Objective evaluation of radiation damage in a nucleoprotein complex

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Radiation damage has been a curse of macromolecular crystallography from its early days. This problem was very acute when diffraction data used to be measured from crystals kept at ambient temperatures. The introduction of cryo-cooling techniques to some extent alleviated the severity of damaging effects incurred by protein and nucleic acid crystals because of irradiation by X-rays, but very intense currently available synchrotron sources may destroy diffracting crystals after minutes or seconds of exposure. Not only does the quality of diffraction data and structure solution processes suffer, but, more importantly, radiation damage may lead to misinterpretation of chemical and biological results and to false mechanistic conclusions. Investigations into the effect of radiation damage, and of the ways of dealing with this effect, is a hot topic of contemporary macromolecular methodology. Dedicated international workshops are regularly organized every two years as a forum for discussion of novel results, with the proceedings published in the *Journal of Synchrotron Radiation* (see <http://journals.iucr.org/s/services/specialissues.html> for a list of radiation damage special issues).

The effects of radiation damage are manifested globally after absorbing doses of several tens of MGy by a decrease in the total crystal diffraction power, a change of unit-cell dimensions, an increase of crystal mosaicity or eventually its cracking and disintegration. However, even after absorbing much smaller energy doses of 1–2 MGy, many specific local effects of damage can be identified within the structures of macromolecules. Those most often observed are breakage of disulfide bonds, decarboxylation of acids, reduction of metal ions and conformational changes of side chains (Ravelli & McSweeney, 2000). Certain effects severely affect SAD and MAD phasing procedures which are based on very accurate measurement of the anomalous signal from crystals preserving a high degree of isomorphism throughout the whole data measurement session. The accuracy of the anomalous signal can deteriorate significantly as a result of the cleavage of Se–C bonds in selenomethionines (Holton, 2007), Hg–S bonds in mercury derivatives (Ramagopal *et al.*, 2005), Br–C bonds in brominated nucleic acid bases or I–C bonds iodinated tyrosine residues (Zwart *et al.*, 2004).

A significant amount of effort has been directed in the last decade to investigating the effects of radiation damage, relating these effects quantitatively to the amount of the absorbed dose or to the X-ray energy (Shimizu *et al.*, 2007; Liebschner *et al.*, 2015), or to finding ways to ameliorate them by the use of scavengers (Allan *et al.*, 2013) or extrapolation of reflection intensities to zero dose (Diederichs *et al.*, 2003). Particularly active in this field is the group at the University of Oxford headed by Elspeth Garman. In this issue of *Acta Cryst. D* this group and their collaborators describe an ingenious method to systematically quantify the effect of increasing absorbed dose on individual atoms of the structure and then apply it to a crystal structure containing simultaneously an uncomplexed protein and its complex with nucleic acid (Bury *et al.*, 2016).

The way they trace the effects of specific radiation damage at the individual atomic sites is simple and objective. They collected a series of ten data sets from the same crystal, each affected by increasing X-ray dose from 1.3 to 25 MGy, and calculated nine sets of difference Fourier maps, $(F_n - F_1, \varphi_1)$ based on differences of reflection amplitudes between the first and each successive data set with phases obtained from model refinement against the first set. A per-atom density loss metric D_{loss} was computed from changes in electron density around each individual site at each dose. All calculations were performed automatically by a pipeline using popular programs, producing objective information about the damage effect at each atom of the structure.

The crystal selected for this work was excellent in two respects. Firstly, the TRAP protein is multimeric, forming a symmetric ring of 11 monomers. The 11-fold non-crystallographic symmetry averaging very highly increases the statistical validity of effects observed at each atomic site. Secondly, there are two TRAP oligomers in the crystal asymmetric unit, of which only one is complexed with a 53-base long RNA chain that completely surrounds the ring of TRAP. This facilitates objective comparison of the radiation damage sensitivity of the protein and the RNA and, in addition, allows one to check if the complexation with RNA affects the damage susceptibility of the protein.

The thorough analysis revealed several interesting and important conclusions. The results confirmed that the most prone to damage are the carboxylic groups, but revealed that this effect is not correlated with the solvent accessibility of the individual Glu and Asp residues. However, the amino-acid side-chain conformations are more stable in the TRAP molecule bound to RNA and the rate of decarboxylation is significantly reduced, especially of the residues that interact directly with nucleic acid. This may be important for ensuring proper execution of various biochemical reactions *in vivo*, diminishing the probability that damaged proteins take part in some crucial cell processes involving interactions between proteins and nucleic acids.

RNA proved to be much less susceptible to radiation damage than the protein, similarly to the previously observed behavior for DNA (Bury *et al.*, 2015). Whereas the signs of decarboxylation were identifiable in maps calculated from the

initial data sets, some density loss could only be seen around the phosphate groups at the largest doses, but the ribose and bases were not affected even after absorbing doses higher than 20 MGy. Moreover, the nucleic acids are not only more resistant, but also act as a kind of chaperone shielding the protein against any detrimental effects of radiation damage.

This paper presents the novel, objective methodology for judging the effects of radiation damage of macromolecular crystals during X-ray data collection that will certainly be extremely helpful for the community of macromolecular crystallographers.

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