Synopsis of Original Research Paper

Enhancement of Antioxidative Activity of Vitamin E using Tetrapeptide Derived from Human Serum Albumin Hydrolysates

Hideo Hatate

National Fisheries University

Antioxidative substances play an important role in depression of undesirable oxidation in biological systems as well as in food processing. Although synthetic antioxidants such as butylated hydroxytoluene are effective for preventing lipid peroxidatione, their safety has been questioned. Therefore, many researchers have been screening for useful antioxidants from various natural materials. At present, vitamin E (α -tocopherol) is widely used as a safe natural antioxidation. Furthermore, it has been demonstrated that peptides from various proteins, such as soybean protein, sardine myofibril protein, and bovine serum albumin, enhanced the anti oxidative activity of α-tocopherol (Toc). These results suggest that peptides, which are produced metabolically and incorporated dietetical!y, will serve as an effective synergist in biological systems. Namely, these peptides are supposed to protect *in vivo* lipid peroxidation in cooperation with antioxidants such as tocopherols. Nevertheless, the investigation on the synergisms of peptides originated from human serum albumin (RSA) is not made yet.

In the present study, S-carboxymethylated HSA (CM-HSA) was employed for the preparation of synergistic peptide, instead of the intact HSA, because the disulfide bridges in the HSA molecule seemed to prevent the proteolytic digestion. The CM-HSA treated with pepsin showed strong synergistic effect towards Toc. From the resulting hydrolysates a peptide, having a strong synergism, was successfully separated by chromatography on a Sephadex G-25 column, and then high-performance liquid chromatography on an ODS column. This peptide, designated as HSA-H-5, was again hydrolyzed with a lysyl endopeptidase to give two peptide fragments. Each fragment thus isolated was tested for its synergistic effect with Toc, and it was found that one fragment had a potent synergism comparable to the original peptide, HSA-H-5, but the other one had little effect. The structure of that active fragment was confirmed as the tetrapeptide, Leu-Gln-His-Lys, by amino acid analysis and sequence determination. In addition, HSA-H-5 and the active fragment corresponded to the amino acid residues 103 to 112 and 103 to 106 of HSA, respectively. Although the role of that active fragment in the HSA was not apparent, it composed of only five amino acid residues. Therefore, it will be advantageous not only for the application as a useful synergist but also for the elucidation of unknown synergistic mechanism of peptide synergists.