

Electron beam-induced electrostatic charging causes spectral changes of an insulating electrochromic material

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ABSTRACT

Electrostatic charging of electrically insulating samples upon electron irradiation plays a large role in the analysis and processing of materials in biosciences and the semiconductor industry. We have analyzed spectral changes of purple membranes (PMs) containing bacteriorhodopsin, an electrochromic biological material, upon irradiation with electrons at an acceleration voltage of 30 kV. We observed a bathochromic shift in the UV/VIS spectrum of PM films, due to internal electric fields generated by charges injected into the films. The experiments demonstrate that spectroscopic changes, accompanying electron beam-induced charge injection into electrochromic materials, enable quantitative analysis of electrostatic charging of insulators upon electron irradiation by optical spectroscopy.

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Understanding electrostatic charging of insulating materials upon electron irradiation has tremendous importance for technological applications of electron beams. It has long been recognized that electron beam-induced charging of frozen hydrated samples in electron cryo-microscopy causes loss of high-resolution information.¹ Furthermore, the balance between primary electrons impinging on an electrically insulating sample, concomitant emission of backscattered and secondary electrons, and charges implanted in the material determine the quality of scanning electron microscopy (SEM) images^{2–5} and are of critical importance in electron lithographic processing of insulating samples.^{6,7} Electrostatic charging of organic photoresist contributes to the edge placement error in electron beam lithography in the semiconductor industry and has been extensively studied in previous work.^{6–9} Furthermore, electron beam-induced charging has been used to fabricate nanostructures by self-assembly of molecules on charged surfaces.^{10–12}

Owing to its importance for research and technology, a quantitative understanding of electron beam-induced charging is important to improve the analysis and processing of insulating materials with electron beams. However, the analysis of time-dependent charge distributions in the bulk of insulating materials remains challenging. Previously, Kelvin force microscopy has been used to analyze the electrostatic surface charges of photoresist upon electron irradiation.⁷

It would be highly desirable to obtain quantitative information about the time-dependent electron beam-induced charging of an insulating material *in situ*. In this work, we have used the protein bacteriorhodopsin (BR) to analyze spectroscopic changes upon electron irradiation. BR is the only protein in the purple membrane (PM) from *Halobacterium salinarum*.^{13–15} The purple color of wild-type BR is due to its retinal chromophore with an optical absorption maximum at 570 nm. Due to its photochromic properties and astonishingly high stability toward extreme environmental conditions, BR has been studied as material for technological applications such as data storage.^{16–22}

In our experiments, we have used PM/gelatine films as well as pure PM films deposited on glass substrates. PM films were mounted in the chamber of a Zeiss Auriga FIB/SEM and irradiated with electrons using an acceleration voltage of 30 kV and a current of 4.08 nA as measured with a Faraday cup. Samples were mounted at a distance of 49.2 mm from the pole piece. It is worth noting that PM films can be exposed to the vacuum of a SEM chamber without any damage to the material.

Figure 1 illustrates the experimental setup.

Upon electron irradiation of PM films, we observed a considerable bathochromic shift of the irradiated area [Fig. 2(a)] accompanied by a visible color shift from purple to blue [Fig. 2(a), inset].

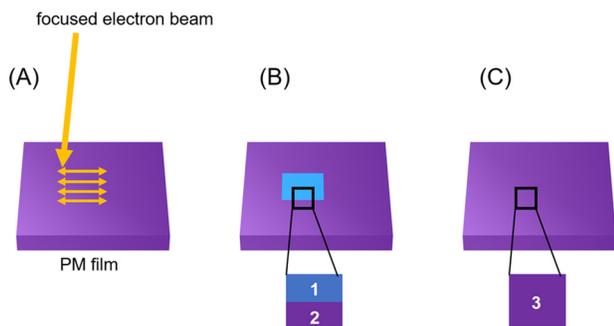


FIG. 1. Experimental setup. (a) A PM film is mounted in the vacuum chamber of a SEM and irradiated with electrons. (b) Upon irradiation with the electron beam, the exposed area of the PM film shows a bathochromic shift of its absorption spectrum and appears blue. (c) The purple color of the PM film recovers indicating a reversible electrochromic spectral change. The numbers resemble the labels in the inset of Fig. 2(a).

Apart from some loss in optical density (OD) due to electron beam-induced bleaching of BR,²³ the spectral changes are fully reversible.

In another experiment, we acquired an UV/VIS spectrum of a PM film, irradiated for 30 min with electrons in the SEM and acquired UV/VIS spectra of the same area, confirming the formation of a bathochromic species [Fig. 3(a)]. Time-dependent UV/VIS spectra demonstrate that the electron beam-induced bathochromic state disappears within 30 min in the dark [Fig. 3(b)].

