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ABSTRACT OF DOCTORAL THESIS

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Title: Characterization of folate and vitamin B_{12} compounds in foods and the mechanisms of oxidative stress generation caused by the nutritional status of these vitamins

(食品中の葉酸とビタミンB12化合物の特徴とこれらビタミンの栄養状態によって生じる酸化ストレス発生メカニズム)

Homocysteine (Hcy), a sulfur-containing amino acid, is primarily formed through methionine demethylation and is an important compound in the thiol compound metabolic pathway. The oxidation of two Hcy molecules results in the formation of reactive oxygen species. Hcy is known to be a risk factor for cardiovascular disease and cerebrovascular diseases, such as Alzheimer's disease. Elevated Hcy levels can be reduced by taking folate and vitamin B_{12} (B_{12}), which can help lower the risk of these diseases. Thus, accurate quantification of folate compounds in foods is important for determining folate nutritional values and clarifying the mechanisms of oxidative stress generation caused by B_{12} and folate compound nutritional status.

I investigated whether *Lactobacillus rhamnosus* strains (ATCC 27773) used for the folate microbiological assays grew similarly or differently when pteroyl-mono-glutamate ($PteGlu_1$) and pteroyl-di-glutamate ($PteGlu_2$) was added. $PteGlu_1$ stimulated ATCC 27773 growth to a greater extent than $PteGlu_2$ at various concentrations. The total folate content of various foods treated with a chicken pancreas folate conjugase was determined using ATCC 27773 bioassays. When $PteGlu_1$ was used as a calibrator, the value was significantly lower than when $PteGlu_2$ was used. These findings suggested that $PteGlu_2$ should be used as the standard folate compound when preparing samples for the ATCC 27773 bioassay using chicken pancreas folate conjugase.

Although plants do not biosynthesize B_{12}, it has been demonstrated that some algae absorb exogenous B_{12}. Some commercially available *Chlorella* tablets were found to contain significant amounts of B_{12} and folate compounds. Therefore, I determined the total B_{12} and folate contents of 13 commercially available *Chlorella* tablets for human dietary supplements to discuss the correlation of both vitamin contents. Although the B_{12} content of *Chlorella* tablets varied significantly, the folate level remained constant. There was no correlation between the B_{12} and folate values of all tested *Chlorella* tablets, but a weak correlation was observed using the values, except for high

B_{12}-containing samples. The average value of total folate compounds in these *Chlorella* tablets was ~1.3-mg/100-g dry weight, with tetrahydrofolate (THF; ~27%) and 5-CH_3-THF (~30%) detected as the major folate compounds.

Many studies have discovered that certain foods contain inactive B_{12} compounds, such as pseudovitamin B_{12}. Therefore, accurate quantification of B_{12} compounds in foods is important for determining folate nutritional values. Small shrimp viscera contain B_{12}-*d*-monocarboxylic acid and dicarboxylic acid, compounds with modified peripheral propionamide side chains on the corrin ring. Thus, I prepared six types of authentic B_{12} monocarboxylic and B_{12} dicarboxylic acids to identify B_{12} compounds from edible viscera extracts of these seafoods and commercially available livestock liver, such as cow, pig, and chicken. The viscera tested contained B_{12}-*b*-monocarboxylic, B_{12}-*d*-monocarboxylic, and B_{12}-*e*-monocarboxylic acids and B_{12}-*be*-dicarboxylic acid modified with the corrin ring's peripheral propionamide side chains. Conversely, livestock liver contained B_{12} (~90%) and various inactive corrinoids (~10%), including factors III, A, S, pseudovitamin B_{12}, and B_{12} monocarboxylic acids.

It has been demonstrated that folic acid is more bioavailable than naturally occurring THF compounds. Thus, folic acid is fortified into cereals in over 80 countries to reduce the risk of neural tube defects in fetuses. Thus, there is a growing concern about the long-term effects of high-dose synthetic folic acid consumption. Using *Caenorhabditis elegans* as a model animal, I evaluated the effects of chronic high-dose folic acid supplementation on physiological events and folate metabolism. High-dose folic acid supplementation significantly reduced the egg-laying capacity, and folate metabolic disorders caused accumulation of unmetabolized homocysteine, resulting in severe oxidative stress in worms. These results were consistent with what has been observed in mammals during folate deficiency.

The short and plump "dumpy" mutant phenotype formed by disordered cuticle collagen biosynthesis, the main component of worm skin (extracellular matrix), was observed in B_{12} deficient worms. To understand how B_{12} deficiency causes physiological dysfunctions, including specific morphological abnormalities, I demonstrated the effects of B_{12} deficiency on the formation of dityrosine crosslinking in collagen maturation. The B_{12} deficient worms exhibited a decreased collagen level of up to ~59% compared with the control. Dityrosine crosslinking is involved in worm collagen extracellular maturation. The oxidative stress caused by B_{12} deficiency leads to dityrosine crosslinking. Moreover, B_{12} deficiency-induced oxidative stress triggers the formation of dityrosine crosslinked amyloid β, which may promote its stabilization and toxic oligomerization in GMC101 mutant worms that express the full-length human amyloid β.

(Note: When approved for publication as a abstract, please specify as follows.)
"* In addition, some of the figures, etc., have been omitted."