METABOLISM AND BIOLOGICAL ACTIVITY OF BENZENESULFONYLTHIAMINE DISULFIDE

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In the previous paper (1) the metabolism of benzenesulfonyl thiamine (BST) was compared with that of thiamine, while in this paper the metabolism of benzenesulfonylthiamine disulfide (BSTDS), disulfide compound of BST, was studied and its metabolism was found to be significantly different from that of BST.

BSTDS is a compound (2) obtainable by the reaction of benzenesulfonyl chloride with alkaline thiamine solution in the cold, and it can also be prepared from thiamine disulfide (TDS) by the similar reaction. Both TDS and thiamine propyl disulfide (TPD) are reduced in animal bodies to thiamine, whereas BSTDS is also reduced to BST via thiol compound (II') of BST (II) or it can also be converted into thiamine anhydride (TA) (III) from II' by ring closure. When BSTDS was injected to rats, the urinary excertion of thiamine (thiochrome-positive compounds are regarded as thiamine) was very low, a finding significantly different from that after administration of TDS or TPD. This finding suggests the occurrence of a reaction of BSTDS to TA. So the urinary metabolic products following the administration of BSTDS were investigated. However, TA could not be separated and identified. BSTDS is degraded by the reaction of rat liver homogenate at pH 6, whereas it was fairly stable at pH 4.5, the recovery being approximately 80 per cent. This reaction proceeds nonenzymatically, and the same result was obtained by the reaction with cysteine or thioglycolic acid. Especially using thioglycolic acid, TA was obtained as crystals, suggesting the reaction of BSTDS (I) to TA (III) via (II'). On the other hand, the increase of the body weight of the rats fed a thiamine-deficient diet was measured as an index of the thiamine activity of BSTDS. Although the compound equivalent to 20 μg of thiamine-HCl daily was given every other day, it was proved to be ineffective to promote the growth of rats. Administration of 10-fold amount of BSTDS, however, resulted in the increase of body weight, showing the partial occurrence of the reduction, of BSTDS to thiamine in vivo.

EXPERIMENTAL

1. Urinary Excretion of Thiamine after Intraperitoneal Injection of BSTDS

One ml each of BSTDS solution (containing the compound equivalent to 5 mg of thiamine-HCl) was injected intraperitoneally to each of the three male
rats, weighing about 230 g, and 9 ml of water was given orally. Then the urine for 5 and 24 hours, respectively, was collected, to which 1 ml of 3 % acetic acid and 0.5 ml of toluene had been added. As controls, the urine excreted by the rats having received TDS or TPD (equivalent to 5 mg of thiamine-HCl) was collected. After adequate dilution of the urine, the amount of thiamine was determined by BrCN method. As shown in Fig. 1, the urinary excretion of BSTDS was 105 µg after 5 hours and 172 µg after 24 hours. It was considerably low as compared with the administration of other thiamine disulfide compounds.

2. Degradation of BSTDS by Rat Liver Homogenate

The liver (approximately 6.2 g) was removed from the male rat weighing approximately 150 g, and it was homogenized with 0.9 % NaCl solution in the cold; it was diluted 10-fold with the salt solution and filtered through a gauze.

To 5 ml of the filtrate 2 ml of acetate buffer (0.1 M, pH 4.5 and 6.0) and 1 ml of thiamine or BSTDS solution containing the amount equivalent to 40 µg of thiamine-HCl were added and the whole was incubated at 37° for 3 hours. As a control, the liver homogenate heated at 80° for 20 minutes was used. After the reaction, the mixture was boiled for 5 minutes, treated with Takadiastase, filled up to 50 ml and filtered. To 5 ml of the filtrate, 2 ml of 0.5 M tartarate buffer (pH 3.0), and 1 ml of 5 mg/ml cysteine solution (neutralized) were added and the whole was incubated at 60° for 30 minutes. Then 1 ml of 10 % hydrochloric acid was added and thiamine was determined by a BrCN method.

As shown in Fig 2, thiamine was practically completely recovered, whereas the recovery of BSTDS was significantly less, suggesting the degradation of the compound by the liver homogenate. The detailed investigation of this result, however, revealed that the recoveries at pH 4.5 and 6.0, 11 and 77 per cent, respectively, are roughly the same as those using the homogenate previously.

