Crystallization of the Fab fragment of the tumour-specific antibody PR1A3

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Abstract

PR1A3 antibody binds specifically to the tumour-associated cell-surface antigen, carcinoembryonic antigen. Crystals of the Fab fragment of the PR1A3 antibody were obtained by vapour diffusion against mother liquor containing Tris–HCl buffer, pH 8.6, magnesium chloride and polyethylene glycol 4000 as precipitating agent. Crystals belong to the monoclinic space group $P2_1$ with cell dimensions $a = 42.2$, $b = 216.7$, $c = 45.9$ Å and $\beta = 95.6°$. Two Fab fragments are present in the asymmetric unit. Diffracted intensities up to 2.9 Å resolution have been measured from frozen crystals.

1. Introduction

PR1A3 is a monoclonal antibody with specificity for colorectal cancer (Richman & Bodmer, 1987). The antibody binds to carcinoembryonic antigen (CEA), but only when this antigen is expressed on cells and not when it is released from tumours into the circulation (Durbin *et al.*, 1994). As a consequence this antibody does not bind to draining nodes in the absence of metastatic spread of the tumour (Granowska, Jass, Britton & Northover, 1989), a problem which can be experienced with conventional anti-CEA antibodies which bind CEA released from the tumour and sequestered by the lymph nodes (Kubo *et al.*, 1992). Moreover, PR1A3 is highly specific for CEA and does not react with other related molecules of the CEA family such as human non-specific crossreacting antigen (NCA) and human biliary glycoprotein (BGP) (Durbin *et al.*, 1994).

The antigen CEA is composed of seven domains attached to the cell-surface membrane through a glycosyl-phosphatidyl-inositol (GPI) linker (Thomson & Zimmermann, 1988). Sequence analysis has shown that each of the seven domains are immunoglobulin folds of class, C2, and are therefore members of the immunoglobulin super family (Williams, 1987). Analysis of chimeric proteins, made from CEA and BGP (Durbin *et al.*, 1994) revealed that PR1A3 binds to the extreme C-terminal domain of CEA the domain next to the GPI anchor. A series of mutations to this last domain refined the location of the epitope to lie on one face of the Ig fold, the CFG β-sheets. The β-strands are labelled by the letters *A–G*. In a C2-type Ig fold, the *D* strand is missing thus the two β-sheets consist of strands *A*, *B*, *E* and *C*, *F*, *G* and are often termed the *ABE* and *CFG* faces of the Ig fold. face (Stewart *et al.*, 1997). Further, three-dimensional atomic models (Stewart *et al.*, 1997) show that the GPI linker is not actually part of the epitope, and is, therefore, not contributing to the affinity of the antibody, but does play a role in the presentation of the epitope. The models of the seven Ig domains of CEA (Bates, Luo & Sternberg, 1992) and of the GPI linker and antibody (Stewart *et al.*, 1997) indicate that the antibody can bind the C-terminal Ig fold, centred on the *CFG* face, without interacting with the GPI linker, but that when the GPI linker is naturally cleaved by phospholipase the residual carbohydrates remaining from the linker (Ferguson, 1991) can freely rotate and block the epitope.

Although molecular modelling in conjunction with experimental results have been able to define the location of this important epitope and rationalize the binding, in order to progress with the antibody in terms of improving its affinity for the antigen or the design of other therapeutic/diagnostic agents based on the antibody structure, highly detailed models, and, therefore, X-ray crystal structures, are needed. To this end we report the crystallization of the PR1A3 antibody. Work is in progress to obtain co-crystals of both the antibody and part of the CEA antigen.

2. Materials and methods

2.1. *Preparation of PR1A3 Fab fragments*

PR1A3 at 7.35 mg ml^{-1} in 100 mM sodium acetate buffer pH 6.5, 3 mM EDTA, 50 mM cysteine was incubated with 220 µg ml^{-1} of papain at 210 K for 4 h. Fab fragments were purified first by gel filtration on Superose 6 in PBS, and then after dialysis into 20 mM Tris pH 8.5 by Mono Q ion-exchange chromatography. The Mono Q fractions were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and the Fab's pooled and concentrated to 4.5 mg ml^{-1} in 10 mM Tris pH 8.0. The final yield was 31%.

