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Antibodies from *Escherichia coli*

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Use of *Escherichia coli* as an expression host has opened up new possibilities in antibody research and its applications. It greatly facilitates rational engineering and random mutagenesis.

THE use of *E. coli* as an expression host brings the arsenal of bacterial gene technology to antibody molecules. The ease with which *E. coli* can be manipulated greatly facilitates antibody engineering of both the antigen binding pocket and the framework. The efficient transformation and transfection permit random mutagenesis experiments and the construction of libraries. The fast growth and easy fermentation of *E. coli* allow rapid, large-scale production of antibody fragments, which is a prerequisite for structural studies. The bacterial metabolism permits easy labelling of antibody protein with isotopes, and one may also devise metabolic selection schemes for binding and catalysis by an antibody.

The expression system developed by us[1,2] and independently by Better *et al.*[3] combines the advantages of antibody expression in the native state with the use of *E. coli* as the host. Expression in the native state is essential for any kind of screening as the individual refolding of many random mutants *in vitro* would not be feasible. Functional expression is also a prerequisite for the one-step purification process of antigen-affinity chromatography[1,2,4]. Finally, different refolding properties of mutants may obscure areas of interest in studies of structure and function.

Expression system

A strategy was developed that consists of the simultaneous secretion of both chains of an antibody fragment (Fig. 1) into the periplasm of *E. coli*. The two chains can therefore fold in each other's presence, and were found to assemble correctly *in vivo*. The disulphide formation within each variable domain, which was found to be essential for stability[5], can occur in the oxidizing milieu of the periplasm. It was noted that the outer membrane can become leaky, in which case the periplasmic content is lost to the medium[2], independent of the choice of signal sequence[5]. Lysis can be prevented, however, by growing the cells at lower temperatures. The final yield can vary depending on the antibody fragments (Fig. 1) used for expression[1,2,4,5].

Our studies were carried out with the anti-phosphorylcholine antibody McPC603[6,7]. This antibody has been extensively characterized, including the determination of its crystal structure with and without bound antigen[8,9].

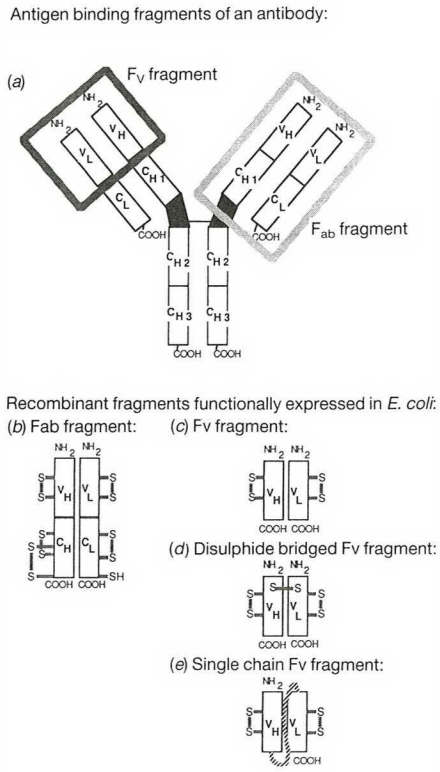

FIG. 1 Arrangement of the various antibody domains in the complete antibody and recombinant fragments functionally expressed in *Escherichia coli*. Note that the disulphide bond structure drawn in the Fab fragment is that of McPC603, a mouse IgA.

Recombinant Ab fragments

Fab fragments (Fig. 1 *a* and *b*) of antibodies, which can easily be prepared by proteolytic cleavage, are well documented as having the same antigen binding affinities as whole antibodies. The same applies to the recombinant Fab fragment of McPC603 from *E. coli*[2,5,10]. Fab fragments are stable and associate well because the constant domains contribute to stability. However, the yield of the functional Fab fragment of McPC603 (ref. 5) is significantly lower than that of the F_v fragment (see below; Fig. 1 *c*) in *E. coli*. An analysis of this phenomenon with several diagnostic framework variants[5] revealed that the problem does not lie in transport or processing, but rather in folding and/or assembly *in vivo*.

