Development of highly sensitive determination methods for α-lipoic acid and lipoyllysine, and their application to biological specimens

Toshimasa TOYO'OKA

Division of BioAnalytical Chemistry, Graduate School of Pharmaceutical Sciences

University of Shizuoka

α-Lipoic acid (LA) and dihydrolipoic acid (DHLA: reduced form of LA) are well known to possess excellent antioxidant activities. The activity is due not only to direct free radical quenching, but also indirectly through the recycling of other cellular antioxidants. In tissues, LA is mainly present in the protein-bound form (lipoyllysine, LLys) and plays important roles in biological systems. LA is covalently bound to the ε-NH_2 group in the lysine residue(s) of proteins. The protein-bound LA essentially plays a role in the energy metabolism as a part of the pyruvate and α-ketoglutarate dehydrogenase complexes which regulate the flow of carbon into the Krebb's cycle, ultimately resulting in the production of ATP.

The determinations of LA, DHLA, and LLys are thus important to understand the physiological role in living systems. The determination methods for LA, DHLA and LLys in biological specimens are described in this presentation. The simultaneous determination of LA and DHLA was carried out by HPLC with fluorescence (FL) detection. DHLA in the sample was first labeled with ABD-F, and then the LA was labeled with SBD-F after conversion to DHLA using the reducing agent, TCEP. The resulting fluorophores were separated by reversed-phase chromatography and detected at 510nm (excitation at 380nm). The proposed method was applied to the determination in human plasma after the oral administration of LA supplement.

The direct determination of LLys in proteins was also carried out by HPLC with FL detection. The proteins containing LA were first hydrolyzed with several enzymes such as pronase E and subtilisin A. The disulfide bond (-S-S-) in LLys liberated from the enzyme digestion was reduced with TCEP to the thiol form (-SH). The reduced LLys was then labeled with SBD-F. The developed method was applied to the determination of LLys in spinach and animal tissues. The direct determination of LLys in protein using the FL labeling method is reported for the first time.