Site Specific Determinants of Thimet Oligopeptidase Oxidative Oligomerization and S-Glutathiolation

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Thimet oligopeptidase (EC 3.4.24.15; EP24.15) is a zinc metallopeptidase involved in the degradation of oligopeptides (5-17 residues). Fifteen Cys residues are present in the rat EP24.15 protein, seven are solvent accessible and two are found inside the catalytic cleft; no intra-protein disulfide bond is described. In a previous work, we showed that mammalian EP24.15 oligomerization is triggered by S-glutathiolation of three Cys residues, identified by mass spectrometry (C46, C175 and C253). In the present work our goal has been to identify amino acid residues in the 3D structure of the wild EP24.15 that might stabilize the thiolate form (CyS^-) of the Cys residues prone to S-glutathiolation because, the CyS^- configuration presumably favors either S-thiolation by oxidized glutathione or the inter-protein thiol-disulfide exchange. These studies have been addressed by site-specific mutations. Results obtained with the R263E mutant, where the Arg residue is vicinal to the C175, this one prone to S-glutathiolation, showed that S-glutathiolation and oligomerization were greatly reduced under incubation with oxidized glutathione concentrations to elicit both modifications in the wild type protein. Most probably, the R263E mutation increased the C175 residue protonation, inhibiting its S-glutathiolation and, consequently, protein oligomerization. Reduced oligomerization of the E683A mutant protein, where the Glu residue is vicinal to a Cys (C682) residue, was also verified. These results are consistent with our hypothesis that EP24.15 oligomerization is dependent on the electron transfer from specific sulfhydryl residues to previously S-glutahionylated Cys residues.

Keywords: Thimet oligopeptidase EP24.15; S-glutathiolation; Oxidative oligomerization; Thiol–disulfide exchange