REPRESSION OF THIAMINASE I IN BACILLUS THIAMINOLYTICUS[1]

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Bacillus thiaminolyticus produces an exoenzyme, thiaminase I, which catalyzes the reaction:

$$\text{thiamine} + \text{base} \rightleftharpoons \text{PyCH}_2\text{Base} + \text{'thiazole'}$$[3]

Nothing is known of the physiological significance of the production of this enzyme by an organism which requires thiamine for growth. Murata (1965) proposed that the enzyme may be responsible for cellular thiamine biosynthesis, although the equilibrium of the in vitro reaction greatly favors thiamine decomposition. Thiaminase I activity of B. thiaminolyticus was found to increase as the concentration of thiamine in the culture medium decreased during the growth of an individual culture (Douthit and Airth, 1966). This observation suggested a possible regulatory role for thiamine in the production of this enzyme. Our data indicate that thiamine represses thiaminase I synthesis in this organism.

Materials and Methods

B. thiaminolyticus was grown in a medium of: NaCl 1.7×10^{-2}M, $MgSO_4$ 2.8×10^{-3}M, $FeSO_4$ 1×10^{-4}M, KH_2PO_4 8×10^{-3}M, Na_2HPO_4 4.2×10^{-2}M, sodium citrate 1.7×10^{-2}M, monosodium glutamate 1×10^{-1}M, $(NH_4)_2HPO_4$ 5×10^{-2}M, glucose 1×10^{-1}M, and, unless otherwise specified, thiamine · HCl

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[3]Abbreviations: $PyCH_2Base$ = N-(4-amino-2-methylpyrimidyl-5-methyl) base; 'thiazole' = 4-methyl-5-(βhydroxyethyl)thiazole.

3×10^{-7}M. The medium (hereafter referred to as BMM-7), was adjusted to a final pH of 7.5.

B. thiaminolyticus, grown at 37°C, were harvested by centrifugation at 23,000 x g for 15 minutes at 0°C, and the culture medium was then dialyzed against glass redistilled water for 4 hours at 4°C. Thiaminase I activity from this crude preparation was determined spectrophotometrically by the procedure of Douthit and Airth (1966). One unit of activity is defined as the amount of enzyme which forms 1 μmole of product in one minute at 25°C. Protein was estimated by the method of Lowry et al. (1951) using bovine plasma albumin (Armour) as a standard. Cell numbers were determined by viable plate counts on Brain Heart Infusion Agar (Difco).

Cellular thiamine content, free and phosphorylated, was assayed by the thiochrome procedure. Twice washed cell suspensions were sonicated (Branson sonifier model S75 operating at 20,000 cycles/min.) for 5 minutes at 1-5°C and then treated with a crude pyrophosphatase preparation (from Aspergillus oryzae) for 1 hour at 37°C. Pyrophosphatase activity was terminated by the addition of trichloroacetic acid (TCA, 4% w/v final concentration) and total thiamine content was determined on the supernatant after centrifugation of the TCA precipitate. Free thiamine was estimated by omitting the pyrophosphatase step.

Results and Discussion

As thiamine concentration was increased in the culture media, there was an increase in cell numbers but a decrease of thiaminase I activity per cell (Fig. 1). Thus, thiamine may either repress thiaminase I production, or inhibit its activity via some type of feedback mechanism. Control experiments demonstrated that the above result could not be attributed to high cell density or to an inhibitor(s) present in cultures at high thiamine concentration.

A culture was grown to mid log phase at low thiamine concentration (30 μmoles/ml) and divided into two equal parts at 23 hours. To one part, 300 μmoles/ml of thiamine was added; the other portion served as a control and received no addition. Aliquots were removed at intervals preceeding and following the division of the culture, and assayed for thiaminase I activity (Figure 2). The increase in activity ceased after the secondary addition of thiamine, thus implying a repression of thiaminase I synthesis.

Chloramphenicol (CM) was used to inhibit protein synthesis in the cells

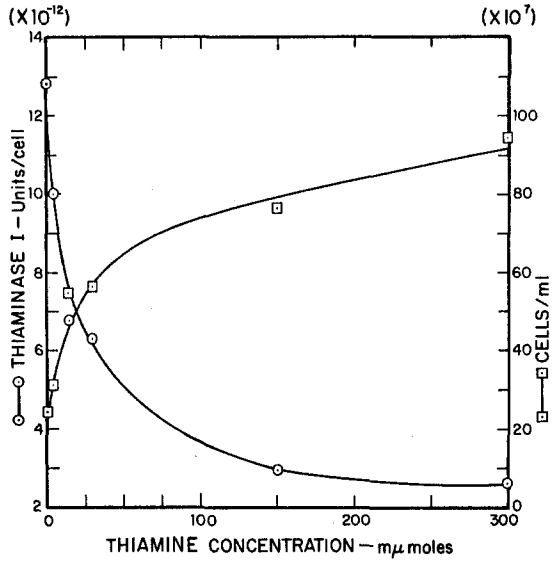

Fig. 1 Effect of thiamine concentration on growth and thiaminase I production. A late log phase culture grown at 3×10^{-8} M thiamine was diluted 5 fold with fresh BMM-7 medium containing varying thiamine concentrations. After 4-1/2 hours of incubation at 37°C, the cultures were harvested, cell numbers determined and enzyme activity assayed as described in the text.

