SHORT COMMUNICATION

Repression of Cytidine Triphosphate Synthetase in *Salmonella typhimurium* by Pyrimidines during Uridine Nucleotide Depletion

By THOMAS P. WEST† and GERARD A. O’DONOVAN*

Department of Biochemistry and Biophysics, Texas A&M University, College Station, Texas 77843, U.S.A.

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Regulation of the synthesis of cytidine triphosphate (CTP) synthetase (EC 6.3.4.2) was investigated in *Salmonella typhimurium*. CTP synthetase appeared to be repressed only when intracellular concentrations of uridine nucleotides were significantly lowered. Under such nucleotide pool conditions, a cytidine compound and, to a lesser degree, a thymidine compound appeared as putative repressing metabolites of enzyme synthesis.

INTRODUCTION

The gene pyrG encodes cytidine triphosphate (CTP) synthetase (EC 6.3.4.2), which is the enzyme directly responsible for the *de novo* biosynthesis of cytidine nucleotides in *Salmonella typhimurium* (O’Donovan & Neuhard, 1970). This enzyme, under physiological conditions, catalyses the irreversible amination of UTP to CTP in a reaction requiring glutamine, ATP and magnesium ions (Chakraborty & Hurlbert, 1961; Long & Pardee, 1967; Savage & Weinfeld, 1970). In addition, GTP serves as an allosteric activator of the amination (Savage & Weinfeld, 1970; Levitzki & Koshland, 1972). The most effective inhibitor of bacterial and mammalian CTP synthetase activity is its product CTP (Long & Pardee, 1967; McPartland & Weinfeld, 1979; Mitchell & Finch, 1979). Our *in vitro* study of CTP synthetase activity in *S. typhimurium* crude extracts also confirmed CTP as a potent inhibitor (T. P. West, unpublished results).

Although CTP synthetase has been investigated with respect to its regulation of enzyme activity in a few organisms, previous studies did not determine whether its synthesis was controlled by pyrimidines. Regulation by pyrimidines would not be totally unexpected in *S. typhimurium* since the synthesis of the six *de novo* pyrimidine pathway enzymes has been shown to be repressed by either a uridine or a cytidine compound (Abd-el-al & Ingraham, 1969; Kelln *et al.*, 1975; Schwartz & Neuhard, 1975; Smith *et al.*, 1980). The present study explored pyrG gene expression in *S. typhimurium*. The results indicate that its expression was not repressed by pyrimidines when normal concentrations of uridine nucleotides were present within the cell. Rather, repression of enzyme synthesis was evident only when CTP synthetase specific activity was examined in a strain containing lowered concentrations of UDP and UTP. In such a strain, the synthesis of CTP synthetase was strongly repressed by a cytidine compound and weakly repressed by a thymidine compound.

METHODS

*Strains.* Bacterial strains used in this study are all derivatives of *Salmonella typhimurium* LT2. Strains KD1275 (cdd-7), JL1269 (pyrH1609 cdd-7), KD1109 (cdd-4) and KD1104 (pyrH11 cdd-4) were isolated in previous

† Present address: Department of Molecular and Medical Microbiology, College of Medicine, University of Arizona, Tucson, Arizona 85724, U.S.A.
those of Sanderson
resuspended cells were sonically disrupted at 0 °C for a total of 90 min. The treated extract was stirred intermittently while standing in ice for 20 min and was then centrifuged for 15 min at 43 500 g.

determined by the Lowry method with bovine serum albumin as the standard.

was then assayed. The CTP synthetase assay was a modification of previously described assays (Long & Pardee, 1967; Levitzki, 1972; Kelln et al., 1975). The assay mixture contained (in 1 ml) 20 mM-imidazole/acetate pH 7.2, 10 mM-glutamine, 10 mM-MgCl₂, 0.3 mM-GTP, 1 mM-ATP and 1 mM-UTP. The reaction was followed at 290 nm: an increase in absorbance of 1.56 is equivalent to the conversion of 1 pmol UTP into CTP. The addition of the substrate (UTP) initiated the reaction, which was followed spectrophotometrically for 10 min at 37 °C. The rate of product formation was linear with respect to time and protein concentration. Protein concentration was determined by the Lowry method with bovine serum albumin as the standard.

