A Monoclonal Antibody Fab Fragment Crystallized With and Without a Peptide Epitope From HIV

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ABSTRACT  The Fab fragment of CB 4-1, a monoclonal murine antibody against HIV protein p24, has been produced. It forms a complex with a synthetic antigen, an epitope of p24 made up of 11 amino acids, with the binding constant $K_d = 3.6 \times 10^{-9}$ M. Crystals of hexagonal and orthorhombic space group have been obtained by cocrystallization of the Fab with the epitope and crystallization without the epitope, respectively. In either case, the crystals are suitable for X-ray structural analysis. Crystals of the Fab fragment cocrystallized with the peptide have the space group $P \overline{6},\overline{2}2$ with cell dimensions of $a = b = 105 \text{ Å}, c = 297 \text{ Å}$. Fab crystals without the epitope are in space group $C 222$ with cell dimensions $a = 110.1 \text{ Å}, b = 110.2 \text{ Å}, c = 150.1 \text{ Å}$. © 1993 Wiley-Liss, Inc.

Key words: peptide antigen, high affinity complex, crystallization, preliminary X-ray data

INTRODUCTION

Monoclonal antibodies which bind to epitopes of the human deficiency virus (HIV) are of special interest because of their potential therapeutic and diagnostic use. Murine monoclonal antibodies have been produced against p24 core protein of HIV and shown to bind to recombinant p24 protein as well as to virus lysate. The murine antibody CB 4-1 was selected for further investigations to characterize in detail the interaction of the antibody binding region with its epitope. The amino acid sequences of the variable parts of the heavy and light polypeptide chain of CB 4-1 are known from nucleotide sequencing of the corresponding gene segments. CB 4-1 binds not only to intact recombinant p24 core protein but also to a synthetic 11-residue peptide epitope of p24 with the amino acid sequence: GAT-PQDLNTML with high affinity ($K_d = 3.6 \times 10^{-9}$ M).

In order to provide detailed insight into the interaction between the p24 epitope and the antigen-binding domain of the antibody, X-ray structural analysis of the antibody cocrystallized with and crystallized without the antigen should be performed. The X-ray structure will be used to confirm and adjust the model which was elaborated for the complex.

MATERIALS AND METHODS

The CB 4-1 is secreted by hybridoma cells and has been purified by protein-A sepharose affinity chromatography. The conditions for proteolytic digestion to obtain the Fab fragment are as described in Porter. For the separation from the Fc fragment, a protein-A sepharose column was used followed by a gel filtration step. The presence of Fab in the eluted fractions was monitored using SDS-page electrophoresis. The fractions containing the Fab fragment were pooled and concentrated by ultrafiltration (Amicon membrane YM 10).

For X-ray studies, single crystals were mounted in thin-walled glass capillary tubes and adjusted on a rotation axis of a rotation camera. All X-ray data were collected on an imaging plate scanner using synchrotron radiation at the European Molecular Biology Laboratory at DESY (Deutsches Elektronen Synchrotron). The beamlines X11 and X31 of the storage ring DORIS were used for the experiments of the hexagonal crystals and X31 was used for the orthorhombic crystals. The images were evaluated with the program DENZO (Z. Otwinowski, personal communication).

RESULTS AND DISCUSSION

The vapor diffusion method in hanging drop set-ups (Linbro plates) was used to scan through a variety of crystallization conditions using different precipitants, pH-values, and additives. For the fol-
lowing conditions we obtained crystals suitable for X-ray characterization.

To get crystals of the Fab/peptide complex, the Fab fragment was cocrystallized with 1.2 times excess of the peptide. The experiments were performed at room temperature. All buffers and solutions used were filtered through 0.45 μm filters. The drops containing a Fab concentration of 10 mg/ml in a solution of 0.3 M NaCl and 1.0 M (NH₄)₂SO₄ in 10 mM phosphate buffer at pH 7.2 were equilibrated against reservoirs of 0.3 M NaCl and 2.0 M (NH₄)₂SO₄ in 0.1 M phosphate buffer at pH 7.2.

Hexagonal crystals appeared after 3 weeks and exhibited dipyrimal shape (Fig. 1). The crystals are suitable for X-ray analysis and grow to maximum size of 0.3 × 0.3 × 1 mm. These crystals have the space group P 6,22 with cell dimensions of a = b = 105 Å, c = 297 Å. The diffraction of the crystals shows a relatively high mosaic spread and a resolution limit of about 3.4 Å using synchrotron radiation (Fig. 2).

Fab crystals without the epitope were grown at 0.1 M Tris buffer pH 8.5, 75 mM NaCl, and 15% polyethylene glycol (PEG) using a precipitant and buffer concentration of 50% of the well concentration. X-ray diffraction showed the Fab crystals to be in orthorhombic space group C 222 with cell dimensions a = 110.1 Å, b = 110.2 Å, c = 150.1 Å. The crystals are relatively unstable in the X-ray beam and diffract to at least 3.1 Å resolution. Assuming that the asymmetric unit consists of a single monomer or two molecules with a molecular mass of 55 kDa each, the Vₘ value is 4.1 Å³/Da and 2.05 Å³/Da, respectively. Data of 3.5 Å resolution were received from a single crystal comprising 12,915 reflections. Because of the decay of diffraction caused by radiation damage we could not collect a complete data set. The 8,670 unique reflections (completeness 80.2%) gave a merging R factor on intensities (R_merge = Σ |I – <I>| / Σ |I|) of 12.6% in the resolution range of 10.0 to 3.5 Å.

We plan to collect a complete data set of improved quality to continue the X-ray structural analysis. Because of the sequence homology observed among the murine antibodies and the identity of the observed tertiary structure generally found between structures of antibody Fab fragments it should be possible to solve the structure by molecular replacement.

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