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# AMINOACYL-tRNA SYNTHETASES FROM *SARCINA LUTEA**

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## SUMMARY

Extracts from *Sarcina lutea* were assayed for aminoacyl-tRNA synthetase activity by determining amino acid-stimulated ATP–$^{32}PP_i$ exchange or hydroxamate formation. Extracts from logarithmically growing cells exhibited a higher specific activity than those from stationary cultures. When amino acid activation was measured both by hydroxamate formation and ATP–$^{32}PP_i$ exchange, the ratio of activities determined by these methods varied with each amino acid tested. This is explained by the finding that hydroxylamine at higher concentrations is an inhibitor of the exchange reaction.

## INTRODUCTION

The activation and subsequent transfer of each amino acid to tRNA has been shown to be catalyzed by a specific enzyme, aminoacyl-tRNA synthetase (amino acid:tRNA ligase (AMP), EC 6.1.1.10), which forms an aminoacyl adenylate intermediate. Although hydroxamic acid formation and ATP–$^{32}PP_i$ exchange both are accepted measures of the formation of this intermediate, data obtained by the two methods generally do not agree when used to compare the relative rates of activation of various amino acids by a crude source of enzymes[1–3]. We have attempted to explain the apparent discrepancy between the two assay methods on the basis of an observed inhibition of the exchange reaction by hydroxylamine. An investigation of certain other factors affecting the activities of these enzymes also was undertaken.

## METHODS

Cultures for individual experiments were obtained by growing *Sarcina lutea* under forced aeration, and subcellular fractions from osmotically lysed protoplasts were prepared as described previously[4,5]. "Membrane" and "cytoplasmic" fractions

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were obtained by centrifugation of protoplast lysates at 10 000 × *g* for 20 min. Disruption of intact cells also was accomplished using the Branson Model-S75 sonifier at maximum output with continuous-flow attachment.

In the hydroxamic acid assay each tube contained 3000 μmoles $NH_2OH$ HCl, 30 μmoles Tris (pH 8.0), 30 μmoles $MgCl_2$, 15 μmoles ATP, 30 μmoles of the L-amino acid to be tested and a limiting amount of enzyme in a final volume of 3.0 ml. The control tube contained no added amino acid. After incubation at 37° for 2 h reactions were terminated by the addition of 0.5 ml of 5.3 M HCl containing 1.1 M trichloroacetic acid. This mixture was allowed to stand in an ice–water bath for 30 min, then centrifuged for 15 min at 12 000 × *g*. Finally, 2.5 ml of the supernatant solution and 0.5 ml 0.6 M $FeCl_3$ were mixed in a 3-ml cuvette, and the absorbance was determined immediately at 520 mμ using a Beckman Model DU spectrophotometer. Tyrosine hydroxamate (Nutritional Biochemical Corp.) was used as the standard for all assays.

The reaction mixture used to measure ATP–$^{32}PP_i$ exchange contained 100 μmoles Tris (pH 8.0), 10 μmoles $MgCl_2$, 10 μmoles L-amino acid, 5 μmoles ATP, 5 μmoles $^{32}PP_i$ as $Na_4P_2O_7$ ($1 \cdot 10^5$–$1 \cdot 10^6$ counts/min, Volk Radiochemical Co.), 10 μmoles KF and a limiting amount of enzyme in a total volume of 1.0 ml. The control mixture lacked only amino acid. Enzyme was added to the other reactants after preincubation at 37° for 10 min. The reaction was terminated and samples collected as described by FANGMAN AND NEIDHARDT[6]. A thin-window, gas-flow counter was used to determine radioactivity, and protein was determined by the method of LOWRY *et al.*[7].

1 munit of aminoacyl-tRNA synthetase is defined as that amount of enzyme which will catalyze the transformation of 1 mμmole of substrate per min under standard conditions. Specific activity is defined as munits per mg protein.

Cell growth was determined turbidimetrically as Klett units at 660 mμ.

