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Original Article

Biotin and its analogs in edible mushrooms measured by *Lactobacillus plantarum*

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Summary

Biotin in foods is generally measured by a microbiological method using *Lactobacillus plantarum* (ATCC 8014). This bacterium responds not only to biotin but also to biotin *d*- and *l*-sulfoxides, which have no effect as biotin in rats. Since these analogs influence measured values, biotin and its analogs in foods were fractionated by column chromatography. Then, the proportion of growth activity of *L. plantarum* responding to these biotin compounds was examined. The results show that in mushrooms, the proportion of a compound presumed to be biotin *d*-, *l*-sulfoxides, or both was relatively high compared with egg yolk, chicken liver, and broccoli. This indicates that the biotin content in mushrooms may be overestimated when determined by *L. plantarum*.

Introduction

Biotin is one of vitamin B groups and functions as coenzymes of carboxylases involved in gluconeogenesis, fatty acid synthesis, and branched-chain amino acid metabolism. Recently, biotin has also been reported to have various physiological functions[1,2)].

This vitamin widely exists in various foods and is especially rich in egg yolk and liver. The contents in foods are described in the food composition table and are used for nutrition management. In Standard Tables of Food Composition in Japan, 2020 (Eighth Revised Edition)[3)], the biotin contents were measured by a microbiological method using *Lactobacillus plantarum* (ATCC 8014). There are several methods for measuring biotin contents, such as high-performance liquid chromatography (HPLC), liquid chromatography with tandem mass spectrometry (LC-MS/MS) and avidin-binding assay[4)]. However, the microbiological method is chosen because it has excellent detectability and can measure many samples simultaneously. Additionally, this method does not require special equipment and highly refined samples compared with other methods.

There are biotin analogs in nature, such as desthiobiotin, biotin sulfoxide, and bisnorbiotin, present *in vivo* and in foods. These biotin analogs may be confounding when measuring biotin. For example, in the binding assay, avidin binds biotin analogs as well as biotin[5)]. In the microbiological assay, *L. plantarum* grows in response to not only biotin but also biotin *d*- and *l*-sulfoxides. These compounds have a biotin activity of 100% and 5% (biotin *d*- and *l*-sulfoxides, respectively) for *L. plantarum*. However, other biotin analogs such as desthiobiotin and bisnorbiotin show no activity. Additionally, it has been reported that biotin *d*- and *l*-sulfoxides are not as effectively used as biotin in rats[6)].

This study separated biotin and its analogs in egg yolk, chicken liver, broccoli, and various mushrooms by column chromatography and then measured the growth activity of *L. plantarum* in each fraction. As a result, it was clarified how much biotin analogs affect the biotin value determined by the microbiological assay using *L. plantarum*.

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Materials and Methods

Foods and chemicals

Foods used in this study were purchased at supermarkets. Egg yolk, chicken liver and broccoli were used as general foods. All chemicals were commercial products of analytical grade.

Sample preparation

The food was homogenized with distilled water, and then acid hydrolysis treatment was conducted to liberate the biotin bound to the protein. An equal volume of 2.25 mol/L sulfuric acid was added to the homogenate and autoclaved at 121℃ for 1 h. After cooling to room temperature, pH was adjusted to 7.0 with sodium hydroxide. The solution was centrifuged (2,000×*g*, 10 min) and the supernatant was stored at −20℃ until chromatographic separation.

Fractionation and measurement of biotin and its analogs

The stored samples were centrifuged (10,000×*g*, 10 min, 4℃), and the supernatant was applied to the column. Column chromatography was performed as follows with reference to the report[7]. The column (0.39 cm^2×15 cm) packed with DOWEX 1 X 2 (formate form, 200–400 mesh) was used. Distilled water (0–20 mL) and 0.012 mol/L formic acid (20–120 mL) were used sequentially as eluents. The effluent was collected using a fraction collector. The microbial growth activity of each fraction was measured using *L. plantarum* (ATCC 8014). The activity was determined using biotin as a standard.

Results and Discussion

Representative calibration line for biotin assay is shown in Fig. 1. The results of typical fractionation of egg yolk, maitake mushroom (*Grifola frondosa*) (maitake), and hanabiratake mushroom (*Sparassis crispa*) (hanabiratake) are shown in Fig. 2. Growth activity peaks were confirmed to be around 40 mL and 90 mL of elution volume, defined as peak I and peak II, respectively. In egg yolk, peak II was detected as the main peak. This peak was derived from biotin, as the standard biotin was detected at this position. On the other hand, in maitake and hanabiratake, peak I was detected as a relatively large peak in addition to peak II. Peak I was presumed to be derived from biotin *d*-, *l*-sulfoxides, or both, as *L. plantarum* grows in response to these analogs as well as biotin.