**RESULTS**

The isolation of *pyrH* strains in *S. typhimurium*, which contain a defective uridine monophosphate (UMP) kinase (EC 2.7.4.4) (Ingraham & Neuhard, 1972), has simplified determining the in vivo regulation of pyrimidine biosynthetic enzymes (Kelln et al., 1975; Schwartz & Neuhard, 1975). Strain JL1269, in which UMP kinase activity is extremely low (Ingraham & Neuhard, 1972; Justesen & Neuhard, 1975), was compared with respect to CTP synthetase specific activity with an isogenic strain, KD1275, which has a fully active UMP kinase (Kelln & O'Donovan, 1976) (Table 1). Strain JL1269 had approximately twice the specific activity of strain KD1275. Another pair of isogenic strains, KD1109 (*pyrH*) and KD1104 (*pyrH*) (Kelln et al., 1975) with respective generation times of 64 and 80 min, were also assayed for CTP synthetase activity. The *pyrH* strain synthetase activity was 1-7-fold higher than the activity of the *pyrH* strain (Table 1). The increased specific activity in the *pyrH* strains may be due to changes either at the level of enzyme synthesis or at the level of enzyme activity.

Unlike strains KD1275, JL1269, KD1109 and KD1104, the wild-type strain, LT2, has a functional cytidine deaminase (EC 3.5.4.5) and is unable to utilize cytidine as a source of cytidine nucleotides effectively (Beck et al., 1972). Therefore, the effect of cytidine in minimal medium on wild-type CTP synthetase activity was not tested. The addition of exogenous pyrimidines did not have a significant effect on the generation times of strains KD1275 (68 min), JL1269 (82 min) or LT2 (48 min). From Table 1, it can be noted that the presence of pyrimidines in the medium of strains LT2 or KD1275, in which UTP levels are normal (Justesen & Neuhard, 1975), did not result in a decrease in specific activity of any consequence. This finding was not indicative of repression by a cytidine, uridine or thymidine compound in these *pyrH* strains.

In contrast, exogenous pyrimidines caused significant decreases in CTP synthetase activity when present in the medium of the *pyrH* strain JL1269. Inclusion of cytidine in the medium produced a large depression in enzyme activity (Fig. 1). When strain JL1269 was grown in increasing concentrations of cytidine, enzyme specific activity decreased to 11-fold below the level observed with no cytidine in the medium (Fig. 1). These data strongly indicated repression of enzyme synthesis by a cytidine metabolite. The presence of uracil in the minimal medium increased the enzyme activity slightly (Table 1). This increase may be due to substrate inhibition of the defective UMP kinase by the accumulating UMP causing the UDP and UTP concentrations to decrease further. Thymidine metabolites were able to diminish the
Table 1. Specific activities of CTP synthetase in pyrH and pyrH+ strains of S. typhimurium cultured under various growth conditions

Growth conditions were as described in Methods. Uracil, cytidine or thymidine was added to AG medium at a final concentration of 50 μg ml⁻¹. Each value is the average of at least two separate determinations.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Relevant genotype</th>
<th>Pyrimidine addition</th>
<th>CTP synthetase activity [nmol min⁻¹ (mg protein)⁻¹]</th>
</tr>
</thead>
<tbody>
<tr>
<td>KD1275</td>
<td>cdd-7 (pyrH⁺)</td>
<td>None</td>
<td>0.893</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cytidine</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Thymidine</td>
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<td>JL1269</td>
<td>pyrH1609 cdd-7</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Cytidine</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Thymidine</td>
<td>1.07</td>
</tr>
<tr>
<td>KD1109</td>
<td>cdd-4 (pyrH⁺)</td>
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<td>0.702</td>
</tr>
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<td>KD1104</td>
<td>pyrH11 cdd-4</td>
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</tr>
<tr>
<td>LT2</td>
<td>wild-type</td>
<td>Thymidine</td>
<td>0.973</td>
</tr>
</tbody>
</table>

Fig. 1. Effect of exogenously added cytidine on the specific activity of CTP synthetase in S. typhimurium strain JL1269 (pyrH1609 cdd-7). The specific activity for growth without cytidine was 1.72 nmol min⁻¹ (mg protein)⁻¹.

