Poster

Myeloperoxidase dimerization in solution is mediated by interface glycans

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The oxidoreductase myeloperoxidase (MPO) plays a key role in phagocytotic killing of invading pathogens by neutrophils as part of the innate immune response [1]. It is a highly stable dimeric protein, that undergoes a number of post-translational processing steps, including proteolytic cleavage at multiple sites, complex glycosylation and formation of an interdimeric disulphide bridge before being packaged into the neutrophil azurophilic granules [2,3]. The structural and biological relevance of some of these modifications, especially dimerization and glycosylation remain to be understood.

Here we investigate the effect of glycosylation and disulphide bridge linking on the structure of native leucocyte MPO. MPO is glycosylated at five sites, most prominently featuring an interface glycan structure that is sandwiched between the covalently linked MPO monomers and shields a highly charged surface patch. We successfully monomerised MPO by DTT reduction of the intermolecular disulfide bridge and iodoacetamide shielding of free cysteines (Figure 1B). While the activity remains undisturbed, the thermostability of the enzyme is severely compromised (Figure 1C). Interestingly, we can show that monomerized glycosylated MPO dimerizes in a concentration dependent manner to form the native dimer using Small Angle X-Ray scattering and HPLC-MALS. The crystal structure of monomeric MPO, which is nearly identical to the native dimer, suggests dimerization is mediated by the interface glycans (Figure 1A). This suggests that glycosylation precedes covalent linkage during biosynthesis and packaging of MPO. Comparison of binding of MPO autoantibody in patient sera with neutrophilic cytoplasmic antibody (ANCA) associated vasculitis to native, monomerized or monomerized and deglycosylated MPO investigates for the first time the distribution of structural features recognized by ANCA-antibodies.

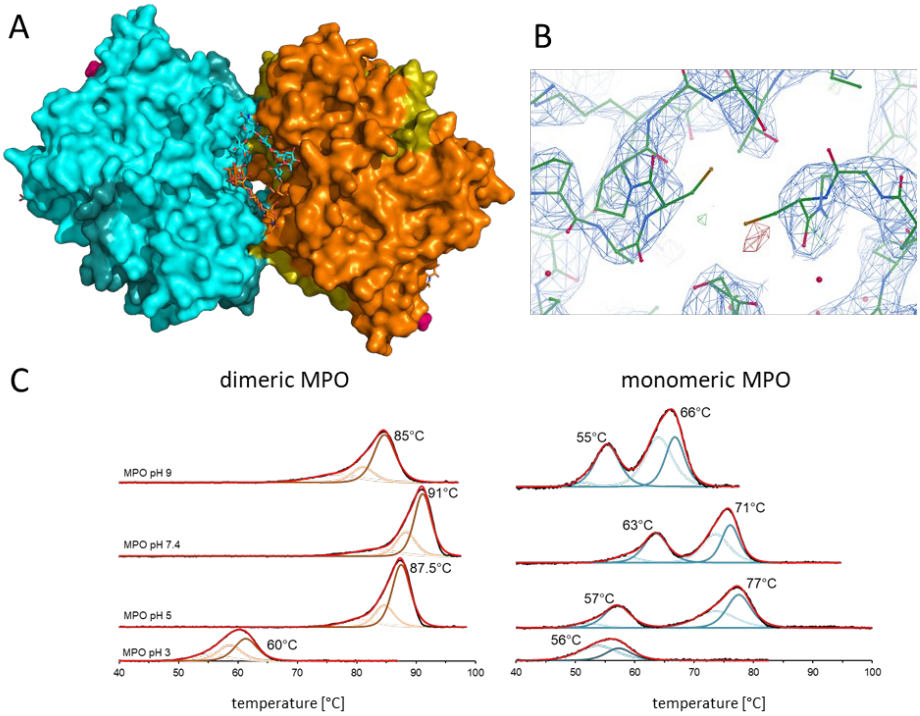

Figure 1. structural characteristics of monomerized native MPO. A) monomeric MPO forms native-like non-linked dimers *in crystallo*, B) electron density around the reduced intermolecular disulfide bridge. C) monomeric MPO has a significantly reduced thermal stability over a large pH range (pH 3-9) and exhibits two distinct unfolding events.

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