Seeing is believing: glycosylation in the crystal structure of human myeloperoxidase

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Glycosylation in the crystal structure of human myeloperoxidase

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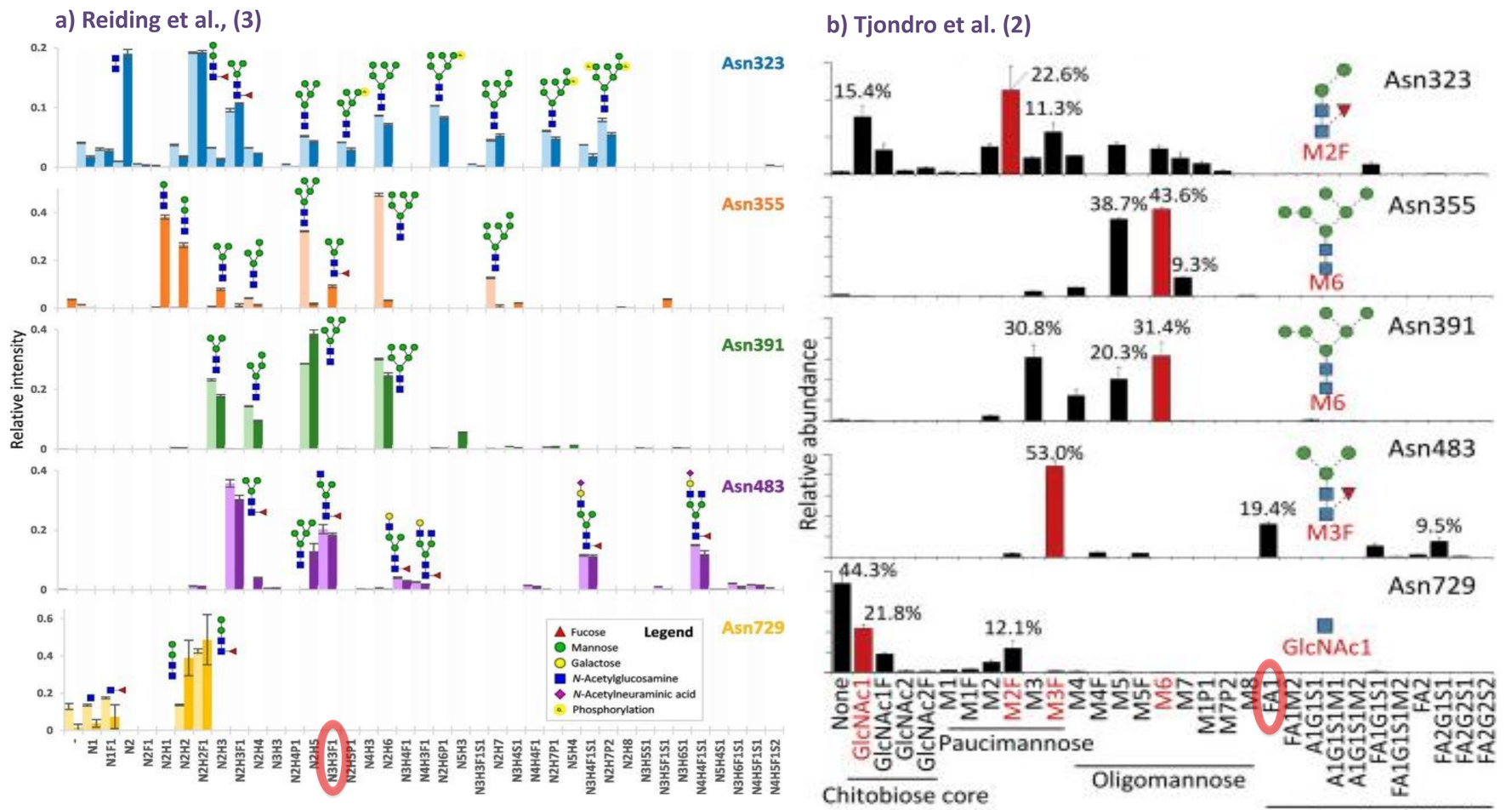
Abstract

