

Mechanical Unfolding of Influenza Hemagglutinin Using Dynamic Force AFM

Yoshiki HIRATA*, Masami KAGESHIMA**, Tatsuya SAKAI***,
Osamu NIWA*, Fumio MIZUTANI* and Takahito INOUE*

*National Institute of Advanced Industrial Science and Technology (AIST),
1-1 Higashi, Tsukuba, Ibaraki, 305-8566, Japan

**Department of Applied Physics, Graduate School of Engineering, Osaka University,
2-1 Yamada-oka, Suita, Osaka, 565-0871, Japan

***Kawasaki Medical School,
577 Matsushima, Kurashiki, Okayama 701-0192, Japan

E-mail: y-hirata@aist.go.jp

The infection of influenza virus begins with the binding of virus to sialic acid, a component of membrane proteins and lipids. The bound virion is then internalized into a cellular endosome by receptor-mediated endocytosis. The viral envelope fuses with the membrane of the mature endosome in response to a drop in pH. Membrane fusion is promoted by the trimeric, viral envelope glycoprotein, hemagglutinin (HA) which also functions in cell attachment by binding to sialic acid. At low pH, HA is sufficient for the fusion of membranes *in vivo* and *in vitro*. Influenza virus hemagglutinin play a key roll for biological membrane fusion mechanisms. Most important recent progress in this field are “spring loaded mechanism” based on a coiled coil of alpha helix[1]. The fusogenic interaction force between hemagglutinin and cell mimic lipid bilayer was measured using dynamic mode atomic force microscopy in physiological environment[2-3]. This approach involves spatially modulating the tip-sample distance and to measure the AC response in the signal. Especially the magnetic activation of the cantilever [4-5] proved to be an useful approach for study in liquid environment in that it does not suffer from considerable difficulty in acoustic excitation caused by spoiled Q-value or from spurious resonance peaks that would appear if the sample is modulated instead of the cantilever. The cantilever is modeled as an harmonic oscillator actuated with a constant AC external force. The stiffness of the target bio-molecule could be obtained by measuring vibration amplitude and phase lag of cantilever using the lock-in technique. The elongation force and mechanical stiffness measurements suggests the conformational change of HA between acidic and neutral pH.

References

- [1] C. M. Carr and P. S. Kim, *Cell*, **73**, 823 (1993)
- [2] C. Yuan, A. Chen, P. Kolb, and V. T. Moy, *Biochemistry*, **39**, 10219 (2000)
- [3] M. Kageshima, M. A. Lantz, S. P. Jarvis, H. Tokumoto, S. Takeda, A. Ptak, C. Nakamura, and J. Miyake, *Chem. Phys. Lett.*, **343**, 77 (2001).
- [4] S.P. Jarvis, H. Yamada, S.-I. Yamamoto, and H. Tokumoto, *Rev. Sci. Instrum.* **67** (1996) 2281.
- [5] M.A. Lantz, S.P. Jarvis, H. Tokumoto, T. Martynski, T. Kusumi, C. Nakamura, J. Miyake; *Chem. Phys. Lett.* **315** (1999) 61.