L. plantarum growth activity in foods derived from peaks I or II is summarized in Table 1. This activity was calculated by summing the activities in each fraction that constitute the peaks I or II. The results showed

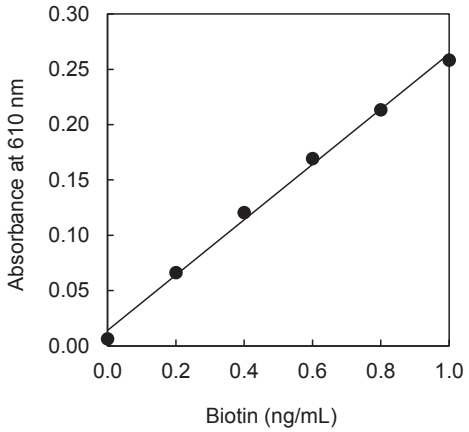

Fig. 1 Growth response of *L. plantarum* to biotin.

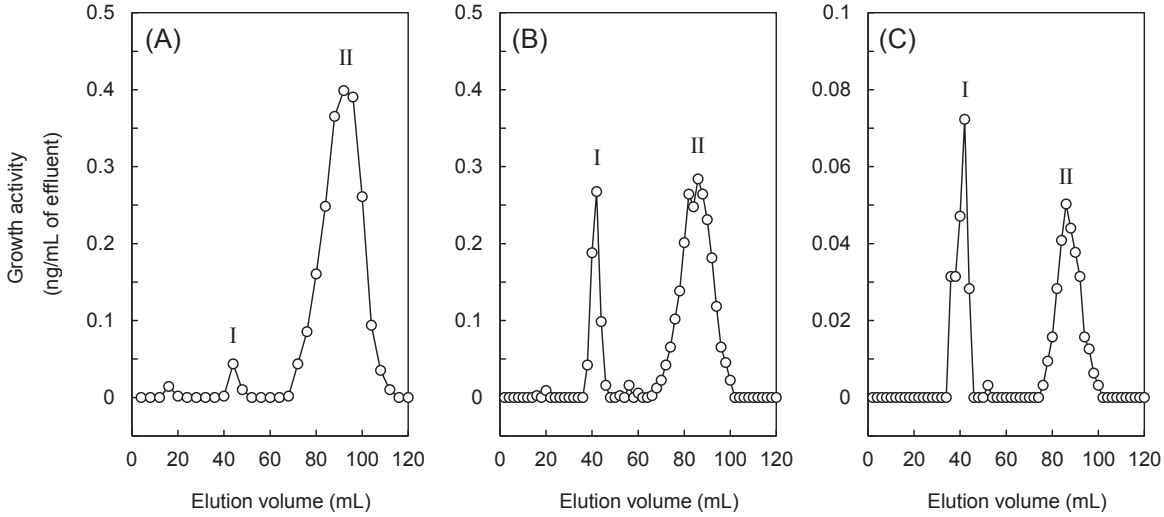

Fig. 2 Fractionation of biotin and its analogs in egg yolk (A), maitake (B), and hanabiratake (C) by column chromatography. These chromatograms are representative of the results. The growth activity in each fraction was measured using *L. plantarum*. This activity was calculated using biotin as a standard.

