

## Abstract

Countless electron transport/transfer (ET) processes occur in living organisms every day. Therefore, their study is a crucial field of modern structural and functional proteomics. In many cases model proteins like azurin from *P. aeruginosa* are utilised in experiments. This blue copper protein is favoured due to a characteristic absorbance maximum at 630 nm in Cu(II) redox state of the central Cu atom. During its oxidation to Cu(I) state the  $A_{630}$  value decreases allowing UV-Vis detection of ET reaction progress.

We have introduced a structural photoinducible analogue of canonical amino acid Met – L-2-amino-5,5-azihexanoic acid (photo-Met) – into azurin structure to study oligomerization in solution *via* photo-induced cross-linking (PIXL). Using previously optimised protocols for recombinant expression in *E. coli* B834 we have inserted photo-Met into azurin moieties: wild type azurin and Az2W mutant where two adjacent W residues with confirmed role in electron hopping across protein-protein interface are present. The incorporation percentage of photo-Met in analysed samples was determined after SDS-PAGE and in-gel protease digestion *via* MALDI-TOF MS. PIXL was employed to study azurin-azurin interaction and oligomerization under different total concentrations of protein (in range of 15–300  $\mu$ M). The samples were exposed to intense UV light and the results were evaluated *via* SDS-PAGE and UV-Vis spectrophotometry. SDS-PAGE protein band relative mobility 28–30 kDa corresponding to dimer was observed and MALDI-TOF MS and LC-MS/MS were employed after in-gel proteolysis to confirm protein moiety and determine XL identity, respectively. We have observed higher dimerization yield with increasing total azurin concentration in solution and generally increased dimer formation in Az2W mutant compared to wild type azurin under lower protein concentrations. Simultaneously, UV-Vis spectra of studied azurins before and after UV irradiation suggest that during PIXL experiment a significant decrease of  $A_{630}$  value occurs, probably implicating a change in redox state of central Cu atom. This intriguing effect was not observed in control Met-containing azurins to such degree.

Our findings support the role of two additional W residues on the interacting surface (formed by a  $\beta$ -sheet close to Cu centre) not only during intermolecular ET hopping but also in azurin oligomerization. They also widen our horizons and introduce a potential application of photo-Met for ET reaction initiation in proteins, a novel and promising technique which warrants further research.

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