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The Catalytic Properties of Tyrosyl Ribonucleic Acid Synthetases from *Escherichia coli* and *Bacillus subtilis**

Richard Calendar and Paul Berg

ABSTRACT: A comparison of the substrate specificity of tyrosyl RNA synthetases from *Escherichia coli* and *Bacillus subtilis* has been made. Of the four common ribonucleoside triphosphates only adenosine triphosphate is utilized by both enzymes. Several alterations in the L-tyrosine structure have been examined for their effect on the formation of aminoacyl adenylate. Removal of the carboxyl group (tyramine) or its reduction (L-tyrosinol), amidation (L-tyrosine amide), or esterification (L-tyrosine methyl ester) yielded inactive substrates although each of these analogs was a competitive inhibitor. Modification of the α-amino group (*N*-acetyl or *N*-glycyl) or substitution of the α hydrogen by a methyl group yielded compounds which were neither substrates nor inhibitors. Quite unexpectedly, D-tyrosine was activated and transferred to t-RNA$_{tyr}$ by both enzymes. But in each case for the exchange reaction the V_{max} was lower and the K_m was higher than with L-tyrosine. A shift of the ring hydroxyl group from the 4 (*para*) to the 3 (*meta*) or 2 (*ortho*) position yielded inactive substrates with both enzymes. Although substitution of chloro, iodo, amino, or nitro groups in position 3 of L-tyrosine eliminated the substrate activity, after introduction of a fluoro or hydroxy group in the same position the derivatives were still active, but the V_{max} was lower and the K_m was higher. Conversion of the benzene ring to a pyridine structure (5-hydroxy-2-(3-DL-alanyl)pyridine) decreased the V_{max} to about half and increased the K_m about 30-fold with both enzymes. The *B. subtilis* and *E. coli* tyrosyl RNA synthetases utilize the t-RNA$_{tyr}$ from either bacterial source; the same amount of either tyrosyl RNA is formed with either enzyme.

In the previous paper (Calendar and Berg, 1966) tyrosyl RNA[1] synthetases from *E. coli* and *B. subtilis* were isolated and several of their physical and chemical properties were compared. These two enzymes have also been examined with respect to several catalytic parameters, particularly those related to the specificity for the substrates and analogs of the substrates, and these results are reported here.

Experimental Section

Materials. Glycyl-L-tyrosine, *N*-acetyl-L-tyrosine, 3-hydroxyl-L-tyrosine, and natural L-amino acids were purchased from California Corp. for Biochemical Research. D-Tyrosine, 3-iodo-L-tyrosine, 2-hydroxy-L-phenylalanine, 3-hydroxy-L-phenylalanine, 3-amino-L-tyrosine, 3-hydroxy-DL-tyrosine, and 4-fluoro-DL-phenylalanine were purchased from Nutritional Biochemicals, Inc., Cleveland, Ohio. 4-Chloro-DL-phenyl-

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[1] Abbreviations used: RNA, ribonucleic acid; t-RNA, transfer RNA; F-tyrosine, 3-fluorotyrosine; DOPA, 3-hydroxytyrosine; HPA, 5-hydroxy-2-(3-DL-alanyl)pyridine; ATP, GTP, CTP, and UTP, adenosine, guanosine, cytidine, and uridine triphosphate; ADP, adenosine diphosphate; dATP, deoxy ATP; dTTP, deoxythymidine triphosphate.

alanine, 3-chloro-L-tyrosine, 3-fluoro-DL-tyrosine, *N*-(4-hydroxyphenyl)glycine, β-(4-hydroxyphenyl)pyruvate, β-(4-hydroxyphenyl)propionate, and tyramine were from K and K Fine Chemicals, Inc., Plainview, N. Y., and 3-nitro-L-tyrosine was obtained from Delta Chemical Works, New York, N. Y. The methyl ester, ethyl ester, and amide of L-tyrosine were purchased from New England Nuclear Corp., Boston, Mass. 4-Amino-DL-phenylalanine was purchased from Sigma Chemical Corp., St. Louis, Mo.

