# NORMAL SYNTHESIS OF AMINOTRANSFERASE PROTEIN IN A PYRIDOXINELESS STRAIN OF S. TYPHIMURIUM

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Imidazolylacetolphosphate aminotransferase (EC 2.6.1.9) is essential for the biosynthesis of the amino acid L-histidine, is coded for by the C gene of the histidine operon, and its rate of synthesis can be altered by controlling the expression of the histidine operon. Synthesis of the aminotransferase during derepression of the histidine operon has been studied in a pyridoxineless strain of S. typhimurium, suspended in growth media containing or lacking pyridoxine. Our results indicate that under both sets of nutritional conditions, synthesis of enzymes from genes on either side of the C gene are normal, that synthesis of aminotransferase protein is normal and that the proposed obligatory role of the cofactor pyridoxal phosphate in the synthesis of wild-type aminotransferase protein is not substantiated.

The enzyme imdazolylacetolphosphate aminotransferase (EC 2.6.1.9) from Salmonella typhimurium is a dimer of identical subunits (1,2) binding only one mole of pyridoxal phosphate per mole of dimer (3). The cofactor is very tightly bound by the protein since resolution of the holoenzyme is only accomplished after extensive dialysis for several days at room temperature (4). In agreement with this conclusion are the findings that there is no stimulation of enzymic activity when exogenous PLP*** is added to cell-free extracts of S. typhimurium; a result in contrast to the behavior observed with the Neurospora crassa enzyme (5). Also, the addition of PLP does not stimulate the enzyme at any stage during its purification (3,6). Although the purified enzyme has a multimeric structure, tests for complementation with abortive transductions have indicated that the

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*** Abbreviations used: Pdx, pyridoxine; PLP, pyridoxal-5-phosphate; TA, 2-thiazole-D, L-alanine

C gene consists of only a single complementation unit, i.e., no intragenic complementation has been seen in greater than 1700 crosses between C gene mutants (7,8). On the basis of these kinds of genetic and biochemical observations, Martin, Voll, and Apella (9) proposed an obligatory role for the cofactor in the biosynthesis of the enzyme. In an effort to delineate the role of the cofactor in the formation of this enzyme, we have investigated the biosynthesis of the aminotransferase in a pyridoxine requiring auxotroph, pdx1. We have attempted to ascertain whether the cofactor is required at the time of synthesis to insure proper release and/or folding of the polypeptide chains.

By carrying out the derepression of the histidine operon in strain pdx1 in the presence and absence of required Pdx, it becomes possible to answer the questions: (1) can the cell synthesize apoaminotransferase, and (2) does the lack of required PLP lead to translational difficulty, i.e., introduce polarity? In Table 1 are shown the data from such experiments. Note that in the presence of $10^{-6}$M Pdx, the enzyme activity of the aminotransferase increases in a coordinate fashion with the enzyme activities of the L-histidinol dehydrogenase and L-histidinol phosphate phosphatase, which are the enzymes coded for by the two genes which bracket the C gene in the operon. This coordinate synthesis is expected since the enzymes of the histidine operon respond as a unit to the levels of histidine available to the cell (10). In the absence of added Pdx, the dehydrogenase and phosphatase activities increase as before, to between 9 and 12 fold those of the repressed values but the aminotransferase activity remains low (only 2 fold derepressed). The order of the genes in the operon in relation to the operator region is dehydrogenase, aminotransferase, phosphatase. These results with the phosphatase rule out any possibility of polarity effects being introduced as the result of the absence of the cofactor, required for the activity of the aminotransferase, during the time of the synthesis of the C gene protein.

Although the use of TA to derepress the histidine operon of pdx1 predicts a sequential mode of translation of the messenger RNA (11), the results presented in Table 1 do not rule out the possibility of a "simultaneous" translation of

Table 1. Cultures of pdx1 were grown at 37° in E-minimal medium (13) supplemented with 0.2% glucose and $10^{-6}$M Pdx. At an optical density of 30 Klett units (determined in a Klett-Summerson photoelectric colorimeter using filter #54), the cells from duplicate cultures were harvested, washed twice with minimal medium and resuspended at the original volume in minimal medium supplemented with glucose. One culture was supplemented additionally with $10^{-6}$M Pdx while the second culture received no Pdx; each culture received 2-thiazolealanine (final concentration, $10^{-4}$M). The cultures were re-incubated at 37° and at the indicated times, cell samples of the two cultures were harvested, resuspended in 0.1M Tris-HCl, pH 7.5 buffer and then cell free extracts prepared. IAP aminotransferase was assayed according to the method of Albritton and Levin (14); L-histidinol dehydrogenase was assayed by the method of Loper and Adams (15); and L-histidinol phosphatase was assayed according to Ames, Garry and Herzenberg (16). Protein was determined by the micro adaption of the biuret method (17).

TABLE 1.

