緑色野菜中の亜硝酸生成における化学生物学の研究

（様式第３号）

論　文　要　旨

論　文　題　目　A study on chemical biology of nitrite formation in green vegetables
緑色野菜中の亜硝酸生成における化学生物学の研究

Nitrite (NO_2^-) contained in dietary foods has long been recognized for its toxicity as the causative agent of methemoglobinemia and also as a source of mutagenic nitrosamines. Because of these potential toxicities, nitrite as well as nitrate (NO_3^-) contained in foods and drinks is strictly limited by regulations in many countries. Recent studies have offered us to update our recognition of nitrite; nitrite is an important precursor for nitric oxide (NO) that is required for fundamental physiological activities in human. Although it is well established that green vegetables contain high amounts of nitrate, there has been controversy regarding the source of nitrite accumulation in fresh green vegetables. To account for the current controversy, this study aimed to investigate the dynamics of nitrite formation in green vegetables, focusing on three issues: nitrite quantification procedure, mechanism for nitrite accumulation in green vegetables, and search for novel nitrite-releasing reactions. We have improved a procedure of HPLC anion chromatography and successfully quantify nitrite and nitrate contents in green vegetables. Using the new protocol we measured nitrate content in retail spinach (*Spinacia oleracea* L.) as 1,900 mg/kg, with 785 mg/kg as minimum and the maximum of 2,544 mg/kg, but did not measure nitrite in fresh spinach leaves. We investigated the dynamics of nitrite and nitrate contents in spinach leaf extracts to verify the mechanisms of nitrite formation. The time course of nitrite production in leaf extracts showed a reciprocal relationship with nitrate degradation, suggesting a conversion from nitrate to nitrite. The reaction strongly depended on temperature and it was suppressed at a low temperature. Enzyme inhibitor experiments suggest that previous reports of nitrite accumulation can be attributed to microbial nitrate reductase activities that occur during the decaying of spinach leaves. In addition to the nitrite formation by bacterial activities we found that NO_2 groups of nitro-compounds can be released as nitrite or nitrate ions through the reaction with hydroxyl radical (·OH), a novel reaction that is associated with reactive oxygen species (ROS). Based on the new findings, beneficial aspects of nitrite contained in green vegetables are discussed in terms of NO bioavailability for human physiology.

氏　名　渡邊　なお子