Inhibition of histidyl-tRNA—adenosine triphosphate phosphoribosyltransferase complex formation by histidine and by guanosine tetraphosphate
(histidine biosynthesis/repression/autogenous protein)

JEANINE E. KLEEMAN AND STANLEY M. PARSONS*
Department of Chemistry, University of California, Santa Barbara, California 93106

Communicated by Thomas C. Bruce, February 10, 1977

ABSTRACT Formulation of the complex between the first enzyme of histidine biosynthesis from *Salmonella typhimurium*, ATP phosphoribosyltransferase [1-(5'-phosphoribosyl)-ATP: phosphoribosyltransferase; EC 2.4.2.17], and histidyl-tRNA is shown to be inhibited by l-histidine and by guanosine-5'-diphosphate-3'-diphosphate in the presence of histidine. Higher histidine levels make guanosine tetraphosphate a more effective inhibitor. Relatively high concentrations of guanosine-5'-triphosphate also inhibit complex formation, but this inhibition is not enhanced by histidine. The possible implications of these observations with respect to the gene regulatory activity of this enzyme are discussed.

An increasing number of autogenous (1) or autoregulatory (2) proteins that regulate their own gene expression are now known. The first enzyme of histidine biosynthesis in *Salmonella typhimurium*, ATP phosphoribosyltransferase [1-(5'-phosphoribosyl)-ATP:phosphoribosyltransferase; EC 2.4.2.17], exhibits properties of an autogenous protein (3). Missense mutants of the enzyme affecting histidine sensitivity alter expression of the histidine operon in *vivo* (4), and purified enzyme binds DNA of the regulatory region of the histidine operon (5) and inhibits transcription of the operon (5) in *vivo*.

Histidyl-tRNA is a negative effector in *vivo* (6), but no Jacob-Monod type of dispensable repressor is known for the histidine operon. The demonstrations by Goldberger and co-workers that histidyl-tRNA forms a complex with ATP phosphoribosyltransferase (7) suggested that the complex might act as a holorepressor. However, recent evidence indicates that the histidine system is positively regulated by an unknown factor that might be activated by histidyl-tRNA and by an independent guanosine-5'-diphosphate-3'-diphosphate (ppGpp) system (8–10). Translation must occur to obtain transcription in *vivo* (9). Since repressed and derepressed levels of other histidine biosynthetic enzymes can be found in *vivo* in strains lacking the ATP phosphoribosyltransferase polypeptide (11), the enzyme must act in an auxiliary role in regulation of the histidine operon.

Recently we reported that ppGpp is a potent inhibitor of ATP phosphoribosyltransferase enzymatic activity, but only in the presence of histidine (12). We report here that both histidine and ppGpp in the presence of histidine inhibit formation of the complex between histidyl-tRNA and ATP phosphoribosyltransferase. The implications of this observation with respect to possible gene regulatory roles of the enzyme are discussed.

Abbreviation: ppGpp, guanosine-5'-diphosphate-3'-diphosphate.
* To whom reprint requests should be addressed.

MATERIALS AND METHODS

L-[2,5-3H]Histidine (55 Ci/mmol) was from Amersham/Searle, sodium GTP from Sigma (Type I), lithium ppGpp from ICN Pharmaceuticals, and nitrocellulose filters (no. B-6) from Schleicher and Schuell. The purity and concentrations of guanine nucleotides were determined by ultraviolet scanning spectroscopy. ATP phosphoribosyltransferase was isolated as described (13) from *S. typhimurium* strain TA2165 grown on glucose, histidine, and biotin minimal salts medium. The major species of tRNA (14) from *Escherichia coli* B, which is identical to tRNA (15) from *S. typhimurium* (14), was a generous gift from Dr. Brian Reid. It was aminocylated to 560 pmol of histidine per A$_{260}$ unit and isolated as described (15). (1 A$_{260}$ unit is that amount of material that gives an absorbance of 1 when dissolved in 1 ml of solvent with a pathlength of 1 cm.) Approximately 10% of histidyl-tRNA is hydrolyzed in 20 min in the absence or presence of ATP phosphoribosyltransferase under binding conditions used below.

ATP phosphoribosyltransferase (0.35 mg/ml, 1700 pmol of hexamer per ml) was incubated at 25° for 1 hr in 10 mM MgCl$_2$/6 mM KCl/1 mM dithiothreitol/10 mM N-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid adjusted to pH 7.5 with KOH. In a volume of 200 μl, 25 pmol of enzyme and variable concentrations of histidine, ppGpp, or GTP when present, were incubated in the same buffer for 20 min. [3H]Histidyl-tRNA (5 pmol in 5 μl of 1 mM sodium acetate/2 mM sodium thiosulfate/1 mM MgCl$_2$ at pH 4.5) was added and incubated 20 min additionally. The total binding solution was filtered through nitrocellulose and the incubation tube was washed with 0.205 ml of cold buffer. The filter was washed with 1.0 ml of cold buffer and dried, and filter-bound radioactivity was determined by liquid scintillation counting in 10 ml of toluene containing 2,5-diphenyloxazole (PPO) and 1,4-bis(2-(5-phenoxo-5-azolyl))benzene (POPOP). Only radioactivity binding to the enzyme that binds to the filter will be detected in this assay. All work was conducted in plastic containers.

