CRYSTALLIZATION AND STRUCTURE DETERMINATION OF THE Fab FRAGMENT OF AN ANTIBODY AGAINST HUMAN RHINOVIRUS

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Since our group decided to start structural studies on proteins by x-ray crystallography in 1986, we have obtained crystals suitable for high resolutio x-ray analysis of two different proteins: catalase HPII from *Escherichia coli*[1] and a Fab fragment of a monoclonal antibody directed against human rhinovirus serotype 2. During the last year (1990), we have completed the resolution of the three dimensional structure for the Fab fragment at 3.0 Å resolution. This is the first protein structure solved by x-ray crystallography in Spain.

Background

Rhinoviruses can be divided into two distinct groups according to their specific receptor binding: the major group, to which at least 78 serotypes (including HRV14) belong, and the minor group, representing at least 10 serotypes (including HRV1A and HRV2). The structures of two serotypes (HRV14, HRV1A) have been determined by x-ray crystallographic studies to about 3-Å resolution.[2,3] Based on the structure of HRV14, a model for the HRV2 structure has been proposed.[4]

In the course of a program aimed to investigate the surface structure of HRV2 and the mechanisms leading to neutralization, monoclonal antibodies against native virions were raised. One of these antibodies (8F5) was found not only to bind to the viral capsid in its native conformation, but also to the viral protein VP2 in Western blots. This property was used to define the region of the binding site by bacterial expression of various deletion mutants of VP2.[5] As this antibody also recognizes synthetic peptides to this region if VP2, the antigenic epitope is most probably contained in the linear sequence and does not require the contribution of residues of other parts of the capsid.[6] In order to investigate the nature of the interaction between the viral capsid and the antibody in more detail, the Fab fragment of monoclonal antibody 8F5 has been crystallized, both in the native form and the complex with a 15-mer oligopeptide containing the antigenic epitope. The crystal structure of the native Fab fragment has been solved by molecular replacement techniques.

Preparation and crystallization of the Fab fragment

The preparation of monoclonal antibody 8F5, obtention, purification, and crystallization of tis Fab fragment have been published.[7] Fab fragments prepared from this antibody by papain digestion were purified to isoelectric homogeneity by ion exchange chromatography and chromatofocusing. The purification yielded four species with isoelectric points in the range of 5.9 to 8.1. The separation of these species was necessary in order to obtain crystals suitable for x-ray analysis.

Crystallization was performed using conventional hanging drops methods of the microvapor diffusion technique.[8] Crystals of the Fab fragment have been obtained using PEG 3350, PEG 6000, and ammonium sulfate as precipitants. The best conditions were found to be 1.8 M ammonium sulfate, 0.1 m potassium phosphate, pH 6.0, at room temperature, with an initial protein concentration of 20 mg/ml. The crystyals are orthorhombic with space group $P2_12_12_1$ and unit cell parameters a = 59.7 Å, b = 86.8 Å, and c = 128.0 Å. There is one Fab molecule per asymmetric unit and the estimated solvent content is around 61%.

Data collection

One set of data was collected at the EMBL outstation (DORIS storage ring at DESY, Hamburg) using synchrotron radiation and the image

plate detector system; 61977 observations yielded 13457 unique reflections. Integration and reduction of the intensities were carried out using the MOSCO program system[9] and the different frames were scaled together giving an overall maerging R_{sym} of 0.071. A resolution cutoff to 3 Å resolution was applied during the processing, and the data set represents 96.8% of the expected number of reflections to this resolution.

Structure determination

The structure was determined by molecular replacement[10] using the program package MERLOT.[11]

Antibody Fab fragments consist of two structural domains, the variable and the constant domains, connected by a flexible hinge or elbow. This elbow angle has been seen to vary from 133° to 180° in known Fab structures.[12] Because of this flexibility between the two domains, it is necessary for the rotation and traslation functions to be calculated separately for each domain.

The molecular models that proved useful in the analysis were the Fv (module containing V_H and V_L, the variable domains of the H and L chains) of antibody R19.9[13] (Protein Data Bank file 1F19), and the C_L/C_H1 (module containing the constant domain of the L chain, and the first constant domain of the H chain) of antibody 17/9 (Rini, J. M. & Wilson, I. A., unpublished results). The fast rotation function[14] was used with 10.0 - 4.0 Å resolution data and a radius of integration of 24 Å. The rotation function of Lattman and Love[15] was used to refine the positions of the peak of each probe. The Crowther-Blow[16] traslation function was then used with 10.0 to 4.0 Å resolutio data and the three Harker secdtions were examined. The orientation and traslation parameters were verified and refined with BRUTE[17] using reflections between 5.0 – 4.0 Å. At this stage, the Fv domain of R19.9 was replaced by the Fv of 17/9 and the model was further subjected to rigid body refinement with XPLOR.[18] During the final cycles of rigid body refinement the variable heavy, variable light, constant heavy, and constant light chains were allowed to move as four separate rigid bodies in order to allow for differences in the imput model in variable heavy to variable light, or constant heavy to constant light pairing. The R factor after rigid body refinement for all data between 10.0 and 3.0 Å resolution was 46.5%.

