Fresenius J Anal Chem (1990) 337:13 – © Springer-Verlag 1990

Recombinant antibodies produced in *E. coli*: Prospects for catalysis

Andreas Plückthun

Genzentrum der Universität München,
Max-Planck-Institut für Biochemie,
D-8033 Martinsried, Federal Republic of Germany

Today, monoclonal antibodies can be produced against almost any chemical structure. The advent of gene technology makes it now possible to go one step further: To produce antibodies not present in the natural pool or to directly obtain smaller fragments of the antibody. While convenient techniques to alter DNA sequences had been firmly established, the production of the antibody protein from the altered genes was not.

Some time ago, we solved this problem by developing an expression system with which fully functional antibody F_v or F_{ab} fragments can be expressed in *E. coli* [1]. Both chains are co-expressed and co-secreted into the periplasm of *E. coli* with correct signal-processing, disulfide formation, and chain association. The F_v and F_{ab} fragments can be purified to homogeneity in a single step by hapten affinity chromatography. The binding constant of the hapten to the F_v fragment was found to be identical to that of the whole antibody.

As a model system, we used the particularly well studied phosphorylcholine binding antibody McPC603. Most importantly, the crystal structure of its F_{ab} fragment with [2] and without [3] bound hapten is known. The genes encoding the variable domains (V_H and V_L) had been obtained by DNA synthesis. In addition, we had constructed genes encoding the variable and the appropriate constant domains of each chain in order to directly express the F_{ab} fragment [4].

The expression system allows an easy access to both F_{ab} and F_v fragments. F_v fragments are very difficult to prepare proteolytically and had, before the availability of this expression system, not been characterized in detail. Since they constitute the smallest antigen binding fragment and since they are promising models for structural studies as well as in cancer diagnostics and therapy, it is essential to have a complete understanding of their physical properties. The association constant between the V_H and V_L domains was determined by crosslinking and fluorescence experiments. We found that V_L dimerizes with itself with an association constant similar to that of the heterodimer, but V_H does not. The binding of the hapten favors the association to the correct F_v fragment and stabilizes the F_v fragment at low concentrations. The dissociation of the two chains also limits their stability at physiological temperatures. From the knowledge of the structure, altered proteins were constructed that are up to 60-fold more stable against irreversible denaturation. These stable variants are covalently crosslinked by an engineered intermolecular disulfide-bond, by a connecting peptide linker or by glutaraldehyde [5]. Such small and stable fragments with full binding activity might extend the range of applications of antibodies in biotechnology and medicine.

We have recently solved the crystal structure of this recombinant V_L dimer [6] and are pursuing the structure determination of the recombinant F_v fragment.

The F_v and F_{ab} fragments of this antibody are very convenient model systems for quantitatively investigating binding interactions by systematic modification of the antigen binding site and the hapten. We have now characterized the binding affinities of a range of mutants. This constitutes the foundation of a database against which to check the results from theoretical calculation of binding constants in this system.

One of the essential features of enzyme catalysis is a structural complementarity of the protein to the transition state of the reaction. Antibodies raised against stable analogs of the transition state have previously been shown to have catalytic function in certain instances. We have now shown that the recombinant F_v fragment of McPC603 possesses catalytic activity toward the hydrolysis of a carbonate ester. The kinetic rate constants were determined. This made it possible to carry out a systematic investigation of this catalysis by site directed mutagenesis, binding studies, kinetics and crystallography to get further insight into the structural requirements of an efficient catalytic antibody.

References

1. Skerra A, Plückthun A (1988) Science 240:1038
2. Segal DM, Padlan EA, Cohen GH, Rudikoff S, Potter M, Davies DR (1974) Proc Natl Acad Sci USA 71:4298
3. Satow Y, Cohen GH, Padlan EA, Davies DR (1986) J Mol Biol 190:593
4. Plückthun A, Glockshuber R, Pfitzinger I, Skerra A, Stadlmüller J (1987) Cold Spring Harbor Quant Biol 52:105
5. Glockshuber R, Malia M, Pfitzinger I, Plückthun A (1990) Biochemistry (in press)
6. Glockshuber R, Steipe B, Huber R, Plückthun A (1990) (submitted)