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“From amino acids to protein - study of the effect of amino acid photo-oxidation on the model protein”

ABSTRACT:

The aim of this work was to investigate the anoxic oxidation of aromatic amino acid residues in model systems and protein – glyceraldehyde-3- phosphate dehydrogenase (GAPDH) in the presence of the sensitizer – 3-carboxybenzophenone (3CB) by examining the mechanisms and stable products. Aromatic amino acids residues in the model compounds were oxidized by 3CB in the presence of different neighboring groups to mimic the environment of the studied protein, GAPDH. The influence of the formed modifications on the activity of the GAPDH was also examined by determining the enzymatic activity assay of the protein. Analysis of transient products formed during quenching of the 3CB* by the aromatic amino acid residues was carried out using nanosecond flash photolysis technique. The influence of blocking either amine and/or carboxylic groups on the resulting species was also determined. The resulting stable products were separated by liquid chromatography and characterized by high-resolution mass spectrometry. Results from the model systems were compared to data obtained for GAPDH, with particular emphasis on modifications of tryptophan, tyrosine, histidine, and phenylalanine. Laser flash photolysis revealed differences in the quenching rate constants and quantum yield for the formation of various transient products. Trends in Tyr, His, and Trp were largely consistent with the literature, showing that the blocking of the amino group significantly affects the two former amino acids but not the latter. Experiments with Phe demonstrated that reactions with other, more reactive amino acids present in peptide, protect the Phe, as it reacts slower with excited triplet state of the sensitizer. Analyses of various amino acid residues in the model systems, demonstrated that dimers are the main product formed during the oxidation of Tyr or Trp, with the number of diTyr/diTrp isomers increasing with the degree of blockage. The presence of Tyr- 3CBH was seen only for some compounds, while Trp-3CBH was only present in peptidic system. Different results were obtained for His and Phe, where unblocked amino group led to the formation of products not assigned to the first two groups. These products are formed through the recombination of 3CBH radicals with methyl-imidazyl or benzyl radicals (as well as between benzyl radicals and Phe derivatives) generated by the homolytic cleavage of either His or Phe. It is proposed that energy transfer from the sensitizer to the N-terminal His derivative leads to homolytic cleavage of the derivative into a methyl-imidazole radical. Another product characterized in the third group of compounds was formation of a C=C double bond in Trp or His. The knowledge gathered from the model compounds was then applied to the photosensitized oxidation of a chosen protein, GAPDH, under anoxic conditions. The formation of a ground-state complex between GAPDH and 3CB was confirmed. SDS-PAGE provided information on the relationship between exposure time and the formation of dimers and higher polymers, allowing the selection of an appropriate exposure time for analysis. The characterization of stable products of GAPDH (by timsTOF) digested in solution and in gel after a 5-minute irradiation confirmed the formation of some products identified in the model products, such as 3CBH-amino acid. Amino acid analysis combined with the Ellman test provided insights into the reactivity of selected amino acids that are included in the sequence of GAPDH. Sulfur-containing amino acids were identified as the main

source of GAPDH damage, while His and Tyr were considered as secondary targets. The activity test showed that although GAPDH forms a ground-state complex with 3CB, it does not affect its activity significantly. Moreover, modifications of the available amino acids in solution do not markedly change the activity. The main differences arise when GAPDH is exposed to light for more than 5 minutes, resulting in more dimers and covalent polymers.