Specific activity of the enzyme by 38% in the pyrH strain (Table 1). This decrease in activity by a thymidine compound was significant since it occurred despite the partial degradation of thymidine to thymine in this deoA⁺ (tpp⁺) strain (Schwan & Holldorf, 1975). Although not as effective as a cytidine compound in exacting repression of CTP synthetase synthesis, a thymidine compound would appear to be involved in the transcriptional regulation occurring in strain JL1269.

CTP, the product of CTP synthetase, was a potent inhibitor of the enzyme in vitro. When CTP (at 0-1 mM final concentration) was added to the standard assay mixture for CTP synthetase about 70% inhibition was observed. This is in accordance with previous reports (Long & Pardee, 1967; McPartland & Weinfeld, 1979; Mitchell & Finch, 1979). Moreover, the 70% inhibition was observed for the enzyme from cells grown at all concentrations of cytidine as well as from cells grown in minimal medium without cytidine.
DISCUSSION

As with the de novo pyrimidine pathway enzymes in S. typhimurium (Kelln et al., 1975; Schwartz & Neuhard, 1975; Smith et al., 1980), CTP synthetase appears to be controlled at the level of enzyme synthesis by pyrimidines. Regulation of CTP synthetase synthesis by pyrimidines, though, was confined to conditions where UDP and UTP intracellular concentrations were limiting in S. typhimurium. Under conditions which were normally obtained for wild-type cells or for the pyrH+ cdd cells, the results were best explained by constitutive synthesis of CTP synthetase. Inclusion of pyrimidines in the minimal medium had only a slight effect on enzyme activity in strains LT2 and KD1275 indicating that CTP synthetase synthesis was not sensitive to fluctuations in pyrimidine nucleotide levels. Rather, it was only by examining abnormal nucleotide pool conditions, such as those found in strains JL1269 and KD1104, that significant variations in CTP synthetase activity were seen. In the pyrH strain, KD1104, with low UTP [2·52 μmol (g dry wt)−1] and low UDP [0·30 μmol (g dry wt)−1] (Kelln et al., 1975), the enzyme activity was elevated 1·7- to 2·0-fold relative to its isogenic relatives.

It appears, therefore, that pyrG gene expression can be repressed by pyrimidines in strain JL1269. In this pyrH strain, low concentrations of cytidine in the medium depressed CTP synthetase specific activity by 70% or more, while thymidine metabolites decreased the activity by 38%. Enzyme synthesis appeared to be highly dependent upon the cytidine and thymidine nucleotide levels when uridine nucleotide concentrations were depleted sufficiently.

A possible explanation of this transcriptional regulation of CTP synthetase might be that at normal uridine nucleotide concentrations, these nucleotides (possibly UDP or UTP) interact with a putative aporepressor in a manner that promotes its inactivation. It is possible that the binding of the uridine nucleotide elicits a conformational change within the protein that substantially decreases its affinity for the pyrimidine corepressors. With the drastically reduced uridine nucleotide levels in the pyrH strains, the concentration of active aporepressor would be increased. The cytidine and thymidine nucleotides could now bind to this protein forming an aporepressor–corepressor complex that blocks CTP synthetase messenger RNA transcription following its association with the appropriate region of DNA. Considering the low UTP concentration in strain JL1269 (Justesen & Neuhard, 1975), it would certainly be logical for the cell to utilize only enough UTP to maintain CTP levels at the appropriate concentration for RNA synthesis to continue. If there is a high concentration of endogenous CTP present there is no need to synthesize additional CTP synthetase especially if UTP levels are rate-limiting for RNA synthesis. CTP synthetase, or a precursor of the enzyme, possibly could act as the proposed aporepressor to autogenously regulate its own synthesis in S. typhimurium when the concentration of its substrate UTP is diminished appreciably.

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REFERENCES


