Abstract

A large number of biological processes depends on dynamics of protein structure and specific protein-protein and protein-ligand interactions occurring under specific native conditions in or outside of cells. Standard methods for protein structure analysis like x-ray crystallography, nuclear magnetic resonance or cryo-EM are able to obtain important atomic or near- atomic resolution protein structures, however these are usually a static snapshot of protein locked in a specific conformation and mostly in non-native conditions.

Structural mass spectrometry on the other hand, allows to describe protein structure dynamics, protein-protein and protein-ligand interactions and obtain inter- and intraprotein distance constraints between amino acid residues, all while working with proteins in their native conditions and needing only a fraction of sample.

In this work, hydrogen/deuterium exchange mass spectrometry (HDX-MS) and classical proteomic approaches were used together with other methods to analyse biotechnologically important proteins of fungal cellulolytic system lytic polysaccharide monooxygenase (LPMO) and cellobiose dehydrogenase (CDH) as well as plant-derived photosensitizer protein LOV2 with potential use in biologically targeted photodynamic therapy.

These methods allowed us to follow cellulolytic reaction of reduced LPMO even in heterogeneous solution of crystalline cellulose, obtaining insights into structural changes accompanying LPMO catalysis, mainly its notorious instability which was determined to be caused by oxidative modification of the protein, as well as verifying and structurally describing previously reported stabilisation of LPMO by suitable substrate. The recently speculated role of hydrogen peroxide as true LPMO cosubstrate was also confirmed.

Analysis of LOV2 protein then explained previously reported gradually increasing production of singlet oxygen upon protein irradiation as caused by a release of flavin cofactor into the solution, with interesting implications for biologically targeted photosensitizers.

Keywords: Structural mass spectrometry, hydrogen/deuterium exchange mass spectrometry (HDX-MS), lytic polysaccharide monooxygenase (LPMO), cellobiose dehydrogenase (CDH), oxidative modification, cellulose degradation, turbidimetry, photosensitizer, light oxygen and voltage sensing domain (LOV)