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Crystallization and preliminary X-ray analysis of herpes simplex virus neutralizing antibody Fab fragment DL11

JOHN J. RUX,[a] SHARON H. WILLIS,[b,c] ROGER M. BURNETT,[a]* GARY H. COHEN[b,c] AND ROSELYN J. EISENBERG[c,d] *at* [a]*The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104, USA, and* [b]*School of Dental Medicine,* [c]*Center for Oral Health Research and* [d]*School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA. E-mail: burnett@wistb.wistar.upenn.edu*

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Abstract

An Fab fragment of a virus-neutralizing monoclonal antibody (DL11) that binds to herpes simplex virus glycoprotein D (HSV gD) has been purified, sequenced and crystallized. The biological activity of the purified Fab was verified by enzyme-linked immunosorbant assay, flow cytometry and by neutralization of HSV infectivity. The crystals have the space group $P1$ with cell dimensions $a = 40.2$, $b = 49.2$, $c = 63.9$ Å, $\alpha = 76.1$, $\beta = 77.4$, $\gamma = 71.6°$. The unit-cell volume is consistent with it containing a single Fab molecule. The crystals grow to a maximum size of $0.7 \times 0.3 \times 0.3$ mm and diffract X-rays to greater than 2.2 Å resolution. The amino-acid sequences of the variable regions of the heavy and light chains of DL11 have been determined. These have been compared to those for other known Fab structures in the Protein Data Bank for selection of a starting model for crystallographic refinement by the molecular-replacement method.

1. Introduction

Herpes simplex virus (HSV) gives rise to a variety of human diseases including cold sores, eye and genital infections, neonatal infections and encephalitis. Both the oral form of the virus, type 1 (HSV-1), and the genital form, type 2 (HSV-2), establish lifelong latent infections that reactivate periodically to produce recurrent infections (reviewed in, Roizman & Sears, 1987). The HSV capsid is surrounded by a lipid envelope that contains at least 11 glycoproteins which are required for viral attachment and for fusion of the viral envelope with the cell plasma membrane (reviewed in, Spear, 1993). After infection, most of these virally encoded glycoproteins are expressed on the host-cell plasma membrane and provide the principle targets for the host's immune response.

Glycoprotein D (gD) is one of the envelope proteins found in both HSV-1 (gD-1) and HSV-2 (gD-2) and is essential for viral entry into mammalian cells (reviewed in, Eisenberg *et al.*, 1994). Animals immunized with either gD-1 or gD-2 produce cross-reactive virus-neutralizing antibodies that protect them from lethal challenge with both serotypes of HSV and from the establishment of latency (reviewed in, Burke, 1993). This property has motivated the use of gD as a vaccine against HSV and human phase III clinical trials are underway (Straus *et al.*, 1994).

A panel of monoclonal antibodies (MAb's) have been raised against gD (reviewed in, Muggeridge, Roberts, Isola, Cohen & Eisenberg, 1990) to determine which epitopes are involved in virus neutralization, as well as to determine which sites on gD are important for its function. These MAb's have been classified as binding to either continuous or discontinuous epitopes and then further mapped to distinct sites on gD (Muggeridge *et al.*, 1990).

The MAb, DL11, binds to a discontinuous epitope on gD that is conserved between HSV-1 and HSV-2 (Muggeridge *et al.*, 1988). Binding of DL11 neutralizes viral infectivity, presumably due to interference with the function of gD in virus penetration. MAb's and their antigen-binding fragments (Fab's) both have potential as HSV therapeutic agents (Burioni, Williamson, Sanna, Bloom & Burton, 1994; Whitley, 1994). Determining the structures of the native DL11 Fab, native gD and the DL11:gD complex will yield the information necessary to understand the nature of this important antigen–antibody interaction. It will also help to localize the functional domains of gD. Knowledge of the structures of DL11 Fab and gD will also aid drug-design efforts to model compounds that interfere with the attachment to or penetration of HSV into human cells.

