Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

J. Mol. Biol. (1992) **225**, 1127–1130

Preliminary X-ray Crystallographic Analysis of Intercellular Adhesion Molecule-1

Prasanna R. Kolatkar[1], Marcos A. Oliveira[1], Michael G. Rossmann[1] Arthur H. Robbins[2], Suresh K. Katti[2], Helana Hoover-Litty[3], Carla Forte[3] Jeffrey M. Greve[3], Alan McClelland[3] and Norman H. Olson[1]

[1]*Department of Biological Sciences*
Purdue University, West Lafayette, IN 47907, U.S.A.

[2]*Institute of Chemistry*
Miles Inc., 400 Morgan Lane, West Haven, CT 06516-4175, U.S.A.

[3]*Institute for Molecular Biologicals*
Miles Inc., 400 Morgan Lane, West Haven, CT 06516-4175, U.S.A.

(Received 20 December 1991; accepted 12 February 1992)

Crystals of the two amino-terminal domains of intercellular adhesion molecule-1, the receptor for the major group of human rhinovirus serotypes, diffract to 3·0 Å resolution. The crystals are trigonal in space group $P3_121$ or $P3_221$ with cell dimensions of $a = b = 55{\cdot}7$ Å, $c = 166{\cdot}3$ Å, with probably six molecules per unit cell.

Keywords: ICAM-1; crystals; rhinovirus receptor; electron microscopy; X-ray diffraction

The roughly 100 characterized human rhinoviruses can be divided into two groups according to the cell surface receptor they recognize (Colonno, 1986). The receptor for the major group has been identified as the intercellular adhesion molecule-1 (ICAM-1†) (Greve *et al.*, 1989; Staunton *et al.*, 1989). The major group of rhinoviruses includes human rhinovirus 14 (HRV14), whose three-dimensional atomic structure has been determined (Rossmann *et al.*, 1985), as well as HRV16, whose structure is being determined (M. A. Oliveira, M. G. Rossmann, W. M. Lee & R. R. Rueckert, unpublished results). The minor rhinovirus group includes HRV1A, whose structure is known (Kim *et al.*, 1989). The surface structures of homologous HRV14 and HRV1A differ in their charge distribution, but both show a large surface canyon that is thought to be the site of receptor attachment (Rossmann *et al.*, 1985; Rossmann, 1989). Solvent exposed residues within the canyon are more conserved (Rossmann & Palmenberg, 1988) and would be inaccessible to larger antibodies, thus conserving the receptor attachment site in the face of host immune pressure for change in the antigenic viral surface. Support for this hypothesis comes from site-specific mutagenesis (Colonno *et al.*, 1988) and conformational changes caused by antiviral drugs (Pevear *et al.*, 1989) or pH (Kim *et al.*, 1990) that affect attachment.

The normal physiological function of ICAM-1 is to serve as the ligand of lymphocyte function-associated antigen-1 (LFA-1) (Kishimoto *et al.*, 1989) and Mac-1 (Diamond *et al.*, 1991), which are members of the $\beta 2$ integrin family of receptors. The expression of ICAM-1 is induced by proinflammatory cytokines such as interleukin 1, tumor necrosis factor and interferon γ. Consequently, ICAM-1 is involved in promoting a variety of cell–cell interactions during inflammation. The binding of ICAM-1 with LFA-1 is divalent cation dependent, while the binding of ICAM-1 with HRV is cation independent (Staunton *et al.*, 1990).

Amino acid sequence homology (Simmons *et al.*, 1988; Staunton *et al.*, 1988; Giranda *et al.*, 1990) suggests that ICAM-1 is a member of the immunoglobulin superfamily. The protein has five immunoglobulin-like extracellular domains, but the first amino-terminal domain (D1) provides the major interaction for binding to the major group of rhinoviruses (Marlin *et al.*, 1990; McClelland *et al.*, 1991; Lineberger *et al.*, 1990). There are several hydrophobic as well as electrostatic interactions that are predicted to mediate the virus–receptor interactions (Giranda *et al.*, 1990). Staunton *et al.* (1990) have observed in electron micrographs that ICAM-1 is a hinged rod with a length of approximately 170 Å

† Abbreviations used: ICAM-1, intracellular adhesion molecule-1; HRV, human rhinovirus; LFA-1, lymphocyte function-associated antigen-1; HIV, human immunodeficiency virus.

