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Scientific Session I

EPR Studies on Free Radical Generation by the Reaction of Methylglyoxal with Amino Acids and Protein

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The formation of -dicarbonyl compounds seems to be an important step for cross-linking proteins in the glycation or Maillard reaction. To elucidate the mechanism for the cross-linking reaction, we studied the reaction between a three-carbon -dicarbonyl compound, methylglyoxal, and amino acids. Our results showed that this reaction generated yellow fluorescent products as formed in some glycated proteins. In addition, three types of free radical species were also produced, and their structures were determined by EPR spectroscopy. These free radicals are 1) the cross-linked radical cation, 2) the methylglyoxal radical anion as the counterion, and 3) the superoxide radical anion produced only in the presence of oxygen. The generation of the cross-linked radical cations and the methylglyoxal radical anions does not require metal ions or oxygens. These results indicate that dicarbonyl compounds cross-link free amino groups of protein by forming Schiff bases, which donate electrons directly to dicarbonyl compounds to form the cross-linked radical cations and the methylglyoxal radical anions. Oxygen can accept an electron from the radical anion to generate a superoxide radical anion, which can initiate damaging chain reactions. Time course studies suggest that the cross-linked radical cation is a precursor of yellow fluorescent glycation end products.

Oxidation-reduction properties of methylglyoxal-modified protein in relation to free radical generation were investigated. Glycation of bovine serum albumin by methylglyoxal generated the protein-bound free radical, probably the cation radical of the cross-linked Schiff base, as observed in the reaction of methylglyoxal with l-alanine or with *N*-acetyl-l-lysine. The glycated bovine serum albumin showed increased electrophoretic mobility suggesting that the basic residues, such as lysine, were modified by methylglyoxal. The glycated protein reduced ferricytochrome *c* to ferrocytochrome *c* in the absence of oxygen or added metal ions. This reduction of cytochrome *c* was accompanied by a

large increase in the amplitude of the electron paramagnetic resonance signal originated from the protein-bound free radical. In addition, the glycated protein catalyzed the oxidation of ascorbate in the presence of oxygen while the protein-free radical signal disappeared. These results indicate that glycation of protein generates active centers for catalyzing one-electron oxidation-reduction reactions. This active center, which exhibits enzyme-like characteristic, was suggested to be the cross-linked Schiff base/the cross-linked Schiff base radical cation of the protein. It mimics the characteristics of metal-catalyzed oxidation system. The glycated bovine serum albumin cross-linked further to the cytochrome *c* in the absence of methylglyoxal. The cross-linked cytochrome *c* maintains its oxidation-reduction properties. These results together indicate that glycated proteins accumulated *in vivo* provide stable active-sites for catalyzing the formation of free radicals.

References

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