![Fig. 1 Urinary Excretion of Thiamine after Intrapertitoneal Injection of BSTDS and Other Thiamine Derivatives (mean of 3 animals)](image)

○, thiamine; ●, TDS; ▲, TPD; ×, BSTDS

![Fig. 2 Change of Thiamine and BSTDS by Liver Homogenate](image)

□, liver homogenate; ■, boiled liver homogenate
inactivated by heating, 3 and 77 per cent respectively, showing the degradation
to be of nonenzymatic nature.

3. Reaction of BSTDS with Cysteine or Thioglycolic Acid.

To 5 ml of the solution of BSTDS containing the compound equivalent to
2 μg of thiamine-HCl per ml were added 1 ml of 0.1 M phosphate buffer (pH 7 to
8) or 0.1 M acetate buffer (pH 3 to 6) and 1 ml of 5 mg/ml cysteine-HCl solution
(neutralized) and the mixture was heated at 50° for 30 minutes. The solution
was then acidified with 1 ml of 10 % hydrochloric acid and the thiamine produc-
ed was determined by a BrCN-thiochrome method. As a control, the same experi-
ment was was carried out with TDS. As shown in Fig. 3, thiamine was recovered
almost quantitatively between pH 5 and 8 in the case of TDS, while the recove-
ry was highest at pH 3 in the case of BSTDS decreasing significantly at pH
levels higher than 6. The recovery of thiamine was decreased as the pH ap-
proached neutrality in the reaction of BSTDS with cysteine possibly due to poor
formation of BST at neutral reaction through the reduction by cysteine may have
taken place. For isolation of the reaction product, the reaction of a somewhat
great amount of BSTDS with cysteine was tried, but it failed owing to the
difficulty of controlling the pH values, so thioglycolic acid was used in stead of
cysteine. 200 mg of BSTDS was added to 20 ml of 1 M acetate buffer (pH 6.1)
containing 500 mg of sodium-thioglycolate and the mixture was heated at 50° for
30 minutes. The crystals were gradually dissolved to light pink solution, leaving
behind a small amount of insoluble matter. It was filtered off with heating and
the filtrate was left standing in a refrigerator overnight. Colorless squamous
crystals formed were filtered and washed with cold water. By recrystallization
from water, colorless blade crystals (mp 110°) were obtained. The mixed melting-
point, when mixed with an authentic sample of TA, showed no depression. Analysis for TA, C_{12}H_{16}O_{4}N_{5}S. Calculated: C, 54.52; H, 6.10 and N, 21.20. Found:
BSTDS is reduced by thioglycolic acid to a thiol form of BST, but in a solution with pH near neutrality, the reaction toward TA predominates over thiazole-ring formation. Therefore the urinary excretion of thiamine in the rats having received BSTDS was considerably lower than that after administration of other thiamine disulfide derivatives and both the reaction with cysteine and the degradation by rat liver homogenate are markedly affected by pH values: The production of TA must be taken into consideration.

4. Urinary Metabolites Following BSTDS Injection

BSTDS, 50 mg, was injected intraperitoneally to the three rats kept on a normal diet and the urine was collected for 5 hours. It was subjected to paper chromatography using three different solvent systems. As shown in Fig. 4, a spot having the same value as thiamine anhydride sulfoxide (TAO) was obtained only when 80% ethanol was used, whereas the values were somewhat different from TAO when two other solvents were used. The paper chromatography of the metabolite in 24-hour urine gave similar results. The main metabolite showed the same $R_f$ values with three different solvents as one of the two spots of the chromatogram of the substance obtained by permanganate oxidation of TA. Since the urinary excretion of this metabolite was poor compared with the dose of BSTDS, it could not be obtained as crystals.