0022-2836/92/121127-04 $03.00/0

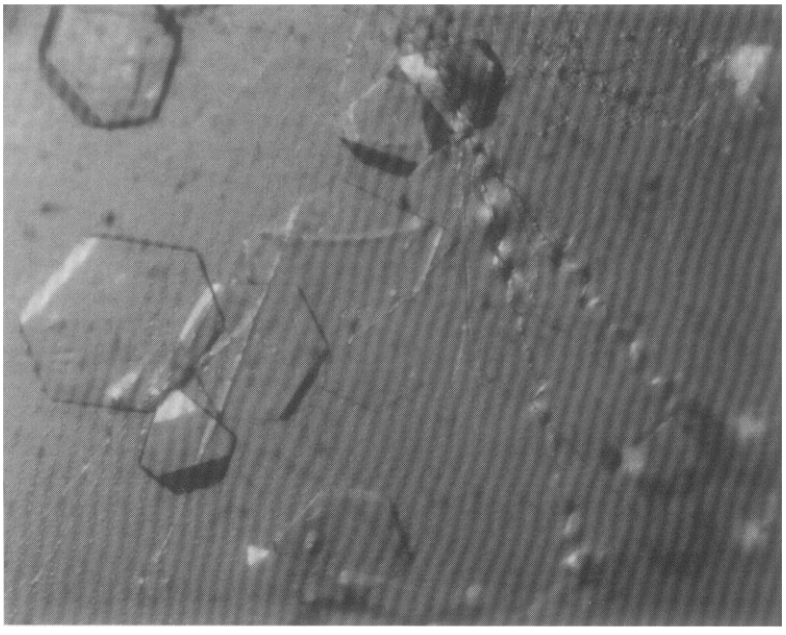

Figure 1. Crystals of the 2 amino-terminal domains of desialated ICAM-1 designated as ntICAM-1(185). Crystal diameter is about 0·4 mm and thickness is about 0·07 mm.

(1 Å = 0·1 nm). The bend might be at the junction of domains D2 and D3. The ICAM-1 molecule has relative molecular mass of 95,000, with 505 amino acid residues and seven to eight N-linked oligosaccharides. However, the amino-terminal domain D1, which contains the virus binding site (Staunton *et al.*, 1990; McClelland *et al.*, 1991), is not glycosylated. A model of this domain, based on its amino acid sequence homology to the constant-type domain of immunoglobulins, has been docked into the HRV14 canyon structure (Giranda *et al.*, 1990).

Rhinoviruses are by no means unique in using an immunoglobulin-like structure as a receptor. For instance, the receptor for polioviruses has three extracellular domains with the amino-terminal domain again providing much of the binding capacity (Mendelsohn *et al.*, 1989; Ren *et al.*, 1990). Human immunodeficiency virus (HIV) uses the cell surface molecule CD4 as a receptor, the structure of whose two amino-terminal domains has been determined (Ryu *et al.*, 1990; Wang *et al.*, 1990). Furthermore, Williams *et al.* (1991) have shown that carcinoembryonic antigen is the receptor for a murine coronavirus.

