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Crystallization of two hCG-specific monoclonal antibody fragments

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Abstract

The Fab fragments of two monoclonal antibodies (Fab3A2, Fab6A) raised against epitopes of human chorionic gonadotrophin (hCG) have been crystallized using the vapour-diffusion technique. The Fab3A2 antibody recognises an epitope on the C-terminal peptide of the β-subunit and the Fab6A a conformational epitope of hCG. Both Fab crystals grow as hexagonal rods from ammonium sulfate solutions. The Fab3A2 crystals belong to space group $P3_121$ with $a = b = 74.84$, $c = 198.2$ Å and diffract to 1.33 Å at the ESRF. The Fab6A crystals are in the space group $P3_221$ with $a = b = 129.53$, $c = 74.40$ Å and diffract to 2.7 Å at the Daresbury SRS. One Fab molecule per asymmetric unit is present in both crystals.

1. Introduction

Human chorionic gonadotropin (hCG) is a member of a family of glycoproteins that show close evolutionary, immunological and biochemical similarities. The family also includes luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyroid-stimulating hormone (TSH). All four hormones have two non-covalently linked glycosylated subunits, α and β (Morgan *et al.*, 1975, for a review see Ryan *et al.*, 1987). Within a given species the shorter α-subunit is encoded by a single gene, is common to the four hormones and is immunologically indistinguishable. The β-subunits are transcribed from different genes (Talmadge *et al.*, 1984), are unique for each hormone and confer specific biological and immunological activity. The β-subunit of hCG has a unique C-terminal peptide containing four O-linked glycosylation sites. Both subunits have two N-linked glycosylation sites.Human chorionic gonadotropin specific antibodies have a number of clinical uses. The hormone is essential for the maintenance of pregnancy and is found in high levels in urine, particularly during the first trimester. Furthermore, increased levels of hCG or its free subunits or fragments of the β-subunit are clinical markers for a number of forms of cancer (Madersbacher *et al.*, 1994; Berger *et al.*, 1993; Marcillac *et al.*, 1992).

The antigenic surface of hCG has been studied extensively (Bidart *et al.*, 1993) and epitopes can be classified (Berger *et al.*, 1990) as being located wholly on the α-subunit, wholly on the β-subunit, or conformational epitopes comprising residues from both subunits.The structure of HF (hydrofluoride) treated hCG has been determined (Lapthorn *et al.*, 1994; Wu *et al.*, 1994). Residues 112–145 of the C-terminal region of the β-subunit are missing from the current model and there is no structure of the complex carbohydrate which is removed with the HF treatment.To study the interactions of hCG with its antibodies and to obtain a more complete and accurate structure of hCG, crystallographic studies of Fab fragments and their complexes with hCG are in progress. We report here the crystallization and characterization of two Fab fragments, one conformational (Fab6A) and one raised against the C-terminal peptide (Fab3A2).

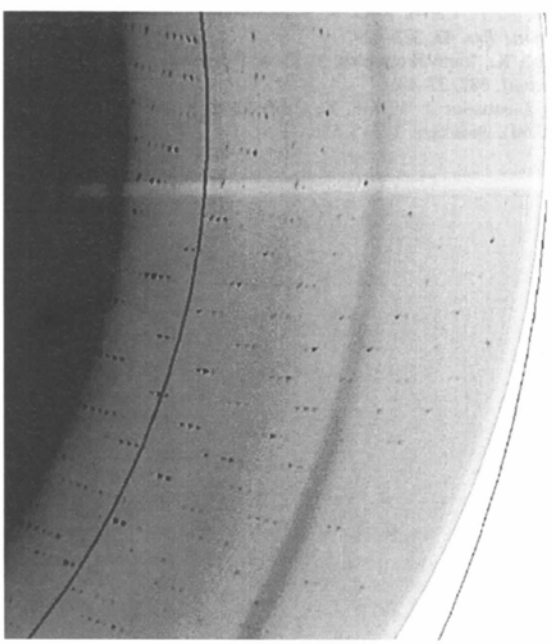

Fig. 1. A section of the Fab3A2 diffraction pattern to 1.33 Å resolution. Outer circle is 1.3 Å, inner circle is 1.7 Å.

