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Effects of Proton Irradiation on Single-Stranded DNA Studied by Using X-ray Photoelectron Spectroscopy

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X-ray photoelectron spectroscopy (XPS) has been employed in order to study the effects of proton irradiation on herring sperm single-stranded DNA. Systematic changes of the chemical shifts in the C, N, O, and P XPS line components as functions of the irradiation dose were observed, indicative of the bonding configurations in the DNA system. While the C 1s XPS lines showed weak blueshifts, the N 1s, O 1s, and P 2p XPS lines showed blueshifts with a marked dependence on the irradiation dose in a prominent manner. Our results show that linear energy transfer by charged particles and photons may have distinct molecular-level effects as the C 1s, N 1s, O 1s, and P 2p XPS lines showed redshifts in our previous study of effects of the γ-ray irradiation on the same system.

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I. INTRODUCTION

A large proportion of cosmic radiation in low-Earth orbit, where astronauts spend extended periods of time, is composed of MeV-energy protons [1]. Thus, understanding the effects on a biological specimen chronically exposed to such conditions is imperative. Protons, like other particles and photons, induce breaks in deoxyribonucleic acid (DNA) strand as well as abasic and oxidized base damages [2,3]. Single-strand breaks (SSBs) on opposite strands within several helical turns can cause DNA double-strand breaks (DSBs) [4, 5]. Protons and alpha particles produce a significant proportion of small DNA fragments [6], the number of which is believed to increase with increasing linear energy transfer (LET) [6, 7]. The number of DSBs increases little with increasing ionization but a large increase in the relative biological effectiveness (RBE) [8, 9] is seen. A comparison of different ionizing radiations showed that charged particles produced a greater number of DSBs compared to the numbers of abasic and oxidized base clusters than ionizing photons, with protons generating the highest ratio of DSBs to abasic and oxidized damage [4]. Different radiation sources that induce strand breaks can have different outcomes in regard to cluster complexity. For example, damage induced by treatment of cells with protons appears to be more serious than that induced by photon irradiation [10–12]. Several studies have now shown that protons produce smaller DNA fragments than γ- or X-rays, suggesting that protons produce more complex DNA lesions than γ- or X-rays [4,6,13,14]. In fact, a suggestion has been made that low-LET protons will have a greater impact on biological systems than photons when compared to high-LET particles [4].

Ionizing radiation results in cell damage and biological effects such as cell apoptosis, mutation, or carcinogenesis [15–17], whose exact natures still need to be elucidated. The radiation damage to DNA and the properties of the bases have recently been studied by means of X-ray photoelectron spectroscopy (XPS) measurements [18,19]. In this work, XPS has been employed in order to study the effects of low-LET proton irradiation at the molecular level on the chemical bonding configurations in herring sperm single-stranded DNA (ssDNA) in view of the results of our previous work employing γ-ray irradiation on the same system [20].

II. EXPERIMENT

The Herring sperm ssDNA films used in this work were prepared as previously reported by drop-casting onto the quartz substrates [20–23]. The DNA films were subsequently irradiated with 3.2-MeV proton beams to doses