Table 1 *L. plantarum* growth activity in mushrooms

Foods		*L. plantarum* growth activity *				peak I / (peak I + peak II)
		peak I		peak II		
[General foods]						
Chicken liver	n=3	7.3	(3.3 - 10.5)	137.8	(99.5 - 210.9)	0.05
Egg yolk	n=4	2.9	(2.0 - 5.6)	69.0	(52.5 - 83.6)	0.04
Broccoli	n=2	0.2	(0.0, 0.4)	8.2	(6.3, 10.1)	0.02
[Mushrooms]						
Maitake (*Grifola frondosa*)	n=6	3.8	(3.0 - 4.5)	18.4	(12.4 - 27.1)	0.17
Brown mushuroom (*Agaricus bisporus*)	n=2	1.5	(1.3, 1.6)	9.1	(8.8, 9.5)	0.14
Enokitake (*Flammulina velutipes*)	n=3	1.1	(0.9 - 1.5)	8.4	(7.9 - 8.9)	0.12
Shiitake (*Lentinus edodes*)	n=3	0.8	(0.4 - 1.1)	5.9	(5.1 - 6.7)	0.12
Bunashimeji (*Lyophyllum ulmarium*)	n=2	1.0	(0.9, 1.2)	3.8	(1.9, 5.7)	0.22
Eringi (*Pleurotus eryngii*)	n=2	0.1	(0.1, 0.2)	3.6	(3.6, 3.6)	0.03
Hanabiratake (*Sparassis crispa*)	n=4	1.7	(1.4 - 2.1)	2.3	(2.0 - 3.3)	0.43
(Without hydrolysis treatment)						
Maitake (*Grifola frondosa*)	n=3	3.2	(2.3 - 3.8)	4.3	(2.8 - 6.2)	0.42
Enokitake (*Flammulina velutipes*)	n=3	0.8	(0.7 - 1.0)	4.6	(3.2 - 5.3)	0.15
Hanabiratake (*Sparassis crispa*)	n=4	1.4	(0.7 - 1.7)	0.1	(0.0 - 0.4)	0.91

*Activity was measured using biotin as a standard and expressed as μg/100 g of food.
*mean (minimum and maximum)

that mushrooms had a relatively high proportion of peak I to total activity (peaks I plus II), compared with general foods, such as egg yolk, chicken liver, and broccoli. Especially for hanabiratake, about 43 % of the total activity was derived from peak I. As a side note, in the food composition table, for example, the value of maitake is 24.0 μg and the value of enokitake is 11.0 μg. These values are almost the same as the total value of peak I and II, and the method in this study is considered to be appropriate.

In this study, acid hydrolysis was performed according to conventional methods to release biotin bound to protein. However, it has been reported that this treatment oxidizes biotin to biotin sulfoxides[8]. Therefore, samples of maitake, enokitake, and hanabiratake mushrooms were prepared without hydrolysis. As shown in the lower part of Table 1, peak I was still detected without acid hydrolysis. These results indicate that mushrooms originally contain compounds that formed peak I.

It has been reported that biotin in mushrooms was measured by a microbial method using *Saccharomyces. cerevisiae*[9]. *S. cerevisiae* responds to various biotin analogs more than *L. plantarum*. In that study, biotin, desthiobiotin, and 7,8-diaminopelargonic acid were detected by the bioautogram method. Therefore, when measuring biotin in mushrooms using the microbiological method, it may be necessary to consider the presence of biotin analogs.

In addition to mushrooms, biotin analogs in marine algae have been reported using the difference in the responsiveness of biotin, biotin *d*-sulfoxide, and biotin *l*-sulfoxide of three microorganisms (*L. casei, S. carlsbergensis* and *Neurospora crassa*)[10]. According to the report, marine algae contained a relatively large amount of biotin *d*- and *l*-sulfoxide compared with biotin.

It has been reported that the concentration of biotin metabolites in human milk was measured by HPLC avidin-binding assay[11]. The study showed that at eight days postpartum, the proportion of biotin was 44 %, bisnorbiotin 48 %, and biotin sulfoxide 8 %. It suggested that substantial amounts of inactive metabolites, bisnorbiotin and biotin sulfoxide, are present in human milk. Therefore, it is not surprising that biotin analogs also exist in foods. The authors also pointed out problems in quantifying biotin, that biotin analogs lead to overestimates of the biotin content in the avidin-binding assay, and biotin sulfoxide influences the biotin content in the microbial assay using *L. plantarum*[11].

Staggs et al. determined the quantity of biotin in foods using HPLC/avidin-binding assay[12]. However, the study showed considerable differences in some foods between the biotin values published in the previous report and those using HPLC/avidin binding assay. Therefore, the authors concluded that biotin values published in previous research might be inaccurate, partially due to the use of the microbiological method.

The standard method of microbiological assay does not separate biotin analogs in food. However, this study separated biotin and its analogs in foods, especially

mushrooms, by column chromatography and then determined by *L. plantarum*. As a result, in general foods, this bacterium was mostly responsive to biotin. However, mushrooms contained a relatively high proportion of compounds other than biotin that allowed *L. plantarum* to grow, suggesting that biotin content in mushrooms was overestimated when determined by *L. plantarum*.

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