Poster Presentation

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Mechanism and X-ray damage: beware of artefacts in Xylose Isomerase

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Radiation chemistry causes structural perturbations with significant impact to the accurate structural understanding of mechanism, especially in metalloproteins. Xylose isomerase (XI) is an example of this issue: it catalyzes the reversible interconversion of the aldoses D-xylose, D-ribose, and D-glucose to the corresponding ketoses. XI requires a divalent metal ion such as Mn2+, Mg2+ or Co2+ as a cofactor [1]. It is an important industrial enzyme; XI is used extensively in the food industry, for example in producing high-fructose corn syrup, and it also holds enormous potential for application in converting biomass into fuels and chemicals. Thus understanding its mechanism and thereby potentially improving its suitability for industrial applications is even more important. The reaction mechanism has been studied in increasingly detailed studies, many discussing the mobility of the catalytic metal ion. We show that this mobility can be driven by the X-rays used to reveal the structure, and that it may not be mechanistically critical.

X-rays generate free radicals when interacting with macromolecule crystals. These radicals propagate changes that result in both global effects and specific structural changes, even when cryogenic temperatures (around 100 K) are used to minimize the effects. Global radiation damage is observed as a loss of diffraction intensity with the weakest higher resolution reflections fading first, an increase in unit-cell volume, higher Wilson B-factors, and often overall higher mosaicity of the crystal [2]. These effects can preclude structure solution. On the other hand, specific damage, contributing to the global effects, is seen from the electron density maps after refinement, and manifests as the breakage of disulfide bonds, decarboxylation of acidic side chains and photoreduction of metal atoms [3] potentially leading to incorrect biological deductions being made from the resultant structural model.

The effects of radiation damage on the metalloprotein, XI, have been investigated. By collecting consecutive synchrotron X-ray diffraction datasets at 100 K on a substrate free XI crystal, radiation induced changes in the structure were tracked as a function of dose. The metal and the surrounding amino acid environment experience a buildup of free radicals, making it more susceptible to damage than the rest of the protein. The radiation damage induced structural perturbations are similar to structural alterations that have been attributed to the mechanism, i.e. the metal ion used as a cofactor occupied several different positions. Thus the X-ray induced metal movement observed with XI appears to be an artifact of the method used to derive the structure, and neutron based structural studies have since illuminated the mechanism. All metals will be sensitive to radiation effects, as they have a high X-ray absorption cross-section. This has profound implications for the high resolution study of metalloproteins in general and is especially pertinent to the study of enzymes, since metalloproteins make up over half their population. Understanding and identifying radiation induced structural perturbation is critical to an accurate structural interpretation of mechanism.

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