THE PYROPHOSPHATE EXCHANGE REACTION OF HISTIDYL-tRNA SYNTHETASE FROM *SALMONELLA TYPHIMURIUM*: REACTION PARAMETERS AND INHIBITION BY TRANSFER RIBONUCLEIC ACID

P. DI NATALE, F. CIMINO and F. DE LORENZO

From First and Second Chair of Biochemistry, Second Medical School, University of Naples, Naples, Italy

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1. Introduction

Aminoacyl transfer RNA synthetases catalyze the formation of specific aminoacyl-tRNA molecules. These enzymes play an essential role in protein synthesis ensuring the correct translation of the nucleotide sequence of messenger RNA into the aminoacyl sequence of protein [1].

The reaction has been considered to occur in two consecutive, separable steps:

$$E^* + AA + ATP \rightleftharpoons E - AA - AMP + PP_i \quad (1)$$

$$E - AA - AMP + tRNA \rightleftharpoons AA\text{–}tRNA + AMP + E \quad (2)$$

The reactions generally require magnesium as a cofactor [2,3]. Reaction 1 is the amino acid activation reaction and is usually studied by the ATP–PP_i isotope exchange reaction. Reaction parameters may be very different for each activating enzyme and also for enzymes from different sources which activate the same amino acid [3].

It has been shown that the first reaction may be affected by the presence of the specific tRNA. However, the effect of tRNA on the reaction is quite different depending on the particular synthetase considered. In some cases there is a strict requirement of tRNA (glutamyl-tRNA-synthetase [4] and arginyl-tRNA synthetase [5] from *E. coli*); in other cases, activation (rat liver isoleucyl-tRNA synthetase [6]) or inhibition (rat liver alanyl tRNA synthetase [7], yeast isoleucyl-tRNA synthetase [8], *E. coli* tyrosyl-tRNA synthetase [9]) can be achieved.

The histidyl-tRNA synthetase from *Salmonella typhimurium* has been extensively studied in regard to its purification procedure, kinetics and physico chemical properties [10,11] and also for its regulatory role in the repression mechanism of the histidine operon [12–14].

In this paper several aspects of reaction 1 catalyzed by the histidyl-tRNA synthetase from *Salmonella typhimurium* and the effect on it of transfer ribonucleic acid are reported.

2. Experimental

2.1. *Materials*

Salmonella typhimurium (LT-2) cells were grown in a 36 liter fermentor in a minimal salt medium [10] containing 0,5% glucose. The cells were harvested by centrifugation in late exponential phase of growth and stored at −20°C.

Histidyl-tRNA synthetase was isolated in apparently homogeneous form [10] and crude tRNA from *Salmonella typhimurium* was prepared and stripped as described earlier [15,16].

Crude tRNA lacking in $tRNA^{His}$ and pure $tRNA^{His}$ (accepting 1.6 nmoles of ^{3}H-L-histidine per 1 OD unit

* Abbreviations: E, an aminoacyl transfer ribonucleic acid synthetase; E–AA–AMP, enzyme-aminoacyl adenylate complex; tRNA, amino acid acceptor ribonucleic acid; AA–tRNA, aminoacyl - tRNA; $tRNA^{His}$, tRNA specific for histidine acceptance; $tRNA^{His}_{ox}$, $tRNA^{His}$ which has been oxidized with periodate.

of the tRNA) were kindly supplied by Dr P. Abrescia. They were purified according to the procedure described by S. Nishimura [17], with slight modifications.

Periodate oxidation of crude tRNA from *Salmonella typhimurium* was performed according to the procedure of Monier et al. [18].

(^{32}P) PP_i (10^3-10^4 mCi/mmole) was obtained from Amersham and stored at 4°C. The crystalline sodium salt of ATP, histidine and Norit A were obtained from Sigma; bovine serum albumine from BDH. All other materials were reagent grade.

2.2. *Assay of enzymatic activity*

The ATP–pyrophosphate exchange was determined as described by Rouget and Chapeville [19] with slight modifications. The incubation mixture contained in a total volume of 0.5 ml : 100 mM cacodylate buffer pH 7.2, 6 mM $MgCl_2$, 2 mM ATP, 2 mM (^{32}P) pyrophosphate (approx. 2×10^5 cpm), 2 mM histidine, 1 mM 2-mercaptoethanol, 0.5 mg of bovine serum albumine and 0.35 μg of purified enzyme. When one or more of the components was varied, all others were held constant at the concentrations listed above. The mixture was incubated at 37°C (enzyme was omitted for controls) and at the appropriate times (15 min for single point assays) stopped by the addition, successively, of 0.7 ml 5% perchloric acid, 0.3 ml of an aqueous suspension of activated Norit A (100 mg/ml) and 1.5 ml of 0.1 M sodium pyrophosphate. After thorough mixing and centrifugation, the supernatant was removed and the Norit was washed twice with 2 ml of 0.1 M sodium pyrophosphate. ATP was eluted with 2 ml of 0.3 M ammonium hydroxide in 50% ethanol. After centrifugation, 1 ml of the supernatant in 2 ml of INSTA GEL scintillation fluid (Packard) was counted in a Packard 3375 liquid scintillation spectrometer with 70% efficiency.