2. Experimental

The monoclonal antibodies used in this study (designated 3A2 and 6A) are of the murine IgG1 subclass. MoAb 3A2 is directed against the C-terminal peptide, whereas MoAb 6A is a β conformational antibody. Fab fragments were prepared according to the method of Porter (Porter, 1959) with minor modifications. The antibodies were incubated for 5 h at 310 K in 0.1 *M* sodium phosphate (pH 6.5), 6 m*M* DTT (dithiothreitol) with papain added to 2% of the amount of IgG. The reaction was stopped by the addition of iodoacetamide to a final concentration of 20 m*M* and the reaction mixture was applied to a Protein G column, equilibrated in 0.15 *M* NaCl; 1 m*M* EDTA; 20 m*M* sodium phosphate buffer (pH = 7.0). Fab fragments are selectively bound to the column, whereas the Fc fragments are not retained. The Fab fragments were eluted with 0.1 m*M* glycine–HCl buffer (pH = 2.7) the eluate being neutralized with 1 *M* Tris during fraction collection.

Prior to crystallization the Fabs were purified further using a Superdex-75 gel-filtration column in a buffer of 20 m*M* K_2HPO_4 (pH = 7.0) plus 50 m*M* NaCl. The purified fractions were pooled, concentrated with 10 kDa cutoff Flowgen centrifuge concentrators and the buffer was exchanged to water (HPLC grade).

The purity of the fractions of gel-filtration chromatography was checked with SDS–PAGE sodium dodecyl sulfate polyacrylamide gel electrophoresis. Values of over 98% pure were common. Crystals suitable for X-ray diffraction analysis were grown at 293 K by vapour diffusion in hanging or sitting drops (McPherson, 1982) using ammonium sulfate as the precipitant.

3. Results and discussion

The Fab3A2 and Fab6A samples were concentrated to 10 mg ml^{-1} and crystallization trials were set up. The optimal crystallization conditions found for Fab3A2 were: 2 *M* ammonium sulfate in the reservoir and 1 *M* in the drop (no buffer was used), and for Fab6A: 1.4–1.8 *M* ammonium sulfate plus 0.1 *M* sodium acetate (pH = 4.6) in the reservoir and 0.8 *M* ammonium sulfate plus 0.05 *M* sodium acetate (pH = 4.6) in the drop. Crystals grew in 2 d as hexagonal rods for both Fabs with size 0.2 × 0.2 × 0.6 mm for the Fab3A2 and 0.1 × 0.1 × 0.4 mm for the Fab6A.

The Fab3A2 crystals have unit cell parameters $a = b = 74.84$, $c = 198.2$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ with diffraction extending to 1.33 Å (Fig. 1) under cryocooled conditions at the ESRF, high brilliance beamline. Unfortunately, saturation of the detector and crystal decay due to the high intensity of the beam prevented the collection of high-resolution data. Data were collected at the Daresbury SRS on station 9.5 at 100 K using 20%(*v*/*v*) glycerol/crystallization well solution as cryoprotectant. These data, 99% complete to 2.0 Å with $R_{\rm merge}$ [$R_{\rm merge} = \sum_h \sum_j |I(h) - I(h)_j| / \sum_h \sum_j I(h)_j$, where $I(h)$ is the mean intensity] of 9.1%, were used for structure solution by molecular replacement.The Fab6A crystals have cell dimensions $a = b = 129.53$, $c = 74.40$ Å, $\alpha = \beta = 90$, $\gamma = 120^\circ$ and diffract to 2.7 Å at Daresbury SRS on station 7.2. A data set 99.4% complete to 3.23 Å was collected at Daresbury SRS on station 7.2, at 100 K with a cryoprotectant of 18% glycerol/crystallization well solution. The $R_{\rm merge}$ is 13.5%.The asymmetric unit of both crystals probably contains one Fab with a molecular weight 50 000 Da resulting in an apparent V_m of 3.77 $Å^3$ Da^{-1} and 66% solvent for the Fab3A2, and V_m of 4.16 $Å^3$ Da^{-1} and 70% solvent for the Fab6A (Matthews, 1968).

The structure determination of both Fab3A2 and Fab6A is in progress. The elucidation of their structures, particularly when complexed with hCG, will aid the understanding of the antigen–antibody recognition mechanism.

The high-resolution data might reveal more about the structure and the dynamics of the antigen binding site of the Fabs.

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