3-Fluoro-L-tyrosine was a gift from the late Dr. Carl Niemann; β-(4-hydroxyphenyl)-DL-lactate was provided by Dr. B. N. LaDu; 4-chlorophenyl-L-cysteine was given to us by Dr. E. T. Bucovaz, and α-methyl-DL-tyrosine was donated by the Merck, Sharpe and Dohme Research Laboratories. L-Tyrosine-*O*-sulfate was a gift of Dr. K. S. Dodgson, and 5-hydroxy-2-(3-DL-alanyl)pyridine was either provided by Dr. William Shive or purchased from Cyclo Chemical Corp., Los Angeles, Calif.

Adenyl-5-methylenediphosphonate was the generous gift of Dr. Lionel Simon. Other nucleotides were purchased from California Corp. for Biochemical Research.

[3,5-^{3}H]L-Tyrosine was purchased from New England Nuclear Corp., Boston, Mass. 3-Fluoro-DL-tyrosine was tritiated at New England Nuclear Corp. by treatment with tritium gas. This material was purified by chromatography at 23° on a cellulose column (Whatman cellulose) using the upper phase of 1-butanol–acetic acid–water, 4:1:5, as eluent. Unlabeled 3-hydroxyl-L-phenylalanine was carried through this chromatographic fractionation to eliminate contaminating tyrosine.

Crotalus adamanteus venom was obtained from Ross Allen's Reptile Institute, Silver Springs, Fla. Purified D- and L-amino acid oxidases and catalase were purchased from Worthington Biochemical Corp., Freehold, N. J. These enzymes were dialyzed *vs.* 0.02 M Tris, pH 7.8, before use. Sephadex G-50, coarse, was purchased from Pharmacia, Uppsala, Sweden. t-RNA was prepared from *E. coli* B by the procedure of Zubay (1962) and purified t-RNA$_{tyr}$ (Muench and Berg, 1966) was a gift from Dr. K. Muench. t-RNA from *B. subtilis* SB19 was isolated by the procedure of Zubay (1962), plus a cetyltrimethylammonium bromide fractionation to remove carbohydrate impurities (Dutta *et al.*, 1953). *E. coli* and *B. subtilis* tyrosyl RNA synthetases were the preparations described by Calendar and Berg (1966).

Methods. Measurement of the tyrosine-dependent exchange of ATP and $^{32}PP_i$ was as described by Calendar and Berg (1966). Aminoacyl RNA synthesis (eq 1) was measured in a reaction mixture (0.4 ml) containing 100 mM sodium cacodylate buffer, pH 7.0, 5 mM $MgCl_2$, 2 mM ATP,[2] 10 mM 2-mercaptoethanol, 100 μg/ml of bovine plasma albumin, and 0.25 mM [^{3}H]amino acid (5×10^6 cpm/μmole).

$$\text{ATP} + [^3\text{H}]\text{amino acid} + \text{t-RNA} \rightleftharpoons [^3\text{H}]\text{aminoacyl RNA} + \text{AMP} + PP_i \quad (1)$$

For measurements of the rate of the reaction, enzyme (0.0005 to 0.015 unit) and an amount of t-RNA[3] capable of accepting 1–2 mμmoles of amino acid were used. For measurements of the extent of aminoacyl RNA synthesis, excess enzyme (3 units) and limiting amounts of t-RNA (an amount capable of accepting 0.05–0.3 mμmole of amino acid) were added. Enzyme dilutions were made in a solution containing 50 mM potassium phosphate, pH 6.5, 10 mM 2-mercaptoethanol, and 100 μg/ml of bovine plasma albumin.

