The Role of Zinc in Replication Protein A

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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.

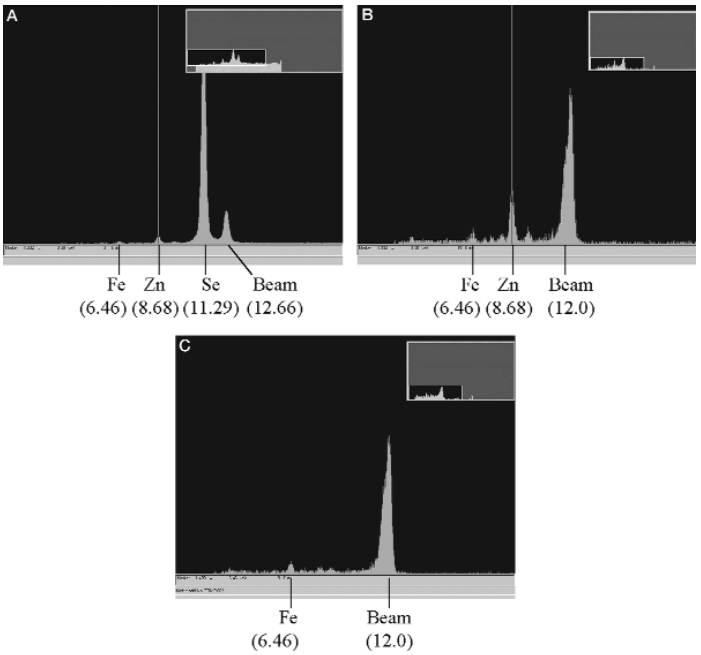

FIG. 1. X-ray fluorescence emission indicates the presence of zinc in RPA14•32-(43-171)•70-(436-616) crystal. (a) An emission spectrum from the crystal was recorded using a beam with an energy of 12.66 keV (selenium absorption K edge). Four peaks, which correspond to the primary beam (Beam), selenium (Se), zinc (Zn), and iron (Fe), are marked. (b) An emission from the same crystal recorded with the energy of 12.00 keV (below selenium absorption edge). Selenium emission has disappeared. (c) The same as (b), but the crystal has been removed. The zinc signal has gone with the crystal, while iron is still present.

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