3. Results and discussion

3.1. *Reaction Parameters*

Under the standard assay conditions, the enzyme activity is proportional to enzyme concentrations in the range 0.01–0.5 μg and is linear for about 20 min of incubation.

The enzyme has a broad pH optimum (between pH 6.9 and 7.5), but displays maximal activity at pH 7.2 in cacodylate buffer. In 0.1 M Tris–HCl or 0.1 M phosphate buffer the reaction rate was only 84% and 40% respectively, of that obtained in cacodylate buffer at the same molarity and pH values. The pH effect on the activity of histidyl-tRNA synthetase in the amino acid activation reaction is the same of that occurring in the aminoacylation of $tRNA^{His}$ [10].

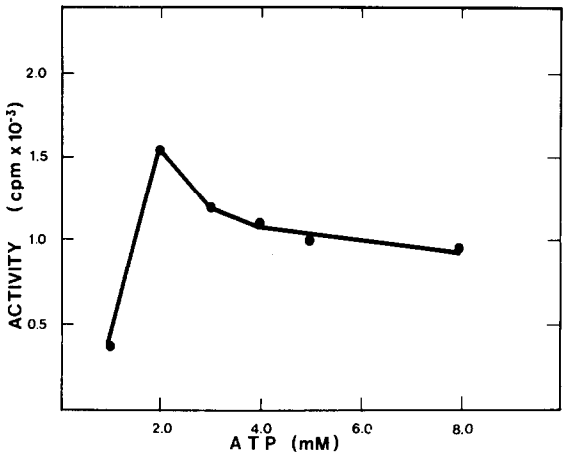

Fig. 1. The ATP–PP_i exchange as a function of ATP concentration. The Mg^{2+}/ATP ratio = 3 is used over entire range of concentration. Maximum exchange occurs at 6 mM Mg^{2+} and 2 mM ATP. Exchange reaction is given in cpm of the labeled ATP as indicated in experimental section.

The reaction does not occur in the absence of Mg^{2+}. The maximal rate of the reaction is observed at a Mg^{2+}: ATP ratio of 3, while the optimal ratio for the aminoacylation reaction is known to be 2 [10]. In the fig. 1 the ATP–PP_i exchange as a function of ATP concentration is reported. The Mg^{2+}: ATP ratio of 3 was used over the entire range of concentrations. Maximum exchange occurs at 6 mM Mg^{2+} and 2 mM ATP.

In table 1 the apparent K_m values for L-histidine, ATP and PP_i are reported. The values were determined from Lineweaver–Burk reciprocal plots (1/V versus 1/S) [20]. The K_m value for L-histidine is very close to that previously obtained with crude enzyme preparation [13]. The differences in the apparent K_m values in the two different reactions, i.e. exchange and acylation, were also observed for other aminoacyl-tRNA synthetases [21,22]. We found, as well, that in the absence of L-histidine the exchange reac-

Table 1
Apparent K_m values for substrates of histidyl-tRNA synthetase

Substrate	K_m for ATP-^{32}PPi Exchange[a] (M)	K_m for aminoacylation of tRNA[b] (M)
L-histidine	1.25×10^{-4}	2.5×10^{-5}
ATP	8.3×10^{-4}	1.4×10^{-4}
PP_i	3.3×10^{-4}	–
tRNA	–	1.1×10^{-7}

a The assay conditions were those described in experimental section except that the substrate concentration was varied. The histidine concentration varied over a range from 5×10^{-6} M to 1×10^{-3} M, the ATP concentration from 8×10^{-5} M to 2×10^{-3} M and the PP_i concentration from 1×10^{-5} M to 2×10^{-3} M.

b The K_m values for the aminoacylation of tRNA are those previously reported [10].

tion does not occur. Thus it would appear reasonable that the release of pyrophosphate occurs after the binding of both histidine and ATP as previously described for other aminoacyl-tRNA synthetases [23,24].