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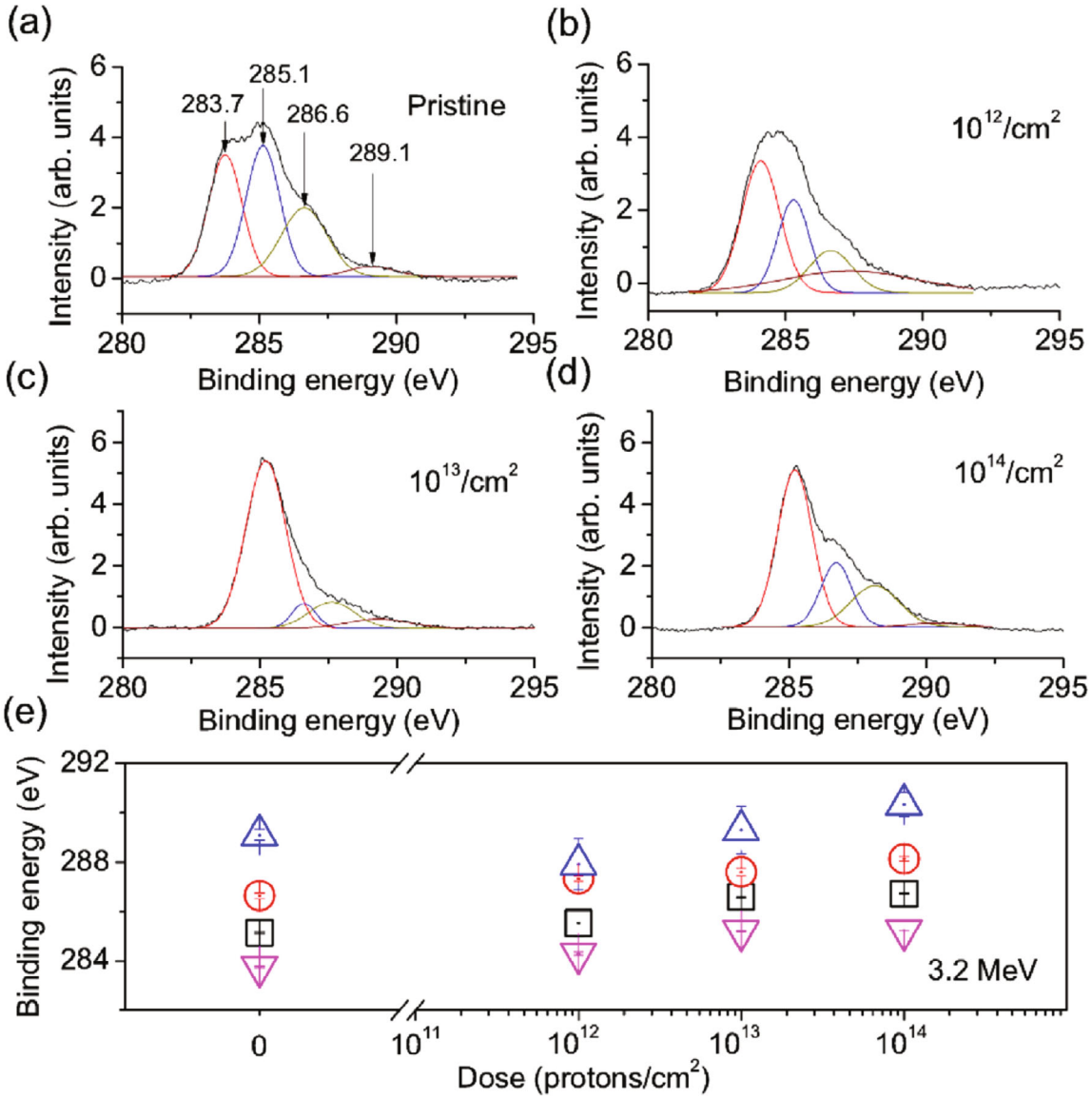

Fig. 1. (Color online) C 1*s* XPS spectra of the herring sperm ssDNA thin film (a) before and after 3.2-MeV proton irradiation at the doses of (b) $1 \times 10^{12}/\text{cm}^{-2}$, (c) $1 \times 10^{13}/\text{cm}^{-2}$, and (d) $1 \times 10^{14}/\text{cm}^{-2}$. (e) Peak positions of the C 1*s* XPS spectra as a function of the proton irradiation dose.

of $1 \times 10^{12}/\text{cm}^{-2}$, $1 \times 10^{13}/\text{cm}^{-2}$, and $1 \times 10^{14}/\text{cm}^{-2}$. The C 1*s*, N 1*s*, O 1*s*, and P 2*p* XPS measurements were made on the pristine and the proton-irradiated samples by using a VG Multilab ESCA 2000 spectrometer with focused monochromatic Mg-K$_\alpha$ X-rays (1253.6 eV) [20].

III. RESULTS AND DISCUSSION

The high-resolution spectra, well fitted by using Gaussian line components, for the pristine and the proton-irradiated ssDNA in the C 1*s*, N 1*s*, O 1*s*, and P 2*p* regions are shown in Figs. 1 to 4, respectively. The assignments of the XPS spectral peaks were made in reference to previous works. The deconvoluted C 1*s* XPS spectrum of the pristine ssDNA (Fig. 1(a)) includes a peak at 286.3 eV (assigned to the C−C and the C−H bonding sites), one at 287.6 eV (C−N, N−C−N, C−O−C, C−OH), one at 288.1 eV (N−C=O), and one at 292.6 eV (N−C(=O)−N) [24–27]. The deconvoluted peaks in the C 1*s* XPS spectrum of the proton-irradiated ssDNA (see Figs. 1(b)−(d)) exhibit weak blueshifts in comparison to those of the pristine sample (Fig. 1(a)). Figure 1(e) shows a weak dependence of the positions of the C 1*s* XPS spectral peaks on the proton irradiation dose.