After 10 min (rate) or 30 min (extent) at 37°, 3 ml of cold 2 N HCl was added, the mixture was filtered through a Whatman GF/C glass filter, washed five times with 3-ml aliquots of cold 2 N HCl, then with 3 ml of cold 95% ethanol. The filter was dried and counted in a scintillation counter using a toluene scintillation medium. A blank without enzyme was run with each set of samples, and its value was subtracted from that of each sample. With 2 mμmoles of *E. coli* t-RNA$_{tyr}$ and up to 0.015 unit (0.02 μg) of purified *E. coli* enzyme (fraction VII), tyrosyl RNA synthesis was proportional to the amount of enzyme added. With limiting quantities of *E. coli* t-RNA$_{tyr}$ and excess enzyme, the extent of tyrosyl RNA synthesis was proportional to t-RNA.

Because we did not have available fractions enriched for *B. subtilis* t-RNA$_{tyr}$, kinetic studies of tyrosyl RNA synthesis were carried out only with purified t-RNA$_{tyr}$ from *E. coli*.

Amino acid concentration was determined with ninhydrin (Moore and Stein, 1954). Paper chromatography was carried out using Whatman No. 3MM paper and chromatographic solvents as follows: (1) 1-butanol–acetic acid–water, 4:1:5, upper phase, and (2) pyridine–isoamyl alcohol–water, 35:35:30.

Tritium was counted in a Nuclear Chicago 720 series or a Packard TriCarb scintillation counter, using Bray's solution (Bray, 1960) for counting aqueous samples and a toluene-based medium for counting dried samples.

Results

Specificity of Tyrosyl RNA Synthetases for Amino Acids. A. ATP–$^{32}PP_i$ EXCHANGE REACTION. Neither the *E. coli* nor *B. subtilis* tyrosyl RNA synthetase catalyzes an ATP–$^{32}PP_i$ exchange (<1% of the rate with L-tyrosine) with any of the naturally occurring L-amino

[2] Where the concentration of ATP was varied, the magnesium concentration was varied to maintain a ratio of Mg/ATP = 5.

[3] Concentrations of t-RNA have been expressed in two ways. The amount of specific tyrosine-acceptor chains (t-RNA$_{tyr}$) is given in molar quantities based on the amount of tyrosine which can be esterified to t-RNA with excess enzyme, ATP, and amino acid. Concentration of bulk t-RNA has been expressed as A_{260} measured at pH 12. Purified t-RNA$_{tyr}$ (Muench and Berg, 1966), about 30% pure, was used to measure the rate of tyrosyl RNA synthesis to avoid adding a very large amount of the crude t-RNA preparation. Aminoacyl RNA formation was <10% of the total capacity of the t-RNA added, and thus the rate of back reaction could be neglected.

