# SYNERGISTIC INHIBITION OF ATP PHOSPHORIBOSYLTRANSFERASE BY GUANOSINE TETRAPHOSPHATE AND HISTIDINE

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Summary. In the presence of partially inhibiting concentrations of the feedback inhibitor histidine guanosine-5′-diphosphate-3′-diphosphate becomes a potent inhibitor of the residual activity of ATP phosphoribosyltransferase from Salmonella typhimurium. Inhibition by guanosine tetraphosphate is higher order and slowly reversible. Inhibition does not occur in the absence of histidine. This effect constitutes an important control on the rate of histidine biosynthesis in response to the general aminoacid nutritional state of the cell.

## Introduction

The first enzyme of L-histidine biosynthesis in microorganisms catalyzes the reaction given in eq.1.[1] ATP phosphoribosyltransferase (E.C.2.4.2.17) from Salmonella typhimurium is a hexameric enzyme under catalytic conditions (1) containing one allosteric site for histidine on

$$\text{ATP} + \alpha\text{PRibPP} \underset{\text{Histidine}}{\overset{\text{Mg}^{+2}}{\rightleftharpoons}} \text{PP}_i + \beta\text{PRibATP}$$

each subunit (2) which does not overlap the active site (3). High concentrations of histidine can totally inhibit the enzymatic reaction in a positively cooperative manner (2,3). The enzyme is of great interest because of its probable involvement in regulation of the histidine operon at the gene level (4,5).

Under conditions of growth limitation caused by any limiting aminoacid in bacteria, the alarmone (6) guanosine-5′-diphosphate-3′-diphosphate increases greatly in concentration, resulting in decreased synthesis of stable

[1] Abbreviations used are as follows: PRibPP, 5-phospho-α-D-ribose-1-diphosphate; PRibATP, $N^1$-(5′-phospho-β-D-ribosyl)adenosine triphosphate; ppGpp, guanosine-5′-diphosphate-3′-diphosphate.

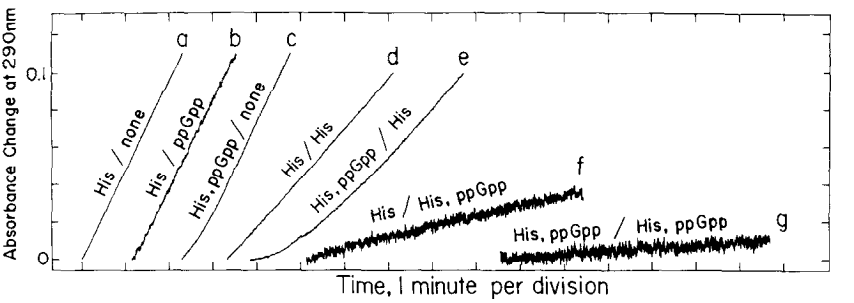


Figure 1. Dependence of assay curve shape on incubation and assay conditions. The ligands indicated parallel to each assay before the slash were present during incubation of the enzyme while those after the slash were present in the assay medium. Histidine (400 μM) was present in all incubations and 350 μM ppGpp also was present in the incubations indicated. Histidine and ppGpp concentrations when present in the assay were 100 μM and 350 μM, respectively. Increased noise in assays containing ppGpp was due to a high absorbance background contributed by the guanosine chromophore.

RNA (7). Recently, it has been shown that ppGpp acts as a positive effector for expression of the histidine operon through a mechanism which is separable from the histidyl-tRNA$^{His}$ specific mechanism (6). We report here that ppGpp functions also as an inhibitor of the first enzyme of histidine biosynthesis, but only in the presence of histidine.

## Materials and Methods

ATP phosphoribosyltransferase and the second and third enzymes of the histidine pathway were isolated and stored as previously described (8). ATP phosphoribosyltransferase was diluted 100 fold into pH 7.5 standard buffer (8) containing 0.1 mg bovine serum albumin/ml, 1 mM dithiothreitol, 0.40 mM histidine and 0.35 or 0.50 mM ppGpp when present and incubated 1 hr at 0° before assay. Assays were conducted as previously described (8) at pH 8.50 with the free $Mg^{+2}$ concentration held at 2.0 mM utilizing a Cary 118 ultraviolet spectrophotometer at 25°. All assays contained excess inorganic pyrophosphatase (Boehringer/Mannheim) and second and third histidine pathway enzymes.

