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Crystallization and Preliminary X-ray Studies of the V_L Domain of the Antibody McPC603 Produced in *Escherichia coli*

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The V_L domain, obtained from a recombinant F_V fragment of the antibody McPC603 expressed in *Escherichia coli*, has been crystallized as a dimer from 2 M-$(NH_4)_2SO_4$ (pH 4·0). The crystals are hexagonal, space group $P6_122$. The cell dimensions are $a=b=86{\cdot}48$ Å, $c=76{\cdot}64$ Å, with a V_L monomer as the asymmetric unit. The crystals diffract to 2·0 Å. The structure was solved by Patterson search using the V_L domain of the F_{ab} fragment of McPC603 and the V_L dimer REI.

Recombinant DNA technology has led to the possibility of generating molecules not present in the natural reservoir for research and applications involving antibodies. While the engineering of antibodies with altered binding specificities has become *technically* feasible, real progress in this area is hampered by the lack of predictability of the structure of the antigen binding sites and their binding properties. Antigen binding sites are formed by six hypervariable loops (3 contributed by each of the variable domains of the light chain (V_L) and of the heavy chain (V_H)) (for a review, see Alzari *et al.*, 1988). The conformation of these loops is a central issue in immunology.

To address some of the structural questions experimentally, we have recently developed an expression system with which fully functional F_V fragments (heterodimers of V_H and V_L: Skerra & Plückthun, 1988) and F_{ab} fragments (Plückthun & Skerra, 1989) can be obtained from *Escherichia coli*. As a model system, we used the well-characterized phosphorylcholine binding antibody McPC603 (Perlmutter *et al.*, 1983), an IgA from mouse. The crystal structure of its F_{ab} fragment with and without bound phosphorylcholine had been determined (Segal *et al.*, 1974; Satow *et al.*, 1986). The F_V or F_{ab} fragment can be purified from *E. coli* with a hapten affinity column in a single step. We have demonstrated that the F_V fragment isolated from *E. coli* had correctly processed chains, correctly formed disulfide bonds in each chain, and a hapten binding constant identical to that of the whole antibody (Skerra & Plückthun, 1988; Plückthun *et al.*, 1988, 1989; Glockshuber *et al.*, 1990). This system permits the rapid production of modified F_V fragments.

We have now separated the two chains, V_H and V_L, using anion-exchange chromatography (Fig. 1(a)). To achieve this separation, the affinity-purified F_V fragment was concentrated by ultrafiltration and denatured by dialysis against 8 M-urea, 25 mM-Mops (pH 7·0). It was then applied to a CM-Sepharose CL-6B column equilibrated with the same urea-containing buffer. The column was eluted with a gradient of 0 to 0·3 M-NaCl in the same buffer.

SDS/polyacrylamide gel electrophoresis (Fig. 1(b)) showed that the two chains had been completely separated and that there was no cross-contamination under these conditions. The fractions containing the separated chains were then renatured by dialysis against 150 mM-NaCl, 50 mM-Tris · HCl (pH 8·0) and concentrated by ultrafiltration.

The structure of the V_L domain was determined crystallographically. Crystallization was achieved with 2 M-$(NH_4)_2SO_4$ at pH 4·0 at a protein concentration of 16 mg/ml using the hanging drop method. A droplet of 5 μl of protein solution was mixed with 1·7 μl of buffer and equilibrated with the reservoir at room temperature. Within five days, crystals in the