The crystallization trials were conducted using the hanging-drop method and a wide range of conditions were tested using commercial screens, namely Hampton Research I and II (Jancarik & Kim, 1991; Cudney, Patel, Weisgraber, Newhouse & McPherson, 1994). The initial screens gave tiny aggregated crystals (conditions No. 6 H I). In subsequent trials we tried to improve the quality of crystals by varying molecular weight polyethylene glycols (PEG's), various buffers with the pH range 7.0–9.5 and divalent metals. The best crystals appeared to grow in the drops equilibrated against the solution of 23% PEG 4000 as a precipitant and 0.1 M Tris–HCl buffer, pH 8.6, and 0.2 M MgCl$_2$. The original crystals were too small for data collection, but larger crystals were obtained by transfer of growing crystals to new drops with fresh protein (macroseeding). For routine growth of PR1A3's Fab fragment crystals the protein was concentrated to 10 mg ml^{-1} and the drops were prepared by mixing 2 µl of the protein solution with 2 µl of the well solution. The crystals appeared after 4–5 d. For macroseeding the well contained the same buffer and salt but only 19% PEG 4000. The drop was prepared as the original one and was equilibrated with the well solution for 1–2 d prior to transfer of the seed. A single crystal was then washed thoroughly in the well solution and transferred to the drop. After 6–7 d, when the crystal stopped growing larger, it was transferred to a fresh drop. After repeating the process three to four times plates of

dimensions $0.5 \times 0.3 \times 0.05$ mm were obtained. Further macro-seeding resulted in twinned crystals. These crystals belong to the monoclinic $P2_1$ space group with the cell dimensions $a = 42.2$, $b = 216.7$, $c = 45.9$ Å, and $\beta = 95.6°$. Assuming two molecules per asymmetric unit of $M_r = 48.2 \times 10^3$ each, the volume per unit molecular weight $V_m = 2.17$ Å^3 Da^{-1} of protein corresponds to a solvent content of 43% (Matthews, 1968).

3. Results and discussion

The monocrystal of dimensions $0.4 \times 0.2 \times 0.03$ mm (Fig. 1) was used for the diffraction measurements on the Rigaku RU-200 rotating anode with Cu $K\alpha$ radiation. Since the number of available crystals was limited, data collection was carried out at 110 K. To enable this the crystal was transferred to a PEG-enriched mother liquor (25% instead original 19%) and then flash-frozen using standard techniques (Teng, 1990) in a stream
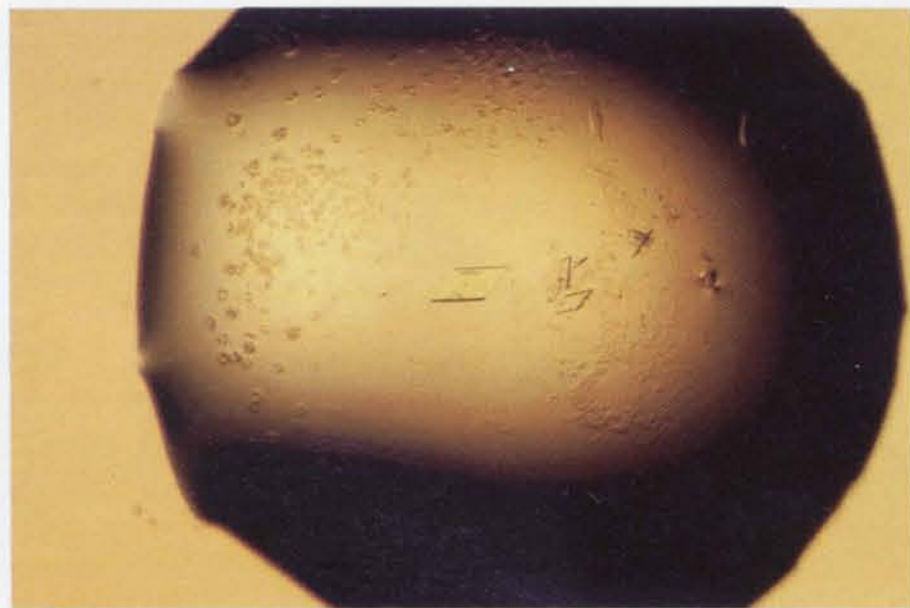

Fig. 1. Crystals of the Fab fragment from the PR1A3 antibody. The largest crystal is of dimensions $0.4 \times 0.2 \times 0.03$ mm.

of nitrogen gas produced by an Oxford Cryosystems Cryostream.

Crystals appeared to diffract highly anisotropically, and only 81.5% of the data were collected up to 3.0 Å resolution and 74.0% up to 2.8 Å. Further collections using other crystals did not improve the completeness.

The data was collected in $1.0°$ oscillation frames over an 180° oscillation range on an R-AXIS IIC image plate. The frames were processed with R-AXIS software resulting in 44 233 observations with $I > 1.0\sigma(I)$, which had been merged into 15 588 independent reflections with an R_{merge} factor of 7.4%.

We are now in the process of determining the structure of the PR1A3 Fab fragment using molecular replacement methods, which should provide insights into the CEA antigen recognition process.

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