CRYSTAL STRUCTURE OF THE COMPLEX FORMED BY A NEUTRALIZING ANTIBODY AGAINST HRV2 AND A VP2 PEPTIDE. BIVALENT DOCKING OF THE ANTIBODY ON THE INTACT VIRUS.

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BACKGROUND

Human rhinoviruses (HRVs), members of the picornavirus familly are small, icosahedral RNA viruses and are the main causative agent of the common cold. The capsid of all picornaviruses is composed of 60 copies of each of four proteins, VP1,VP2,VP3, and VP4 arranged on a T=1 icosahedral surface[2]. The three large capsid proteins VP1-VP3 share a common core structural motif, an eight-stranded β-barrel. The secondary-structural elements of this barrel are connected throughout loops of dissimilar length and structure, that decorate the surface of the virus. Binding sites for neutralizing antibodies are generally located in these hypervariable loops and flank the «canyon» which has been proposed to contain the recognition site for the extracellular receptors of HRVs[3]. Three neutralizing antigenic sites, designated A, B, and C, have been defined for HRV2[4]by analysis of escape mutants and using structural information available for another member of the minor receptor group, HRV1A[1].

The monoclonal antibody 8F5, raised against native virions, not only binds to the viral particle in its native conformation, but also to the viral protein VP2 on Western blots[5]. This property was used to define the region of the binding site by bacterial expression of various deletions mutants of VP2. It was found that

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the binding site lies between residues 153 and 164. This polypeptide segment is located in the region of site B, analogous to the NIm-II antigenic site on HRV14 which appears as a «puff» on the viral particle surface between strands of the eight-stranded β-barrel. As antibody 8F5 also recognizes peptides bearing this sequence[6], an extensive analysis of the recognition site was carried out with a set of overlapping peptides[7]. These experiments defined the minimal binding site as the sequence (AE)TRLNPD corresponding to residues 159-165 of VP2. Furthermore all sequenced mutans that escape neutralization by 8F5 are also localized in this minimal site[4].

In this work we report the 2.5 Å resolution crystal structure of the complex formed by a synthetic peptide and an Fab fragment from the monoclonal antibody 8F5. The 15 amino acids long, synthetic peptide, used in this crystallographic work has the sequence (VKAETRLNPDLQPTE-NH2) that corresponds to residues 156 to 170 of VP2 and includes the minimal binding site of 8F5. The structure of the Fab in the complex is compared to the crystal structure of the uncomplexed 8F5 antibody which had previosly been determined at 2.8 Å resolution in our laboratory (Tormo et al. in press).

Crystallization and structure determination

The complex of Fab 8F5 with the 15 amino-acid oligopeptide (representig residues 156 to 170 of HRV2 VP2) was crystallized by the hanging-drop vapor diffusion method. 7 ml droplets containing 7.0 mg/ml of Fab, 1.1 mg/ml of oligopeptide, 0.45 M sodium citrate, 25 mM NaCl, 50 mM Tris, pH 7.75, were equilibrated against a reservoir containing 1 ml of a solution 0.9 M sodium citrate, equally buffered, at 4°C. The space group was $P2_12_12_1$, with unit cell dimensions of a=71.1 Å, b=75.5 Å, and c=91.4 Å. X-ray diffraction data were collected with a Siemens-Nicolet-Xentronics area detector and reduced with the XENGEN package of programs. Tha data set was 96.7% complete to 2.5 Å resolution, 93.2% reflections with $Fo>2\sigma(Fo)$. The structure was solved by molecular replacement using the MERLOT package[8]. The starting model was taken from the structure of the uncomplexed Fab fragment of 8F5 which was solved at 2.8 Å resolution in our laboratory. The correctly oriented and positioned model was subjected to rigid body refinement with XPLOR[9] and the resulting R factor for data with $Fo>2\sigma(Fo)$ between 8.0 Å and 3.0 Å resolution was 36.5%. At this stage, a 2Fo-Fc electron density map was calculated. This map clearly showed extra density corresponding to the oligopeptide occupying the antigen binding site. This map also showed poor density for some parts of the three CDRs of the heavy chain that were removed from the model and gradually rebuilt during the course of the refinement. After alternative cycles of least-squares refinement with

