HYDROXYL RADICAL FOOTPRINTING OF BOVINE MYOGLOBIN USING PLASMA INDUCED MODIFICATION OF BIOMOLECULES (PLIMB)

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I. OBJECTIVES

The study objective was to utilize Plasma Induced Modification of Biomolecules (PLIMB) to examine solvent access to the distal and proximal cavities of bovine oxymyoglobin. Tentative mechanisms of myoglobin oxidation and heme dissociation are predicated on solvent access to the heme pocket which can potentially be examined using PLIMB.

II. MATERIALS AND METHODS

PLIMB: Fifty hertz and 10 volts was generated and amped to 10 kHz and 10 kV, which was then discharged from a steel needle above protein in buffer. Fifty microliters of protein (30 μM) in sodium phosphate buffer (50 mM, pH 6.5) was used per exposure and excess hydroxyl radicals quenched with 25 mM methionine solution.
Orbitrap tandem mass spectrometry (MS/MS): After a trypsin/LysC digest, samples were cleaned with a C18 OMIX tip. Samples were injected into a Pepmap C18, 3 μM, 100 A, 25 μM ID, 15 cm reversed phase column and ionized with an EASY-Spray Ion Source. Samples were analyzed with an Orbitrap Elite in data-dependent MS/MS mode and with Protein Metrics Byos software. Plasma-induced modifications of side chains included (I) mono-oxidation as +15.99 Da, (II) di-oxidation as +31.99 Da, (III) His to Asp as −22.032 Da, (IV) His to Asn as −23.016 Da, (V) Lys to Arg as +28.006 Da, (VI) Lys to Asn as −14.05, (VII) Thr to Asp as +13.979 Da, (VIII) Carbamidomethyl as +57.021 Da, (IX) Gln to pyro-Gln as −17.026 Da, (X) Glu to pyro-Glu as −18.010 Da, and (XI) Ser to Asp as +27.99 Da.

III. RESULTS

There are 17 amino acids within 4 angstroms of the heme protoporphyrin nine ring that collectively form the distal and proximal pockets. Notably, the distal residues at E7 and CD3 (His64 and K44) and the proximal residue at C7 (K42) were modified by PLIMB after 1 s of exposure. Histidine modifications resulted in mass shifts of −22.03 Da, −23.016 Da, and +15.99 Da. Lysine modifications resulted in a mass shift of +28.006 Da. One experiment conducted in duplicate was done, which limited power of the statistical evaluations. There was no significant difference between the summed modifications of the proximal residues compared to the distal residues, even though the absolute value was higher for the distal residues compared to the proximal residues (0.37% vs. 0.04%). There was no significant increase in modification in response to time, though the absolute value was higher at 1 s compared to 0 s (0.29% vs. 0.12%). It should be noted that the −22.03 and −23.016 Da modifications were more numerous than the +16 Da even though additive reactions generally occur faster with hydroxyl radical footprinting. Indeed, there was no validated +15.99 Da modification of lysine, nor of the other 15 amino acids examined as part of the experiment.

IV. CONCLUSION

The study demonstrates potential for the use of PLIMB in tandem with MS/MS to study solvent access to myoglobin. More distal amino acids were modified compared to proximal, consistent with solvent access to the distal pocket facilitating Mb oxidation. Further experiments will be required to better assess the utility of PLIMB to measure solvent access and differential modification of the distal and proximal amino acids.

Keywords: heme, myoglobin, oxidation, protein footprinting