

# Histidine protonation and the activation of viral fusion proteins

Daniela S. Mueller<sup>\*†1</sup>, Thorsten Kampmann<sup>‡</sup>, Ragothaman Yennamalli<sup>§</sup>, Paul R. Young<sup>†||</sup>, Bostjan Kobe<sup>†||¶</sup> and Alan E. Mark<sup>\*†||</sup>

<sup>\*</sup>Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Nijenborgh 4, 9747AG Groningen, The Netherlands,

<sup>†</sup>School of Molecular and Microbial Sciences, University of Queensland, Brisbane, QLD 4072, Australia, <sup>‡</sup>Department of Theoretical and Computational

Biophysics, Max Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany, <sup>§</sup>Centre for Computational Biology and

Bioinformatics, School of Information Technology, Jawaharlal Nehru University, New Delhi-110067, India, <sup>||</sup>Institute for Molecular Bioscience, University of

Queensland, Brisbane, QLD 4072, Australia, and <sup>¶</sup>Special Research Centre for Functional and Applied Genomics, University of Queensland, Brisbane, QLD

4072, Australia

## Abstract

Many viral fusion proteins only become activated under mildly acidic condition (pH 4.5–6.5) close to the  $pK_a$  of histidine side-chain protonation. Analysis of the sequences and structures of influenza HA (haemagglutinin) and flaviviral envelope glycoproteins has led to the identification of a number of histidine residues that are not only fully conserved themselves but have local environments that are also highly conserved [Kampmann, Mueller, Mark, Young and Kobe (2006) *Structure* **14**, 1481–1487]. Here, we summarize studies aimed at determining the role, if any, that protonation of these potential switch histidine residues plays in the low-pH-dependent conformational changes associated with fusion activation of a flaviviral envelope protein. Specifically, we report on MD (Molecular Dynamics) simulations of the DEN2 (dengue virus type 2) envelope protein ectodomain sE (soluble E) performed under varied pH conditions designed to test the histidine switch hypothesis of Kampmann et al. (2006).

## Low-pH-dependent viral fusion proteins

Many enveloped viruses (e.g. influenza virus, vesicular stomatitis virus, rhabdoviruses, alphaviruses and flaviviruses) enter the host cell by endocytosis and fusion of the viral envelope with the endosomal membrane [1,2]. This fusion is triggered by a decrease in pH in the endosomal compartment to pH 4.5–6.5 and mediated by viral envelope proteins [3,4]. Specifically, acidification triggers large-scale conformational changes within the envelope glycoprotein [3,5]. In the case of flaviviruses and alphaviruses, the initially dimeric protein disassembles into monomers, which undergo a conformational change and then irreversibly assemble into trimers [6].

Low pH also plays a role in the furin-dependent cleavage of the envelope protein precursor prM (precursor of M) during maturation of the virion prior to its release from the host cell [2,7]. The initial maturation of the virion and the later triggering of conformational changes that lead to membrane fusion require a pH range that suggests that the protonation of one or more histidine residues is critical in both processes. Identification of these critical histidine residues thus presents an important step in understanding the mechanism of fusion in detail.

## The histidine switch hypothesis

As noted above, pH-induced fusion occurs close to the  $pK_a$  of histidine in water ( $pK_a = 6$ ). This strongly suggests that the process is triggered by the protonation of one or more histidine residues. Viral fusion proteins have been separated into two classes based on structural and functional properties [5]. An analysis of HA (haemagglutinin) (class I) sequences from 16 influenza serotypes shows that a number of histidine residues are indeed fully conserved [8]. Analysis of 12 flaviviral envelope protein sequences (class II) likewise showed a number of fully conserved histidine residues. Furthermore, homology mapping of the aligned sequences on to the X-ray crystal structure of the pre-fusion DEN2 (dengue virus type 2) sE (soluble E) protein (1OKE [10]; Figure 1) showed that although certain histidine residues were not fully conserved they were compensated by other mutations. The specific conserved histidine residues considered to be of interest included His-144, His-244, His-261 and His-317 [8].

To act as effective pH-sensitive switches, histidine residues must be protonated only when the pH becomes sufficiently low and without being too readily deprotonated again [8]. In fact, in dengue viruses, fusion occurs at approximately pH 5, i.e. 1 pH unit below the  $pK_a$  of histidine in water. Such  $pK_a$  shifts can be associated with the residue being buried or with stabilization of the deprotonated form by hydrogen-bonding or charge–charge interactions. Calculations using a Poisson–Boltzmann approach [10] predict that His-144, which is buried and has very low solvent accessibility, would

