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X-ray induced photoreduction of cobalamins

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Cobalamin cofactors crystallize very well and their crystals diffract to very high resolution. This makes them ideal model systems to study the mechanisms of (X-ray induced) photoreduction of redox sensitive corrine systems which play an important role in many metallo-proteins.

The photoreduction of several cobalamin cofactors in enzymes has been investigated extensively. The generally accepted mechanism in case of adenosylcobalamin is a homolytic cleavage of the Co(III)-C-bond going along with the formation of a 5'-deoxyadenosyl radical. [1], [2].

Radiation damage of biological samples is a major impediment to the sucIn our study we have further investigated the X-ray induced photoreduction of cobalamins by XANES and high resolution X-ray diffraction experiments. One mechanism proposed for X-ray photoreduction of metal organic compounds and metallo-proteins is X-ray induced water photolysis generating free electrons which then reduce the metal atom. [1],[3].

Our XANES measurements revealed that the presence or absence of water in the sample has only minor influence on the photoreduction. Lowering the temperature reduces the susceptibility to photoreduction of cyanocobalamin. This further indicates that no direct photoreduction by the photo electrons is taking place. In such a case a temperature independent susceptibility would be expected.

In our X-ray diffraction measurements of methylcobalamin and cyanocobalamin specific bond length changes as function of dose could be observed, which can be attributed to X-ray induced photoreduction. In case of cyanocobalamin, analysis of the thermal displacement parameters showed a strong B-factor increase for one specific hydrogen atom. This increase was observed in all of 6 independent measurements. Such an intramolecular hydrogen abstraction might be of importance for the X-ray induced photoreduction of cobalamins and could explain the observed temperature dependence.

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Kewords: cobalamin, photoreduction, specific radiation damage

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Phasing Selenomethionine proteins using UV induced radiation damage

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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 when no tunable X-ray source is available.

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Crystal structures of two archaeal Pelotas reveal inter-domain structural plasticity

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Dom34 from *Saccharomyces cerevisiae* is one of the key players in no-go mRNA decay, a surveillance pathway by which an abnormal mRNA stalled during translation is degraded by an endonucleolytic cleavage. Its homologs called Pelota are found in other species. We showed previously that *S. cerevisiae* Dom34 (domain 1) has an endoribonuclease activity, which suggests its direct catalytic role in no-go decay. Pelota from *Thermoplasma acidophilum* and Dom34 from *S. cerevisiae* have been structurally characterized, revealing a tripartite architecture with a significant difference in their overall conformations. To gain further insights into structural plasticity of the Pelota proteins, we have determined the crystal structures of two archaeal Pelotas from *Archaeoglobus fulgidus* and *Sulfolobus solfataricus*. Despite the structural similarity of their individual domains to those of *T. acidophilum* Pelota and *S. cerevisiae* Dom34, their overall conformations are distinct from those of *T. acidophilum*