Poster Presentation

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Crystallographic evidence for oxidative Met→Asp conversion in human hemoglobin

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The mutants HbA Bristol-Alesha (βV(E11)67M) and HbF Toms River (γV(E11)67M) [1,2] are examples of a 'silent' posttranslational modification in which the side chain of the substituted amino acid is chemically modified (Met→Asp) resulting in a disparity between the DNA and protein sequences. In both cases the patients' hemolysate contained both V67M and V67D isoforms. But in the analogous α subunit mutant, Hb Evans αV(E11)62M, the conversion to Asp was not identified and DNA sequencing confirmed the Met replacement [3]. Our crystal structures of the three (ferrous) CO-bound recombinant V(E11)M mutants show the MetE11 side chain in similar conformations. But the air-oxidized β mutant crystals clearly showed a 'bifurcated' and smaller electron density pattern for the E11 side chain, indicating the appearance of Asp. Also, the ligand electron-density at the iron atom in the oxidized β subunit appears to be an oxoferryl Fe4+=O rather than a Fe3+OH2 ferric complex. In contrast, there was little change in the electron density for αMetE11 in oxidized αV62M crystals. The ligand in the ferric α subunit is clearly a coordinated water molecule. But again, a ferryl Fe4+=O complex appears to occur in the wild-type β subunit. This strongly suggest that β subunits have a greater propensity to form highly reactive ferryl species, and that the ferryl species play a role in the Met→Asp conversion. Our autoxidation and proteomics studies showed that although all three recombinant VE11M mutants had similar, high rates of autooxidation and a strong H2O2 dose dependence on sulfoxide and sulfone formation, no Asp formation was detected in α subunits whereas MetE11 is converted to Asp to levels as high as 15% in vitro in β and γ subunits. We propose that the Met→Asp conversion specifically involves H2O2 mediated oxidation of the ferrous heme to an oxoferryl state, and because the transient ferryl intermediates are much less stable in the α subunits, there is no oxidative conversion.

[1] *D.C. Rees, J. Rochette, C. Schofield et al., Blood, 1996, 88, 341-348,* ***[2]*** *M.A. Crowley, T.L. Mollan, O.Y. Abdulmalik et al., N Engl J Med, 2011, 364, 1837-1843,* ***[3]*** *M.I. Zanotto, K. Calvo, G. Schvartzman et al., Archivos argentinos de pediatria, 2010, 108(6), 130-133*

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