Several physical and chemical conditions have been described, which favor the reversible or irreversible formation of bathochromic species in BR, among them are the removal of divalent cations,²⁴ the protonation of aspartic acid 85,²⁵ and two-photon-induced decarboxylation of aspartic acids in the vicinity of the protonated Schiff base of the retinal chromophore.^{21,26,27} However, none of these conditions match the conditions of the experiments described in this work. An 8 μm thin aluminum foil, placed on top of the PM film during electron irradiation, suppressed the formation of the bathochromic species (data not shown). This excludes electron beam-induced generation of x-rays as a source for spectral changes in BR.

It has been observed in previous work that strong external electric fields can induce a reversible bathochromic shift in PM films.²⁸ We propose that the bathochromic shift of PM films observed in our experiments is caused by electric fields generated by charges injected into the films upon electron irradiation. In other words, the retinal chromophore of BR is an electrochromic probe, which enables quantitative analysis of internal electrostatic fields by optical spectroscopy. PM films are electrical insulators. When a PM film is irradiated with electrons at an acceleration voltage of 30 kV, the number of secondary electrons escaping from the sample is lower than the number of electrons implanted in the material leading to a net negative charge.²⁹ Previous work has demonstrated that electron beam-induced electrical charges remain trapped in insulating substrates, long enough to generate patterns of adsorbed biomolecules and nanoparticles.^{10,11,30–32} Figure 4 shows a model of electron beam-induced electrochromic changes in BR. As the spectral ground state of BR recovers in the dark, we conclude that charge neutralization in the film is driven thermally.

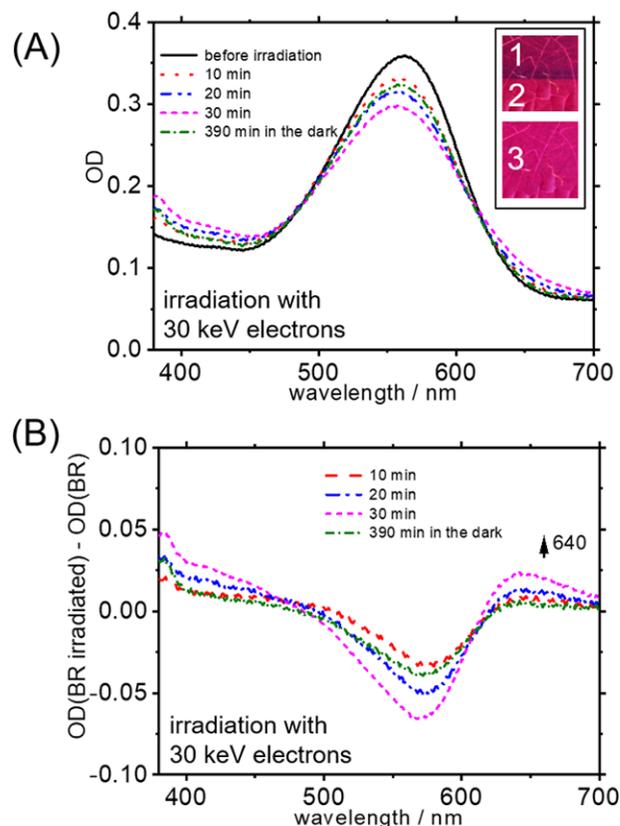


FIG. 2. Bathochromic shift of PM films upon electron irradiation. (a) Shown are UV/VIS spectra of a PM film after 10, 20, and 30 min irradiation corresponding to cumulative electron doses of 3.6, 7.2, and 10.8 $\mu\text{C}/\text{cm}^2$. After 10 min of irradiation in the SEM chamber, PM films were transferred to a UV/VIS spectrometer and spectra were acquired in transmission mode. Subsequently, the films were transferred back into the SEM chamber and irradiation was continued. Finally, the PM film was kept in the spectrometer in the dark for 390 min. Apart from some loss in OD due to bleaching, the purple ground state of BR recovers. (Inset, top) photograph of a PM film. The top part of the film (labeled 1, also see Fig. 1) was irradiated for 30 min with the electron beam and appears blue, whereas the bottom part was not irradiated (labeled 2, also see Fig. 1) (inset, bottom, labeled 3, also see Fig. 1). After 300 min, the bathochromic state was reversed to the initial state and the color difference between the areas has disappeared. (b) Time-dependent difference spectra of a PM film upon electron irradiation, revealing the formation of a bathochromic species absorbing at 640 nm at the expense of the ground state.