PROLSQ[10] and manual model building using TOM-FRODO[11], the model refined to an R value of 24.7% for data between 7.0-2.55 Å resolution. A difference electron density map was used to locate the peptide. The high quality of this electron density allowed us to recognize and build the sequence KAETRLNP. These residues correspond well to the minimal binding site for 8F5. The rest of the peptide did not show clear side-chain density and was not build at this stage. After a refinement cycle, most of the electron density for the peptide residues was clearly interpretable and those residues were added to the model. The current R factor for the model inclouding all the peptide residues and ~75 well ordered water molecules is 17.3% for 15581 reflections with $Fo>2\sigma(Fo)$ between 7.0-2.50 Å resolution. The root-men-square deviation for bond lenghts is 0.20 and for bond angles is 2.1°.

Complex structure

The disposition of CDRs in the Fab creates a pocket occupied by the peptide in the complex.The Fab residues in direct contact with the peptide involves the six CDRs and a few framework residues: 12 residues are from the light chain (among which three framework residues) and 13 from the heavy chain (two outside the CDRs). The framework residues Tyr_{255} and Asp_{497} are making hydrogen bonds with the peptide Arg_{2161} side chain. All the peptide residues are directly involved in interactions with the antibody. A diversity of hydrogen bonds (20) between the Fab and the peptide, some of them (5) with an ionic character, indicates that polar interactions are important during the specific recognition in the antibody-antigen complex. The extent of the contacting surface (12) in the amino acid peptides correlates well with epitope mapping results from an earlier inmunological study. Some water molecules have been located and placed in the vicinity of the peptide in the final complex model. A few of these water molecules trapped at the antigen-antibody interface mediate some of the specific interactions.

The peptide in the complex has a compact folded conformation (figure 1). The Cα atoms of residues 2157 and 2168 are only 6.9 Å appart. Several turns are observed in the peptide structure. The folded conformation of the peptide is further stabilized by side chain interactions. Thus Glu_{2159} and Arg_{2161} form two lateral salt bridges while the hydrophobic part of their side chains pack together with Leu_{2162} and Leu_{2166} from the peptide. The side chain of Gln_{2167} loops backwards making well defined hydrogen bonds main chain atoms of residues Ala_{2158} and Arg_{2161}. Pro_{2168} has been built with cis conformation, though the alternative trans configuration cannot be, at present, completely discarded.

Peptide residues, in the minimal binding site, appear to be strongly involved in the specific recognition interactions and show the lower peptide temperature