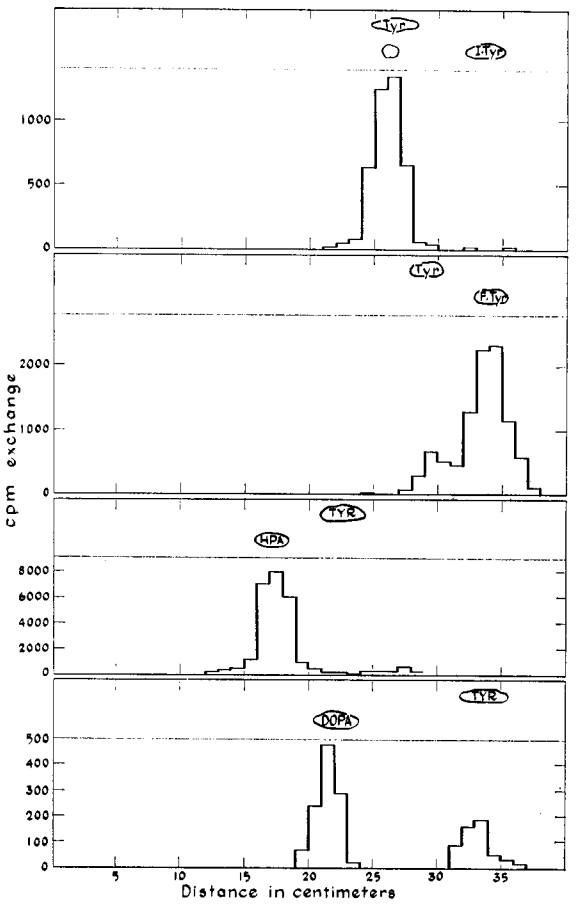

FIGURE 1: Test for activity of tyrosine analogs in ATP–$^{32}PP_i$ exchange reaction. Tyrosine analog (1 μmole) was spread on Whatman No. 3MM paper and chromatographed in parallel with the markers shown. Markers were sprayed with ninhydrin and 1-cm strips were eluted with water. This eluate was tested for activation of the ATP–$^{32}PP_i$ exchange reaction.

acids (at 1 mM) other than L-tyrosine. Of a variety of tyrosine analogs tested with each enzyme (Table I), only D-tyrosine, 3-fluorotyrosine (F-tyrosine), 3-hydroxytyrosine (DOPA), and 5-hydroxy-2-(3-DL-alanyl)-pyridine (HPA) were active in replacing L-tyrosine in supporting the ATP–$^{32}PP_i$ exchange (Table I; kinetics parameters are presented in Table IV).

In making these tests many of the compounds listed showed some activity, but except for the compounds mentioned above, the activity was always due to contamination by L-tyrosine. This was shown as follows: each analog (1 μmole) was chromatographed with parallel markers of the analog and tyrosine, and after the markers were visualized by spraying with ninhydrin, the material active in the enzyme assay was located by assaying the eluates of 1-cm strips of the chromatogram (Figure 1). The top panel shows that all the activity attributed to 3-iodo-L-tyrosine migrates with tyrosine and not with iodotyrosine. The second and fourth panels show that, although the F-tyrosine

TABLE I: The Ability of Tyrosine and Tyrosine Analogs to Support or Inhibit ATP–$^{32}PP_i$ Exchange.[a]

Amino Acid	Activator	Inhibitor
L-Tyrosine	+[b]	
D-Tyrosine	+	
2-Hydroxy-L-phenylalanine	—[c]	—
3-Hydroxy-L-phenylalanine	—	—
3-Fluoro-L-tyrosine	+	
3-Chloro-L-tyrosine	—	
3-Iodo-L-tyrosine	—	
3-Amino-L-tyrosine	—	—
3-Nitro-L-tyrosine	—	
3-Hydroxy-L-tyrosine	+	
4-Amino-DL-phenylalanine	—	—
4-Fluoro-L-phenylalanine	—	—
4-Chloro-DL-phenylalanine	—	—
L-Tyrosine-*O*-sulfate	—	—
α-Methyl-DL-tyrosine	—	—
N-Acetyl-L-tyrosine	—	—
N-Glycyl-L-tyrosine	—	—
β-(*p*-Hydroxyphenyl)propionate	—	—
β-(*p*-Hydroxyphenyl)-DL-lactate	—	—
β-(*p*-Hydroxyphenyl)pyruvate	—	—
N-(*p*-Hydroxyphenyl)glycine	—	—
(*p*-Chlorophenyl)-L-cysteine	—	—
L-Tyrosinol	—	+
L-Tyrosine amide	—	+
L-Tyrosine methyl ester	—	+
Tyramine	—	+
5-Hydroxy-2-(3-DL-alanyl)pyridine	+	

[a] Tyrosine analogs were tested in the ATP–$^{32}PP_i$ exchange reaction at a concentration of 1 mM for activation and at the same concentration for inhibition of the exchange reaction supported by 0.02 mM L-tyrosine. All tyrosine analogs which showed activation of the exchange reaction were further tested chromatographically as illustrated in Figure 1. Analog preparations contaminated with tyrosine were not tested for inhibition. [b] + denotes that the analog is activated or inhibits activation of tyrosine. The kinetic constants are given in Table IV. [c] — indicates that the analog is not activated (<1% of the rate of L-tyrosine) or does not inhibit activation of tyrosine (K_I >2.5 mM).

and DOPA preparations contain tyrosine, both compounds also support the ATP–$^{32}PP_i$ exchange. HPA (third panel, Figure 1) is free of tyrosine and all the material capable of being activated chromatographs with the HPA marker. This emphasizes the necessity for establishing the purity of amino acid analogs before testing their ability to be activated, particularly in measurements of the ATP–$^{32}PP_i$ exchange.

