STUDIES ON THIAMINE DISULFIDE

VI. THE ACTION OF THIAMINASE ON O-BENZOYL THIAMINE DISULFIDE DERIVATIVES

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Although the microbiological activities on thiamine disulfide (TDS) derivatives will be reported elsewhere, it would be important to study the action of thiaminase on TDS derivatives, such as *O*-benzoylthiamine disulfide (BTDS), having thiamine activity in living organisms. In this paper, the actions of thiaminase on BTDS, *O*-acetylthiamine disulfide (ATDS) and thiamine disulfide cetylsulfate (TDS-CS) using the crude thiaminase obtained from both *Bacillus thiaminolyticus* Matsukawa et Misawa (BMM) and *Bacillus aneurinolyticus* Kimura et Aoyoma (BKA) are described as compared to those on thiamine hydrochloride (B_1 -HCl) and TDS by the same enzymes (1, 2).

EXPERIMENTAL

1. Materials

B_1 -HCl, TDS, TDS-CS, BTDS and ATDS were used as the substrates.

2. Enzyme Solutions

Crude Solution of Thiaminase I—In a 50-ml Erlenmeyer flask, BMM was inoculated to a peptone medium containing 1% peptone, 1% meat extract and 0.1% NaCl (pH 7.0), and after incubation for 5 days at 37°, the culture was centrifuged at 5,000 rpm for 20 minutes. The supernatant fluid was used as the crude enzyme after dilution.

Crude Enzyme Solution of Thiaminase II—As given above, BKA was incubated for 3 days at 37°, followed by centrifugation. The supernatant fluid was used as the crude enzyme after dilution.

3. Condition for Enzyme Reaction—TDS derivatives were used as the substrates in amounts equivalent to 20 μ g B_1 -HCl. Incubation was made at 37° for one hour in the following reaction mixture.

Substrate solution (equivalent to 20 μ g/ml B_1 -HCl),	1 ml
Buffer solution (for thiaminase I, McIlvaine buffer, pH 5.4; and for thiaminase II, Sørensen buffer, pH 7.0)	1 ml
Crude enzyme solution	3 ml
Total	5 ml

4. *Decomposition of Thiamine and its Derivatives*—After completion of the reaction, 10 ml of McIlvaine buffer (pH 7.0) and 1 ml of 10 mg/ml L-cysteine-hydrochloride solution were added to 5 ml of the reaction mixture, containing a thiamine derivative equivalent in amount to 20 μ g B₁-HCl and the mixture was incubated at 37° for one hour. By this procedure, B₁-HCl remained intact, but other TDS derivatives were completely reduced to thiamine or its derivatives. To the reaction mixture 5 ml of 25 % KCl-HCl was added and water was added to the volume of 25 ml. A 5-ml aliquot of the mixture was used to the determination of thiamine by the thiochrome method using cyanogen bromide. The recoveries of thiamine and its disulfide derivatives throughout this procedure were 95–100 %. The remaining substrate was determined by subtracting the value with intact enzyme from that with heat-inactivated enzyme.

RESULTS

1. *Decomposition by Crude Thiaminase I*

The 5- or 10-fold diluted solution of the supernatant liquid of BMM culture was used as the crude enzyme solution. Table I shows the results of the experiment in which the derivative was subjected to decomposition by the enzyme under the condition of about 90 and 50 % decomposition of B₁-HCl. TDS and its derivatives were all found to be definitely resistant to thiaminase I as compared with B₁-HCl. Especially TDS-CS, BTDS and ATDS were proved to be more resistant than TDS which had been reported to be resistant to thiaminase I.

TABLE I
Decomposition by Thiaminase I of B. thiaminolyticus

Sample	Remaining thiamine derivative after reaction with the enzyme	
	5-fold diluted	10-fold diluted
	<i>per cent</i>	<i>per cent</i>
B ₁ -HCl	10.2	53.2
TDS	74.6	85.0
TDS-CS	97.6	99.0
BTDS	85.0	95.3
ATDS	84.5	92.1

TABLE II
Decomposition by Thiaminase II of B. aneurinolyticus

Sample	Remaining thiamine derivative after reaction with the enzyme	
	30-fold diluted	60-fold diluted
	<i>per cent</i>	<i>per cent</i>
B ₁ -HCl	18.6	38.7
TDS	92.0	92.8
TDS-CS	97.2	98.0
BTDS	97.3	98.0
ATDS	97.9	98.2

2. *Decomposition by Crude Thiaminase II*

The results of the experiment with the crude enzyme (30- and 60-fold diluted) of BKA capable of degrading about 80 and 40 % of B₁-HCl under the condition described above are summarized in Table II. TDS derivatives were shown to be definitely resistant to both thiaminase I and II.

The heat-inactivated enzymes of both thiaminase I and II showed no decomposition of the vitamin. After incubation of the reaction mixture of each TDS derivative and the enzyme, no thiamine was detected by the thiochrome method without cysteine treatment.

SUMMARY

The action of the thiaminases produced by *Bacillus thiaminolyticus* Matsukawa et Misawa and *Bacillus aneurinolyticus* Kimura et Aoyama on various thiamine disulfide derivatives were investigated, and thiamine disulfide cetylsulfate, *O*-benzoylthiamine disulfide and *O*-acetylthiamine disulfide were found to be definitely resistant to the enzymes.

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