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Comparative Essential Dynamics of Protein-Protein Interactions

（京大・福井セ）○Chung Wilfredo Credo，石田 俊正

Introduction. The Epstein-Barr virus (EBV) is a herpes virus that is the major cause of infectious mononucleosis. It is known to infect the salivary gland cells where the virus growth cycle is completed and the B lymphocyte where the virus cycle is abortive. Even in the latter case, the affected B lymphocytes can multiply excessively to produce a cancer to the lymphatic system. The EBV is associated with a variety of malignancies. Chronic active EBV infection is a severe systemic disease associated with high rates of mortality and morbidity.

EBV-encoded latent membrane protein 1 (LMP1) is the major transforming protein of EBV for transforming primary B lymphocytes into lymphoblastoid cell lines. To help EBV evade the cell death machinery of the human host, LMP1 mimics a tumor necrosis factor receptor protein CD40. CD40 is a costimulatory protein that, after ligation, interacts with cellular signaling intermediate tumor necrosis factor receptor (TNFR)-associated factor 3 (TRAF3) to control B cell proliferation, growth and differentiation. LMP1 is also known to bind to TRAF3, and LMP1, therefore, can be seen as a decoy of CD40.

Wu *et al.* [J. Biol. Chem. 280 (2005) 33620] claimed from their X-ray structure analysis that LMP1's Asp^{210} may be a key residue in the structural decoy action.

The essential dynamics (ED) is undertaken to separate a few essential degrees for freedom that are relevant to the function of the protein from irrelevant local fluctuations. ED is an eigenvalue/vector analysis of covariant matrix of positional deviations. The method was used to identify important structural distortions in CD40 and LMP1 that relate to binding with TRAF3 and structural decoy action.

Methodology. The TRAF3-LMP1 and TRAF3-CD40 complexes were subjected to classical MD trajectory simulation. Initial complexes are derived from Protein Data Bank crystal structures 1ZMS and 1FLL, respectively. Only one part of the 1ZMS or 1FLL trimer was utilized for each simulation. Each complex was solvated with water molecules and neutralized with chloride ions. Periodic boundary conditions were applied. Initially, the entire protein was kept frozen and only the energy of the water molecules was minimized. Subsequently, the energy of the whole system was minimized. The system was then slowly heated at constant volume to 300 K over a period of 10 ps. The system density was then equilibrated at constant pressure to a final density around 1 g/cm^3. It took 120 ps to stabilize the density. Production MD runs were done at 300 K for 500 ps. The trajectory points recorded every 20 fs and the trajectory within the 300 – 470 ps interval were analyzed for ED.
The structure at 300 ps was used as the reference structure for ED. The simulations were done using the Amber 9.0 suite of MD programs.

The trajectories of the TRAF3-LMP1 and TRAF3-CD40 complexes were subjected to essential dynamics analysis to gain insight on the structural decoy action of LMP1 over CD40. The covariance matrix $\boldsymbol{C}$ from the trajectory $\mathbf{x}(t)$ is

$$\boldsymbol{C} = \mathbf{cov}(\mathbf{x}) = \langle(\mathbf{x} - \langle\mathbf{x}\rangle)(\mathbf{x} - \langle\mathbf{x}\rangle)^{\mathbf{T}}\rangle$$

Diagonalization of $\boldsymbol{C}$ yield eigenvalues and eigenvectors where the larger eigenvalues represent eigenvectors that correspond to more flexible directions of the trajectory.

Initially, all water molecules and neutralizing ions were excluded. Both TRAF3-CD40 and TRAF3-LMP1 trajectories were subjected to ED analysis under similar conditions. We note that the CD40 fragment is much longer (21 residues) compared to LMP1 (7 residues). The hairpin configuration of CD40 is easily discernible from the trajectories whereas the seven residues of LMP1 are inadequate to determine if CD40's hairpin configuration is mimicked by LMP1.

