Selenium is the most widely used heavy atom for experimental phasing, either by single anomalous scattering (SAD) or multiple-wavelength anomalous dispersion (MAD) procedures. The use of the single isomorphous replacement (SIR) or single isomorphous replacement with anomalous scattering (SIRAS) phasing procedure with selenomethionine (Mse) containing proteins is not so commonly used, as it requires isomorphous native data.

Several non-redundant X-ray diffraction data sets from various Mse derivatised protein crystals were collected at energies far below the absorption edge before and after exposing the crystal to ultraviolet (UV) radiation with 266 nm lasers. A detailed analysis revealed that significant changes in diffracted intensities were induced by ultraviolet irradiation whilst retaining crystal isomorphism. These intensity changes allowed the crystal structures to be solved by the radiation damage-induced phasing (RIP) technique [1]. These can be coupled with the anomalous signal from the dataset collected at the selenium absorption edge to obtain SIRAS phases in a UV-RIPAS phasing experiment [2].

Inspection of the crystal structures and electron-density maps demonstrated that covalent bonds between selenium and carbon at all sites located in the core of the proteins or in a hydrophobic environment were much more susceptible to UV radiation-induced cleavage than other bonds typically present in Mse proteins. The rapid UV radiation-induced bond cleavage opens a reliable new paradigm for phasing at synchrotron [1,2] and in house X-ray source[3].


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