Human myeloperoxidase (MPO) was first isolated in 1941 from purulent pleuritis fluid from tuberculosis patients. When neutrophilic polymorphonuclear leukocytes (neutrophils) entrap microbial or other invasive particulates, they release MPO during degranulation. In a respiratory burst of highly reactive oxygen species, MPO catalyzes the production of hypohalous acids, primarily hypochlorous acid in physiologic situations, from hydrogen peroxide. Mammalian MPO crystal structures were progressively acquired and entered in the Protein Data Bank (PDB) with partial glycosylation identification. Actually, the N-glycan composition of native MPO had been thoroughly investigated with mass spectrometry and shows 5 *N*-glycans at positions 323, 355, 391, 483 and 729 (1). Recently, MPO's enzymatic activity was reported to depend on the hyper-truncation of 2 out of 5 *N*-glycosylation sites (2).
In our obtained crystal structure at 2.6 Å resolution containing 4 disulfide-linked homodimers of MPO, an interesting collection of glycans have been characterized. We compared those with the glycans from proteomics studies (1- 3) and from 18 human MPO structures in the PDB. We made use of the Symbol Nomenclature for Glycans (SNFG) to illustrate congruence in the experimental data. We found each of the 5 glycosylation sites either non-glycosylated or glycosylated with paucimannosidic, high-mannose and complex N-glycans, with the hyper-truncated N-acetyl-β-D-glucosamine core-type asparagine-linked glycans on Asn355 or Asn391 sites gate keeping the funnel towards the ROS-activated heme group. More experimental data on the glycosylation of MPO is hereby provided.

Results



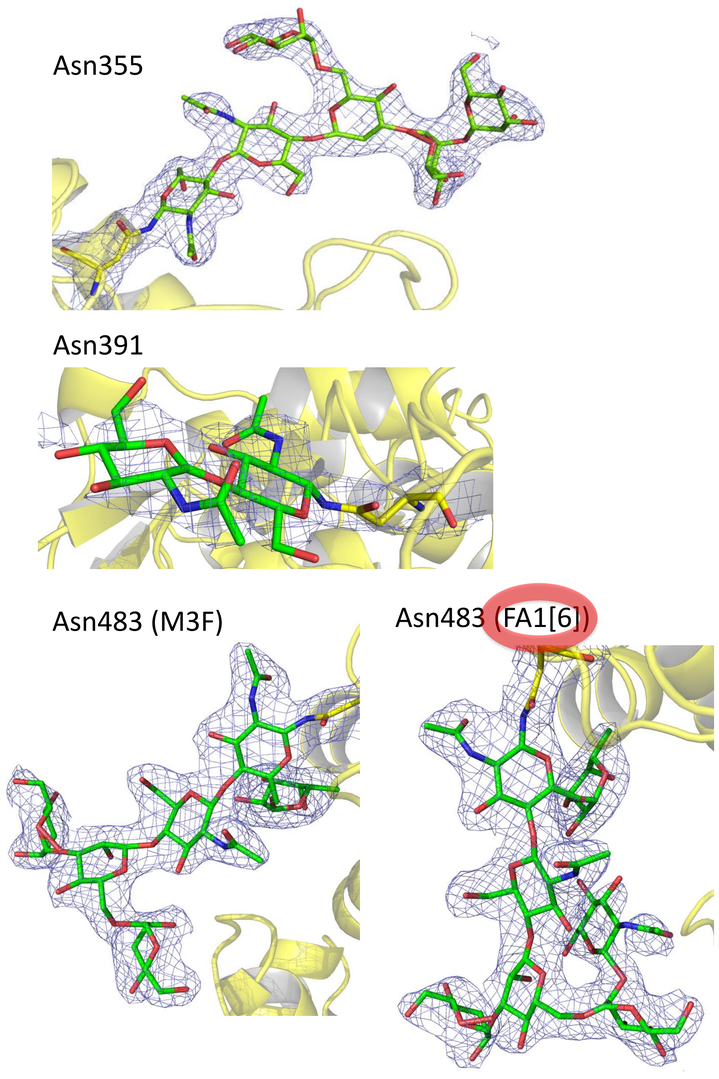


Figure 2: Electron density (blue) defining the *N*-glycans in our MPO crystals (PDB 7OIH).