Results and Discussion. Figure 1 shows equilibrated structure of the TRAF3-LMP1 complex at 300K. The critical contact made by LMP1 Asp^{210} with TRAF3 Arg^{393} is clearly shown. Figure 2 shows the components of the concerted motion of atoms within the PXQXTXX moiety which is revealed both in LMP1 and in CD40 (^{204}PQQATDD^{210} in LMP1 and ^{250}PVQETLH^{256} in CD40). Sharp peaks in the graph indicate large fluctuations of atomic motion. It can easily be seen in Figure 2 that most of the large atomic fluctuations in LMP1 (black curve) are concentrated within atoms number 3154 to 3164, corresponding to atoms of residue Asp^{210}. This residue was reported to make a unique contact with residues in TRAF3 that enable LMP1 to compete CD40 in the structural decoy action, based on the analysis of X-ray crystal structure of the LMP1-TRAF3 complex by Wu *et al.* [J. Biol. Chem. 280 (2005) 33620] Thus, Asp^{210} is an important residue in the structural decoy action of LMP1 over CD40. On the other hand, the parallel residue His^{256} in CD40 (atoms number 3409 to 3426; red curve, Figure 2) shows low components of atomic vibrations thus underscoring the ability of Asp^{210} to compete with His^{256}.

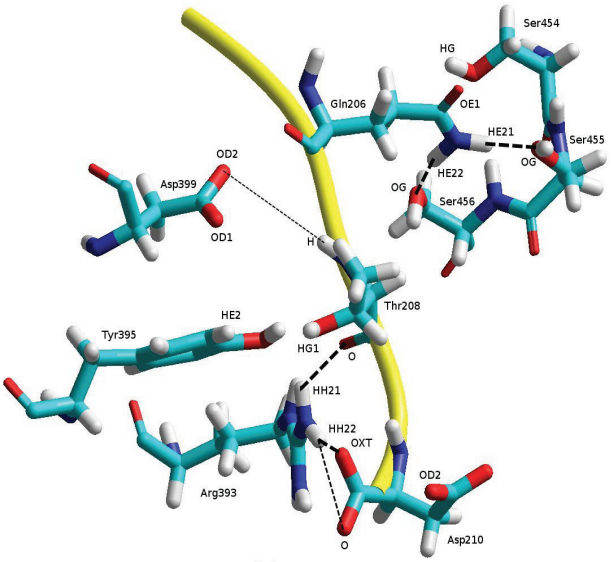

Fig. 1. A snapshot of equilibrated structure of LMP1-TRAF3 complex at 300 K.

While Asp^{209} in LMP1 also is flexible in this context, the CD40 residue Leu^{255} in CD40 is approximately equally flexible and is therefore expected to be able to compete with Asp^{209}.

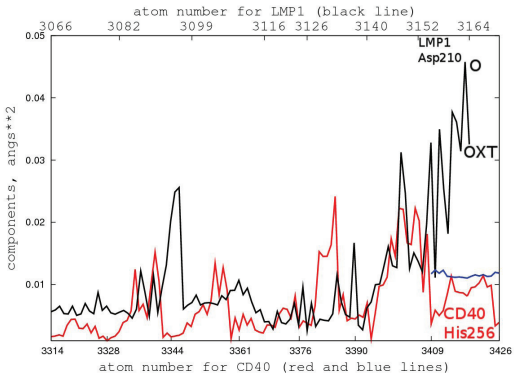

Fig. 2. Components of concerted motion of selected atoms in CD40 and LMP1.

The highest peak (atom number 3163 in LMP1; black curve, Figure 2) corresponds to Asp^{210} carbonyl oxygen ('O' of Asp^{210} in Fig. 1) which is originally H-bonded to Arg^{393} η^2-guanidino hydrogen ('HH22') in the X-ray crystal structure albeit this interaction is found to be slightly weakened after thermal equilibration and solvation. Not far behind in peak prominence is that of atom number 3164, which is Asp^{210} 'OXT' oxygen. The oxygen is strongly H-bonded to Arg^{393} η^2-guanidino hydrogen 'HH22' (see the black curve, Figure 2). This hydrogen bond was reported in the X-ray structure by Wu et al.

One may suspect that Asp^{210} in LMP1 fluctuates wildly because the residue is located at the terminal. We examined the flexibility of the counterpart His^{256} in CD40 when we shorten CD40 artificially and make His^{256} the terminal one. The blue curve in Figure 2 shows the flexibility of His^{256} in the shortened CD40. This artificial termination still offers limited flexibility of His^{256}. Thus, the large fluctuations Asp^{210} would be not artificial, but rather due to its unique function in EBV infection.

Conclusion. Combined MD simulation and comparative ED analysis of solvated complexes of TRAF3 with LMP1 and CD40 confirm the key residue status of Asp^{210} in LMP1 in the structural decoy action that characterizes Epstein-Barr virus infection. Thus, drug design for remediation and prevention of EBV infection should consider suppression of the interaction of Asp^{210} in LMP1 with TRAF3.