Note

Evidence for the Occurrence and Distribution of a New Type of Vitamin B₆ Conjugate in Plant Foods[†]

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Vitamin B₆ is widely distributed in foods. In plant foods, the predominant forms of B₆ vitamers are pyridoxine (PN) and PN conjugates¹⁻⁴⁾ such as pyridoxine 5'-phosphate (PNP) and 5'-O-(β-D-glucopyranosyl)pyridoxine (PNG).^{5,6)} Kabir *et al.*⁷⁾ suggested that a substantial part of total vitamin B₆ exists as unidentified vitamin B₆ conjugate(s) in plant foods, especially in grains and legumes. We found a new type of vitamin B₆ conjugate (B₆X) in some plant foods. B₆X released free vitamin B₆ (FB₆) only when treated with alkali and then by β-glucosidase. This paper describes the occurrence and distribution of B₆X in some plant foods.

A vitamin B₆-depleted basal medium for microbiological assay was bought from Nissui Pharmaceutical Co., and food samples were from markets unless otherwise mentioned. PNG was obtained as described previously.⁸⁾ Sweet almond β-glucosidase from Sigma Chemical Co. was contaminated with phosphatase activity hydrolyzing pyridoxal 5'-phosphate (PLP) under the present conditions. Microbiological assay was performed by using *Saccharomyces uvarum* (ATCC 9080) as a test organism according to the method of Atkin *et al.*⁹⁾ as modified by Fukui *et al.*¹⁰⁾ turbidity being measured at 660 nm. The high-performance liquid chromatography (HPLC) described in a previous paper¹¹⁾ was modified for the sensitive detection of PN, a fluorescence detector—JASCO FP-210—being used (excitation at 345 nm and emission at 397 nm).

Rice bran (Kōkin Chemical Co., 100 g) was extracted with water (500 ml) in a boiling water bath for 30 min. The extract was adjusted to pH 7.15 with 2 M KOH, and put on a column (1.6 × 10 cm) of Dowex 1 × 4 (acetate form). B₆X was eluted with 0.5 M AcOH, and the fraction containing B₆X was concentrated to 25 ml *in vacuo* below 40°C. The

concentrate was analyzed by HPLC before and after the following treatments: (a) The concentrate (1 ml) was adjusted to pH 5.0 with KOH, and incubated with 10 units of β-glucosidase at 37°C for 3 hr. (b) The concentrate was brought to a 0.7 M KOH concentration by addition of 6 M KOH, and allowed to stand at room temperature for 3 hr in the dark. (c) The concentrate (1 ml) was subjected to treatment (b), adjusted to pH 5.0 with dil. HCl, and subjected to treatment (a). (d) The concentrate (1 ml) was submitted to treatment (a) and then (b). The untreated sample gave two peaks on a HPLC chromatogram. B₆X was eluted at a time of about 13 min. On treatments (a), (b) and (d), a peak attributable to PN did not appear, indicating that B₆X was not PNG or PNP. Treatment (c), however, gave PN (elution time 27 min), but not PL (21.6 min) or pyridoxamine (58 min). The released PN was confirmed by paper chromatography.⁸⁾ There was no practical difference between the released-PN values obtained by bioassay and HPLC. These results show that B₆X is a new type of PN conjugate. In B₆X, the glucose moiety of PNG may be esterified with an organic acid. Similar results were obtained in wheat bran (Torigoe Milling Co.).

Total vitamin B₆, B₆X, FB₆, glycosylated vitamin B₆⁷⁾ (B₆G) and FB₆ 5'-phosphate (FB₆P) in some plant foods were analyzed by bioassay. A food sample (0.5~5 g), finely divided if necessary, was rapidly treated as follows: for total vitamin B₆, the sample was hydrolyzed according to the method of AOAC¹²⁾; for FB₆, the sample was extracted with water (50 ml) in a boiling water bath for 15 min. The extract was filtered through paper, and the debris washed with water. The filtrate and the washings were combined, and filled up to 100 ml with water; for B₆G + FB₆P, the suspension of the sample with 50 mM acetate buffer of pH 5.0 (50 ml) was heated as described above, followed by incubation with 60 units of β-glucosidase by the method of Kabir *et al.*,⁷⁾ B₆G and FB₆P being hydrolyzed. After heating the mixture, it was filtered, and filled up to 100 ml as described above; for B₆X, the suspension (50 ml) of the sample with 0.7 M KOH was allowed to stand at room temperature for 3 hr in the dark, adjusted to pH 5.0 with dil. HCl, and incubated with β-glucosidase as described above. An appropriate volume of each solution obtained in the above treatments was subjected to bioassay.

Table I shows the contents of total vitamin B₆, FB₆, B₆G + FB₆P, and B₆X in the 8 plant foods. Recovery of FB₆, B₆G and FB₆P was assessed by using PN, PNG and PLP, respectively, as standards to be 96 ± 11%. The total vitamin B₆ content was in fairly good agreement with the values reported in the literature. Although B₆X was also present in defatted soybeans (Kyōwa FD Foods Co.) and immature podded peas, its content was highest in rice bran. The sum of FB₆, B₆G + FB₆P and B₆X was close to the total vitamin B₆ content in the foods, except for

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TABLE I. CONTENTS OF TOTAL VITAMIN B₆ AND VITAMIN B₆ COMPONENTS IN PLANT FOODS

Food	Total vitamin B ₆ ($\mu\text{g}/100\text{ g}$ fresh weight) ^a		Vitamin B ₆ components ($\mu\text{g}/100\text{ g}$ fresh weight) ^a		
	Present work	Reported	Free (FB ₆)	Glycosylated + 5'-phosphate (B ₆ G + FB ₆ P)	Conjugate (B ₆ X)
Cauliflower	104 $\pm$ 5	20 ¹³⁾ 150 ⁷⁾	36 $\pm$ 1 (35) ^b	61 $\pm$ 3 (59) ^b	ND ^c
Spinach	104 $\pm$ 13	102 ¹³⁾	39 $\pm$ 1 (38)	79 $\pm$ 10 (76)	ND
Pumpkin	351 $\pm$ 22	114 ¹⁰⁾	76 $\pm$ 9 (22)	243 $\pm$ 19 (69)	ND
Broad beans, immature	113 $\pm$ 15	—	67 $\pm$ 6 (59)	19 $\pm$ 3 (17)	ND
Podded peas, immature	70 $\pm$ 9	—	20 $\pm$ 2 (29)	31 $\pm$ 3 (44)	15 $\pm$ 1 (21) ^b
Soybeans, defatted	478 $\pm$ 21	440 ¹⁴⁾ 550 ¹³⁾	125 $\pm$ 10 (26)	260 $\pm$ 5 (54)	128 $\pm$ 21 (27)
Rice bran	4828 $\pm$ 157	2120 $\sim$ 5050 ¹³⁾	384 $\pm$ 15 (8)	427 $\pm$ 17 (9)	1822 $\pm$ 225 (38)
Wheat bran	1039 $\pm$ 119	1400 ¹³⁾ 903 ⁷⁾	369 $\pm$ 14 (36)	431 $\pm$ 40 (41)	199 $\pm$ 27 (19)

^a Values are the means $\pm$ S.D. of 3 determinations and represented as pyridoxine equivalents.

^b % of vitamin B₆ components in total vitamin B₆.

^c Not detected.

immature broad beans and rice bran.

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