## Results

Figure 1 shows typical assay curve shapes for various combinations of histidine and ppGpp present during incubation of the enzyme and assay of the enzyme. In all cases an identical concentration of enzyme was diluted 120 fold upon addition to the assay medium. Thus ligands present during in-

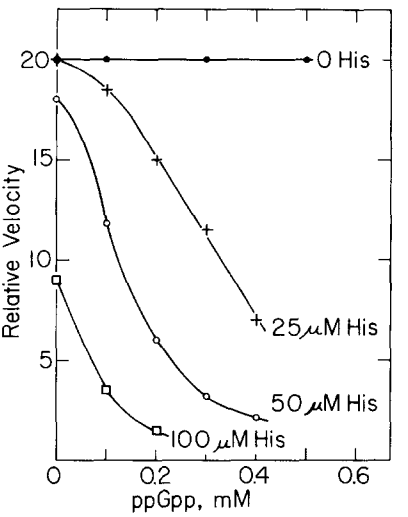


Figure 2. Guanosine tetraphosphate inhibition of ATP phosophoribosyltransferase in the presence of histidine. Enzyme incubated in the presence of 400 μM histidine and 500 μM ppGpp was assayed in the presence of the indicated concentrations of histidine and ppGpp. Maximal reaction velocity attained after recovery from any lag period is reported in all cases.

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cubation of the enzyme were diluted effectively to zero during the assay if the ligand was not already present in the assay solution.

Assay a contained no ligands other than substrates in the assay and represents the fully active enzyme. Assay b contained a high concentration of ppGpp in the assay but showed no effect on the enzyme activity. Assay c contained no ligands other than substrates in the assay but the enzyme was incubated in the presence of ppGpp plus histidine. A lag in expression of enzyme activity is apparent. The enzyme became fully active after several minutes. Assay d contained sufficient histidine in the assay solution to give 35 percent inhibition. Assay e was identical to d except that enzyme incubated in the presence of ppGpp plus histidine was assayed. A very pronounced lag in expression of the activity is apparent, but again the same final level of activity as in the absence of incubation ppGpp was attained after several minutes. Thus, ppGpp plus histidine in the absence of sub-

TABLE 1 - Guanosine Tetraphosphate Apparent Inhibition Constants

| Histidine Concentration (μM) | Apparent ppGpp Constant (μM) | Apparent ppGpp Hill Coefficient[a] |
|---|---|---|
| 0 | ∞ | - |
| 25 | 321 | 2.2 |
| 50 | 139 | 1.9 |
| 100 | 76 | 1.6 |

[a]Hill coefficients were obtained from the slopes of replots of the data for each concentration of histidine in Fig.1 in the form log $v_i/(v-v_i)$ versus -log ppGpp, where $v_i$ is the velocity in the absence of ppGpp and $v_i$ is the velocity in its presence.

strates produces an inhibited form of the enzyme which requires several minutes to activate upon removal of ppGpp.

Assay f contained ppGpp plus histidine in the assay solution and should be compared to b and d. The presence of a partially inhibitory concentration of histidine in the assay induced ppGpp to become an inhibitor in the assay. Substantial inhibition by ppGpp was rapidly established since the zero time velocity of assay f was greatly decreased. However, the slight downward curvature in f suggested that full inhibition was not established at zero time, but was established progressively upon exposure to ppGpp in the assay solution. In agreement with this inference, enzyme incubated in the presence of ppGpp plus histidine and assayed in the presence of these two ligands was essentially totally inhibited as shown in assay g. Thus ppGpp is a total inhibitor of ATP phosphoribosyltransferase in the presence of a moderate concentration of histidine.

