Abstract

Heterotrimeric human single-stranded DNA (ssDNA)-binding protein, replication protein A (RPA), is a central player in DNA replication, recombination, and repair. The C terminus of the largest subunit, RPA70, contains a putative zinc-binding motif and is implicated in complex formation with two smaller subunits, RPA14 and RPA32. The C-terminal domain of RPA70 (RPA70-CTD) was characterized using proteolysis and x-ray fluorescence emission spectroscopy. The proteolytic core of this domain comprised amino acids 432-616. X-ray fluorescence spectra collected from RPA14•32-(43-171)•70-(436-616) crystal at SBC CAT, APS, revealed that RPA70-CTD possesses a coordinated Zn(II). The trimeric complex of RPA70-CTD, the ssDNA-binding domain of RPA32 (amino acids 43-171), and RPA14 had strong DNA binding activity. When properly coordinated with zinc, the trimer’s affinity to ssDNA was only 3- to 10-fold less than that of the ssDNA-binding domain in the middle of RPA70. However, the DNA-binding activity of the trimer was dramatically reduced in the presence of chelating agents. Our data indicate that (i) Zn(II) is essential to stabilize the tertiary structure of RPA70-CTD; (ii) RPA70-CTD possesses DNA-binding activity, which is modulated by Zn(II); and (iii) ssDNA binding by the trimer is a synergistic effect generated by the RPA70-CTD and RPA32.

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