A secreted form of the first two domains (185 amino acid residues) of human ICAM-1, designated tICAM-1(185), has been purified from culture supernatants of a stable CHO cell line (Greve *et al.*, 1991) to permit structural and functional analysis. This protein contains four N-linked oligosaccharide chains and, upon native isoelectric focusing, runs as a smear at a pI of 4·6 to 4·8. It has been desialated by treatment with neuraminidase (Genzyme, EE. 3.2.1.18; 8 h at 37 °C in 100 mM-sodium acetate (pH 6·5) at 10 mg substrate/ml and 0·1 enzyme unit/ml), and separated from undigested material and enzyme by dialysis against 10 mM-Tris, 25 mM-NaCl (pH 6·0) and passage through a Mono-Q column (Pharmacia), where it appears in the flow-through fraction. The desialated tICAM-1(185), designated ntICAM-1(185), had a slightly reduced relative molecular mass of around 43,000 and formed a major species with a pI of 5·8 upon isoelectric focusing. Based on the amino acid sequence and the carbohydrate analysis (M_r = 6000), the total relative molecular mass is around 27,000. It was crystallized (Fig. 1) in hanging drops by means of vapor diffusion with conditions similar to those used for crystallizing the soluble two-domain protein of CD4 (Ryu *et al.*, 1990; Wang *et al.*, 1990). A five microliter drop of ICAM-1 (17 mg/ml) was mixed with a five microliter drop of the well solution containing the precipitant polyethylene glycol 3350 (24 to 27%) in buffer. The buffer consisted of 10 mM-Tris, 25 mM-NaCl, 1 mM-$MgCl_2$ and 1 mM-$CaCl_2$. The crystals grew rapidly as hexagonal plates of bipyramids with dimensions of approximately 0·2 mm × 0·2 mm × 0·07 mm. They diffract to about 3·0 Å resolution on "still" photographs using an Elliott GX6 rotating anode source. Oscillation photographs showed that there is a 3-fold axis, the lattice is primitive and the cell dimensions are $a = 55{\cdot}7$ Å and $c = 166{\cdot}3$ Å. When crystals were soaked with 40% (v/v) polyethylene glycol 3350 in Tris buffer the cell dimensions shrank to $a = 54{\cdot}0$ Å, $c = 144{\cdot}5$ Å without change in space group, but with a substantial improvement in crystalline stability towards radiation damage. The space group was confirmed by data collection on a San Diego Multiwire Systems area detector with a Rigaku X-ray generator. An 80% complete data set to 4 Å resolution was collected using 0·1° frames every two minutes. These data show the space group to be $P3_121$ or $P3_221$. Assuming six molecules per unit cell, the Matthews coefficient, V_M (Matthews, 1968), is 2·7 Å^3/Da,

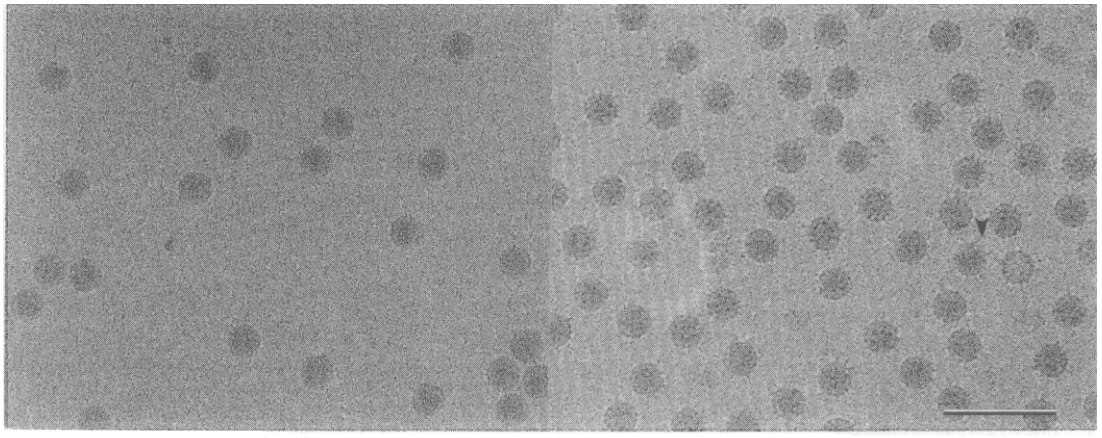

Figure 2. Electron micrographs of frozen hydrated (a) HRV16 and (b) HRV16 complexed with ntICAM-1(185). The ntICAM-1(185) molecules (arrowhead) may be observed as protrusions on the periphery of the particles. The length of the protrusions is about 100 Å, or about one-third of the particle diameter. The length of the related CD4 molecule (Ryu *et al.*, 1990; Wang *et al.*, 1990) is around 90 Å. The bar represents 1000 Å.

which implies one molecule per asymmetric unit. A $Pt_2(NH_3)_2(NO_2)_2$ heavy-atom derivative diffraction data set has also been collected to 5 Å resolution and interpreted in terms of one major Pt site.

Aqueous samples were prepared for cryomicroscopy, without the addition of stains or fixatives, by quickly plunge freezing them into liquified ethane (Dubochet *et al.*, 1988). The samples were maintained at liquid nitrogen temperatures during transfer and while in the electron microscope. Micrographs were recorded at 49,000 × and minimal dose procedures were utilized to reduce specimen damage by the electron beam.