The incubation conditions of assay g then were adopted to explore the synergistic relationship between ppGpp and histidine. Figure 2 presents the results. At increasing concentrations of histidine lower concentrations

of ppGpp are required to obtain inhibition of 50 percent of the residual enzymatic activity. At 100 μM histidine only 78 μM ppGpp is required (Table 1). Furthermore, at a concentration of histidine insufficient to show inhibition by itself, 25 μM, ppGpp can still be an effective inhibitor (Fig.2, Table 1).

Guanosine tetraphosphate inhibition is a positively cooperative process. Table 1 lists Hill coefficients obtained from separate Hill plots. The values of about 1.6 to 2.2 are similar to those often obtained for the histidine Hill coefficient (2).

## Discussion

The observation that ppGpp reversibly inhibits ATP phosphoribosyltransferase in the presence of histidine establishes a new class of ligand that binds to this versatile enzyme. In addition to substrates and histidine the enzyme also binds AMP and ADP (3), histidyl-$tRNA^{His}$ (4), and histidine operon attenuator DNA (4). Even though ppGpp is a total inhibitor it cannot be determined from the data presented here whether the ppGpp binding site overlaps the substrate binding sites. If it does the apparent ppGpp inhibition constant would be a function of substrate concentration, with higher concentrations of substrate causing higher apparent ppGpp inhibition constants.

Although the simplest interpretation of the histidine requirement for ppGpp inhibition is that bound histidine creates the ppGpp binding site, these data do not prove this model. Guanosine tetraphosphate could bind to the enzyme in the absence of histidine but not affect enzymatic activity if the ppGpp binding site was distinct from the active site. Binding of histidine could cause a conformational change in the enzyme which made bound ppGpp inhibitory and promoted further reversible binding of ppGpp in a cooperative manner.

Inhibition by ppGpp is effective at concentrations of histidine, ppGpp, and substrates which are comparable to estimated *in vivo* levels for bacteria

growing in glucose-minimal salts (6,9). Upon starvation for any aminoacid the ppGpp concentration increases greatly (7). This should have the following consequences for histidine biosynthesis. If the limiting amino-acid was not histidine, the intracellular histidine concentration would rise since histidine could not be utilized as rapidly due to slowed protein biosynthesis. The increase in histidine concentration would coincide with an increased concentration of ppGpp due to the stringent response. Since both histidine and ppGpp inhibit ATP phosphoribosyltransferase in a positively cooperative manner, ATP phosphoribosyltransferase would be very effectively inhibited. Thus the general aminoacid nutritional state of the cell could exert important control over the rate of histidine biosynthesis at the metabolite level by means of ppGpp and histidine synergism.

Acknowledgement

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References

1. Parsons, S. M., and Koshland, D. E., Jr. (1974), J. Biol. Chem. 249, 4119-4126.
2. Bell, R. M., Parsons, S. M., Dubravac, S. A., Redfield, A. G., and Koshland, D. E., Jr. (1974), J. Biol. Chem. 249, 4110-4118.
3. Martin, R. G. (1963), J. Biol. Chem. 238, 257-268.
4. Goldberger, R. F., and Kovach, J. S. (1972), Curr. Top. Cell. Reg. 5, 285-308.
5. Scott, J. F., Roth, J. R., and Artz, S. W. (1975), Proc. Natl. Acad. Sci. (US) 72, 5021-5025.
6. Stephens, J. C., Artz, S. W., and Ames, B. N. (1975), Proc. Natl. Acad. Sci. (US) 72, 4389-4393.
7. Cashel, M., and Gallant, J. (1974), in Ribosomes, eds., M. Nomura, A. Tissieres, and P. Lengyel, Cold Spring Harbor Press, Cold Spring Harbor, N.Y., pp.733-745.
8. Morton, D. P., and Parsons, S. M. (1976), Arch. Biochem. Biophys. 175, 677-686.
9. Brenner, M., and Ames, B. N. (1971), in Metabolic Pathways V, eds., D. M. Greenberg and H. J. Vogel, Academic Press, New York, pp.349-387.