EVALUATING ELECTRON BEAM DAMAGE IN PROTEIN CRYSTALS USING TRANSMISSION ELECTRON MICROSCOPY

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Electron beam damage in proteins have been studied for about 40 years. Early studies showed that proteins are damaged at very low doses, e.g. $10^{-2}$ Å\textsuperscript{2}, but in the last years, reports applying total doses up to $80\times10^{-2}$ Å\textsuperscript{2} have been shown. Diffraction in transmission electron microscopy has been used as a tool for measuring damage in protein crystals, were intensities of spots are associated to the protein integrity. However, these intensities combine protein and crystal integrity information. This work aims to improve this technique, to determine the damage to the protein associated to different electron doses. Catalase crystals obtained from sodium chloride aqueous suspension followed by dialysis against 50 mM sodium phosphate pH6.3 were used as standard sample. Diffraction patterns were acquired with total accumulated radiation up to $48\times10^{-2}$ Å\textsuperscript{2} and resolution between 1.2-2.9 Å\textsuperscript{-1}. It was observed that the intensity of diffraction spots decreases 50\% with $5\times10^{-2}$ accumulated radiation, suggesting that periodicity in protein crystals is strongly affected in the very beginning of the radiation exposure. Applying $10\times10^{-2}$ s\textsuperscript{-1} dose, the spots disappear with total accumulated radiation of $20\times10^{-2}$ Å\textsuperscript{2}. The use of $20\times10^{-2}$ s\textsuperscript{-1} dose increases the disappearance rate of diffraction spots, suggesting that dose rate is an important variable in the process, which could explain for the use of total doses of up to $80\times10^{-2}$. As a result, the fading of spots with accumulated radiation is initially related to loss of periodicity in the catalase crystal caused by electron beam damage and later to protein disrupt. The methodology applied here ensures reliability and accuracy on the damage quantification caused by electron beam radiation in protein crystals.

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