BRIEF COMMUNICATIONS

nature
structural &
molecular biology

Structure of the measles virus hemagglutinin

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Measles virus is a highly pathogenic virus that infects roughly 20 million people per year. We report here the crystal structure of the measles virus hemagglutinin, the surface glycoprotein responsible for the binding of measles virus to its host cell receptors. Although the protein lacks neuraminidase activity, its structure resembles a 'dead' neuraminidase fold, presenting spatially distinct receptor-binding sites for its receptors CD46 and SLAM.

Measles is a highly contagious viral disease and remains a substantial health risk despite extensive vaccination efforts. As a member of the *Morbillivirus* genus in the Paramyxoviridae family, measles virus expresses a hemagglutinin protein (MVH) that lacks neuraminidase activity, an activity essential to the life cycles of many viruses[1]. Rather, morbilliviruses attach to host cells through direct protein-protein interactions with host receptors, without the involvement of sialic acid. MVH uses the host cell surface receptors SLAM (CD150) and/or CD46 for attachment[2], indicating a unique host-receptor cross-reactivity. We solved the structure of MVH to better understand its role in host cell receptor attachment and entry.

MVH is a 617-residue type II integral membrane glycoprotein. Its C-terminal globular head extends from the viral core on a long stalk[3] (**Fig. 1a**). We produced the globular head region without the disulfide-linked stalk and found that this isolated headpiece, which is functionally active in receptor binding[4], is a monomer both in solution and in the crystal (**Supplementary Methods** online). There is no prior evidence that the MVH headpiece itself is a dimer in the absence of the disulfide-linked stalk.

We solved the structure of MVH at a resolution of 2.7 Å (**Supplementary Table 1** online). The overall fold of MVH is that of a β-propeller with six blades surrounding a large cavity, similar to predicted structural models[5–7] (**Fig. 1a,b** and **Supplementary Fig. 1** online). Each of the blade modules, B1–B6, contains four antiparallel β-strands, S1–S4 (**Supplementary Fig. 2** online). The blades are connected sequentially through extended loops between S4 of one module and S1 of the next (**Supplementary Figs. 1** and **2**). The interstrand loops have been implicated in mediation of ligand binding[8–10]. The S1-S2 interstrand loops seal the base of the cavity (**Fig. 1b**). The N and C termini of MVH are connected through a disulphide linkage. The propeller structure is stabilized by extensive hydrogen-bonding between the S1 strands, which line the center of the internal cavity (**Supplementary Fig. 1**). Asparagine-linked glycosylation has been reported to facilitate MVH folding and stabilization[11], and the MVH used in our structural studies (Edmonston strain) contains four potential N-linked glycosylation sites; of these, we were able to model the glycan electron density for Asn200 and Asn215 (**Fig. 1b**). The *N*-acetylglucosamine (NAG) moieties extending from Asn215 interact with Glu235, Met251, Asp283, Gly592 and His593, stabilizing the folds of B1, B2 and B6.

The MVH diverges from the hemagglutinin of influenza virus in both structure and function. A structural similarity search with the Dali server reveals that MVH most closely resembles the hemagglutinin/neuraminidase (HN) fold, as seen in the parainfluenza virus (PIV) HN (PDB 1V3C)[10], with a *Z*-score of 23.1. Nonetheless, MVH and PIV HN differ by an r.m.s. deviation of 3.9 Å over their Cα atoms; such large positional differences indicate considerable structural divergence despite similar global folds (**Fig. 2a**). PIV HN also has the highest sequence similarity to MVH of any known structure (12% identity). In comparison with MVH, the Newcastle disease virus HN (PDB 1E8T) and influenza hemagglutinin (PDB 2QWC) have *Z*-scores

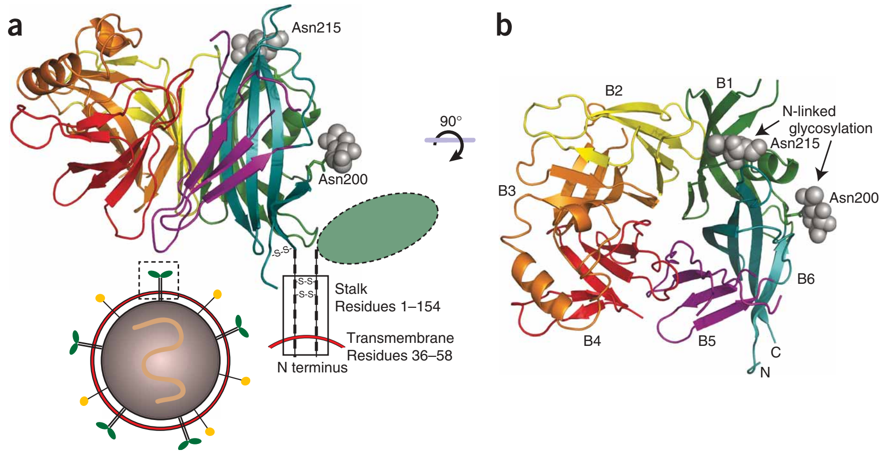

Figure 1 Structure of the measles virus hemagglutinin. (**a**,**b**) Two views of a cartoon of MVH, with a schematic of the measles virus particle and surface. Each blade of the β-propeller is colored differently (as in **Supplementary Fig. 2**). Gray spheres, glycan moieties.

