Abstract

The human mitochondrial single-stranded DNA binding protein (HmtSSB) binds single-stranded DNA (ssDNA) with high affinity and defines the nucleoprotein substrate upon which DNA replication and repair processes must act. It is, therefore, indispensable for mitochondrial and cell survival. We used optical tweezers to measure the binding properties of the HmtSSB proteins to long ssDNA molecules and their elastic and energetic properties. Modeling of the force extension curves of individual nucleo-protein complexes revealed that the HmtSSB proteins use two binding modes to organize the ssDNA, which strongly depend on the ionic strength and protein concentrations. Since different binding modes may be related to different functions, we next investigated if any of these two main binding modes is preferentially used when protein binding is coupled to DNA replication. Under these conditions, only one binding mode was selected for all experimental conditions. These results reveal the crucial role of the gradual release of ssDNA during replication on regulating the HmtSSB binding mode and consequently, on generating the appropriate nucleoprotein structure for subsequent replication of the displaced strand. Similarly, the gradual release of ssDNA during other DNA metabolic processes is expected to regulate the binding modes of SSB proteins exposing multiple OB folds.