**Key words:** dengue virus, envelope glycoprotein, flavivirus, influenza virus, membrane fusion, pH-dependent fusion.

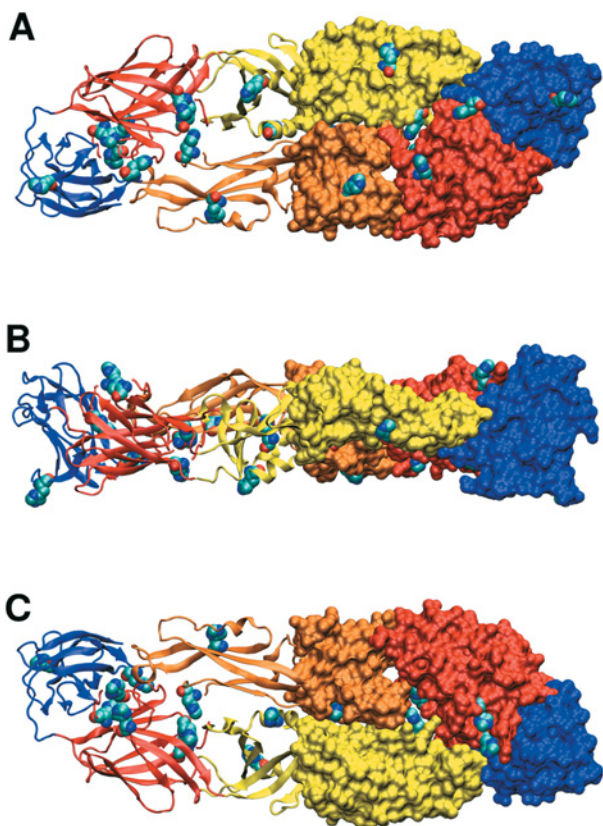
**Abbreviations used:** DEN2, dengue virus type 2; MD, Molecular Dynamics; sE, soluble E.

<sup>1</sup>To whom correspondence should be addressed (email [d.s.mueller@rug.nl](mailto:d.s.mueller@rug.nl)).

Biochem. Soc. Trans. (2008) **36**, 43–45; doi:10.1042/BST0360043

### Figure 1 | X-ray crystal structure of the DEN2 envelope glycoprotein ectodomain sE in its pre-fusion conformation

Locations of histidine residues in the homodimeric structure (1OKE [10]) are highlighted by CPK (Corey–Pauling–Koltun) models. **(A)** View of the inner envelope surface (*in situ* facing the viral membrane), **(B)** rotated by 90° and **(C)** top view of the outer envelope surface. The solvent-accessible surface was rendered for one-half of the structure, whereas the other half is shown in cartoon representation. Domains are coloured red (domain I), yellow and orange (II) and blue (III). Images were prepared using VMD software (<http://www.ks.uiuc.edu/Research/vmd/>) [16].



have a  $pK_a$  value of  $< 5$ , making His-144 a prime candidate for a potential switch. A number of other histidine residues, in particular His-261 and His-317, which interact closely

with polar and charged residues, were assessed as further potential trigger residues due to the significant changes that their environments undergo during fusion activation. One difficulty in determining which residues in the DEN2 sE protein specifically play a critical role in fusion activation is that low pH also plays a role in the cleavage of the envelope protein precursor [7]. However, the structure of the uncleaved precursor and therefore its histidine environments are not known.

### MD (Molecular Dynamics) simulations test histidine functions in a flaviviral fusion protein

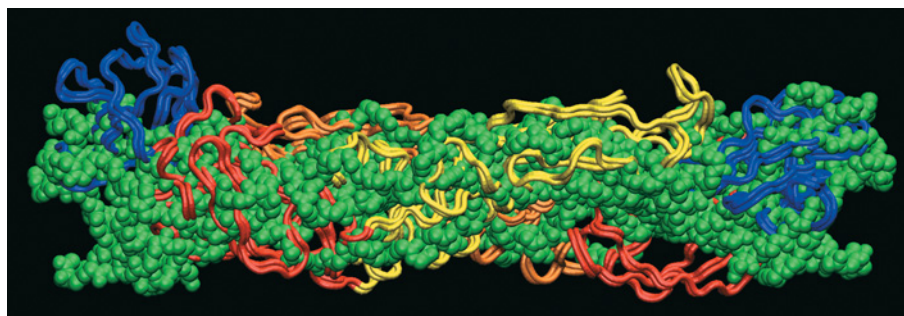
Experimentally, protein-mediated membrane fusion cannot be observed with either the spatial or temporal resolution required to investigate the role of specific histidine residues. Instead, to selectively test the effect of histidine protonation, MD simulations have been performed of the DEN2 sE protein in its pre-fusion, dimeric form 1OKE (Figure 1) in water [8]. The simulations were performed in the NPT ensemble using the Gromos96 force field 43A1 [11,12].

From the X-ray crystal structures of the pre- (1OKE, 1OAN) and post- (1OK8) fusion conformations of the DEN2 sE protein [9,13], it is known that the primary difference between the conformations is a displacement of domain III by 33 Å (1 Å = 0.1 nm) and associated changes of the domain interfaces [14]. After trimerization, intermolecular interactions give rise to the exceptionally thermostable post-fusion complex [13]. The simulations were examined with regard to initial stages of activation, i.e. to dimer separation and domain III displacement.