3.2. *Inhibition of ATP–PP_i exchange by tRNA*

Transfer RNA is not required for the histidine-dependent ATP–PP_i exchange. Indeed the presence of tRNA markedly decreases the initial rate of exchange with an inhibition of about 45% at a concentration of 40 A_{260} units per ml of crude tRNA (fig. 2). The inhibition seems to be specific for $tRNA^{His}$ since the addition of a crude preparation containing most tRNAs other than $tRNA^{His}$ does not influence the rate of the reaction (fig. 2). Furthermore with pure $tRNA^{His}$ (accepting 1.6 nmoles of ^{3}H-L-histidine per 1 A_{260} unit of the tRNA) the same pattern of inhibition was obtained with 50-fold less tRNA (fig. 2). This means that about 45% inhibition was observed at a $tRNA^{His}$ concentration of 2.24 μM that is very close to the intracellular concentration of this molecule (approx. 3 μM) [25].

The addition of crude $tRNA^{His}$ to the exchange reaction should lead to the formation of histidyl-$tRNA^{His}$. In fact, in parallel experiments where ^{3}H-histidine was used and acylation reaction was measured together with ATP–PP_i reaction under the standard

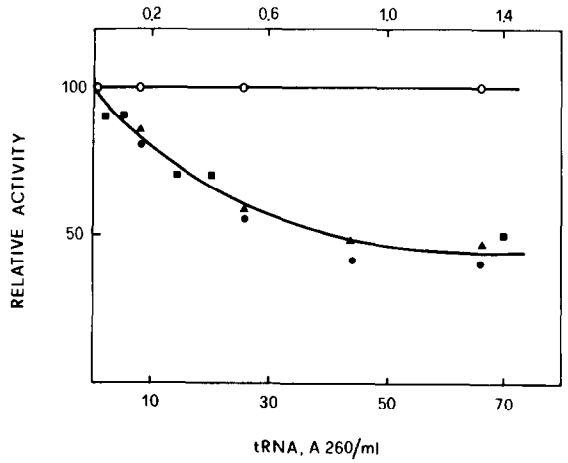

Fig. 2. ATP–PP_i exchange rate in the presence of crude tRNA lacking in $tRNA^{His}$ (○), crude $tRNA^{His}$ (●), crude $tRNA^{His}_{ox}$ (▲) and pure $tRNA^{His}$ (■). Values are expressed as percentage of the activity of enzyme reaction without added tRNA. The upper scale indicates the A_{260} units per ml of pure $tRNA^{His}$ (■). Exchange reaction is given in cpm of the labeled ATP as indicated in experimental section.

assay conditions, the added $tRNA^{His}$ was completely acylated. To check if the inhibition of ATP–PP_i exchange reaction was due to accumulation of histidyl-$tRNA^{His}$, a crude preparation of $tRNA^{His}$ was

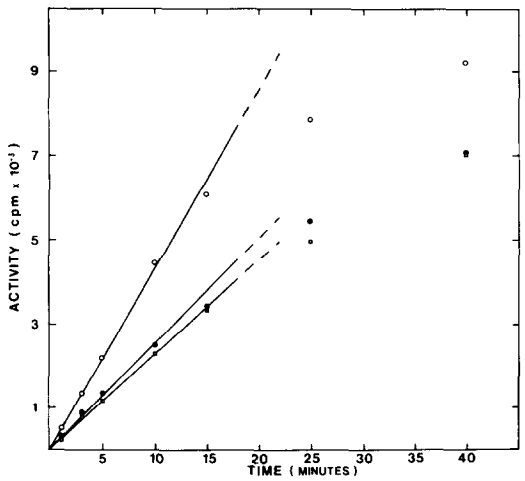

Fig. 3. Time course of the ATP–PP_i exchange reaction in the absence or presence of *Salmonella typhimurium* crude tRNA. No tRNA (○), $tRNA^{His}$ 32 A_{260} units per ml (●), $tRNA^{His}_{ox}$ 32 A_{260} units per ml (□). Exchange reaction is given in cpm of the labeled ATP as indicated in experimental section.

oxidized with periodate and its effect on exchange reaction examined; also shown in fig. 2, $tRNA^{His}_{ox}$ which is completely devoid of acceptor activity, produces an inhibitory effect very similar to that observed with native $tRNA^{His}$. Furthermore a time course of the exchange reaction with either unacylated (periodate treated tRNA) or crude $tRNA^{His}$ was similar (fig. 3), indicating that acylation is not required for inhibition.