5. Thiamine Activity of BSTDS

Twenty-three male rats, Wistar strain, weighing about 60 g, were preliminarily kept on a thiamine-deficient diet for 8 days and they were divided into following 4 groups. The first group consisting of 6 animals received an intraperitoneal injection of BSTDS. The second group of 7 animals orally received BTDS. The third group of 4 animals received thiamine. The fourth group of 6 animals was fed a thiamine-deficient diet. All the samples were given every other day. The first group received 0.5 ml of 50 $\mu$g/ml of BSTDS (equivalent to 40 $\mu$g of thiamine-HCl) till the 18th day, then 0.5 ml each of 500 $\mu$g/ml of BSTDS from 20th to 28th day. The second group orally received the same amount of BSTDS as the first group. The third group received 0.5 ml of 40 $\mu$g/ml thiamine solution every other day till the 18th day and of 80 $\mu$g/ml thiamine from the 20th day. The fourth group received only the thiamine-deficient diet and 0.5 ml of water every other day till the 18th day. Therefore, they were divided into two groups, 4--1 and 4--2 group, each consisting of 3 animals, and 0.5 ml each of 5 mg/ml BSTDS solution (equivalent to 4 mg of thiamine-HCl) was intraperitoneally injected and orally given, respectively, every other day. They were weighed before each administration which was carried out between 9:00 and 10:00 a.m. As shown in Fig. 5, the body weight increased gradually in the thiamine group, whereas the group of BSTDS showed about the same change in body weight as thiamine-deficient group: It was decreased in about 2 weeks. However, following the administration of 10- and 100-fold amounts after the 20th day, the body weight began rapidly to increase. With this dose, thiamine activity was clearly observed regardless of the mode of administration.

It was reported in the previous paper that BST was not hydrolyzed in rats, being
DISCUSSION

excreted in the urine per se but disulfide compounds such as BSTDS can be metabolically reduced to the thiol form of BST, which, in turn, can be converted to TA. Since TA is excreted in the urine after being oxidized to TAO (5) the urinary metabolites were investigated by paper chromatography, taking the change, BSTDS → TA → TAO, into consideration, but neither TA nor TAO could be detected. However, considering the findings that BSTDS is readily degraded by liver homogenate nonenzymatically at pH 6.0, BSTDS produces TA by the reaction with thioglycolate, and the metabolite coincided in paper chromatography with the oxidation product of TA by permanganate, the main metabolites might be deformylated thiamine anhydride sulfone (TAO₂) or TAO. Deformylated TAO₂ was therefore prepared according to Yonemoto (3). TA was oxidized by permanganate, and after concentration it was dissolved in ethanol. The solvent was evaporated and the residue was crystallized from water. The crystals melted at 188°, but the analysis was not in agreement with deformylated TAO₂. Calculated for deformylated TAO₂: C, 49.23; H, 6.01; N, 0.288. Found: C, 47.90; H, 5.23; N, 18.80. It remains to be settled by further investigation. On the other
hand, the thiamine activity of BSTDS for rats was less than one-tenth as active as thiamine, and different effects of oral and intraperitoneal administrations were observed when 10-fold amount of BSTDS (equivalent to 200 µg thiamine, given every other day) was given. Daily Oral administration of 100 µg BST was also found to be effective (6) but its injection was ineffective. The cause of the different effects of BSTDS and BST after injection is obscure, but in each case, the oral administration resulted in greater increase of body weight, possibly due to the occurrence of the reaction, BSTDS → TDS → thiamine or BST → thiamine in the intestine.

SUMMARY

1. Urinary excretion of thiamine in 24 hours after intraperitoneal injection of benzenesulfonylthiamine disulfide (BSTDS), equivalent to 5 mg of thiamine-HCl, to rats was about 3 per cent (as thiamine or benzenesulfonyl-thiamine). It is markedly low as compared with thiamine disulfide or thiamine propyl disulfide, suggesting the conversion of BSTDS to thiamine anhydride (TA) in the body. BSTDS was degraded nonenzymatically by rat liver homogenate and it was converted to TA by the reaction with thioglycolate. Its low urinary excretion is possibly due to the conversion of BSTDS to thiamine anhydride. However, thiamine anhydride or its sulfoxide could not be demonstrated as the urinary metabolite.

2. The thiamine activity of BSTDS was studied in rats by determining the increase of the body weight. Daily administration of BSTDS equivalent to 100 µg of thiamine showed a significant increase but its administration equivalent to 10 µg of thiamine was ineffective. Oral administration resulted in more rapid rise of body weight than after intraperitoneal injection.

REFERENCES