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PROTEIN-CROSS-LINKING BY METHYLGLYOXAL

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To elucidate the mechanism for the cross-linking reaction in the glycation or Maillard reaction, we studied the reaction between proteins, and a three-carbon α-ketoaldehyde, methylglyoxal. When Cu,Zn-SOD was incubated with 200 mM of methylglyoxal , the peroxidase activity as well as the superoxide dismutase activity was reduced. This reduction is accompanied by the decrease of the anion binding affinity of the enzyme. Modification also led to loss of copper, which was another reason of partial inactivation of the enzyme. Cu,Zn-SOD changed in its epr spectrum from rhombic to axial configuration upon incubation with methylglyoxal. It means that one of four histidyl imidazole ligands bound to Cu(II) ion in a distorted square planar geometry was replaced by other molecule such as water. The electrophoretic mobility of the modified Cu,Zn-SOD was altered in 10% native gel. SDS-polyacrylamide gel electrophoresis also showed the cross-linked species of Cu,Zn-SOD. When the modification reaction was carried in the presence of $NaCNBH_3$ which reduces Schiff base in neutral pH, cross-linking of protein was completely inhibited. This means that a following reaction of Schiff base formation is a requisite step for cross-linking of protein. Bovine serum albumin (BSA) was also cross-linked upon incubation with methylglyoxal when analyzed by SDS-polyacrylamide gel electrophoresis and gel permeation chromatography. Through EPR spectroscopy we detected radical signals, broad singlets in the course of and after modification of BSA. The glycated BSA readily reduced cytochrome *c* accompanying an increased EPR signal, and still had cross-linking ability in spite of long term storage.