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Radiation damage within nucleoprotein complexes studied by macromolecular X-ray crystallography

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HIGHLIGHTS

- We review radiation damages nucleoprotein complexes during X-ray crystallography.
- We detect radiation-induced chemical changes from electron density difference maps.
- We use a systematic pipeline to track electron density loss with increasing dose.
- Nucleic acids are radiation-insensitive compared to protein within crystals at 100 K.
- RNA protects key RNA-binding residues from radiation-induced decarboxylation.

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ABSTRACT

In X-ray crystallography, for the determination of the 3-D structure of macromolecules, radiation damage is still an inherent problem at modern third generation synchrotron sources, even when utilising cryo-crystallographic techniques (sample held at 100 K). At doses of just several MGy, at which a typical diffraction dataset is collected, site-specific radiation-induced chemical changes are known to manifest within protein crystals, and a wide body of literature is now devoted to understanding the mechanisms behind such damage. Far less is known regarding radiation-induced damage to crystalline nucleic acids and the wider class of nucleoprotein complexes during macromolecular X-ray crystallography (MX) data collection. As the MX structural biology community now strives to solve structures for increasingly larger and complex macromolecular assemblies, it essential to understand how such structures are affected by the X-ray radiation used to solve them. The purpose of this review is to summarise advances in the field of specific damage to nucleoprotein complexes and to present case studies of MX damage investigations on both protein-DNA (C.Esp1396I) and protein-RNA (TRAP) complexes. To motivate further investigations into MX damage mechanisms within nucleoprotein complexes, current and emerging protocols for investigating specific damage within $F_{obs}(n) - F_{obs}(1)$ electron density difference maps are discussed.

1. Introduction

Over the past century, macromolecular X-ray crystallography (MX) has proved an invaluable tool within structural biology, permitting the three-dimensional visualisation of proteins and nucleic acids at near atomic resolution. As of early 2016, a total of 104,244 structures of proteins and nucleoprotein complexes have been deposited within the Protein Data Bank (PDB); this world-wide effort has been instrumental in the correct characterisation of the molecular interactions underpinning the function of highly biologically-important macromolecules (Ferreira et al., 2004; Voorhees et al., 2009).

During a standard MX experiment, crystalline macromolecular samples are exposed to a monochromatic beam of ionising X-rays (with typical energies 8–15 keV) in order to generate a series of diffraction images, from which a temporal and spatial average of the macromolecular electron density throughout a crystal can be derived. However, for a typical 100 μm thick protein crystal exposed to a 12.4 keV X-ray beam, only a small proportion (2%) of incident photons are predicted to actually interact directly with the crystal, with only 8% of these elastically scattering to contribute to the desired diffraction pattern. The photoelectric and Compton effects are the dominant processes by which the incident

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photon energy is absorbed by the crystal (84% and 8% respectively) (Garman, 2010). Consequently, for the current third generation high-brilliance synchrotron sources with photon fluxes in the range of 10^{10}–10^{13} ph/s into a 5–100 μm diameter spot, this results in a large deposition of energy within typical macromolecular crystals upon an X-ray exposure of a few frames of data collection.

The dose is defined as the energy absorbed in the crystal per unit mass (with units of gray, Gy=J/kg); protein crystals typically accumulate doses of the order of several MGy within the course of one diffraction dataset collection at 100 K. Increasing dose is coupled with increasing crystal non-isomorphism, eventually leading to *global radiation* damage effects: an overall reduction in the mean diffraction intensity, loss in resolution, and increases in both crystal mosaicity and unit cell volume (Murray and Garman, 2002; Ravelli and Garman, 2006; Weik et al., 2000). An experimental dose limit ($D_{0.7}$) of 30 MGy has been determined, cited as the upper dose at which the average diffraction intensity for *any* protein crystal held at 100 K will have decayed to 70%, and at which the biological information derived from the inferred structure of the crystalline protein may be compromised (Owen et al., 2006). Due to these various effects, radiation damage is still an inevitable hindrance to successful MX data collection and structure determination, even with the crystal held at cryocooled temperatures (100 K) and with modern advances in multi-crystal data collection methods (Stellato et al., 2014), high X-ray flux density nano- and micro-beamlines (I24) and faster pixel based detectors, also at room temperature (RT) (Owen et al., 2014). Such issues are exacerbated as experimenters push towards structure determination of larger and more complex macromolecules, which in turn typically involve collecting data from smaller crystals (< 1–5 μm). These have fewer unit cells compounded with intrinsic crystal disorder, resulting in weaker diffraction. There must always be a compromise between collecting more diffraction data and the inevitable progression of radiation damage with increasing dose.

