MAPPING OF THE AMINO ACIDS AT THE HEMIN NEIGBORING BY PHOTO-OXIDATION OF NON-COVALENT COMPLEXES

Dias C. F. B. Mascio, P. and Nantes, I. L. [1]
CIIB-UMC, S. P., Brazil;
[2]Depto Bioquim, IQ, USP, São Paulo, Brazil.

The capacity free base hemin to promote photo-oxidation of amino acids preferentially located at the neighboring of the prosthetic group in a non-covalent complex was studied as a potential method for protein structure determination. Two non-covalent complexes were probed: apocytochrome c/hemin and a synthetic peptide (A-A-W-A-A-A-A-K-N-D-D-D-W-D-D)/hemin. The complexes were submitted to photosensitizing with a 500 Watts halogen lamp during 90 min and analyzed by mass spectrometry in comparison with the same apoprotein photooxidized by methylene blue. The pattern of oxidation was completely different for the two probed conditions suggesting that the oxidized amino acids in the non covalent complex are that located at the neighboring of the hemin. The non covalent complex formed by the association of free base hemin with the synthetic peptide revealed changes in the electronic absorption spectrum of the prosthetic group compatible with the chromophore in a hydrophobic microenvironment. Accordingly, the complex exhibited blue shift and quencher of tryptophan fluorescence spectrum. This result was expected considering the hydrophobicity of the porphyrin ring. The photosensitization of the peptide resulted in the oxidation of tryptophan to *N*-formylkynurenine attested by mass spectrometry and fluorescence of the photoproduct. Compatible with the preferential oxidation of tryptophan residue located at the hydrophobic side of the peptide, the formation of the oxidized photoproduct resulted in the complex dissociation with recover of the fluorescence of fluorescent amino acid located at the hydrophilic side of the peptide. Furthermore, the oxidation of one of the two triptophan residues of the peptide was corroborated by mass spectrometry analysis.

Supported by: FAPESP CNPq and FAEP-UMC

Key Words: Photo-oxidation, tripthophan, *N*-formylkynurenine, free base hemin.