The principal N 1*s* core-level XPS spectra can be deconvoluted into two distinct peaks (Fig. 2) [24, 26, 28, 29]. The larger peak at 404.4 eV attributed to

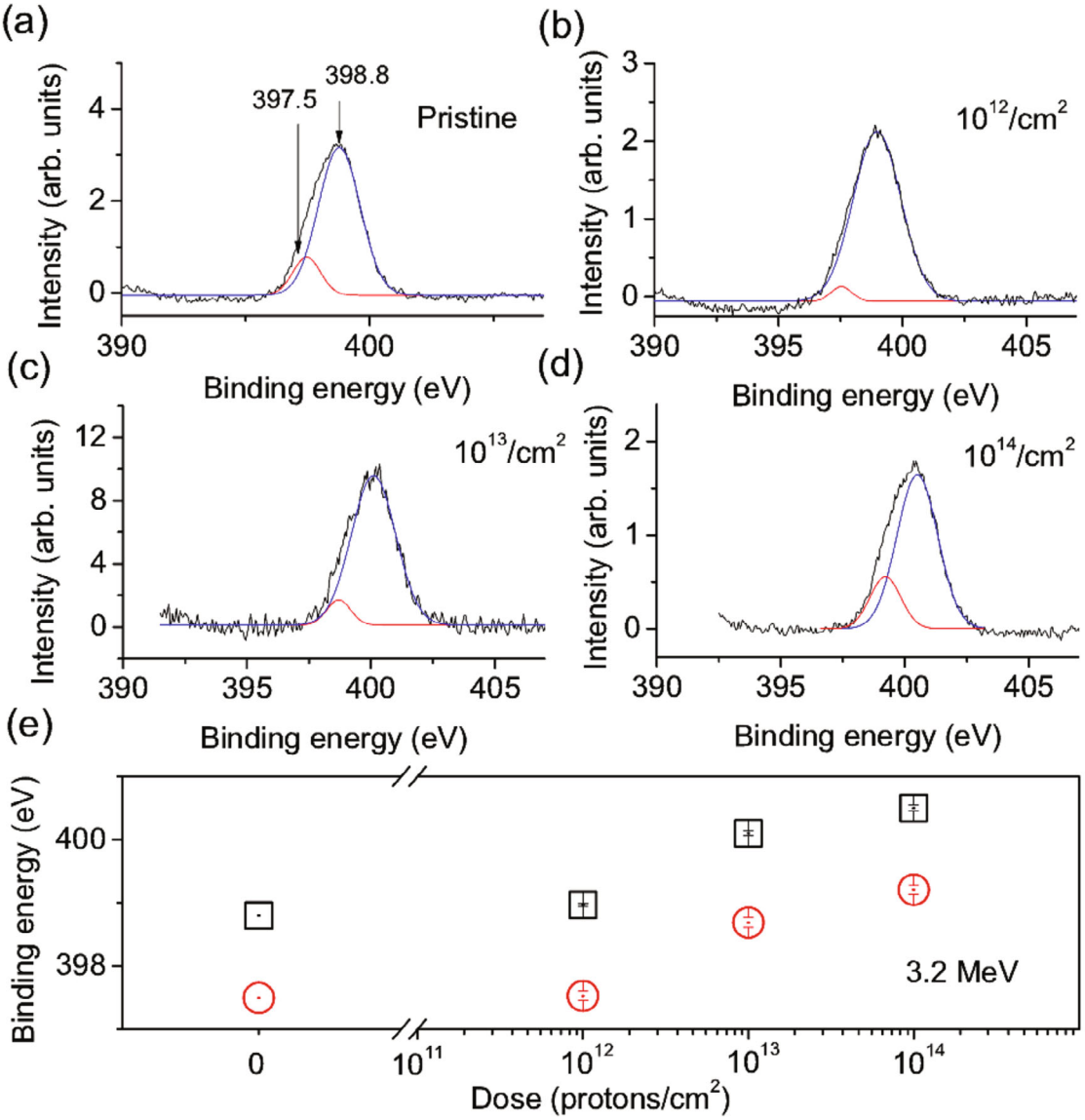

Fig. 2. (Color online) N 1s XPS spectra of the herring sperm ssDNA thin film before (a) before and after 3.2-MeV proton irradiation at the doses of (b) $1 \times 10^{12}/\text{cm}^{-2}$, (c) $1 \times 10^{13}/\text{cm}^{-2}$, and (d) $1 \times 10^{14}/\text{cm}^{-2}$. (e) Peak positions of the N 1s XPS spectra as a function of the proton irradiation dose.

the -NH_2 group and the smaller one at 401.9 eV attributed to the >C=NH bonding in the pristine ssDNA (Fig. 2(a)) undergo blueshifts after proton irradiation (see Figs. 2(b)−(d)) in contrast to the case of the C 1s XPS spectra [30,31]. Figure 1(e) shows the peak positions of the N 1s XPS spectra as a function of the proton irradiation dose, with the N sites displaying similar, noticeable blueshifts with increasing irradiation energy.

Figure 3 shows the O 1s XPS spectra of the ssDNA [28], consisting of a peak at 532.9 eV (O=C, P=O, P-O) and one at 533.9 eV (O−C, P−O−C, C−O−C) in the pristine ssDNA. Figures 3(b)-(d) show that both peaks undergo blueshifts after proton irradiation [32], and Fig. 3(e) shows the irradiation energy dependence of the blueshift for the O bonding sites.

Figure 4 shows that the P 2p spectrum consists of a single Gaussian line attributed to the phosphate group ($PO_4{}^{-2}$). It also undergoes a blueshift arising from the proton irradiation (see Figs. 4(b)−(d)), and Fig. 4(e) shows a prominent increase in the chemical shift with increasing irradiation dose.

We note that the XPS data for pristine ssDNA for C, N, O, and P are different from the ones reported previously [20] because different samples containing different numbers of bases and sugar units were used in this work. In addition, the proton and the γ-ray irradiations gave quite distinct effects even though the XPS data of the sample proton-irradiated to a dose of $1 \times 10^{14}/\text{cm}^{-2}$

