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Crystallization and Preliminary X-ray Diffraction Study of the Bacterially Expressed Fv from the Monoclonal Anti-lysozyme Antibody D1.3 and of its Complex with the Antigen, Lysozyme

Ginette Boulot[1], Jean-Luc Eiselé[1], Graham A. Bentley[1], T. Narayana Bhat[1]
E. Sally Ward[2], Greg Winter[2] and Roberto J. Poljak[1]

[1]*U.R.A. 359 C.N.R.S.*
Département d'Immunologie
Institut Pasteur, 75724 Paris-cedex 15, France

[2]*MRC Laboratory of Molecular Biology*
Hills Road, Cambridge, CB2 2QH, U.K.

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The associated heavy (V_H) and light (V_L) chain variable domains (Fv) of the monoclonal anti-lysozyme antibody D1.3, secreted from *Escherichia coli*, have been crystallized in their antigen-bound and free forms. FvD1.3 gives tetragonal crystals, space group $P4_12_12$ (or $P4_32_12$), with $a = 90{\cdot}6$ Å, $c = 56{\cdot}4$ Å. The FvD1.3–lysozyme complex crystallizes in space group $C2$, with $a = 129{\cdot}2$ Å, $b = 60{\cdot}8$ Å, $c = 56{\cdot}9$ Å and $\beta = 119{\cdot}3°$. The crystals contain one molecule of Fv or of the Fv–lysozyme complex in their asymmetric units and diffract X-rays to high resolution, making them suitable for X-ray crystallographic studies.

Monoclonal antibody (mAb†) D1.3 is one of a series of BALB/c anti-hen egg-white lysozyme (HEL) mAbs (Harper *et al.*, 1987), made to study the molecular structure of antigen–antibody interactions by immunochemical and X-ray crystallographic analyses. For this purpose, the antibodies were proteolytically cleaved with papain to obtain Fab fragments that could be crystallized free or bound to the specific antigen, HEL. The structure of one such Fab (D1.3) was solved in complex with HEL (Amit *et al.*, 1986).

Proteolysis of immunoglobulins can lead to poor yields of homogeneous Fab fragments. Furthermore, the constant (C) domains of the Fab fragment may introduce some degree of disorder into the crystals, owing to heterogeneous proteolysis and higher thermal mobility of the carboxy terminus. Additional mobility may also arise at the "elbow" that joins the V and C-domains. Thus, the C-domains of Fab fragments are usually more difficult to trace in electron density maps than the V-domains. Since antigens interact entirely with the antibody V-domains (Amit *et al.*, 1986), the Fv fragment could provide an attractive alternative for structural studies of antigen-binding sites (Skerra & Plückthun, 1988; Riechmann *et al.*, 1988). Indeed, for crystallographic analyses Fv fragments may offer certain advantages over Fab fragments because of their smaller molecular weight.

Although Fv fragments are difficult to obtain by proteolysis of whole antibody molecules (Inbar *et al.*, 1972), they have recently been derived by gene technology. The genes encoding the V_H and V_L domains of antibodies have been expressed, and the domains correctly associated and folded, were secreted from either mammalian (Riechmann *et al.*, 1988) or bacterial (Skerra & Plückthun, 1988; Ward *et al.*, 1989) cells. In future, Fv fragments may also be made by directly tapping the *V*-gene repertoire (Orlandi *et al.*, 1989; Ward *et al.*, 1989; Huse *et al.*, 1989). Fv fragments have great potential in biotechnology and medicine. For example, they may offer a convenient means of targeting drugs and cytotoxic proteins (Chaudhary *et al.*, 1989) in therapeutic treatment.

In view of the potential utility of Fv fragments, we have undertaken to study the X-ray crystallographic structure of the Fv fragment of the D1.3 antibody (FvD1.3) and its complex with HEL. This should allow comparisons with the tertiary and quaternary structures of the corresponding FabD1.3 and its complex with HEL (Amit *et al.*, 1986; Bentley *et al.*, 1990).

In previous work, the FvD1.3 fragment was

† Abbreviations used: mAb, monoclonal antibody; HEL, hen egg-white lysozyme.

