4 - 71 Influence of Phototoxicity of the Live Cell Imaging System at IMP Microbeam Facility

Liu Wenjing, Guo Jinlong, Wu Ruqun, Wei Junzhe, Chen Hao, Li Yaning, Zhao Jing, Li Xiaoyue and Du Guanghua

To investigate the spatiotemporal dynamics of DNA damage and repair after particle irradiation, an online live cell imaging system has been established based on the microbeam facility at Institute of Modern Physics (IMP). In the experiment of live HT1080 cells (RFP tagged XRCC1) imaging, XRCC1 foci were formed without irradiation, which were mainly caused by the phototoxicity of fluorescence imaging XRCC1 (X-ray repair cross complementing group 1) is a base excision repair protein which is required for DNA single-strand break repair in human cells[1] and XRCC1 is really a sensitive marker to characterize the phototoxicity of the live cell imaging system. In this work, we discussed the influence of phototoxicity of the live cell imaging system. The phototoxicity was investigated through the evaluation of DNA repair protein XRCC1 foci formed in HT1080-RFP cells during the imaging exposure.

The foci induced by phototoxicity started to appear after several minutes of the observation and then the foci emerged constantly and the number increased continuously (as shown in Fig. 1(a)). This was different from the appearance of foci induced by ion hits. The foci induced by ions hits appeared immediately after the irradiation, and the number remained constant (as shown in Fig. 1(b)). However, the relative intensity of foci induced by phototoxicity is about 1.1 (as shown in Fig. 1(c)), which is close to the diffused XRCC1 intensity inside the nucleus. So the foci induced by phototoxicity are difficult to be recognized. Actually, they were not observed in the irradiated cells. Each heavy ion induced focus contains much more SSBs than those in the focus induced by phototoxicity[2], so the most of free XRCC1 molecules in the nucleus were recruited to foci induced by heavy ion hits. The measurements of relative intensity of the foci induced by phototoxicity and ion hits were done in different cell samples.

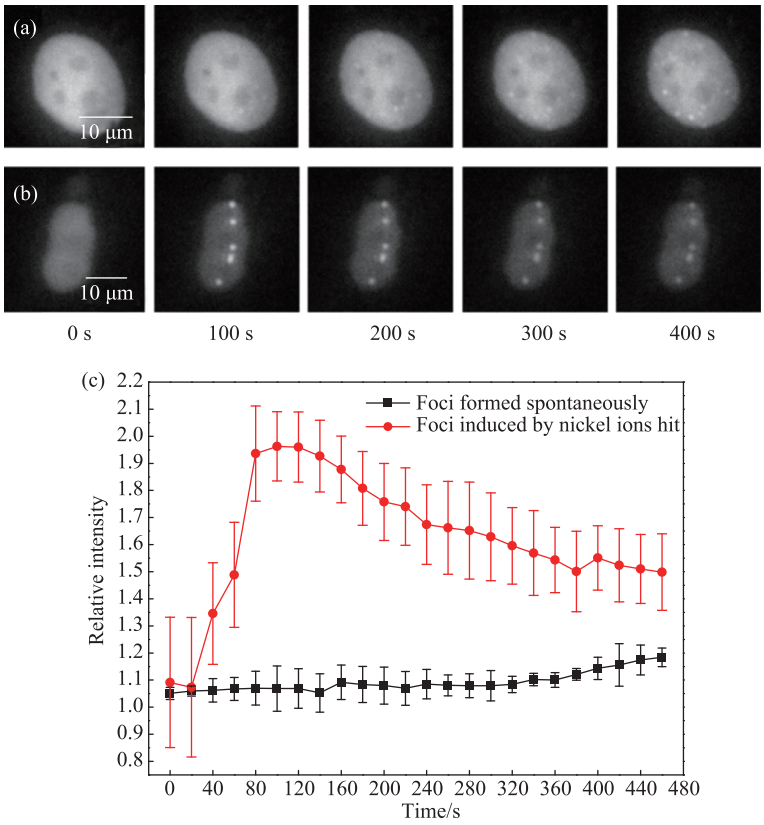

Fig. 1 The fluorescence images and kinetics of DNA repair protein XRCC1 in the foci caused by phototoxicity and heavy ion hits. (a) The fluorescence images of foci caused by phototoxicity. (b) The fluorescence images of foci caused by Ni ion hits. (c) The fluorescence kinetics of XRCC1 in the foci caused by heavy ion hits (solid cycle), compared with those formed spontaneously in non-irradiated cells (solid rectangle). The ion irradiation started at about 15 s. The time when the relative intensity began to rise was defined as zero for the foci induced by phototoxicity, because it usually took several minutes to appear after exposure.