The F_v fragment (Fig. 1 *c*) is the smallest conceivable fragment that still contains the complete binding site. F_v fragments are difficult, often impossible, to prepare by proteolysis, and little was known about their properties before they became available through gene technology[1]. Analysis has shown[1,4] that the F_v fragment does have the same binding properties as the Fab fragment or whole antibody. However, F_v fragments have a tendency to dissociate into V_H and V_L upon dilution[4]. The exact equilibrium constants will vary from antibody to antibody, as the hypervariable loops contribute to this interaction. For McPC603, the dissociation constant was found to be about 10^{-6} M (ref. 4). This problem does not interfere with all uses, and the relatively small size of the molecule makes it an attractive target for structural studies by X-ray crystallography and nuclear magnetic resonance. F_v fragments may have important applications in the diagnosis and treatment of tumours, where their small size may facilitate the penetration of dense tumour tissue and provide for low antigenicity.

Several strategies have been developed to overcome the dissociation problem while maintaining the small size[4]. First, the two chains can chemically be cross-linked with glutaraldehyde. With McPC603, this can be accomplished without any loss of binding affinity. Second, an intermolecular disulphide bond can be created (Fig. 1 *d*). It has been shown that the additional disulphide bridge forms spontaneously in the periplasm and the molecule can, therefore, be obtained in fully functional form from *E. coli*[4]. Antigen binding affinity was only marginally affected. Third, the two domains can be connected by a peptide linker (Fig. 1 *e*). Such single chain F_v fragments had been obtained previously as insoluble inclusion bodies that had to be refolded *in vitro*[11,12]. The single chain F_v fragment can also be secreted in functional form with almost unaltered binding affinity[4]. Linking the domains in the secreted single-chain fragment does not significantly influence the yield of functional fragment as compared to the secreted F_v fragment, and it is, therefore, unlikely that the association of V_L and V_H is a kinetic problem in *E. coli*. Consequently, the linking of the two domains is optional, and not essential. It remains to be seen whether the linker may obstruct other antigen binding sites: it does not in McPC603.

The properties of the isolated domains V_H and V_L were also investigated. Again,

their properties will vary depending upon the antibody under study. With McPC603, no binding of V_H to the antigen could be shown. The V_H domain also shows low solubility at temperatures above about 4 °C. Consequently, the use of V_H domains as a general substitute for antibody-combining sites[13] presents some technical challenges. While the solubility problem may be alleviated by engineering the framework, it remains to be shown that satisfactory binding and, most importantly, narrow specificity can be maintained in such modified fragments.

On the other hand, V_L has a tendency to dimerize with itself. The precise dissociation constant again varies from antibody to antibody, but is usually between 10^{-3} M and 10^{-6} M. V_L of McPC603 does not bind the antigen either, but this recombinant domain does give rise to well ordered crystals of the dimer[14]. This offers the exciting prospect of rapidly obtaining a structural database of characteristic complementarity-determining regions.

Libraries

The expression of antibody libraries[13,15] is an important extension of the expression of defined single species of antibodies[14]. The advent of polymerase chain reaction (PCR) technology has provided rapid access to the family of antibody genes as they contain conserved sequences in the 5′ and 3′ portion of variable genes, the framework region and the whole constant region. In one approach, a library of λ phages was generated, recombined from individual PCR products obtained from mRNA for antibody heavy and light chains isolated from an immunized animal[15]. In the recombinant phage, the genes were arranged exactly as in the expression vectors described previously[1-3]. Fab fragments with a desired binding activity can be found by a 'reversed' immunoscreening with labelled antigen, although phage may not be such a good production vehicle.

The real challenge with large antibody libraries, however, is screening. While conventional screening technology may be useful with smaller libraries, for example, from the highly enriched mRNA of an immunized animal, the task of finding a binding species with high affinity and narrow specificity out of the complete spectrum of conceivable antibodies (that is, of a non-immunized animal) is formidable indeed. Therefore, the potential substitution of monoclonal antibody technology by *E. coli* is likely to depend mainly on developing screening and enrichment systems for narrow binding specificities that measure up to the efficient multistep processes that the immune system uses.

Human antibodies are one of the obvious targets for this technology. Such human antibody fragments should significantly reduce the problem of antigenicity in the clinical use of antibodies. Yet, there is another hurdle. If, however, antibodies with high affinity and high selectivity cannot be found with *E. coli* screening, problems with crossreactivity against undesired targets in the body might lead to severe clinical consequences, especially if a low-affinity antibody is given in high doses.

There are, therefore, many challenges ahead in antibody research. □

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