RESULTS

Fig. 1 demonstrates that binding of histidyl-tRNA to the enzyme was decreased by increasing concentrations of l-histidine. The midpoint for the histidine effect occurred at approximately the concentration equal to the histidine dissociation constant determined by other methods at pH 7.5 (13). Thus, histidine competes with histidyl-tRNA for the enzyme, but very high histidine concentrations appear not to eliminate all histidyl-tRNA binding. The nature of partial inhibition requires further characterization.
When ppGpp was tested for a possible effect on formation of the histidyl-tRNA ATP phosphoribosyltransferase complex in the absence of histidine, little or no change in the amount of complex was seen up to 10 μM ppGpp (Fig. 2). However, in the presence of histidine, low concentrations of ppGpp inhibited complex formation (Fig. 2). High concentrations of histidine made ppGpp a potent inhibitor with no residual complex remaining at high ppGpp levels in this experiment. Even a concentration of histidine too low to inhibit significantly by itself allowed higher levels of ppGpp to inhibit formation of some of the complex. However, guanosine tetraphosphate appeared incapable of eliminating all histidyl-tRNA binding at lower concentrations of histidine since a plateau was reached at high concentrations of ppGpp and the plateau level increased at lower histidine concentrations.

At intermediate concentrations of histidine, ppGpp inhibition of complex formation is a positively cooperative process with maximal apparent Hill coefficients of approximately 2.0. Also, a linear relationship exists between the logarithms of the concentration of ppGpp giving 50% effect and the logarithm of the histidine concentration (Fig. 3). Thus, the apparent ppGpp dissociation constant is nearly inversely proportional to the histidine concentration.

Inhibition of complex formation by ppGpp was shown to exhibit specificity by examination of the effect of guanosine-5'-triphosphate. Fig. 4 shows that GTP inhibits complex formation in a positively cooperative manner, but much higher concentrations are required. Also, histidine is not synergistic with GTP inhibition. A full examination of the nucleotide specificity for inhibition of complex formation will be presented elsewhere.

**DISCUSSION**

In pioneering work Goldberger and coworkers (16) were unable to demonstrate any effect by histidine and little effect by the substrates ATP and 5-phospho-α-ribosyl-1-diphosphate on the
complex formed between histidyl-tRNA and ATP phosphoribosyltransferase at pH 6.5. We have made recent observations suggesting that histidine either does not bind to ATP phosphoribosyltransferase or is ineffectual in inducing a conformational change at pH 6.5 (J. D. Allen and S. M. Parsons, unpublished). Thus, it is necessary to work at pH 7.5 in order to detect histidine-coupled ligand behavior. This also is the estimated pH in vivo (17) and thus of greatest interest in any case. A full examination and demonstration of validity for the nitrocellulose filter assay procedure used here at pH 7.5 for detection of the complex will be presented elsewhere.

The estimated level in vivo of histidine in minimal salts/glucose medium (15 μM, ref. 17) is insufficient to inhibit formation of the histidyl-tRNA complex under the conditions used here, but this concentration should potentiate inhibition by ppGpp. The concentrations of ppGpp required to inhibit enzymatic activity are about 30 times higher (12) than those required to inhibit histidyl-tRNA complex formation. Thus, it is likely that ppGpp affinity for ATP phosphoribosyltransferase is decreased in the presence of substrates. In the cell the apparent dissociation constant for ppGpp inhibition of complex formation between histidyl-tRNA and ATP phosphoribosyltransferase likely will be comparable to the basal level of ppGpp in wild-type bacteria growing in minimal salts/glucose medium, about 0.1 mM (10), and be dependent upon the cellular histidine level. Synergistic inhibition by ppGpp and histidine is at least partly specific since GTP does not behave similarly and much higher concentrations of GTP are required for inhibition.

The observation that formation of this complex is inhibited by relatively low concentrations of ppGpp plus histidine establishes biochemical signal-responsivity of the complex. Furthermore, the enzyme now has been shown to interact with every regulatory signal known to affect expression of the histidine operon.

Inhibition of complex formation by these two metabolites makes it unlikely that the complex itself acts negatively in expression of the operon. Simultaneously high levels of ppGpp and histidine should constitute a negative signal to the histidine operon, yet this condition inhibits formation of the complex. The behavior leaves open the possibilities that enzyme bound to histidine plus ppGpp or to uncharged tRNA might be a negative factor, or free enzyme might be a positive factor, in operon expression. If some form of ATP phosphoribosyltransferase does participate in gene regulation, it is probable that its action is modulated by the levels of cellular histidine plus guanosine tetraphosphate.

One speculative role that now can be suggested for the complex is that it might function as a regulated reservoir for histidyl-tRNA. This reservoir could modulate the intensity of the negative signal arising from histidyl-tRNA in response to the relationship between the histidine and general amino-acid nutritional states of the cell. A stoichiometric role of this type for the enzyme, rather than its catalytic role, might explain the 3-fold greater translation of the ATP phosphoribosyltransferase gene compared to the other genes of the histidine operon (17). The enzyme constitutes 1.8% of soluble protein in fully derepressed cells (18). Furthermore, the amount of histidyl-tRNA in S. typhimurium normally is at least twice that required to sustain protein synthesis at a wild-type doubling time (6). The "extra" histidyl-tRNA might be available for formation of the ATP phosphoribosyltransferase complex. A similar possible involvement in gene regulation of a histidyl-tRNA reservoir contained in a complex with histidyl-tRNA synthetase has been discussed (19). Proof of such a role for the ATP phosphoribosyltransferase complex, however, must await demonstration that a significantly large proportion of histidyl-tRNA is bound to the enzyme in vivo under some conditions and that modulation of this reservoir specifically alters regulation of the histidine operon.

We thank Dr. Brian Reid, Department of Biochemistry, University of California at Riverside, for a generous gift of tRNA^His. This work was supported by U.S. Public Health Service Grant GM23031.