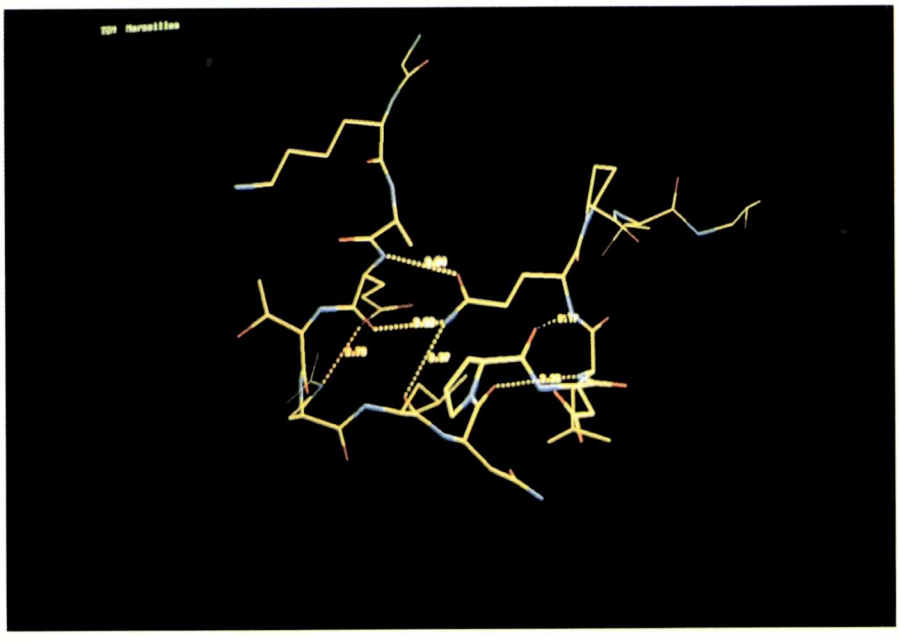

Figure 1. Peptide structure represented with the program TOM-FRODO. Some intrapeptide hydrogen bonds are shown as discontinous lines. Side chains from the N and C terminal residues have been omitted.

B values. These values are similar to some of the best defined residues of the Fab fragment, indicating that the occupancy of the peptide in the crystal is essentially 100% and that the movility of residues most involved in the recognition mechanism is very limited in the complex. In the final (Fo-Fc) omit map there is clearly visible electron density for the 15 peptide residues. However the N and C terminal residues appear to be quite shakies.

The comparison of the Fab fragment structure in the absence and in the presence of the peptide ligand shows three kinds of structural changes:

1) The elbow angle has opened more than 30°. Thus while in the complex the elbow angle observed is 158° in the uncomplexed Fab structure was only 127°. This change likely reflects the flexibility of the antibody arms and does not appear to be directly related to the interactions with the ligand.

2) The relative rotation of the variable domains with respect to each other is around 3.5°. This kind of movement had already been defined as the «interface adaptor».

3) The movement of a few specific residues concentrated in the CDRs from the heavy chain. Both main and side chain atoms in these residues show important

displacements. The movements of residues in the CDRs from the light chain appear to be only slightly above the average movements observed in between the two Fab structures. The heavy chain CDRs movements could be seen as a rigid body rotation around some pivotal points, in such a way, that the conformation of these CDRs loops remains identical in both Fab structures. In all cases the direction of the Fab atomic displacements in the complex are towards the antigen binding pocket.

The last two kind of movements are necessary to accomplish the optimal surface complementarity attained in the complex. Thus the antibody binding surface is defined by both the CDRs conformation and their relative locations.

DOCKING OF THE ANTIBODY ON THE VIRION

Antibody 8F5 was raised against intact virions. Is the peptide structure in the complex related to the conformation adopted in the intact virion, by the corresponding sequence of VP2? The three dimensional structure of HRV2 has not yet been determined. So the atomic coordinates of HRV1A[1], closely related

