Investigating Oxidation-Induced Formation and Structure of Disulphide-Linked Conjugates by p62-SQSTM1

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The selective autophagy receptor p62/SQSTM1 recruits cellular waste for degradation by forming a flexible filamentous helical scaffold, allowing it to bind to both ubiquitinated cargoes and the autophagy core machinery protein LC3. p62 self-oligomerisation is mediated by the PB1 domain, however recent evidence suggests that in oxidative stress conditions, p62 can also form disulphide-linked conjugates (DLCs). This allows p62 to increase autophagic recruitment in response to oxidative stress conditions, including recruitment of dysfunctional mitochondria producing excess reactive oxygen species, thereby restoring redox homeostasis. However, whilst the structure of the PB1 domain-mediated p62 oligomer has already been characterised, the structure of p62 DLCs, and whether they differ from PB1 oligomers, remains uncertain.

To investigate the formation of p62 DLCs in vitro, recombinant p62 proteins, consisting of Maltose Binding Protein (MBP) joined to different lengths of p62, have been expressed and purified. Treatment of recombinant protein with the oxidising agent hydrogen peroxide results in DLC formation, as seen on both SDS-PAGE gels and analytical size exclusion chromatography. DLC formation is independent of PB1 oligomerisation, as mutations in the PB1 domain that perturb oligomer formation do not affect the response to hydrogen peroxide treatment. These DLCs can be observed as filamentous structures by negative stain electron microscopy, and more detailed imaging by cryo-EM is currently in progress. By characterising how p62 responds to oxidation and forms DLCs, the mechanism by which selective autophagy is upregulated in response to oxidative stress can be better understood.