Structural dynamics and physical properties of single-chain Fv antibodies against (4-hydroxy-3-nitrophenyl)acetyl

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An antibody can recognize its antigen, and can evolve toward the immunogen to increase binding strength, in a process referred to as affinity maturation. We generated single-chain Fv (scFv) antibodies against (4-hydroxy-3-nitrophenyl)acetyl on the affinity maturation process, such as germline-type N1G9 and affinity-matured-type C6, and analyzed their structural dynamics and physical properties using biophysical methods. The scFvs were designed to comprise the variable regions of light and heavy chains connected by a $(GGGGS)_3$ linker peptide. The scFvs were expressed in *Escherichia coli* in the insoluble fraction, solubilized in the presence of urea, and refolded by stepwise dialysis. Differential scanning calorimetry (DSC) experiments showed that thermal stabilities increased upon antigen binding, due to favorable enthalpic contributions. Antigen binding kinetics of scFvs were comparable to those of the parent intact antibodies, and the antigen binding affinity of C6 was higher than that of N1G9, mainly due to the dissociation rate. Structural dynamics analysis using the diffracted X-ray tracking (DXT) method showed that fluctuations were suppressed upon antigen binding. The antigen binding energy determined from the angular diffusion coefficients was in good agreement with that calculated from the binding kinetics analysis, indicating that the fluctuations detected at single-molecule level are well reflected by antigen binding events.