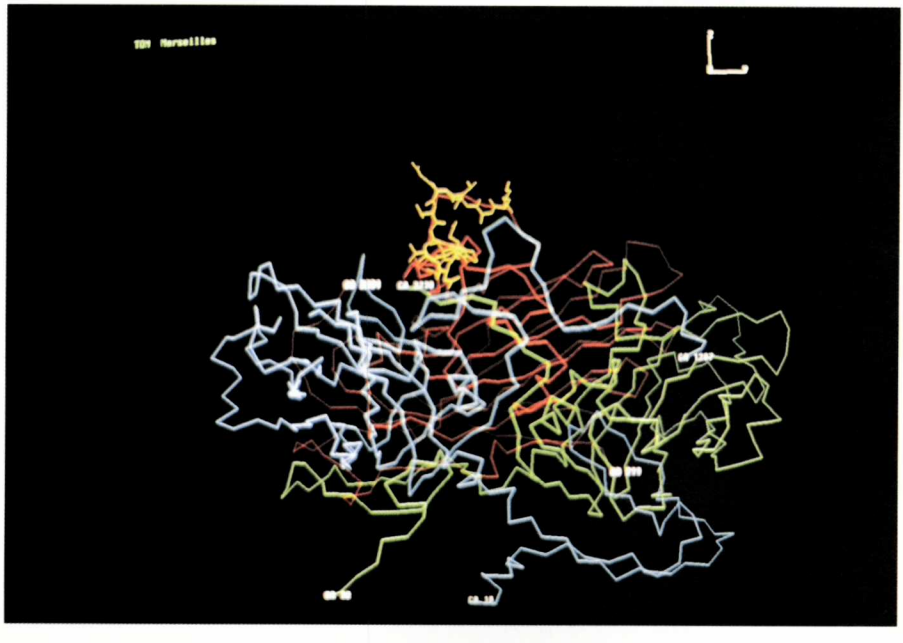

Figure 2. Superposition of the peptide structure (yellow) on the corresponding loop of the viral protein VP2 (red) from the HRV1A structure. Viral proteins VP1 (blue) and VP3 (green) are also shown to indicate the location of the «puff» on VP2.

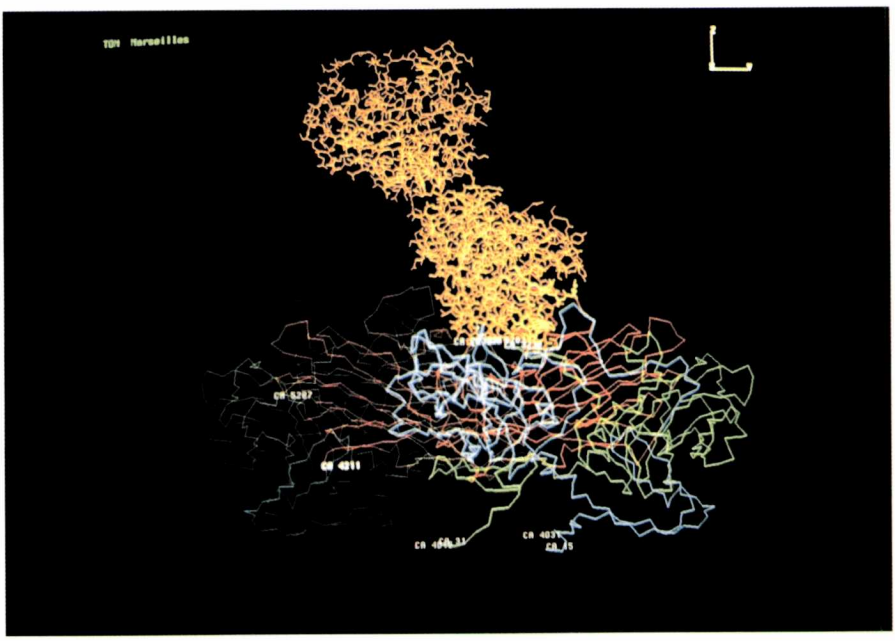

Figure 3. Using the superposition matrix found for the peptide the whole Fab (orange) is placed on the viral capsid proteins. The viral proteins VP1, VP2 and VP3 for both the reference and a two fold related protomers are shown.

to HRV2, were used in the search for structural homologies. The least squares supperposition of C^{α} atoms from residues 2157 until 2164 in the peptide with residues 2159 till 2166 in VP2 gives a root mean square deviation (r.m.s.) of 0.9 A. The corresponding r.m.s. for C^{β} atoms between equivalent residues is 1.4Å. Thus both main chain atoms and side chain orientations , for those residues, are well superimposed in this alignement . With this homology , HRV2 appears to have an insertion of three residues (Pro_{2164}-Asp_{2165}-Leu_{2166}) and a deletion of two residues (Ser_{2157}-Gln_{2158}) with respect to HRV1A. The side chains of Gln_{2167} and Gln_{2166}, whose C^{α} atom is close to the position of C^{α} of Pro_{2164} , could play structurally equivalent roles. The cis conformation assigned to Pro_{2168}, that allows the peptide to attain a compact conformation, does not seem to be related with the conformation adopted in the protein by Pro_{2167} and, as a consequence, the following peptide residues (Thr_{2169} and Glu_{2170}) are progresivelly farther appart from residues Ser_{2168} and Asp_{2169}. An alternative alignement of peptide residues 2157 till 2164 with residues 2157 till 2164, that requires only a single insertion in HRV2 with respect to HRV1A, gives an r.m.s. of 3.5 Å. From the relationship found in between VP2 and the peptide in the complex, docking studies of the Fab fragments and the 8F5 antibody on the viral capside are now in progress (figures 2-4).