Even before any observed reduction in diffraction resolution, at doses of several MGy, synchrotron X-ray irradiation has been widely reported to induce site-specific chemical and conformation changes to crystalline proteins held at 100 K. Such damage has been directly observable within electron density maps reconstructed from sequential diffraction patterns obtained with increasing dose (Weik et al., 2000) (Fig. 1), and a reproducible order of susceptibility has now been established within a variety of proteins at 100 K: metallocentre reduction, disulphide bond elongation and cleavage, acidic residue decarboxylation, and reported Tyr –OH group disordering and methionine S_δ–C_ε bond cleavage (Burmeister, 2000; Weik et al., 2000; Yano et al., 2005). The prevalence of these *specific radiation damage* (SRD) effects are suspected to be highly dependent on local crystalline protein environment, with factors such as solvent accessibility, proximity to high X-ray cross-section atoms, acidic residue protonation state and packing density (Fioravanti et al., 2007; Gerstel et al., 2015) predicted to affect the damage rates. However, investigations into a number of protein systems have revealed minimal correlation between such individual factors and SRD events, and it is thus suggested that a multitude of parameters contribute to a particular residue's susceptibility to SRD (Holton, 2009). For instance, the addition of a single ordered nitrate anion (NO_3^-) proximal to a disulphide bond will result in substantial stability of the otherwise highly reducible bond (De La Mora et al., 2011). At doses below 1 MGy, disulphide bond radicalisation (a precursor for disulphide breakage) has been detected by electron paramagnetic resonance (EPR) and *in situ* UV–visible absorption microspectrophotometry (Sutton et al., 2013). Radiation-induced structural changes within active sites of photosensitive proteins have also been detected at doses as low as 0.06 MGy (Borshchevskiy et al., 2014). As such, it is unclear whether a universal safe dose is achievable in MX before the onset of protein specific damage events.

Somewhat paradoxically, given the wealth of radiation damage studies on nucleic acids conducted by radiation chemists (e.g. (Alizadeh et al., 2015; Cadet et al., 1999; Michaud et al., 2012)), crystallographic investigations regarding MX radiation-induced changes to nucleic acids and the larger class of nucleoprotein complexes have been substantially less comprehensive to date, and a governing MX specific damage *rulebook* for them has not yet been established. Nucleic acid and nucleoprotein complexes now comprise approximately 6.4% of MX-derived structures deposited within the PDB. The structural biology community are currently pursuing increasingly large (> 200 kDa) and complex macromolecular systems encompassing those bound to nucleic acids, and a thorough characterisation of MX radiation damage within such complexes is essential to ensure correct structural interpretations at the atomistic scale provided by crystallography.

Nucleic acids have diverse roles in information exchange and control but also make up some of the fundamental structural and catalytic components of large macromolecular machines such as ribosomes and spliceosomes. A multitude of studies have investigated both nucleic acids and nucleoprotein complexes in solution at RT, where secondary diffusive hydroxyl radicals produced through solvent radiolysis can add to double covalent bonds within both DNA and RNA bases to induce SSBs and base modification (Chance et al., 1997; Spotheim-Maurizot and Davidkova, 2011), and oxidise protein residues with differential degrees of susceptibility (O'Neill et al., 2002). However many radical species (such as hydroxyl radicals) are immobilised below 110 K (Allan et al., 2013; Owen et al., 2012); since most modern MX experiments take place at 100 K, such hydroxyl-mediated damage is not

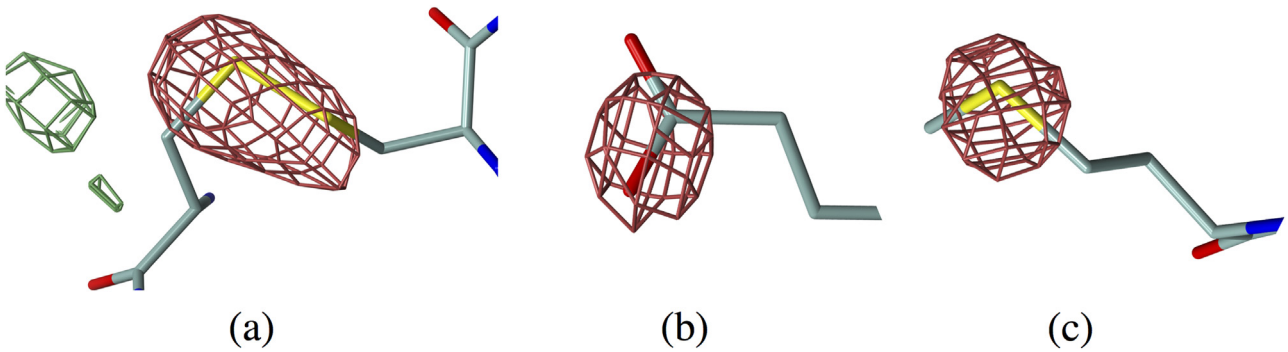

Fig. 1. (a) Disulphide bond cleavage, (b) Glu decarboxylation, and (c) Met sulphur disordering within *Torpedo californica* acetyl-cholinesterase (TcAChE) pdb: 1QID (Weik et al., 2000). $F_{obs}(5)-F_{obs}(1)$ Fourier difference maps between dataset 1 and 5 collected on the same crystal are shown, contoured at $\pm 4\sigma$, with negative difference density (red) indicating disordering of the atomic positions with accumulated dose. F_{obs} is the set of observed structure factors, proportional to the square root of the observed intensities recorded on the diffraction pattern. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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