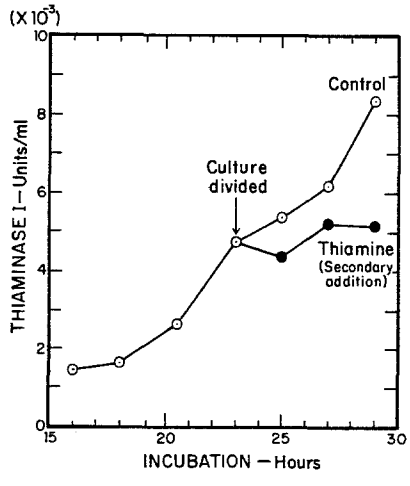

Fig. 2 The effect of thiamine addition on thiaminase I production. See text for details.

and thiaminase I activity was measured as a function of incubation time. Protein synthesis was greatly reduced and no increase in thiaminase I activity was observed on exposure of the cells to CM (Fig. 3),thus suggesting a repression of enzyme production. The level of CM used inhibited protein synthesis by 88%. Longer incubation resulted in the death of some of the cells. This antibiotic did not inhibit the base exchange reaction in vitro.

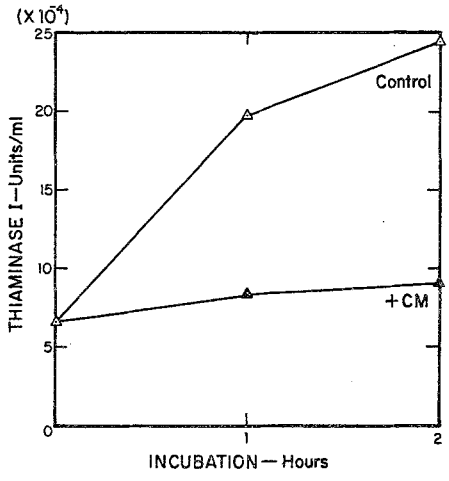

Fig. 3 Effect of chloramphenicol on thiaminase I production. A late log phase culture was diluted 5 fold with fresh medium containing no thiamine. CM (20 μg/ml) was added to one part immediately after dilution. Samples were taken for viable cell counts and thiaminase I assay before CM addition and after 1 and 2 hours of incubation. The cell count remained relatively constant through out the 2 hours incubation period in the CM culture.

It was of interest to determine the thiamine concentration of the cells under different degrees of repression. Table 1 shows that the free thiamine content of the cells from both cultures is approximately the same, but there is 53% more phosphorylated thiamine in those grown at a high thiamine level. From the increased level of thiamine phosphates in the repressed cells, it appears that either thiamine monophosphate or pyrophosphate (or both) is more directly involved in the repression of thiaminase I biosynthesis.

In general, the end product of a reaction sequence serves as the repressor for the production of the enzyme(s) involved. Several examples which suggest direct or indirect stimulation of repressor formation by a vitamin(s) have been

Table 1

Thiamine content of B. thiaminolyticus[1]

Condition	Total thiamine $\frac{\text{cell}}{(\times 10^{-12}\ \mu g)}$	Free thiamine $\frac{\text{cell}}{(\times 10^{-12}\ \mu g)}$	Thiamine phosphates[2] $\frac{\text{cell}}{(\times 10^{-12}\ \mu g \text{ as thiamine})}$
Control	8.8	2.6	6.2
Thiamine	12.0	2.5	9.5

[1] The same experimental protocol was used as in Fig. 2. At mid log phase (17 hours) the culture was divided into two parts. To one 300 μmole/ml of thiamine was added and the other part (control) received no addition. Both portions were harvested after 5 additional hours of incubation.

[2] The values in this column were obtained by the difference between columns 1 and 2.

studied by Ghambeer and Blakley (1966), Kaback et al. (1966), Katzen and Buchanan (1965). It is of interest to note that all these organisms require the particular vitamin(s) in question for growth. The participation of thiamine as a cofactor in decarboxylation and transketolation reactions has been well elucidated. It is not known whether thiamine or its phosphorylated form(s) acts directly to repress thiaminase I production, or exerts its effect indirectly as a result of stimulating the synthesis of a metabolic product(s).

Our finding may simply be the result of typical repression in that the reaction product, i.e., $PyCH_2$Base and or 'thiazole', is the repressor. However, a decrease in enzymatic activity was only found when both substances were present in the culture medium. This agrees with the interpretation that thiamine has a direct or indirect role in regulating thiaminase I biosynthesis.

It is also possible that the results obtained are not due to repression, but a failure of the cells to release the enzyme into the medium at high thiamine concentration. Enzymatic assay of cell sonicates does not suggest

this to be the case. Preliminary experiments using rabbit anti-thiaminase I indicated the presence of an enzymatically inactive cross-reacting material in the derepressed cells. This observation is currently under investigation.

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