STUDIES ON HYDROXYETHYLTHIAMINE AND RELATED COMPOUNDS

II. ACTION OF THIAMINASE ON HYDROXYALKYLTHIAMINE

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In the preceding paper, it was demonstrated that the biological activity of α -hydroxyalkyl-2-thiamine is identical with that of thiamine when tested by the growth-promoting effect on the thiamine-deficient rat (1).

As a part of the investigation on biological properties of α -hydroxyalkyl-2-thiamine, study was made of the action of two types of bacterial thiaminase on this class of compounds and it was found that, while α -hydroxyalkyl-2-thiamine is readily decomposed by thiaminase I (EC 2.5.1.2) of *Bacillus thiaminolyticus* as is the case with thiamine, it is fairly resistant to the action of thiaminase II (EC 3.5.99.2) derived from *Bacillus aneurinolyticus*.

EXPERIMENTAL

1. Thiaminases

Bacillus thiaminolyticus Matsukawa et Misawa and *Bacillus aneurinolyticus* Kimura et Aoyama were used as the source of thiaminase I and II respectively.

The organisms were grown in a medium containing 1% meat extract, 1% peptone and 0.1% NaCl (pH 7.0) at 37°. After five days of incubation, the cells were removed by centrifugation and the supernatant was used as the enzyme preparation after appropriate dilution with distilled water.

2. Materials

DL- α -Hydroxyethyl-2-thiamine (HET), mp 218° (dec); DL- α -Hydroxypropyl-2-thiamine (HPT), mp 207° (dec); DL- α -Hydroxyisobutyl-2-thiamine (HIBT), mp 217° (dec); DL- α -Hydroxyethylthiamine propyl disulfide (HPD), mp 129°.

The first three compounds were the same as described in the preceding paper. HPD was synthesized in this laboratory from HET and it is structurally similar to thiamine propyl disulfide.

3. Action of Thiaminase I on Hydroxyalkylthiamine

The reaction mixture contained the following components in a final volume of

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5.0 ml; 2 μ g substrate, 67 μ moles phosphate buffer, pH 6.0, 5 μ moles aniline and 1.0 ml of 20-fold diluted supernatant of the *B. thiaminolyticus* culture.

The reaction was started by adding the enzyme preparation and incubation was carried out at 37°. At intervals, the reaction was stopped by addition of 1.0 ml of 10% metaphosphoric acid (HPO_3) and then the reaction mixtures were filled up to 20 ml with 25% KCl-0.1 *N* HCl. Aliquots of 5.0 ml were used for the determination of the remaining substrate by ferricyanide thiochrome method.

In case of HPD, the reaction was stopped by heating the solution over steam for five minutes and after pH was adjusted to 7.0 with 0.1 *N* NaOH, 2 mg cysteine in 1 ml water was added and the mixtures were kept at 37° for one hour. During this treatment, HPD was quantitatively converted to HET. Thereafter, they were made up to 20 ml with 25% KCl-0.1 *N* HCl and treated in the same manner.

4. Action of Thiaminase II on Hydroxyalkylthiamine

The reaction mixtures consisted of the following components in a final volume of 5.0 ml: 2 μ g substrate, 67 μ moles phosphate buffer, pH 7.0, and 1.0 ml of 10-fold diluted supernatant of the *B. aneurinolyticus* culture. Subsequent procedure was the same as that described above for thiaminase I.

RESULTS AND DISCUSSION

As is shown in Fig. 1 and 2, considerable decomposition resulted when hydroxyalkylthiamine was submitted to the action of thiaminase I, although the decomposition seemed to be a little slower than that of thiamine.

FIG. 1 Action of Thiaminase I on Hydroxyalkylthiamine (1)

○, thiamine; ×, HET.

FIG. 2 Action of Thiaminase I on Hydroxyalkylthiamine (2)
 ○, thiamine; ×, HPT; △, HIBT.

FIG. 3 Action of Thiaminase II on Hydroxyalkylthiamine
 ○, thiamine; ×, HET; △, HPT; ●, HIBT.

On the contrary, these compounds were fairly resistant to the action of thiaminase II (Fig. 3) and the stability seemed to become more pronounced with the increase of the size of alkyl substituent.

These facts might be taken as an indication that substitution at the C-2 position of the thiazole moiety does not count much for the action of thiaminase I, whereas in thiaminase II the same site plays a vital role for the enzyme action. Thus, the substitution at C-2 of the thiazole moiety seems to affect the two types of bacterial thiamine differently.

HPD, a disulfide type compound derived from HET, is biologically as active as thiamine in the growth-promoting effect on the thiamine-deficient rat (unpublished observation by the authors). The present study showed that, like thiamine propyl disulfide, it is quite stable to both types of thiaminase (Fig. 4).

FIG. 4 Action of Thiaminase on Hydroxyethylthiamine Propyl Disulfide

Solid line, thiaminase I; dotted line, thiaminase II.

○, thiamine; ×, HPD.

SUMMARY

1. Investigation was made of the action of two types of bacterial thiaminase on α -hydroxyalkyl-2-thiamine (hydroxyethyl, hydroxypropyl and hydroxyisobutyl) and hydroxyethylthiamine propyl disulfide.

2. While hydroxyalkylthiamine was readily decomposed by thiaminase I of *Bacillus thiaminolyticus* Matsukawa et Misawa, it was fairly resistant to the action of thiaminase II produced by *Bacillus aneurinolyticus* Kimura et Aoyama.

3. Hydroxyethylthiamine propyl disulfide was remarkably resistant to both thiaminases.

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REFERENCE

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