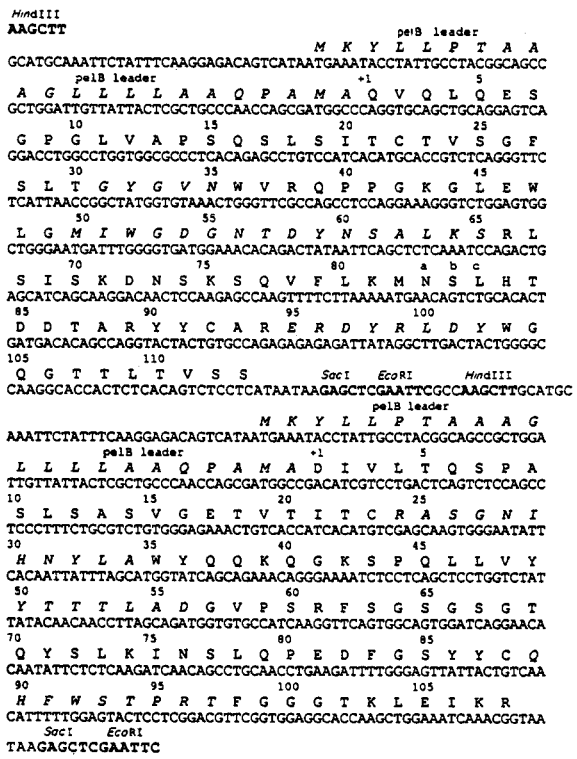

Figure 1. The nucleotide sequence encoding the heavy and light chain variable domains of FvD1.3 for expression and secretion from *E. coli*. To facilitate the assembly of the genes (Ward *et al.*, 1989), *Pst*I and *Pvu*II sites were introduced into the D1.3 V_H and V_L genes in the region encoding the first few amino acids of the mature protein. This changes the amino acid sequence (M. E. Verhoeyen, C. Berek & G. Winter, unpublished results) of both V_H and V_L domains in this region (V_H, Lys5 to Gln; and V_L, Gln3 and Met4 to Val and Leu, respectively). The Fv fragment described by Ward *et al.* (1989) differs slightly from the Fv fragment described here: it has 2 further amino acid substitutions (V_H, Leu109 to Val; V_L, Val3 to Glu) due to the creation of a *Bst*EII site in the V_H gene, and a *Sac*I site in the V_L gene. However, the substitutions do not seem to affect the affinity for lysozyme. In the Figure the upper part corresponds to V_H, the lower to V_L. The amino acid sequences are numbered according to Kabat *et al.* (1987), and the synthetic pelB leader and the complementarity-determining regions (V_H, 31 to 35, 50 to 65 and 95 to 102; V_L, 24 to 34, 50 to 56 and 89 to 97), are shown in italics.

cloned and expressed by secretion into *Escherichia coli* periplasm. The fragment was abundantly expressed (up to 10 mg/l of bacterial culture), and the domains were stably associated and bound the antigen HEL. The dissociation constant was similar to that of the parent antibody, about 3 nM (Ward *et al.*, 1989). The details of the nucleotide sequence of the construct and encoded amino acid sequence of the V_H and V_L domains are given in Figure 1.

The FvD1.3 fragment was harvested from the culture medium of recombinant *E. coli* cells grown in media containing ampicillin, and purified by affinity chromatography on a HEL-Sepharose column (Ward *et al.*, 1989). Further purification was achieved by size exclusion chromatography on a ZORBAX fast protein liquid chromatography column. The purified Fv was complexed with HEL by the addition of a small molar excess of the antigen and overnight incubation at 4°C. The uncomplexed and the HEL-complexed Fv were set to crystallize in hanging drops (Wlodawer & Hodgson, 1975) at an initial concentration of about 5 mg/ml.

The free Fv crystallized from 1·9 M-ammonium sulfate solution, 0·1 M-sodium acetate (pH 4·2), the same conditions that were used to crystallize FabD1.3 (Bentley *et al.*, 1990). The crystals grew as bipyramids, reaching a size of up to about 0·3 mm × 0·2 mm × 0·15 mm. Macro-seeding (Thaller *et al.*, 1981) in capillaries was used to obtain crystals which were about twice as large for each of the linear dimensions given above. The crystals were examined by X-ray diffraction using the precession cameras and CuKα radiation. Their space group is tetragonal $P4_12_12$ (or $P4_32_12$), with unit cell dimensions $a = 90{\cdot}6$ Å and $c = 56{\cdot}4$ Å (1 Å = 0·1 nm). They diffract X-rays beyond 2·5 Å resolution. The volume of the unit cell is compatible with the presence of one Fv molecule per asymmetric unit. Electrophoresis in SDS/15% (w/v) polyacrylamide gels (Laemmli, 1970), showed that the crystals contain both the V_H and V_L domains.

The FvD1.3–HEL complex was crystallized by vapor diffusion against 25% (v/v) polyethylene glycol 8000, 0·1 M-potassium phosphate (pH 5·85). The initial crystals measured up to 0·3 mm × 0·10 mm × 0·05 mm. Larger crystals were subsequently obtained by macro-seeding as described above. They were shown to contain V_H and V_L domains and HEL by SDS/polyacrylamide electrophoresis as described above. The space group and unit cell dimensions, determined as for the unliganded Fv fragment, are monoclinic $C2$, with $a = 129{\cdot}2$ Å, b (unique axis) = 60·8 Å, $c = 56{\cdot}9$ Å and $\beta = 119{\cdot}3°$. The volume of the unit cell is about 75% of the FabNew, which crystallizes in the same $C2$ space group (Avey *et al.*, 1968), indicating that there is one molecule of the Fv-HEL complex in the asymmetric unit. Reflections with spacings smaller than 2·5 Å can be seen in "still" photographs. Thus, these crystals are excellent material for X-ray diffraction studies. They should allow a structural comparison with the free and antigen-complexed FabD1.3 and possibly, higher resolution analysis given the quality of their diffraction patterns.

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