Binding of ICAM-1 or its amino-terminal fragments to major group human rhinoviruses causes their capsids to partially disassemble, presumably representing the first steps in viral host cell penetration. These ICAM-1 fragments also bind to HRV16, but without virion disintegration (J.M.G. & H.H.-L., unpublished results). Figure 2 shows a comparison of HRV16 and HRV16 complexed with ntICAM-1(185). The ICAM-1 decorations are clearly visible on the viral surface, giving promise of a three-dimensional, 25 Å resolution, reconstruction of the complex structure. The crystalline structure of HRV16 is currently being investigated (M.A.O., unpublished results). Thus, knowledge of the ICAM-1 and HRV16 structures at atomic resolution, plus a low resolution reconstruction of the complex, will aid in the analysis of the mode of binding of the receptor to a major rhinovirus serotype. Comparison of HRV14, HRV16 (both major receptor rhinovirus serotypes) and HRV1A (a minor receptor rhinovirus serotype of known structure (Kim *et al.*, 1989)) might suggest the mechanism that determines the specificity for the major rhinovirus group. Such observations are also likely to enhance understanding of how other viruses might interact with receptors belonging to the immunoglobulin superfamily (e.g. the receptors for poliovirus and HIV) and how this interaction initiates endocytosis.

We thank Helene Prongay and Sharon Wilder for help in preparation of the manuscript. These studies were supported by National Institutes of Health grants to M.G.R. and Timothy S. Baker and a Lucille P. Markey grant for the expansion of structural studies at Purdue University.

References

Colonno, R. J. (1986). Cell surface receptors for picornaviruses. *BioEssays*, **5**, 270–274.

Colonno, R. J., Condra, J. H., Mizutani, S., Callahan, P. L., Davies, M. E. & Murcko, M. A. (1988). Evidence for the direct involvement of the rhinovirus canyon in receptor binding. *Proc. Nat. Acad. Sci., U.S.A.* **85**, 5449–5453.

Diamond, M. S., Staunton, D. E., Marlin, S. D. & Springer, T. A. (1991). Binding of the integrin Mac-1 (CD11b/CD18) to the third immunoglobulin-like domain of ICAM-1 (CD54) and its regulation by glycosylation. *Cell*, **65**, 961 971.

Dubochet, J., Adrian, M., Chang, J.-J., Homo, J.-C., Lepault, J., McDowall, A. W. & Schultz, P. (1988). Cryo-electron microscopy of vitrified specimens. *Quart. Rev. Biophys.* **21**, 129–228.

Giranda, V. L., Chapman, M. S. & Rossmann, M. G. (1990). Modeling of the human intercellular adhesion molecule-1, the human rhinovirus major group receptor. *Proteins*, **7**, 227–233.

Greve, J. M., Davis, G., Meyer, A. M., Forte, C. P., Yost, S. C., Marlor, C. W., Kamarck, M. E. & McClelland, A. (1989). The major human rhinovirus receptor is ICAM-1. *Cell*, **56**, 839–847.

Greve, J. M., Porte, C. P., Marlor, C. W., Meyer, A. M., Hoover-Litty, H., Wunderlich, D. & McClelland, A. (1991). Mechanisms of receptor-mediated rhinovirus neutralization defined by two soluble forms of ICAM-1. *J. Virol.* **65**, 6015–6023.

Kim, S., Smith, T. J., Chapman, M. S., Rossmann, M. G., Pevear, D. C., Dutko, F. J., Felock, P. J., Diana, G. D. & McKinlay, M. A. (1989). Crystal structure of human rhinovirus serotype 1A (HRV1A). *J. Mol. Biol.* **210**, 91–111.

Kim, S., Boege, U., Krishnaswamy, S., Minor, I., Smith, T. J., Luo, M., Scraba, D. G. & Rossmann, M. G. (1990). Conformational variability of a picornavirus

capsid: pH-dependent structural changes of Mengo virus related to its host receptor attachment site and disassembly. *Virology*, **175**, 176–190.

