Studies of HIV-1 Gag Protein Assemblies by Solid-State MAS NMR
Sameer K. Singh, Luis Möckel, Marc Wittlich, Dieter Willbold, Bernd W. Koenig.

Human CD4 is a 433 residue transmembrane protein involved in the body’s adaptive immune response. The extracellular domain of CD4 serves as a primary receptor of the human immunodeficiency virus (HIV-1) and binds the viral glycoprotein gp120. The cytoplasmic domain of the 81 residue Virus protein U (VpU) plays an important role in downregulation of CD4. It directly binds the cytoplasmic domain of CD4 in the endoplasmic reticulum, which initiates a series of events resulting in CD4 degradation in the proteasome. We characterized the CD4 - VpU interaction by liquid state NMR spectroscopy utilizing paramagnetic relaxation enhancement (PRE). The PRE effect is a distance-dependent enhancement of the spin relaxation in magnetically active nuclei in the vicinity of a paramagnetic centre. PRE leads to line broadening or complete quenching of NMR signals and indicates spatial proximity to the probe. We recorded NMR spectra of the 15N-labelled cytoplasmic domain of VpU in the presence of varying amounts of a single-cysteine-mutant of CD4(372-433), which was labelled with the active PRE-probe, methanethiosulfonate (MTSL). This CD4 poly-peptide contains the single transmembrane and the C-terminal cytoplasmic domain of CD4. Experiments were conducted in membrane-mimicking decyl phosphocholine (DPC) micelles. Spectra recorded in the presence of paramagnetic MTSL showed reduced NMR signal intensities of certain amino acids of 15N-labelled VpU. Additionally, we studied chemical shift perturbations (CSP’s) of VpU resonances observed on titrating increasing concentrations of unlabelled CD4. Such chemical shift changes are expected for residues in the binding interface but may also occur at remote sites due to allosteric effects. Our data reveal VpU residues involved in CD4 binding, provide insight into the exchange regime, and yield an estimate of the binding affinity.

Structural Studies of RNase II Domain to Develop HIV-1 Reverse Transcriptase Inhibitors using Solution NMR
Lakshmi Menon, Qingguo Gong, Jinwoo Ahn, Michael A. Parniak, Rieko Ishima.

The RNase II-like domain of the intact RT RNase H domain fragment has been expected to undergo significant conformational changes upon Mg2+ interaction and exhibits different dynamics upon slight mutations (Pari et al., 2003). Thus, we have used a series of 1H,15N HSQC and Single Quantum Coherence (HMQC) experiments in combination with three-dimensional solution NMR experiments to clarify whether the p15-EC shows similar structural features to that of the intact RT RNH domain. We also identified the binding site of one of inhibitors, and compared Mg2+ effects on the inhibitor interaction.

Daiisuke Takahashi, Yunjeong Kim, Kyeong-Ok Chang, Asokan Anbanandam, Om Prakash.

Noroviruses are the leading cause of acute food- or water-borne gastroenteritis outbreaks in humans with an estimated 23 million annual cases in the US alone and show high diversity with at least five genogroups (GI-GV). Norwalk virus (NV) is a prototype strain classified as GI strain. Noroviral RNA genome is composed of three open reading frames (ORFs), and the ORF1 encodes a polyprotein that is cleaved by the viral 3C-like cysteine protease (Pro) into 6 non-structural proteins, which makes the Pro as an

References

Solution Structure Determination of Norwalk Virus 3C-Like Cysteine Protease
Arai Takahashi, Kyeong-Ong Chang, Asokan Anbanandam, Om Prakash.

Noroviruses are the leading cause of acute food- or water-borne gastroenteritis outbreaks in humans with an estimated 23 million annual cases in the US alone and show high diversity with at least five genogroups (GI-GV). Norwalk virus (NV) is a prototype strain classified as GI strain. Noroviral RNA genome is composed of three open reading frames (ORFs), and the ORF1 encodes a polyprotein that is cleaved by the viral 3C-like cysteine protease (Pro) into 6 non-structural proteins, which makes the Pro as an

Solution Structure Determination of Norwalk Virus 3C-Like Cysteine Protease
Daisuke Takahashi, Yunjeong Kim, Kyeong-Ong Chang, Asokan Anbanandam, Om Prakash.

Noroviruses are the leading cause of acute food- or water-borne gastroenteritis outbreaks in humans with an estimated 23 million annual cases in the US alone and show high diversity with at least five genogroups (GI-GV). Norwalk virus (NV) is a prototype strain classified as GI strain. Noroviral RNA genome is composed of three open reading frames (ORFs), and the ORF1 encodes a polyprotein that is cleaved by the viral 3C-like cysteine protease (Pro) into 6 non-structural proteins, which makes the Pro as an