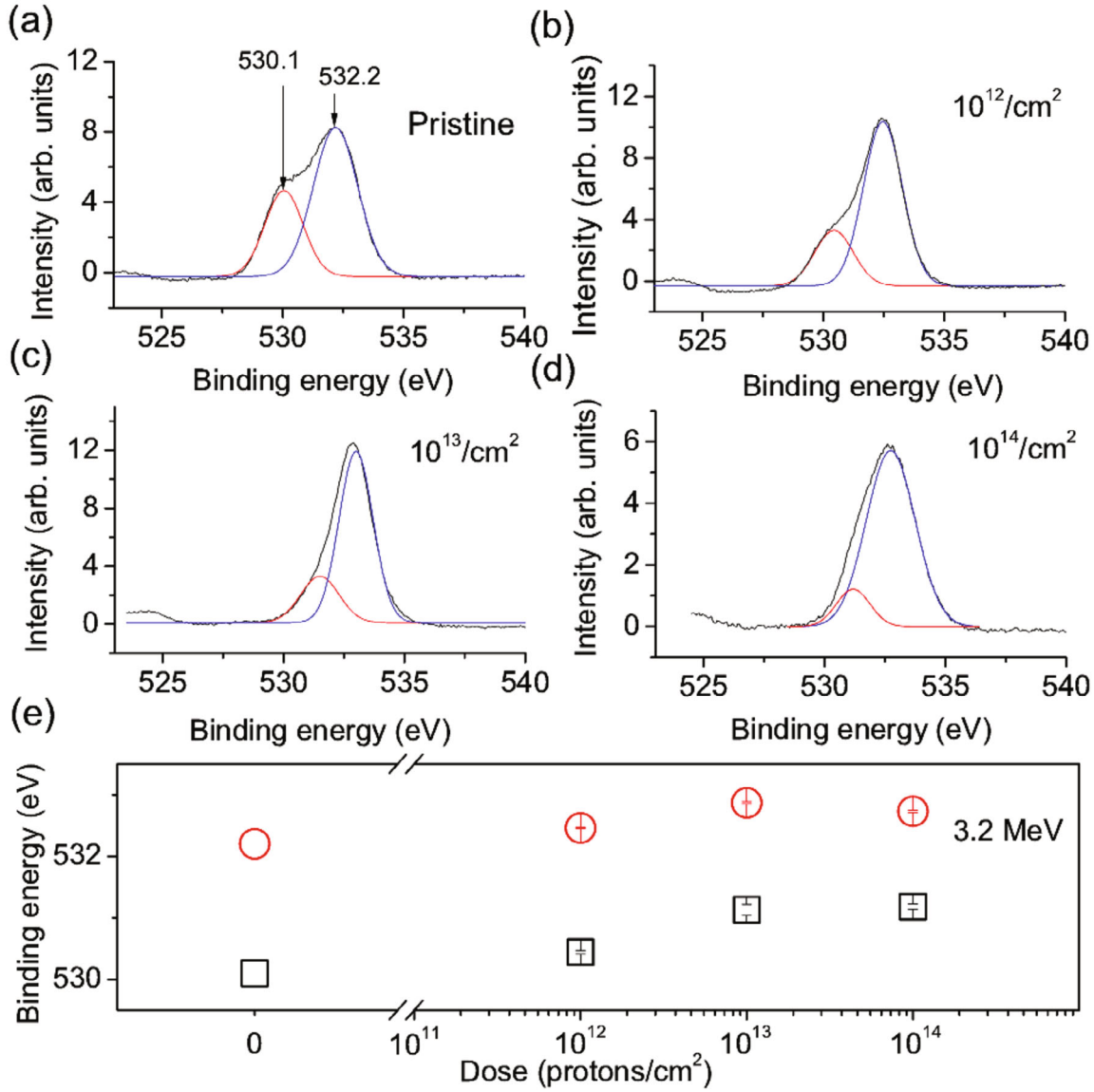

Fig. 3. (Color online) O 1*s* XPS spectra of the herring sperm ssDNA thin film before (a) before and after 3.2-MeV proton irradiation at the doses of (b) $1 \times 10^{12}/\text{cm}^{-2}$, (c) $1 \times 10^{13}/\text{cm}^{-2}$, and (d) $1 \times 10^{14}/\text{cm}^{-2}$. (e) Peak positions of the O 1*s* XPS spectra as a function of the proton irradiation dose.

may look similar to those of the sample γ-ray irradiated to the dose of 20 Gy [20]. Nonetheless, comparing the results of the present work with the results of our previous XPS work, in which the C, N, O, and P XPS line components showed redshifts in the γ-ray-irradiated ssDNA system would be interesting and illuminating. In this work, different energy sources for the ssDNA samples were observed to exhibit opposing energy shifts. In other words, charged massive particles (protons in this work) and massless photons (γ-rays in our case) appear to have distinct effects on the C, N, O, and P bonding sites of the ssDNA samples. This may be due to the collision process of the DNA molecules with particles with mass (protons) or without mass (γ-rays), resulting in distinct changes in the chemical bonding structures and the bonding energies. This may shed new light on the effects of LET on biological systems.

In summary, X-ray photoemission spectroscopy has been employed in order to study the effects of proton irradiation on herring sperm single-stranded DNA. As a result, systematic changes in the chemical shifts in the XPS spectra were identified in the line components of the carbon, nitrogen, oxygen, and phosphorous XPS spectra as functions of the irradiation dose. While the positions of the C 1*s* XPS lines showed blueshifts with a weak dependence on the irradiation dose, the N 1*s*, O 1*s*, and P 2*p* XPS line components showed blueshifts with a marked dependence on the irradiation dose. Thus, distinct be-