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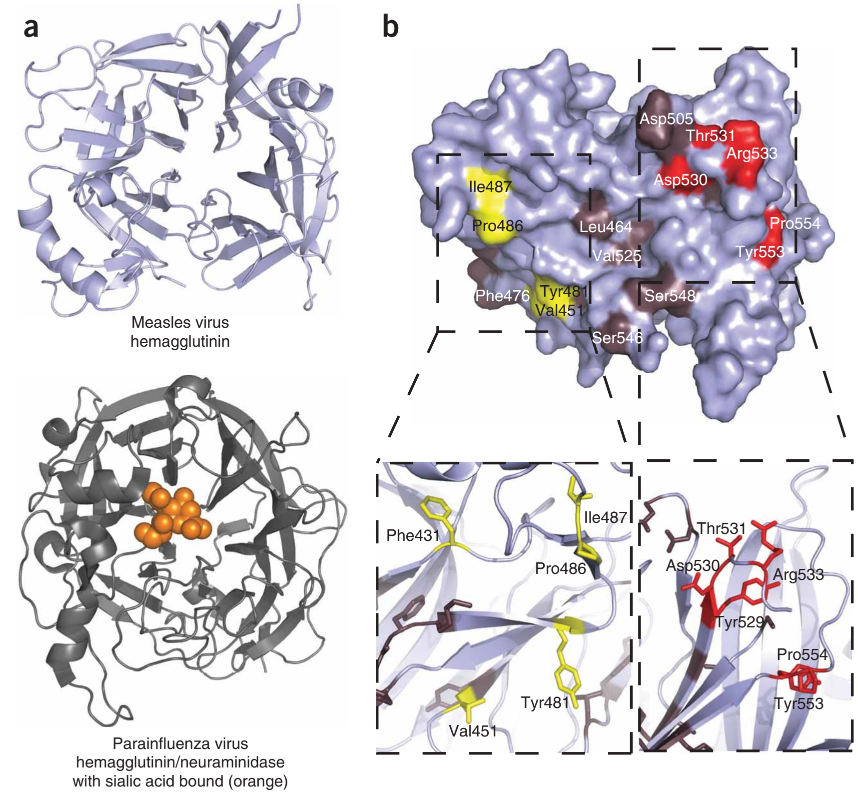
Received 22 September; accepted 30 October; published online 18 November 2007; doi:10.1038/nsmb1342

of 22.8 and 11.5, r.m.s. deviations of 3.9 and 4.4 Å, and sequence identities of 11% and 6%, respectively. Other proteins with weak structural similarities include neuraminidases from humans, parasites, bacteria and leeches[12].



Measles virus hemagglutinin

Parainfluenza virus hemagglutinin/neuraminidase with sialic acid bound (orange)

Figure 2 MVH receptor-binding sites. (**a**) Comparison of measles hemagglutinin structure (top) with human PIV HN structure (PDB 1V3C; bottom), viewed looking directly into the central cavity. Orange, sialic acid bound in the active site of PIV HN. (**b**) Putative binding sites for SLAM (red) and CD46 (yellow) receptors on MVH. Brown, residues making minor contributions to receptor binding.

Sequence similarities of hemagglutinins in the paramyxovirus family are as low as 7%. However, a sequence alignment of morbilliviruses indicates that all cysteine residues forming disulphide bonds are well conserved[3], as are most SLAM-binding residues (**Supplementary Fig. 3** online). Residues essential for CD46 binding are conserved only among measles vaccine strains. The conservation of fold and SLAM-binding site indicates that the MVH structure reported here represents the norm for hemagglutinins of *Morbillivirus* spp.

Even though it retains the overall neuraminidase fold, MVH does not have neuraminidase activity, making it effectively a dead neuraminidase. Furthermore, although binding to sialic acid is not required for its viral entry into host cells[13], the deep cavity analogous to that used by other HNs for sialic acid binding remains in the MVH structure[8,10] (**Fig. 2a**). Instead of sialic acid binding, MVH has developed the ability, unique among known hemagglutinins, to bind the host cell receptors SLAM and CD46 (ref. 3). Three hypothetical mechanisms of receptor recognition by MVH have been proposed[4,6,7]: (i) the sialic acid–binding pocket found in neuraminidase and HN structures has evolved to accommodate protein receptors instead of carbohydrates; (ii) an overlapping surface area on MVH engages both the SLAM and CD46 receptors; or (iii) the SLAM and CD46 receptors interact with MVH at two distinct binding sites but sterically prevent one another's simultaneous engagement of MVH. We mapped the available mutagenesis and antibody-blocking data onto our MVH structure (**Fig. 2b** and **Supplementary Fig. 3**), including both crucial (mutation abolishes binding) and minor (mutation is mildly detrimental) binding determinants reported in the literature[6,7,14]. The deep pocket used for carbohydrate binding in other HNs is approximately 28 Å from the putative SLAM-binding site and 51 Å from the putative CD46-binding site. Residues identified as crucial for SLAM and CD46 binding are located outside the rim of the cavity on spatially distinct surfaces of MVH, separated by approximately 35 Å (**Fig. 2b**).

In summary, we have determined the structure of the measles virus hemagglutinin, which has a structural fold similar to neuraminidases despite limited sequence and functional similarities. We find that the binding surfaces of the SLAM and CD46 cellular receptors are far apart, as well as distinct from the location analogous to the sialic acid–binding site in HNs. Hemagglutinins are chief targets of drugs to prevent viral entry, and our structure now provides a template for structure-based drug design aimed at blocking measles virus entry.

Accession codes. Protein Data Bank: Coordinates and structure factors have been deposited with accession code 2RKC.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

ACKNOWLEDGMENTS

We thank M. Oldstone, L. Martin and B. Hahm (The Scripps Research Institute) for complementary DNA encoding MVH. L.A.C. is supported by a US National Science Foundation graduate research fellowship. K.C.G. is supported by the Howard Hughes Medical Institute and the US National Institutes of Health.

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