At present, the strength of the electric field, generated by trapped electrostatic charges in PM films, is not known. In previous experiments, external electric fields with a strength of 10^4 – 10^5 $\text{V} \cdot \text{cm}^{-1}$ were enough to induce the reversible formation of a bathochromic species.²⁸ We point out that further experiments are required to quantitatively elucidate the charge distribution in the insulating PM film. Future experiments should be performed using a spectrometer integrated into the vacuum chamber of the SEM to enable *in situ* spectroscopic analysis of spectral changes upon electron irradiation. Another interesting question is how the trapped charges move in the substrate and become neutralized, as the spectral ground state of BR recovers.

Spectral changes of electrochromic materials upon electron irradiation have not been described before. Apart from BR, it would be

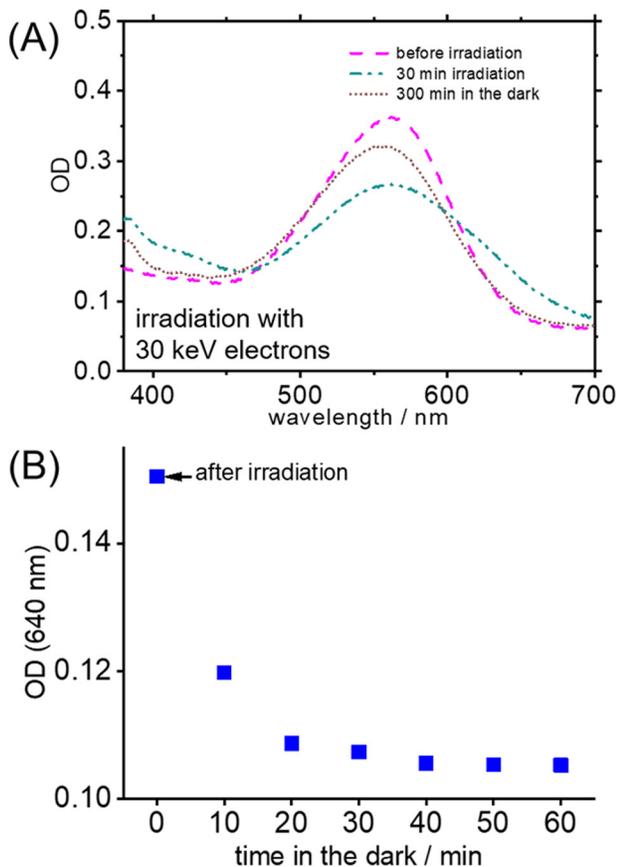


FIG. 3. Time-dependent UV/VIS spectroscopy of PM films after electron irradiation. (a) Electron irradiation of a PM film for 30 min, corresponding to an electron dose of $10.8 \mu\text{C}/\text{cm}^2$, leads to the formation of a bathochromic species. (b) Time-dependent UV/VIS spectra acquired from a PM film, irradiated with electrons for 30 min, after transfer into the UV/VIS spectrometer. Shown is the OD of the bathochromic species. After 30 min in the dark, the electron irradiation-induced bathochromic state has disappeared.

interesting to study electron beam-induced spectral changes in other electrochromic materials.³³ From a technological point of view, insulating electrochromic materials could be used to study time-dependent electrostatic charging of materials upon irradiation with ionizing

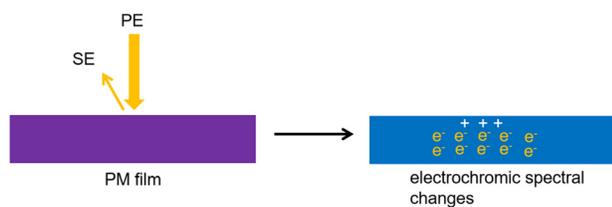


FIG. 4. Model for electron beam-induced electrochromic changes in PM films. An electrically insulating PM film is irradiated with an electron beam. The primary electrons (PE) generate secondary electrons escaping from the surface, leading to positive charging of the surface, whereas negative charges are trapped deep in the film. The electrical field, generated by trapped charges, causes electrochromic spectral changes in BR.

radiation. A quantitative understanding of radiation-induced electrostatic charging of insulators would be of tremendous importance for a variety of technologies used in the semiconductor industry, such as electron lithography and extreme ultraviolet lithography,^{6–9} electron beam inspection of wafers,³⁴ and photomask repair using electron beam-induced processes.³⁵ Furthermore, we conclude that it should be possible to use electron beams to induce spectroscopic changes in electrochromic materials on small length scales, which might find applications in data storage.

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An application for a patent on the method has been filed.

DATA AVAILABILITY

The data that support the findings of this study are available within this article.

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