THE ROLE OF MAGNESIUM IN THE NUCLEOTIDE BINDING PROCESS OF *SULFOLOBUS SOLFATARICUS* ELONGATION FACTOR 1α

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In the process of protein biosynthesis a crucial role is played by the elongation factor Tu (EF-Tu) in eubacteria and by the elongation factor l α (EF-1a) in eucarya and archaea; it carryies the aa-tRNA to the ribosome, switching from an active GTP bound to an inactive GDP bound form. In the last decade a number of three-dimensional structures of EF-Tu have shed light on the structural details of the elongation cycle in eubacteria. By contrast, very little is known about the structure of the proteins involved in the elongation cycle of eucarya and archaea. Very recently, the 3D structure of the complex of EF-1α with the C-terminus of EF-1β from yeast and the structure of EF-1α from the archaeon *Sulfolobus solfataricus* in complex with GDP have been reported (1,2). The structure of EF-1αGDP complex also showed that, in contrast to EF-Tu, the magnesium (II) ion is not required for the GDP binding whereas it remained essential for the GTPase activity. To further investigate the role of the magnesium in the GDP binding to EF-1 α s, we have also determined the crystal structure of the Sulfolobus solfataricus EF-1αGDP in the presence of an excess of this ion. The features of magnesium coordination in the refined structure provide clues on the different role played by magnesium in EF-1α and EF-Tu.

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INVESTIGATION OF POSSIBLE FREE RADICAL SCAVENGERS IN PROTEIN CRYOCRYSTALLOGRAPHY

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Although little can be done to reduce the primary damage to protein crystals caused by X-rays, the effects of the secondary free radicals can be mitigated. The now-standard practice of cooling crystals to 100K reduces the mobility of these radicals and leads to vastly increased crystal lifetime. However even at 100K, specific damage to protein crystals occurs, e.g. to disulfide bonds, indicating that secondary damage processes are occurring. Free radical scavengers in the crystal could compete with the protein for the secondary radicals, preventing secondary damage. Documented scavengers include styrene, ascorbic acid, cysteine, ethanol and glucose. We present evidence for the efficacy of scavengers at cryogenic temperatures. We have cocrystallized proteins with various free-radical scavengers and irradiated them in an intense synchrotron beamline, monitoring the effects both on the ultraviolet (UV) absorbance spectrum with a microspectrophotometer and the effect on the structure. Hen egg-white lysozyme cocrystallized with ascorbate shows reduced radiation damage compared to native crystals as measured by crystallographic statistics and UV absorbance spectra. In damaged crystals a peak at 400 nm due to a putative disulfide radical anion is seen. Crystals with ascorbate do not show this peak. It may be possible to mitigate the effects of secondary radiation damage to protein crystals at synchrotron beamlines by introducing free radical scavengers into the crystal lattice. It is possible to monitor radiation damage using the UV absorption spectrum of the crystal.

Keywords: RADIATION DAMAGE, FREE RADICAL, CRYOCRYSTALLOGRAPHY

SAMPLE HEATING AT THIRD GENERATION SYNCHROTRONS: MYTH AND REALITY

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Since the beginning of using of third generation synchrotron radiation sources for macromolecular crystallography, concerns have been voiced that sample heating in the intense beams may accelerate radiation damage. A detailed treatment of sample heating has been provided by Kuzay, Kazmierczak and Hsieh (Acta Cryst. (2001), D57, 69-81). Here, we apply their method to the sample arrangement and sizes, x-ray fluxes and flux densities, and exposures typical for biological cryo-crystallography. In particular, we give proper consideration to the mother liquor in which the crystal is suspended in the cryo-loop. For most macromolecular crystallography data acquisition, the exposure needed for a data frame increases the temperature of the crystal by less than 10 K. The final temperature is reached within a few seconds of exposure. As pointed out by Kuzay et al., the temperature gradient within the crystal/vitreous ice volume is insignificant. Also, the particular shape and size of the incident beam has little effect. For a given incident flux, the volume of the material suspended in the loop determines the rate of initial temperature rise, and its surface determines, in first approximation, the final, steady-state temperature. Current work on the effect of gas flow speed and the shape of the vitreous ice/ crystal / loop volume will be presented. We will present results for different situations of macromolecular data acquisition and show the limits where sample heating may start to be a concern. We will present recipes to help reduce sample heating.

Keywords: SAMPLE HEATING SYNCHROTRON RADIATION CRYO-CRYSTALLOGRAPHY

SEEING THE HEAT: STUDIES OF CRYOCRYSTALLOGRAPHY USING INFRARED IMAGING

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As preparation for an extensive study in which we image the cryocooling process of macromolecular crystals we have demonstrated the ability to thermally image solid objects and liquids at temperatures far below 273 K. In the case of a large lysozyme crystal qualitative measurements show the cooling process to take about 0.6s with the cooling taking place in a wave from the face of the crystal nearest to the origin of the cryostream, to the point furthest away from the origin. Annealing of this lysozyme crystal, cooled under good cryoprotectant conditions, showed cold striations formed perpendicular to the cooling stream. These striations became more pronounced after successive annealing. Cryocooling of a non-cryoprotected crystal of glucose isomerase displayed an S-shaped cold front wave traveling across the sample. Our first results were qualitative and the approaches taken to achieve quantitative imaging will be presented along with the results. The results show the power of infrared imaging as a new tool for fundamental and practical cryocrystallography studies.

Keywords: CRYOCRYSTALLOGRAPHY INFRARED IMAGING MACROMOLECULES