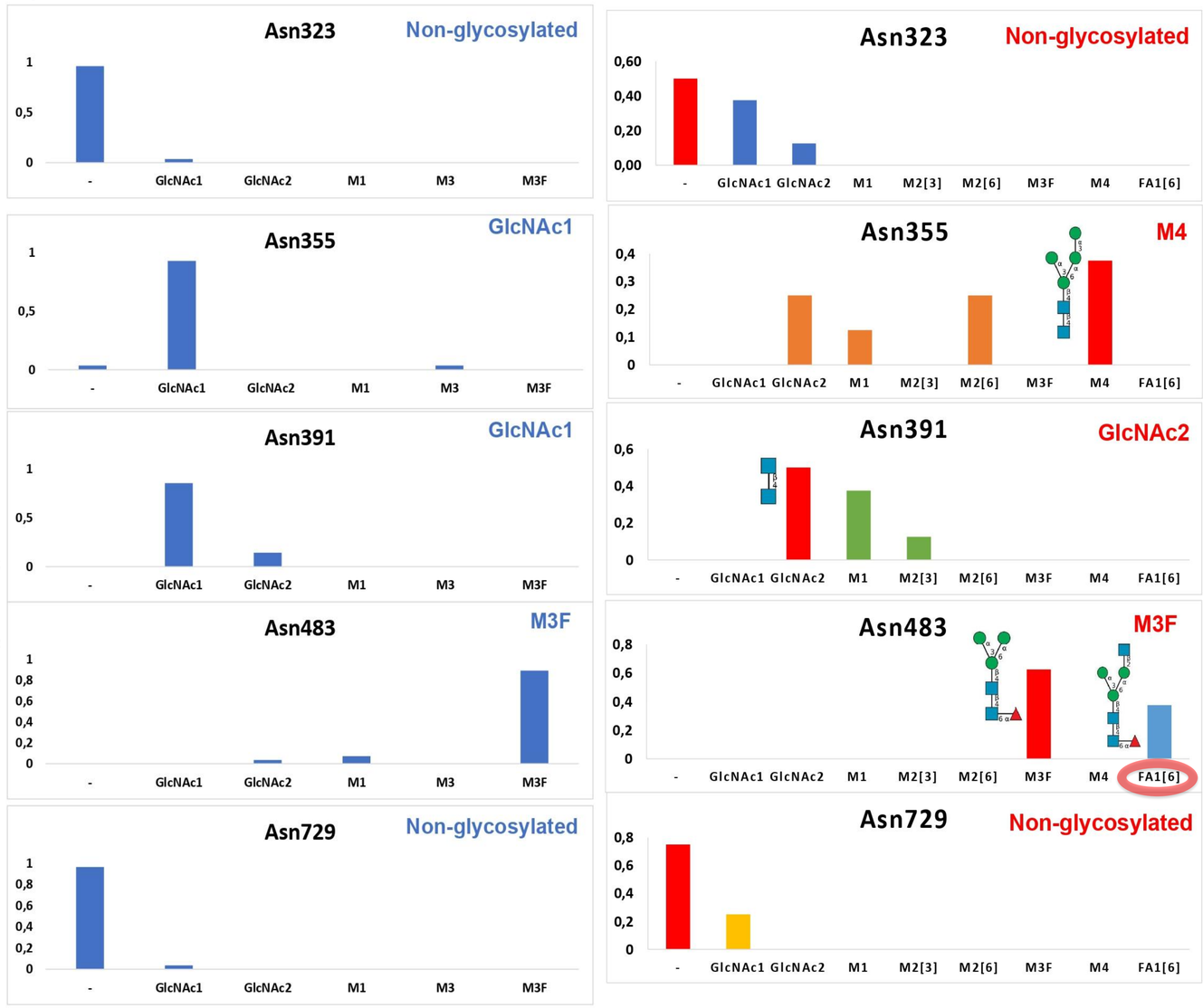


Figure 3: Comparison of the N-glycans found in MPO between the structures available in the PDB and those found in our crystal structure. Fig. 2a shows the repartition of the different glycans on each site for 28 MPO monomers found in the PDB. Fig 2b shows the repartition of the glycans for the 8 monomers of our MPO crystal structure, the major glycosylation type being highlighted in red and displayed for each site.

Conclusions

FA1[6] is a hybrid structure on Asn483, not yet observed in MPO crystal structures but actually well represented also in the two independent proteomics studies under the names N3H3F1 (Figure 1a) (3) and FA1 (Figure 1b)(2), similarly to what we see (Figures 2, 3b). The "plus" of our study is that we can distinguish the isomer because we obtain the 3D-structure of the *N*-glycan in the crystal structure. Therefore we can designate it as FA1[6].
Compared to what has previously been shown in human MPO structures, our MPO crystal structure shows larger *N*-glycans that get closer to the results obtained using mass spectrometry, perfectly illustrating the improvement by crystallographic resolution and its capacity to resolve molecular structure including glycosylation.

References

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