This model is now being refined using PROLSQ,[19-21] the present R factr is around 0.25.

The domain structure of 8F5 is consistent with all the immunoglobulin cryustal structures published to date. However, the elbow angle of this

molecule is around 127° (Figure 1). This constitutes the closest elbow ever observed in a Fab fragment.[12]

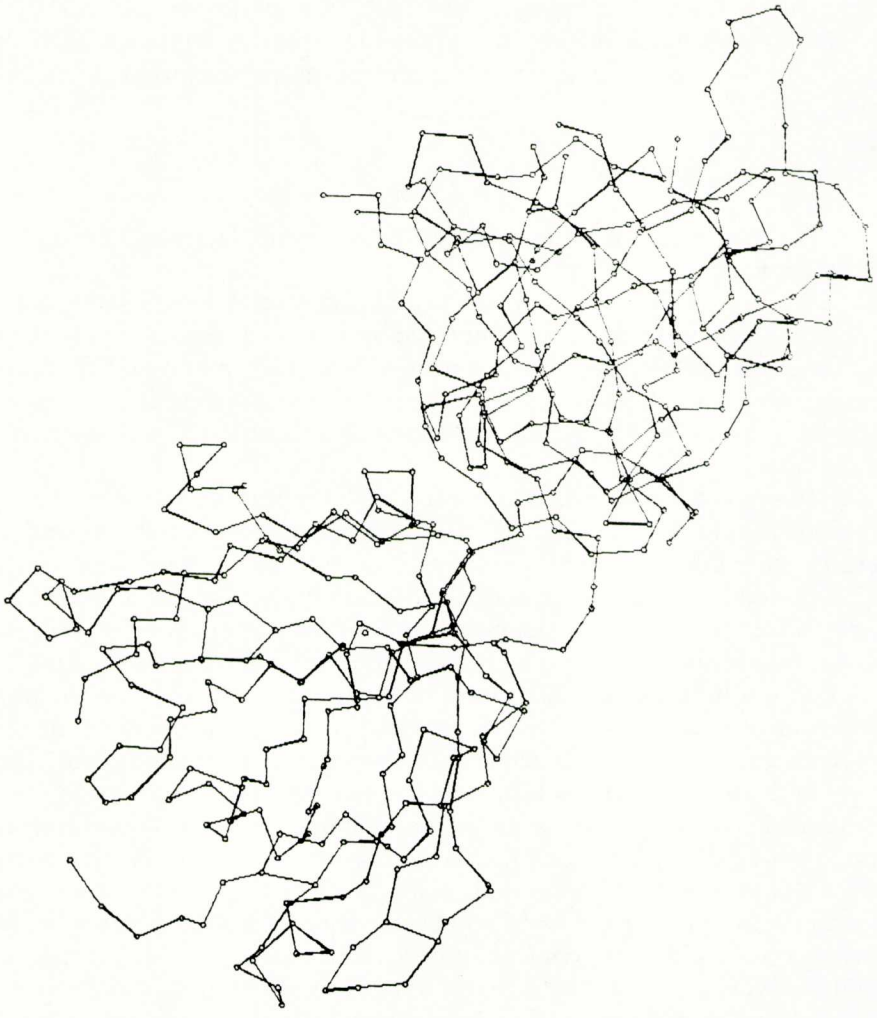

Figura 1. C_α tracing of the Fab fragment of monoclonal antibody 8F5. The closed bend angle between the domains (127°) is clear in this orientation. Some residues of the complementary determining regions (CDR), situated in the variable domain (upper right), are missing in this drawing.

ABSTRACT

Since our group decided to start structural studies on proteins by x-ray crystallography in 1986, we have obtained crystals suitable for high resolutio x-ray analysis of two different proteins: catalase HPII from *Escherichia coli*[1] and a Fab fragment of a monoclonal antibody directed against human rhinovirus serotype 2. During the last year (1990), we have completed the resolution of the three dimensional structure for the Fab fragment at 3.0 Å resolution. This is the first protein structure solved by x-ray crystallography in Spain.

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