The recruitment kinetics of DNA repair protein XRCC1 in the foci caused by phototoxicity and heavy ion hits were investigated. Fig. 1(c) shows that the intensity of foci induced by heavy ion hits rises rapidly and reaches the maximum (about 200% to the original intensity) within 80 s after irradiation, then declined slowly. This was because the free XRCC1 molecules in the nucleoplasm were recruited to the DNA strand breaks caused by nickel ion hits immediately after irradiation. Then the XRCC1 molecules were released when the repair was fished, and the free molecules can be recruited to other damages[3]. By contrast, the intensity of foci caused by phototoxicity increased very slowly (increase about 15% within 400 s, almost no change within 80 s). This demonstrates that the XRCC1 intensity at the SSBs formed by phototoxicity was much lower and had slower recruitment than the heavy ion induced DNA damage foci, and the spontaneous XRCC1 foci had little effect on the live cell imaging study of XRCC1 protein kinetics after ion irradiation. The phototoxicity induced XRCC1 foci provide a useful method to study the XRCC1 kinetics at single SSBs and can be used to verify the reliability of a live cell imaging system.

In conclusion, a few foci may form spontaneously due to the phototoxicity but this had little impact on the analysis of the XRCC1 recruitment induced by the ion irradiation.

References

[1] R. Brem, J. Hall, Nucleic Acids Research, 33(2005)2512.

[2] A. Hauptner, W. Friedland, S. Dietzel, et al., Royal Danish Academy Sciences & Letters, Copenhagen V, (2006)59.

[3] N. Guo, G. H. Du, W. J. Liu, et al., Rev. Sci. Instrum., 87(2016)5.

4 - 72 Interpretation of the Rectification of Single Conical Nanopores

Wei Junzhe, Du Guanghua, Liu Wenjing, Li Yaning, Guo Jinlong, Wu Ruqun and Artem Ponomarov

Due to the symmetry breaking, single conical nanopores show characteristics of ion-current rectification, which has a great potential in applications to ion separation, power supplier and chemical valve[1−5]. It is of great importance to study the electrical properties of nanopores and the modification methods of conical nanopores in order to understand the ion transport properties in nanoscale structures and to improve the application performance of conical nanopores.

Single conical nanopores were etched in tracked PET membranes (irradiated by single 6.9 MeV/u $^{58}Ni^{19+}$ ion in LIHIM). One side of the membrane was bathed in etchant, 5 mol/L sodium hydroxide solution at 50 ℃, and the other side was in a stop solution, 1 mol/L HCOOH and 1 mol/L KCl. Single conical nanopore with tip diameter of 9 nm and base diameter of 1 200 nm was used in the experiments.

Fig. 1 (a∼c) show the I-V curves of the same conical nanopore measured in the LiCl, NaCl, KCl solutions at different concentrations. The result shows that all the I-V curves were significant asymmetry and nonlinearity, which demonstrates the ion-current rectification in single conical nanopores. Fig. 1(d) shows the rectification coefficient γ in the solutions of LiCl, NaCl and KCl at different electrolyte concentration at 2 V. The ionic concentration-γ relationships of KCl, NaCl and LiCl solutions were similar. The rectification coefficient γ increased from about 2 to about 12 with the electrolyte concentration from 0.0001 mol/L to 0.0316 mol/L, and decreased from about 12 to about 3 with the electrolyte concentration from 0.0316 mol/L to 1 mol/L at 2 V.

To explain the experimental phenomena, a new theory was proposed. The influence of pore wall adsorption charge on potential depth is considered in this new theory, which is neglected by other theories. The potential in the single conical nanopores is shown in Fig. 2[1], and there is a deep potential well in tip. When the single conical nanopores is in the electrolyte solution, the distribution of charge in nanopores is shown in Fig. 3(a). The adsorption of positive charges on the pore wall will change the potential well depth (shown in Fig. 3(d)). “0” curve indicates the potential well depth of no voltage, “+” curve indicates the potential well depth of positive voltage and “-” curve indicates the potential well depth of negative voltage. “+” well potential depth is shallower than “-” potential well depth, this is because the positive charge is accumulated in the pore wall at the positive voltage (shown in Fig. 3(b)), while it is depleted at the negative voltage (shown in Fig. 3(c)). The existence of the potential well in tip can accelerate the cation through the nanopores, and the difference of the potential well depth under the positive and negative voltage leads to difference of the current.

The trends of current rectification coefficient first increased and then decreased with the electrolyte concentration from 0.0001 mol/L to 1 mol/L. A small amount of cations in the low concentration solution results in little difference in the potential well depth (ΔV) under the positive and negative voltage, so the rectification coefficient γ is small. When the solution concentration increases and approaches the optimum concentration, ΔV will raises with the increase of cations in the solution, so the γ becomes larger. When the concentration exceeds the optimum concen-