity of a CDR-derived peptide is too low for detectable binding, or that antigen-binding is achieved by cooperative binding of several CDRs. So far, the Fv fragment of an antibody molecule is the shortest possible antigen-binding antibody fragment[2], because it possesses all six CDRs. In exceptional cases[3,5-7] it may be possible to obtain smaller antibody fragments that are able to mimic the antibody-binding site.

The interaction between glycoprotein D of herpes simplex virus type 1 with MAb A16 was also used in the study of the size reduction of an antigen molecule. A previously identified linear epitope of glycoprotein D[8] was shortened to its minimal size, i.e. gD 11-17. SPR measurements showed that the binding constant of this peptide with MAb A16 is similar to that of glycoprotein D. The importance of the residues Asp-13, Arg-16 and Phe-17 for antibody binding was shown by screening a phage display library with MAb A16 in a previous study[4]. A 15-amino acid residue mimotope peptide was identified, which has residues Asp-13, Arg-16 and Phe-17 in common with glycoprotein D. The contribution of these residues to antibody binding was determined in a kinetic analysis with an SPR biosensor. Kinetic parameters were determined of the interaction of MAb A16 with gD 9-19 epitope peptides and gD 9-19 mimotope peptides. It appeared that only one residue, i.e. Arg-16 is critical for binding and that two residues, i.e. Asp-13 and Phe-17 contribute to the secondary structure (probably a β-turn) of the gD 9-19 peptide. These two residues are contact residues as defined by Getzoff *et al.*[1] The three residues mainly determine the strength of interaction between gD and A16, because equal binding constants for gD 9-19 epitope and gD 9-19 mimotope with MAb A16 were found.

In the reverse system, the interaction of a synthetic CDR-derived peptide with an antigen, no specific binding could be achieved. A possible explanation for this difference is that in case of synthetic mini-antibodies the large surface complementarity between antibody and antigen is lost, while surface complementarity is maintained in the interaction of synthetic epitope peptide with an antibody. This explanation is also an important theoretical consideration for the difficulties that can be encountered in the design and development of synthetic mini-antibodies.

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