## RESULTS AND DISCUSSION

The location of aminoacyl-tRNA synthetases in micro-organisms is not well defined. Activation of 20 commonly occurring amino acids has been reported using both soluble and particulate fractions prepared from bacteria[8,9]. Extracts prepared by the osmotic lysis of protoplasts of *S. lutea* had significant synthetase activity associated with both the membrane and cytoplasmic fractions. However, activity was completely removed from the membrane fraction by two washings with dilute buffer. Most of the activity of the cytoplasmic fraction was retained in a soluble fraction prepared from it by centrifugation at 100 000 × *g* for 2 h. It was concluded that the aminoacyl-tRNA synthetases of *S. lutea* were located primarily in this fraction, but the possibility that some were loosely bound to the membrane could not be ruled out.

NURMIKKO, HEINONEN AND LAMMINMAKI[10] reported that the activity of certain streptococcal synthetases reached a maximum during exponential growth. Extracts obtained from *S. lutea* at various stages of growth were tested for synthetase activity related to five amino acids over a period of three generations before cells entered the resting phase. With respect to all amino acids tested the specific activity increased to a maximum in the middle of the second generation. The data are shown in Fig. 1.

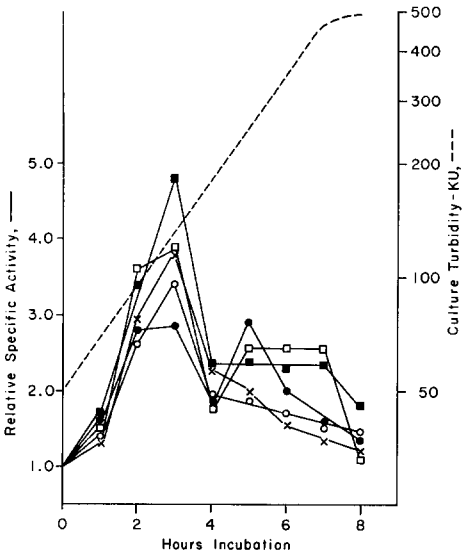


Fig. 1. Aminoacyl-tRNA synthetase activity of sonic extracts at various times. Samples of the culture were removed at hourly intervals and sonic extracts prepared from them. Synthetase activity was determined by ATP-$^{32}PP_i$ exchange. The data are averages of two experiments. The specific activity of each synthetase at various times was compared with that of the extract from the 50-Klett units culture (specific activity = 1.00). The dashed line represents culture turbidity (Klett units (KU) at 660 mμ) at various times. The solid lines represent synthetase activity with respect to isoleucine (■), valine (○), methionine (□), threonine (×) and cysteine (●).

STERN *et al.*[11] found that all of the synthetases from *Escherichia coli* except lysyl-tRNA synthetase were strongly inhibited by *p*-chloromercuribenzoate and have concluded that sulfhydryl groups probably are not involved as a part of the "active site". We have obtained similar results in our studies of synthetases from *S. lutea*. However, in this instance the methionyl- and not the lysyl-enzyme appears to be the one exception.

As shown in Table I stimulation of ATP–$^{32}PP_i$ exchange and hydroxamic acid formation by all twenty naturally occurring amino acids could be demonstrated using crude extracts from *S. lutea*. It is widely recognized that when amino acid activation is measured both by hydroxamic acid formation and ATP–$^{32}PP_i$ exchange, the ratio of activities determined by these two methods is not constant but varies with each amino acid tested[1-3]. Inspection of Table I will document this variability in the case of *S. lutea*. We were interested in correlating the ratio of activities as determined by the two assays to some variable of each enzyme system. Seven amino acids were chosen for further testing because of the high activity of the corresponding synthetases and because of differences in the ratio of activities determined by the two methods. The general reaction between amino acid (*AA*) and enzyme (*E*) in the presence of $PP_i$ or $NH_2OH$ may be formulated as:

$$\mathrm{ATP} + AA + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} E - \mathrm{AMP} - AA + \mathrm{PP_i}$$

$$E - \mathrm{AMP} - AA + \mathrm{NH_2OH} \xrightarrow{k_2} AA\mathrm{NHOH} + E + \mathrm{AMP}$$