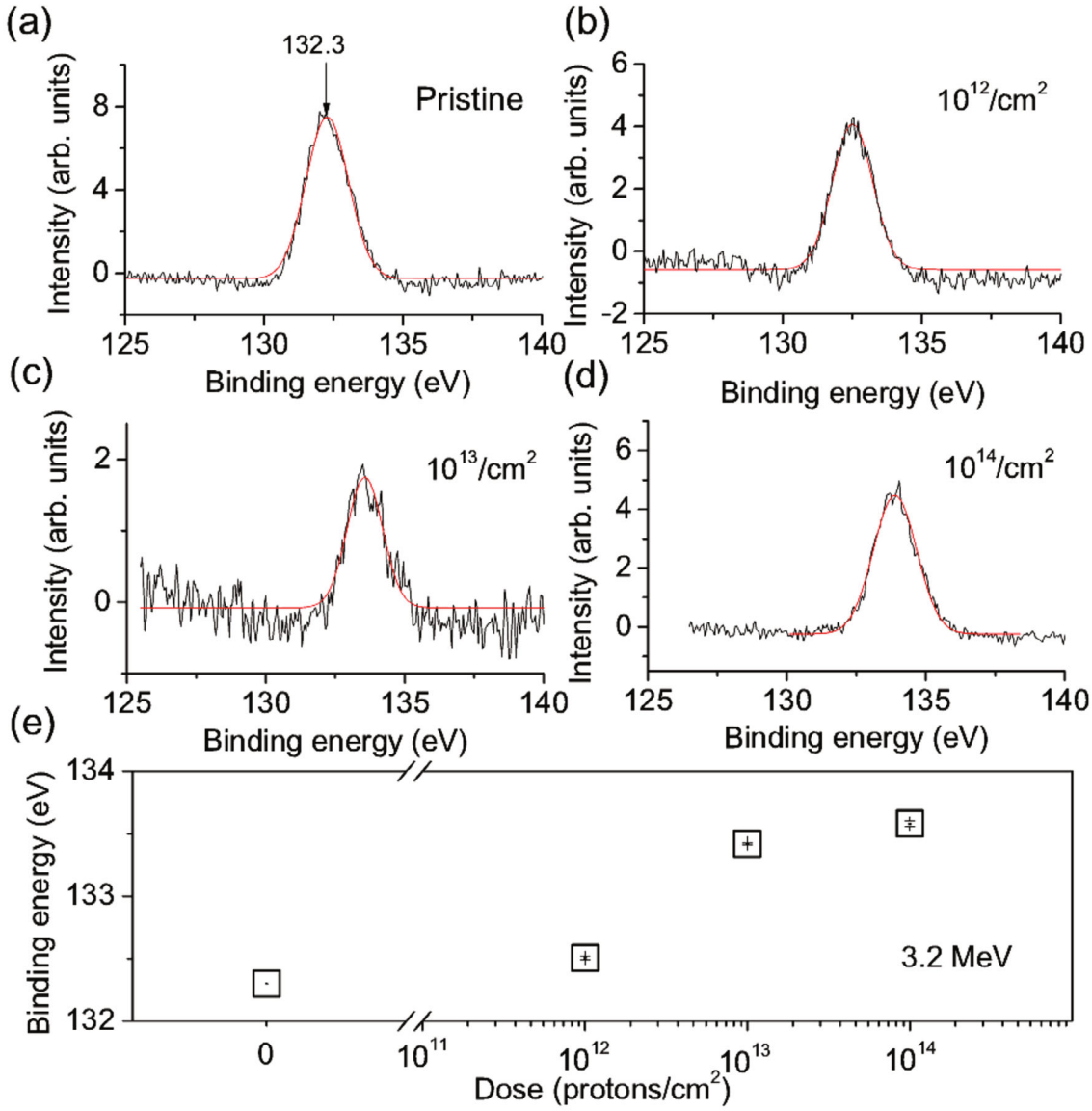

Fig. 4. (Color online) P 2*p* XPS spectra of the herring sperm ssDNA thin film before (a) before and after 3.2-MeV proton irradiation at the doses of (b) $1 \times 10^{12}/\mathrm{cm}^{-2}$, (c) $1 \times 10^{13}/\mathrm{cm}^{-2}$, and (d) $1 \times 10^{14}/\mathrm{cm}^{-2}$. (e) Peak position of the P 2*p* XPS spectra as a function of the proton irradiation dose.

haviors of the XPS line shifts were indicated for irradiations employing protons (charged particles) and γ-rays (photons). Our results may be useful in understanding the mechanisms of LET-induced cell damage and cancer therapy employing proton beams.

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