KINETICS OF DEREPRESSION OF ENZYMES OF THE HISTIDINE OPERON IN pdx1.

| | | Relative Specific Activity | | |
|---|---|---|---|---|
| Time after Derepression | Additions to Culture | Dehydrogenase | Aminotransferase | Phosphatase |
| 0 | $10^{-6}$M Pdx | 1.0 | 1.0 | 1.0 |
| 60 min. | " | 5.6 | 4.0 | 3.0 |
| 120 min. | " | 11.7 | 9.4 | 5.2 |
| 180 min. | " | 15.0 | 10.9 | 9.0 |
| 0 | 0 Pdx | 1.0 | 1.0 | 1.0 |
| 60 min. | " | 7.6 | 2.3 | 6.4 |
| 90 min. | " | 11.5 | 2.3 | 9.6 |
| 120 min. | " | 12.8 | 2.1 | 10.2 |
| 150 min. | " | 11.3 | 1.9 | 9.6 |

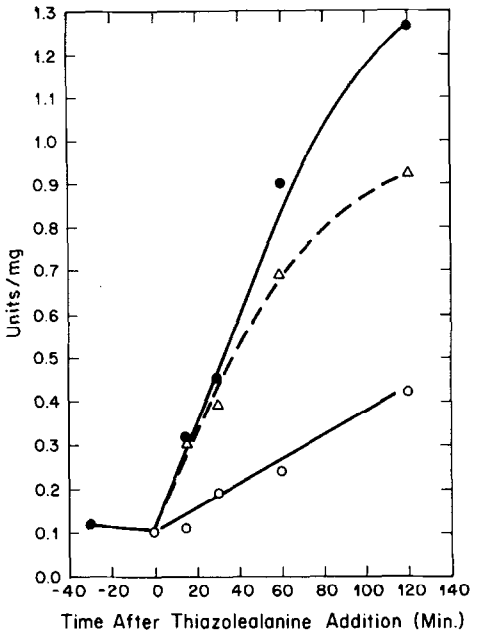


Figure 1. A Fernbach flask containing 500 ml E-minimal medium supplemented with 0.2% glucose and 1.7 x $10^{-8}$M Pdx was inoculated with 10 ml of washed pdx1 cells which had been grown to population limit with $10^{-6}$M Pdx. The flask was shaken at 37°; at an optical density of 70 Klett units, the culture was divided. Each subculture was made $10^{-4}$M with respect to TA and one subculture was made 8.75 x $10^{-7}$M with respect to Pdx. Cells were allowed to continue shaking and production of the aminotransferase was monitored by removing 40 ml aliquots at various times, and preparing cell free extracts. Enzyme activity was measured before and after incubation with $10^{-4}$M PLP. Specific activities of IAP aminotransferase from the cultures suspended in the presence of 8.75 x $10^{-7}$M Pdx (●); Suspended in the absence of Pdx, assayed before (o) and after (Δ) incubation of the extracts with PLP.

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the dehydrogenase and phosphatase gene messages with a lack of translation or blocked translation of the C gene message. If one can show an increase in aminotransferase after derepression then lack of translation of the C gene message can be ruled out. In Figure 1 are shown the results of an experiment similar in design to the one described in Table 1. Only the aminotransferase

activity was monitored in this experiment. Within 2 hours after TA addition, a 12-13 fold derepression of the aminotransferase had occurred in the culture supplemented with $8.75 \times 10^{-7}$M Pdx (●). In contrast, only a 4 fold increase in the enzyme activity was observed in the culture which was derepressed in the presence of $1.7 \times 10^{-8}$M Pdx (○). However, aminotransferase activity in the cell-free extracts prepared from this latter culture could be stimulated up to 2.9 fold by incubation with $10^{-4}$M PLP (△). These results demonstrate that the apoprotein can be made and released into the soluble phase of the cell in the absence of the cofactor, and that it can be reconstituted *in vitro* by incubation with PLP.

It should be noted that the reconstituted activity did not reach the activity value found in the culture supplemented with the higher Pdx concentrations (Figure 1). One factor responsible for the lower specific activity of the reconstituted extracts is the destruction of some reconstitutable activity caused by our method of preparing cell-free extracts. When PLP is present during sonication of the cells one consistently records higher specific activities in the cell extracts compared to extracts prepared in the absence of PLP but subsequently incubated with PLP. A similar observation of apoenzyme instability in a histidine *C* gene mutant of *S. typhimurium* has recently been reported (4). The positive role of PLP in the stabilization of apoproteins against various perturbations has been recently reviewed (12).

The fact that the endogenous holoenzyme activity plus the reconstituted apoenzyme activity reaches a level of specific activity 75% or greater than the specific activity of the aminotransferase from the control culture suggests that the apoenzyme is synthesized at a nearly normal rate and is relatively stable *in vivo*, at least for the time period studied here. A more definitive study regarding the rate of synthesis of the apoaminotransferase and its stability would involve direct comparisons with another enzyme in the histidine operon. Such a study is underway. It still remains to be shown whether the reconstituted enzyme has the same properties as the native holoenzyme and what possible roles the

cofactor may play in the determination of quaternary structure. Our results however seem to rule out an obligatory role of the cofactor in the synthesis and release of the wild-type C gene aminotransferase protein.

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