TABLE I

AMINO ACID ACTIVATION BY SONIC EXTRACTS FROM *S. lutea*

| *Amino acid* | *Hydroxamic acid (munits)* | *ATP–$^{32}PP_i$ exchange (munits)* |
|---|---|---|
| Ala | 1.63 | 29.0 |
| Arg | 0.38 | 13.2 |
| Asn | 6.06 | 18.6 |
| Asp | 11.81 | 26.2 |
| Cys | 13.13 | 53.0 |
| Glu | 2.47 | 15.8 |
| Gln | 0.59 | 18.6 |
| Gly | 0.57 | 21.8 |
| His | 4.68 | 27.6 |
| Ile | 3.37 | 40.4 |
| Leu | 2.56 | 24.0 |
| Lys | 0.38 | 19.6 |
| Met | 6.81 | 44.2 |
| Phe | 0.17 | 9.8 |
| Pro | 4.33 | 14.6 |
| Ser | 0.40 | 17.0 |
| Thr | 0.95 | 41.0 |
| Trp | 0.83 | 3.2 |
| Tyr | 2.27 | 24.2 |
| Val | 2.09 | 61.6 |

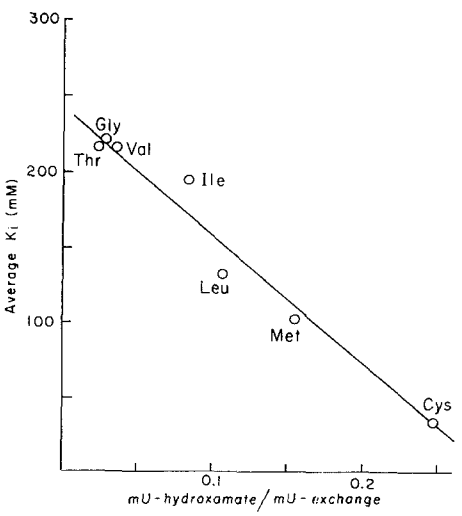


Fig. 2. The variation of $K_i$ values for hydroxylamine with the ratio of hydroxamic acid formation to ATP–$^{32}PP_i$ exchange, expressed in munits (mU). Lineweaver–Burk plots were used to determine $K_i$ values. The ATP–$^{32}PP_i$ exchange assay system was used as described in the methods section except that the concentration of the amino acid was varied. Control and hydroxylamine-inhibited reactions were assayed simultaneously. The $K_i$ values reported are the average of determinations performed using two different sonic extracts.

Under the conditions employed in the assay procedures, we are assuming that the reaction is being forced either toward hydroxamic acid formation or $^{32}PP_i$ exchange. Therefore, we omit consideration of the formation of aminoacyl-tRNA. The exchange reaction is a measure of $k_{-1}$, and hydroxamic acid formation measures $k_2 - k_{-1}$. Thus, the hydroxamic acid assay measures a competition between $PP_i$ and hydroxylamine for the enzyme-bound aminoacyl adenylate. The amount of hydroxamic acid formed will be dependent upon the varying reactivity of each with the aminoacyl adenylate–enzyme complex. The ratio of the rate of hydroxamic acid formation to the rate of $PP_i$–ATP exchange then should be a measure of the relative reactivity of the enzyme–aminoacyl adenylate complex with hydroxylamine and $PP_i$.

To test this hypothesis we measured the effect of increasing amino acid concentration on the rate of $PP_i$–ATP exchange in the presence and absence of hydroxylamine. It was found that hydroxylamine at 0.1 M and 0.2 M was a competitive inhibitor of the $PP_i$–ATP exchange stimulated by each of the amino acids tested and that the $K_i$ values for hydroxylamine were related to the ratio of activities determined by the two methods. This is shown in Fig. 2. Thus, the differences observed in the two assays of aminoacyl adenylate formation were related to the reactivity of the enzyme complex with hydroxylamine, and this varied with each of the enzymes tested. Although the reactivity with hydroxylamine is low, the high concentration of hydroxylamine used in the aminoacyl hydroxamate formation assay is more than sufficient to allow it to act as an inhibitor of the exchange reaction.

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