Kishimoto, T. K., Larson, R. S., Corbi, A. L., Dustin, M. L., Staunton, D. E. & Springer, T. A. (1989). The leukocyte integrins. *Advan. Immunol.* **46**, 149–182.

Lineberger, D. W., Graham, D. J., Tomassini, J. E. & Colonno, R. J. (1990). Antibodies that block rhinovirus attachment map to domain 1 of the major group receptor. *J. Virol.* **64**, 2582–2587.

Marlin, S. D., Staunton, D. E., Springer, T. A., Stratowa, C., Sommergruber, W. & Merluzzi, V. J. (1990). A soluble form of intercellular adhesion molecule-1 inhibits rhinovirus infection. *Nature (London)*, **344**, 70–72.

Matthews, B. W. (1968). Solvent content of protein crystals. *J. Mol. Biol.* **33**, 491–497.

McClelland, A., deBear, J., Yost, S. C., Meyer, A. M., Marlor, C. W. & Greve, J. M. (1991). Identification of monoclonal antibody epitopes and critical residues for rhinovirus binding in domain 1 of ICAM-1. *Proc. Nat. Acad. Sci., U.S.A.* **88**, 7993–7997.

Mendelsohn, C. L., Wimmer, E. & Racaniello, V. R. (1989). Cellular receptors for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. *Cell*, **56**, 855–865.

Pevear, D. C., Fancher, M. J., Felock, P. J., Rossmann, M. G., Miller, M. S., Diana, G., Treasurywala, A. M., McKinlay, M. A. & Dutko, F. J. (1989). Conformational change in the floor of the human rhinovirus canyon blocks adsorption to HeLa cell receptors. *J. Virol.* **63**, 2002–2007.

Ren, R., Costantini, F., Gorgacz, E. J., Lee, J. J. & Racaniello, V. R. (1990). Transgenic mice expressing a human poliovirus receptor: a new model for poliomyelitis. *Cell*, **63**, 353–362.

Rossmann, M. G. (1989). The canyon hypothesis. Hiding the host cell receptor attachment site on a virus surface from immune surveillance. *J. Biol. Chem.* **264**, 14587–14590.

Rossmann, M. G. & Palmenberg, A. C. (1988). Conservation of the putative receptor attachment site in picornaviruses. *Virology*, **164**, 373–382.

Rossmann, M. G., Arnold, E., Erickson, J. W., Frankenberger, E. A., Griffith, J. P., Hecht, H. J., Johnson, J. E., Kamer, G., Luo, M., Mosser, A. G., Rueckert, R. R., Sherry, B. & Vriend, G. (1985). Structure of a human common cold virus and functional relationship to other piconaviruses. *Nature (London)*, **317**, 145–153.

Ryu, S. E., Kwong, P. D., Truneh, A., Porter, T. G., Arthos, J., Rosenberg, M., Dai, X., Xuong, N., Axel, R., Sweet, R. W. & Hendrickson, W. A. (1990). Crystal structure of an HIV-binding recombinant fragment of human CD4. *Nature (London)*, **348**, 419–426.

Simmons, D., Makgoba, M. W. & Seed, B. (1988). ICAM, an adhesion ligand of LFA-1, is homologous to the neural cell adhesion molecule NCAM. *Nature (London)*, **331**, 624–627.

Staunton, D. E., Marlin, S. D., Stratowa, C., Dustin, M. L. & Springer, T. A. (1988). Primary structure of ICAM-1 demonstrates interaction between members of the immunoglobulin and integrin supergene families. *Cell*, **52**, 925–933.

Staunton, D. E., Merluzzi, V. J., Rothlein, R., Barton, R., Marlin, S. D. & Springer, T. A. (1989). A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. *Cell*, **56**, 849–853.

Staunton, D. E., Dustin, M. L., Erickson, H. P. & Springer, T. A. (1990). The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. *Cell*, **61**, 243–254.

Wang, J. Yan, Y., Garrett, T. P. J., Liu, J., Rodgers, D. W., Garlick, R. L., Tarr, G. E., Husain, Y., Reinherz, E. L. & Harrison, S. C. (1990). Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature (London)*, **348**, 411–418.

Williams, R. K., Jiang, G. S. & Holmes, K. V. (1991). Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. *Proc. Nat. Acad. Sci., U.S.A.* **88**, 5533–5536.

Edited by R. Huber