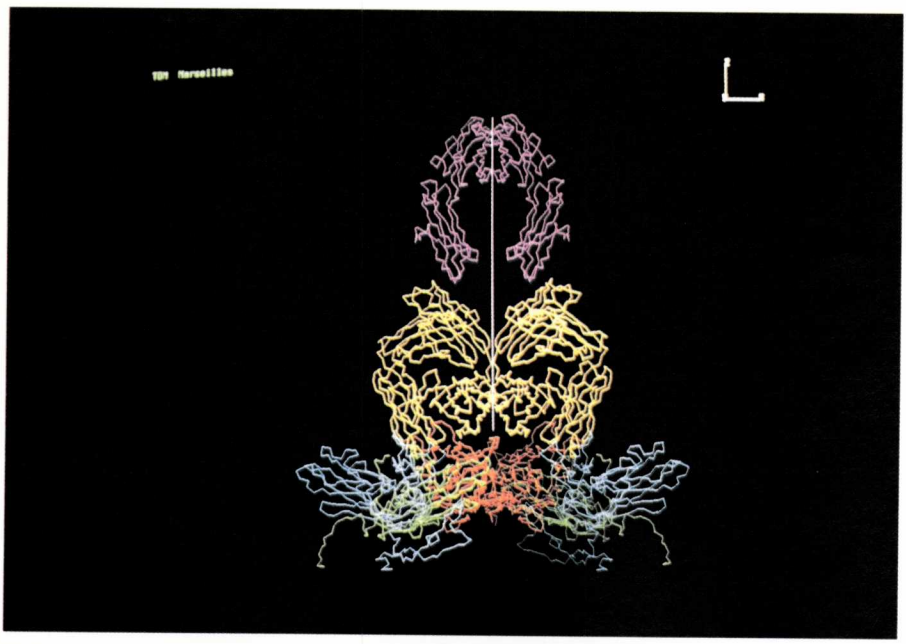

Figure 4. The C^{α} tracing of the two closest Fab structures (yellow) positioned according to the virus symmetry strongly suggest that they can be the arms of the same antibody molecule. The virus and antibody molecular two fold axis (white) can then be coincident. The Fc antibody fragment (purple) is shown only to give an orientative view of the likely docking of a complete antibody molecule .

ACKNOWLEDGEMENTS

We thank D.Campbell and D.Rowlands for providing the peptide used in the complex and S.Sheriff for a modified version of the MS program. This work was supported by the CICYT (Spain). J.T. received a fellowship from the «Ministerio de Educación y Ciencia» (Spain).

ABSTRACT

This work is a preliminary report of the 2.5 A crystal structure of the complex formed by a synthetic peptide and an Fab fragment from a monoclonal antibody (8F5) that neutralizes HRV2. This structure is compared to the crystal structure of the uncomplexed 8F5 antibody which had previosly been determined at 2.8 Å resolution in our laboratory. The peptide shows high structural homology (r.m.s. 0.9 Å) with the corresponding peptide in the HRV1A

structure. This homology is used to analyze the docking of the antibody on the virus capsid. Bivalent attachement with the virus and antibody molecular two fold axis coincident appears to be the most likely arrangement.

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