Salt effect on diffusion signal reveals selective photoinduced dimerization site of a BLUF domain of EB1

Masahide Terazima, Kosei Shibata, Yusuke Nakasone
Department of Chemistry, Graduate School of Science, Kyoto University, Kyoto 606-8502, Japan
mterazima@kuchem.kyoto-u.ac.jp

Conformation changes of proteins and biomolecular interactions are important for biological functions. For the detection of these processes, a variety of techniques have been developed. UV/vis absorption spectroscopy or emission spectroscopy are very powerful to trace the time development of reactions. However, these techniques have a limitation to detect the conformation changes of proteins and biomolecular interactions. Our group found that the translational diffusion coefficient (D) can be a useful and sensitive probe to detect the conformation change as well as the intermolecular interaction changes. Although many techniques, e.g., dynamic light scattering, Taylor dispersion, capillary method, NMR spectroscopy, have been developed to monitor molecular diffusion, D has never been considered as a time dependent property during reactions. We have been developing a method based on the pulsed-laser induced transient grating (TG) technique to detect the time-dependent diffusion. Here, we studied the dimerization reaction of eBLUF, which is a photosensor BLUF domain from EB1, using the time-resolved diffusion method. EB1 is a C-terminal construct of Bldp1 from the magnetotactic bacterium *Magnetococcus marinus*. We found a significant salt effect on the dimer stability using transient grating (TG) and dark recovery measurements, indicating that electrostatic interactions play a critical role in mediating the stability of protein complexes. These results will be very useful to produce a new protein tool for optogenetics.

The TG method can sensitively detect the diffusion coefficients in the time-domain. The principle of the TG method and experimental methods to measure time-dependence of D have been reported previously [1].

To detect the dimerization dynamics, the TG signal of eBLUF was measured at a sufficiently weak light pulse (~a few μJ/pulse). Figure 1 shows typical TG signals. The signal intensity immediately rose after excitation (<ns) representing light (L)-state formation. After the thermal grating signal, a decay-rise component in the milliseconds time range (Fig. 1) is observed, which is attributed to a protein diffusion process (diffusion signal). Comparing the changes in the refractive index of the thermal grating ($\delta n_{th}<0$) and of the diffusion signal enabled us to determine the signs of the refractive index changes of the rise and decay components as negative and positive, respectively. From these signs, the rise and decay components of the diffusion signal are assigned to the diffusions of the reactant and product, respectively. Because the rate of the rise component is faster than that of the decay component, the diffusion coefficient of the product (D_P) is apparently smaller than that of the reactant (D_R). This change is reasonable for the assignment of the reaction, that is, dimerization. On the basis of these observations including the cncentration dependence measuermens, we found that eBLUF mostly exists as a monomer in the dark state (D-state). Upon photoexcitation, two types of dimers are formed: one that consists of two L-protomers (LL-dimer), and another that consists of L- and D-protomers (LD-dimer). The laser power dependence of the TG signal indicated that the stability of the LD-dimer is much higher (selective dimerization). Moreover, the dark recovery of the LD-dimer is approximately 20 times slower than that of the LL-dimer and L-monomer. These characteristic features are particularly interesting as potential optogenetic tools, because the LD-dimer is a heterodimer.

Using this heterodimer, we could selectively induce the interaction of different proteins. The dissociation constant Kd of the LD-dimer (Kd(LD)) of eBLUF was ~25 μM in a buffer solution

containing 300 mM NaCl. However, for applications involving light control in cells, a smaller Kd may be desired. Moreover, identifying the dimerization site is important for future improvement of the protein using site-directed mutation techniques. For improving these aspects, we studied the salt-effect on the dimer formation of eBLUF in detail using the TG method and multi-angle light scattering (MALS) method. It was found that the presence of salt decreased Kd by more than 100 times compared with that in a buffer without salt, and this salt effect mostly originated from the cation but not from the anion. These results indicate that the monomer–dimer equilibrium is determined by a balance between the hydrophobic interactions at the dimer interface, which favor the dimer formation, and electrostatic repulsion between the negatively charged sites, which favors the monomer formation. This implies that the addition of salt decreases the electrostatic repulsion by cation binding and results in stabilization of the dimer. On the basis of this finding, we succeeded in identifying a possible dimerization site close to the end of the C-terminal helix. Using this information, we prepared a mutant of D137N, and found that it exhibited much higher stability of the LD-dimer and higher selectivity for dimer formation than the wild-type (WT)-eBLUF.

The results and discussions in more detail will be presented.

REFERENCES

[1] M.Terazima,Time-Dependent Intermolecular Interaction during Protein Reactions, Phys.Chem.Chem.Phys., 13, 16928-16940 (2011).

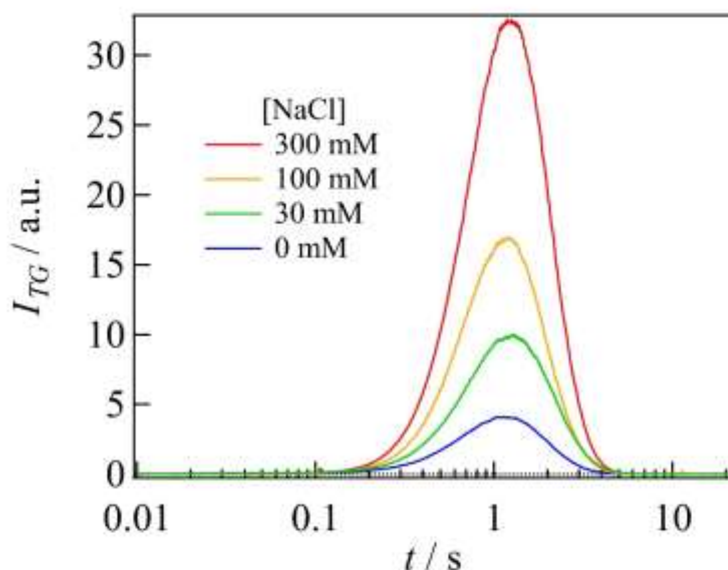

Figure 1: Diffusion signals from the TG measurement at $q^2 = 1.1\times10^{10}$ m^{-2} after the photoexcitation of eBLUF (50 µM) at various NaCl concentrations. The concentrations are indicated in the legend.