The inhibition by $tRNA^{His}$ is noncompetitive with L-histidine (fig. 4) and seems to be uncompetitive with ATP (fig. 5). Since in our crude tRNA preparation 11 pmoles of $tRNA^{His}$ per A_{260} unit are present, we calculated a K_i value for $tRNA^{His}$ of 0.34 μM when L-histidine is the variable substrate (fig. 4) and a K_i value of 0.25 μM when ATP is the variable substrate (fig. 5). The observation that $tRNA^{His}$ is a noncompetitive inhibitor with respect to L-histidine and uncompetitive with respect to ATP in the ATP–PP_i exchange reaction might indicate the presence on the enzyme of distinct sites for both substrates and

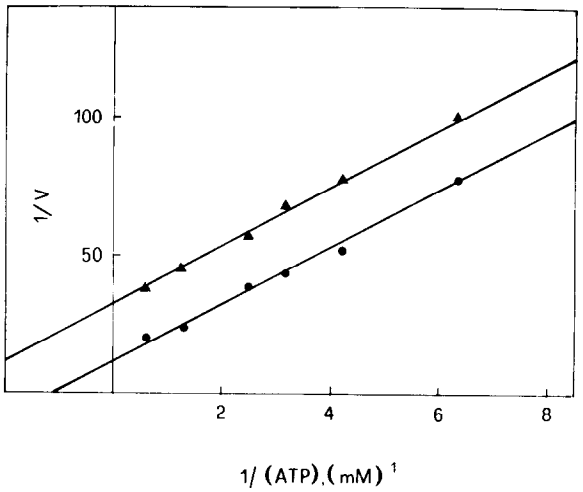

Fig. 5. Double reciprocal plot of histidyl-tRNA synthetase activity as a function of ATP concentration in the absence or presence of *Salmonella typhimurium* crude tRNA. No tRNA (•), $tRNA^{His}$ 40 A_{260} units per ml (▲). 1/V is 10^5 times the reciprocal of cpm in ATP in 15 min. 1/S is given as 1/mM.

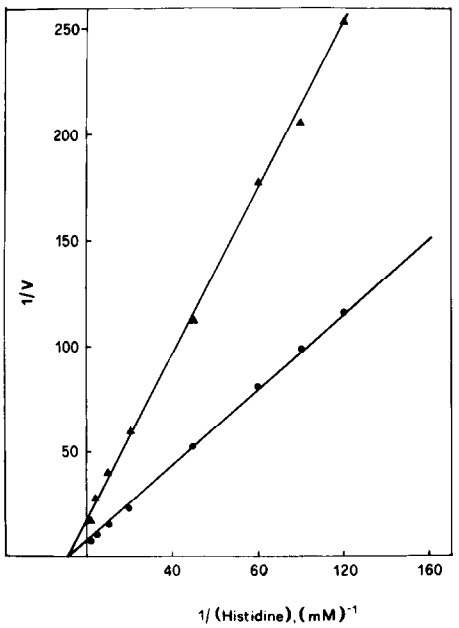

Fig. 4. Double reciprocal plot of histidyl-tRNA synthetase activity as a function of histidine concentration in the absence or presence of *Salmonella typhimurium* crude tRNA. No tRNA (•), $tRNA^{His}$ 40 A_{260} units per ml (▲). 1/V is 10^5 times the reciprocal of cpm in ATP in 15 min. 1/S is given as 1/mM.

for tRNA. We do not know if the binding site(s) for tRNA is the same for the inhibition as the substrate-binding site(s).

Our studies demonstrate that the interaction between histidyl-tRNA synthetase and tRNA inhibits the rate of histidine-dependent ATP–PP_i exchange. This inhibitory effect is specific for $tRNA^{His}$ and doesn't require an intact 3′-adenosine terminus on $tRNA^{His}$ molecule. This observation could suggest that in the interaction between $tRNA^{His}$ and its specific aminoacyl tRNA synthetase the 3′-adenosine terminus is not implicated. Similar results were obtained for $tRNA^{Arg}$ and $tRNA^{Glu}$ where periodate-treated tRNA(s) were still able to bind their specific aminoacyl-tRNA synthetases [26,27]. Conversely $tRNA^{Val}$ after periodate oxidation no longer was bound by valyl-tRNA synthetase [28].

On the basis of the reported results it is not easy to assign a physiological role to the observed inhibition by $tRNA^{His}$ on the histidine-dependent ATP-PP_i exchange reaction. However, if the in vitro effect corresponds to an in vivo effect leading to a modulation of the intracellular level of histidyl-$tRNA^{His}$, then this phenomenon might have a regulatory meaning in view of the fact that the concentration of histidyl-

$tRNA^{His}$ is correlated with the degree of the repression of the histidine biosynthetic enzymes [14]. It must be noted that for the esterification of tRNA the obligatory formation of an intermediate aminoacyl adenylate has been doubted by Loftfield and Eigner [29]. In this case, of course, the inhibition by $tRNA^{His}$ of the histidine-dependent ATP-PP_i exchange reaction would not be of physiological significance.

Acknowledgements

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