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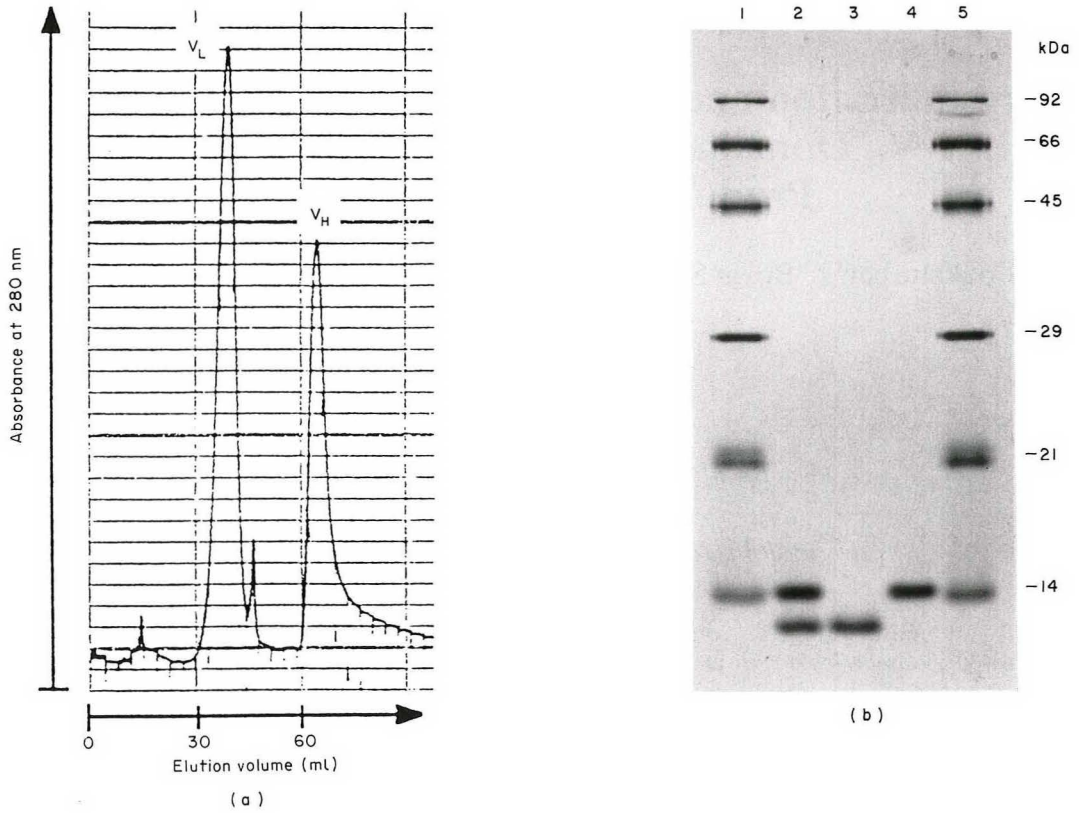

Figure 1. (a) Separation of V_H and V_L on CM-Sepharose CL-6B. Affinity-purified recombinant F_V fragment was obtained essentially as described (Skerra & Plückthun, 1988 and unpublished results) and was used as the starting material. (b) SDS/14% polyacrylamide electrophoresis gel stained with Coomassie brilliant blue. Lanes 1 and 5, molecular weight markers; lane 2, affinity-purified F_V fragment; lane 3, purified V_L; lane 4, purified V_H.

form of hexagonal prisms with pyrimidal caps grew up to 0·5 mm.

X-ray precession photographs showed hexagonal symmetry, space group $P6_122$ with unit cell dimensions of $a=b=86{\cdot}48$ Å, $c=75{\cdot}64$ Å (1 Å=0·1 nm) and $\gamma=120°$. A complete dataset to about 2·0 Å resolution was recorded on a FAST diffractometer (Enraf Nonius, Delft) using the MADNES software (Messerschmidt & Pflugrath, 1987) with one crystal. A total of 118,460 measurements were made and corrected for relative scale, temperature factors and absorption (Huber & Kopfmann, 1969; Steigemann, 1974; Messerschmidt *et al.*, 1990), giving an R_{merge} value of 0·092 (defined as $R_{merge}=(\Sigma I-<I>)/\Sigma I$) and an R_{merge} value of averaged Friedel pairs of 0·027. The crystal symmetry and calculated packing density indicate a monomer as the asymmetric unit, implying that the molecular dyad is crystallographic. The crystals are nearly isomorphous to the V_L dimer AU (Fehlhammer *et al.*, 1975).

The crystal structure was solved by Patterson search methods (Huber, 1969; Crowther & Blow, 1967) (Fig. 2). The V_L dimer REI and the V_L monomer taken from the McPC603 F_{ab} fragment were used as a trial model giving the same solutions. The orientation search in direct space was performed with 5140 peaks within resolution limits of 6·0 to 2·5 Å of the model and experimental Patterson maps. The highest (and correct) peak was 12·5 (arbitrary units) compared to 12·0 for the second. This orientation places the molecular dyad of the REI model along the crystallographic x-axis as required by the space group. Translation was determined in all 11 Harker sections. A consistent solution, which also established the correct enantiomorphic space group, had the highest peak in five calculations and was among the ten highest in others. The structure clearly resembles that of other V_L dimers. Crystallographic refinement is in progress. The crystallographic R value at 2·0 Å is presently 0·21.