A primary objective for this work is to obtain the structural information necessary to design effective therapeutic agents against HSV-1 and HSV-2. The biological properties of DL11 make it an excellent potential therapeutic agent. A necessary step towards this goal is 'humanization' of the mouse antibody to reduce its immunogencity in a human host. This can be achieved either by splicing the entire mouse variable regions into human constant regions to create a chimeric antibody, or by reshaping a human antibody by grafting only the mouse complementarity-determining regions (CDR's) into the human variable regions (Kettleborough, Saldanha, Heath, Morrison & Bendig, 1991).

In this study, we have isolated and crystallized the Fab portion of the MAb DL11. The cDNA coding for the variable regions of the antibody have been sequenced, and X-ray diffraction data have been collected.

2. Preparation of DL11 Fab and confirmation of biological activity

MAb DL11 (Eisenberg *et al.*, 1985) was digested with immobilized papain using the ImmunoPure Fab Kit (Pierce). The reaction was allowed to proceed for 17 h at 310 K with constant agitation. The papain-conjugated beads were removed by centrifugation. The digest was loaded onto a Protein A Sepharose 4 Fastflow column (Pharmacia). The column was washed with 20 m*M* phosphate-buffered saline (0.15 *M* NaCl, pH 7.4), and all of the Fab was contained within the wash. The wash was concentrated and dialyzed against 25 m*M* CHES buffer, pH 9.5 (Sigma). The sample was applied to a Mono Q anion-exchange column (Pharmacia) and eluted by a pH gradient from 25 m*M* CHES, pH 9.5 to 25 m*M* HEPES pH 7.0. Fractions from the major peak were pooled and

examined by isoelectric focusing (IEF) and sodium dodecyl-sulfate polyacrylamide gel electrophoresis. Greater than 90% of the purified Fab migrates as a single isoform and runs as a single silver-stained band on an IEF gel with a p*I* of 8.0. The biological activity of the purified Fab was demonstrated in three different ways: (1) by enzyme-linked immunosorbant assay (ELISA) against a truncated form of gD from HSV-1 [gD-1(306t)] produced using a baculovirus expression system (Sisk *et al.*, 1994); (2) by flow cytometry (FACS) against unfixed HSV-1 infected cells; and (3) by neutralization of HSV infectivity measured by a plaque-reduction assay to a 50% endpoint (Cohen, Ponce de Leon & Nichols, 1972).

3. Sequencing of variable regions of heavy and light chains of DL11

The mRNA coding sequence for the heavy and light chains of DL11 was extracted from 6×10^5 DL11 hybridoma cells, and cDNA was amplified using the appropriate primers ($V_H5'.1$ and $C_\gamma X$ for the heavy chain, and L5 and CK1a for the light chain) (Caton *et al.*, 1991; Kavaler, Caton, Staudt, Schwartz & Gerhard, 1990). The PCR products were gel purified on 0.6% agarose, and the double-stranded DNA encoding the variable region was sequenced (R. King, R. J. Eisenberg & G. H. Cohen, unpublished results) in both directions using the same primers used for amplification (Caton *et al.*, 1991). The deduced amino-acid sequences were combined with the known sequences for the constant regions of the mouse IgG2A heavy and K light chains to yield the complete DL11 sequence, which will be published elsewhere.

4. Crystallization

Large single crystals of DL11 Fab were obtained by the hanging-drop vapor-diffusion method, using 10 µl crystallization droplets (5 µl, 21 mg ml^{-1} DL11 Fab in 50 m*M* NaP_i, pH 6.5 and 5 µl reservoir solution) equilibrated against a 1 ml reservoir solution containing 0.1 *M* sodium cacodylate, pH 7.2, 0.2 *M* $(NH_4)_2SO_4$, 20% PEG 8000 (Fig. 1). Crystals form at 298 K after 1–3 d.