First, the overall effect of different pH conditions on the structure of the pre-fusion dimer was investigated by either single (pH 7) or double protonating (pH 5) all 22 histidine residues present in DEN2 sE protein. While the system simulated at pH 7 remained close to the starting structure throughout 60 ns of simulation, at pH 5 the dimer underwent significant structural changes (Figure 2, and see Supplementary Movies 1 and 2 at <http://www.biochemsoctrans.org/bst/036/bst0360043add.htm>). Within a few nanoseconds, the two subunits twisted around the

### Figure 2 | A snapshot of DEN2 sE protein after 70 ns of MD simulation

The snapshot is superimposed as domain-coloured ribbons (cf. Figure 1) on the backbone of the simulation's starting structure (1OKE [9]; green spheres); the view is as in Figure 1(B).



longitudinal axis of the dimer and folded out towards the surface that *in situ* faces the viral membrane. To further examine the specific roles of His-244, His-261 and His-317, more simulations were performed in which combinations of these residues were selectively protonated. To test the mechanical side-chain function of these histidine residues, another series of simulations of a triple mutant was performed in which these histidine residues were mutated to alanine. It was found that when only a subset of histidine residues was protonated, or mutated to alanine residue, no significant structural changes were observed within 20 ns of simulation. Given the time scales accessible to the simulations, it was therefore not possible to determine whether the specific residues identified were primarily responsible for inducing the initial stages of activation.

## Outlook

The conformational changes observed in the simulation of DEN2 sE protein at pH 5 are consistent with the system undergoing initial stages of the transition from dimeric to monomeric form. However, the time scales currently accessible to MD simulations do not allow the precise identification of the role of specific histidine residues in this process. Nevertheless, the work does highlight the fact that MD simulations in atomic detail can increasingly be used to probe large-scale functional conformational changes induced in protein systems by environmental conditions such as pH. Thereby, MD simulations can shed light on processes that cannot be directly observed experimentally.

## References

- 1 Marsh, M. (1984) The entry of enveloped viruses into cells by endocytosis. *Biochem. J.* **218**, 1–10

- 2 Stiasny, K. and Heinz, F.X. (2006) Flavivirus membrane fusion. *J. Gen. Virol.* **87**, 2755–2766
- 3 Zimmerberg, J., Vogel, S.S. and Chernomordik, L.V. (1993) Mechanisms of membrane fusion. *Annu. Rev. Biophys. Biomol. Struct.* **22**, 433–466
- 4 White, J., Helenius, A. and Gething, M.J. (1982) Hemagglutinin of influenza virus expressed from a cloned gene promotes membrane-fusion. *Nature* **300**, 658–659
- 5 Schibli, D.J. and Weissenhorn, W. (2004) Class I and class II viral fusion protein structures reveal similar principles in membrane fusion. *Mol. Membr. Biol.* **21**, 361–371
- 6 Wengler, G. and Wengler, G. (1989) Cell-associated West Nile flavivirus is covered with E + pre-M protein heterodimers which are destroyed and reorganized by proteolytic cleavage during virus release. *J. Virol.* **63**, 2521–2526
- 7 Allison, S.L., Schlich, J., Stiasny, K., Mandl, C.W., Kunz, C. and Heinz, F.X. (1995) Oligomeric rearrangement of tick-borne encephalitis virus envelope proteins induced by an acidic pH. *J. Virol.* **69**, 695–700
- 8 Kampmann, T., Mueller, D.S., Mark, A.E., Young, P.R. and Kobe, B. (2006) The role of histidine residues in low-pH-mediated viral membrane fusion. *Structure* **14**, 1481–1487
- 9 Modis, Y., Ogata, S., Clements, D. and Harrison, S.C. (2003) A ligand-binding pocket in the dengue virus envelope glycoprotein. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6986–6991
- 10 Gordon, J.C., Myers, J.B., Folta, T., Shoja, V., Heath, L.S. and Onufriev, A. (2005) H<sup>++</sup>: a server for estimating pK<sub>a</sub>s and adding missing hydrogens to macromolecules. *Nucleic Acids Res.* **33**, W368–W371
- 11 van Gunsteren, W.F., Billeter, S.R., Eising, A.A., Huenenberger, P.H., Krueger, P., Mark, A.E., Scott, W.R.P. and Tironi, I.G. (1996) Biomolecular Simulation: The GROMOS96 Manual and User Guide, Vdf Hochschulverlag AG an der ETH Zürich, Zürich
- 12 Lindahl, E., Hess, B. and van der Spoel, D. (2001) Gromacs 3.0: a package for molecular simulation and trajectory analysis. *J. Mol. Model.* **7**, 306–317
- 13 Modis, Y., Ogata, S., Clements, D. and Harrison, S.C. (2004) Structure of the dengue virus envelope protein after membrane fusion. *Nature* **427**, 313–319
- 14 Bressanelli, S., Stiasny, K., Allison, S.L., Stura, E.A., Duquerry, S., Lescar, J., Heinz, F.X. and Rey, F.A. (2004) Structure of a flavivirus envelope glycoprotein in its low-pH-induced membrane fusion conformation. *EMBO J.* **23**, 728–738
- 15 Humphrey, W., Dalke, A. and Schulten, K. (1996) VMD: visual molecular dynamics. *J. Mol. Graphics* **14**, 33–38

Received 14 September 2007  
doi:10.1042/BST0360043