V_L domains have been purified previously as so-called Bence–Jones proteins from the urine of multiple-myeloma patients. All previous structures (ROY, Colman *et al.*, 1977; AU, Fehlhammer *et al.*, 1975; WAT, Stevens *et al.*, 1981; REI, Epp *et al.*, 1975; RHE, Furey *et al.*, 1983) were determined from proteins of human patient origin and the present experiments constitute the first crystal structure determination of a recombinant antibody domain.

This well-characterized model system should permit us to rapidly determine experimental structures of genetically modified antibody variable domains and thus to contribute to the understanding of antibody structure and protein loop

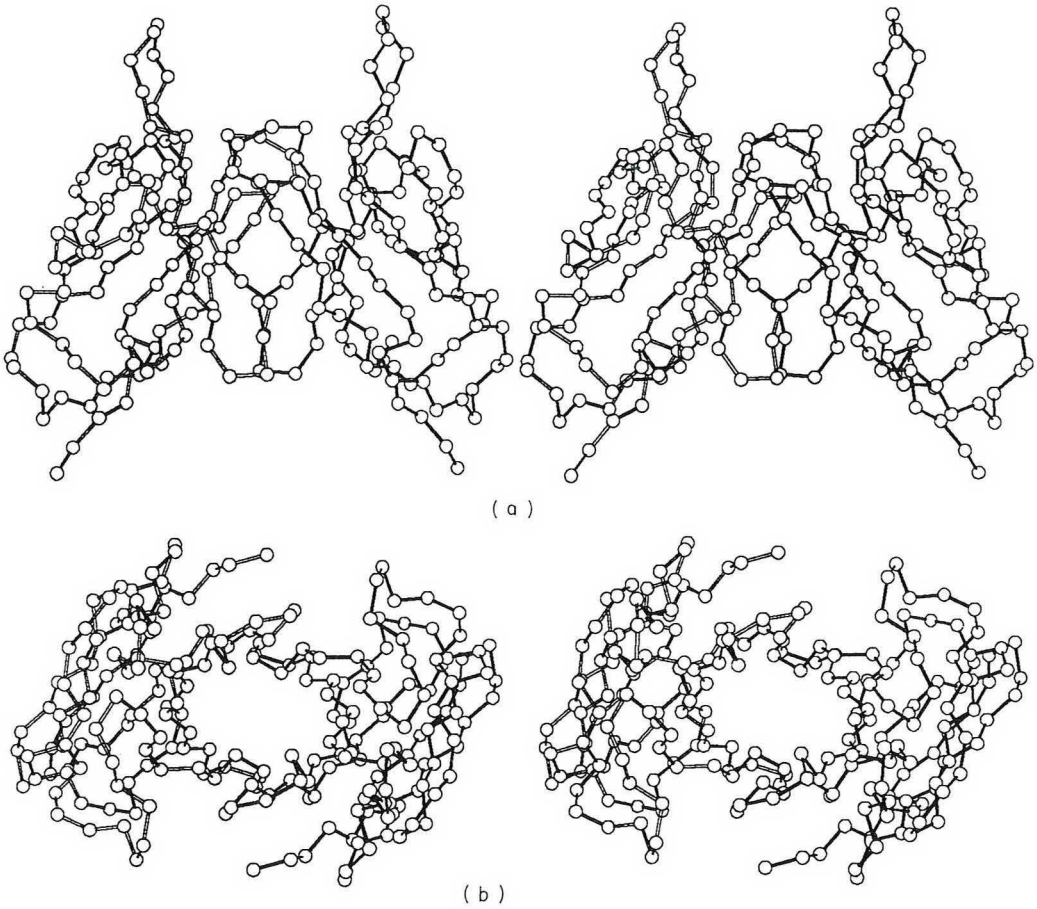

Figure 2. Stereo drawing of the α-carbon backbone of the recombinant V_L domain of McPC603. (a) View perpendicular to the 2-fold axis; (b) view along the 2-fold axis.

folding in general. Since the crystal packing does not involve the hypervariable loops, we expect isomorphous crystals for loop variants.

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