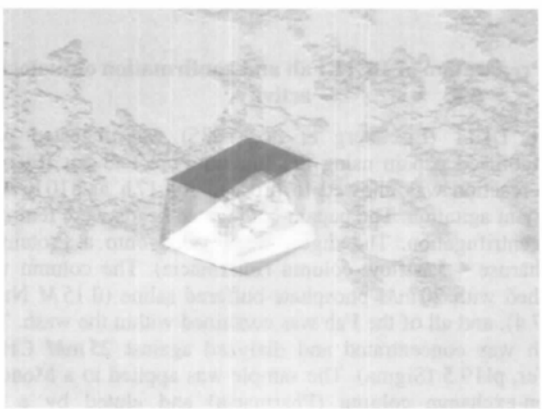

Fig. 1. DL11 Fab crystal, 0.54 × 0.40 × 0.35 mm, grown from 0.1 *M* sodium cacodylate, pH 7.2, 0.2 *M* $(NH_4)_2SO_4$, 20% PEG 8000.

5. Results and discussion

X-ray diffraction data from single crystals of DL11 Fab were collected using a Siemens–Nicolet X100-A multiwire area detector. The Cu $K\alpha$ X-rays were produced by a Rigaku RU200 rotating-anode generator, equipped with a double-mirror focusing system (Supper) and operated at 45 kV and 55 mA. The data were collected with a 15 cm crystal-to-detector distance, a 2θ setting of 15°, an oscillation range of 0.2°, and an exposure time of 4 min per frame. The Harvard software (Blum, Metcalf, Harrison & Wiley, 1987) was used for data collection, and the program *XDS* (Kabsch, 1988) was used for data reduction. The crystals grow as large as 0.7 mm in the longest dimension and diffract to greater than 2.2 Å resolution (Fig. 2). The volume of the unit cell, 115 000 Å^3, is only consistent with it containing a single Fab molecule (~35 500 Da). Combining the diffraction data from four crystals yielded a unique data set that is 89.5% complete to 2.75 Å resolution. The $R_{\rm merge}$, defined as $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$, is 10.7%.

The structure determination is being carried out by molecular replacement using other known IgG2A,K structures (Protein Data Bank, PDB) as starting models. The amino-acid sequences of the DL11 variable regions were compared to the mouse IgG2A Fab variable regions available in the PDB. In several cases, the variable regions show > 70% identity with those of the DL11 Fab. We are starting with Fab 1BBD (Tormo *et al.*, 1992), derived from a neutralizing MAb to human rhinovirus, because it exhibits the highest amino-acid identities of 78 and 75% for the variable regions of the light and heavy chains of the DL11 Fab, respectively.

The three-dimensional DL11 Fab structure will identify the key residues in the hypervariable and framework regions that

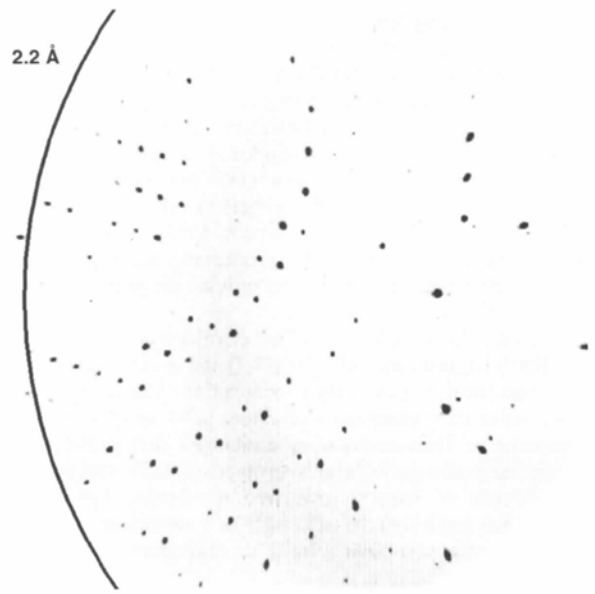

Fig 2. A superposition of 20 consecutive area-detector data frames (0.2° oscillation per frame) collected from a DL11 Fab crystal. The detector is offset from the direct X-ray beam with a swing angle of 15°. The arc on the pattern indicates 2.2 Å resolution. Produced by the program *ELOP* from the *PHASES* program package (Furey & Swaminathan, 1996).

directly effect the conformations of the CDR loops (Chothia *et al.*, 1989). This will make possible the rational design of humanized antibodies. The development of therapeutics based upon neutralizing antibodies, such as DL11, will provide an additional and alternative approach to the prevention and management of HSV infections, and so offer hope for improving the treatment of the disease.

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