D-Tyrosine stimulated the ATP–$^{32}PP_i$ exchange reaction with both *E. coli* and *B. subtilis* enzymes. This is not due to L-tyrosine contamination, since the activity

of D-tyrosine is destroyed by D-amino acid oxidase, but there is no detectable loss of activity if the D-tyrosine is incubated with L-amino acid oxidase. Similarly, although L-amino acid oxidase destroys the activity of L-tyrosine, it does not completely destroy the activity of DL-tyrosine, but leaves an amount of activity which is expected from the residual D-tyrosine (Table II).

TABLE II: The Effect of D- and L-Amino Acid Oxidases on the Activity of D- and L-Tyrosine Supporting ATP–$^{32}PP_i$ Exchange.[a]

Amino Acid	Treatment	ATP–$^{32}PP_i$ Exchange (μmoles/15 min) *E. coli* Enzyme	*B. subtilis* Enzyme
L-Tyrosine	...	0.150	0.056
L-Tyrosine	D-Amino acid oxidase	0.130	0.056
L-Tyrosine	L-Amino acid oxidase	0.001	<0.001
D-Tyrosine	...	0.031	0.004
D-Tyrosine	L-Amino acid oxidase	0.030	0.004
D-Tyrosine	D-Amino acid oxidase	<0.001	<0.001
DL-Tyrosine	...	0.150	0.038
DL-Tyrosine	L-Amino acid oxidase	0.030	0.004

[a] D- or L-Tyrosine (0.1 μmole) or DL-tyrosine (0.2 μmole) was treated in 0.02 M Tris, pH 7.8, at 37° for 3 hr with the specified oxidase preparation. The reaction mixture was boiled for 2 min and then examined for its ability to stimulate the ATP–$^{32}PP_i$ exchange reaction. Control experiments showed that boiled oxidase preparations neither activated nor inhibited the tyrosine-dependent ATP–$^{32}PP_i$ exchange reaction. Purified L-amino acid oxidase (0.25 mg/tube) was used in the experiments in the second column while crude lyophilized *C. adamanteus* venom (1 mg/tube) was used in the experiments in column 1. Purified D-amino acid oxidase (0.5 mg/tube) was used for experiments listed in both columns.

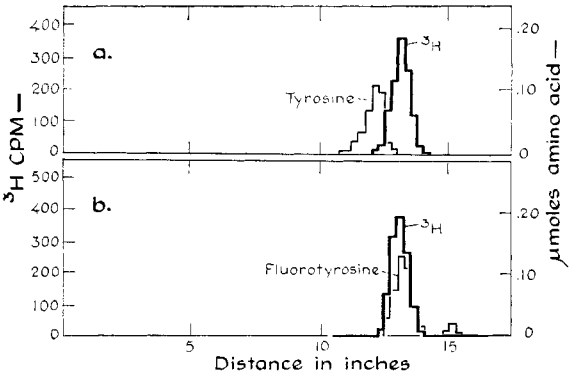

FIGURE 2: Chromatography of F-tyrosine recovered from F-tyrosyl RNA. F-Tyrosyl RNA was prepared as described in the Methods section with enzyme and t-RNA from *E. coli*. The product was precipitated with 67% ethanol, 100 mM KCl; the precipitate was redissolved and passed over a column of Sephadex G-50 in 50 mM sodium acetate, pH 5.0, 100 mM KCl. The peak of A_{260} corresponding to large molecular weight material was collected, and the radioactivity was determined. Without enzyme, no label (1%) accompanied the t-RNA. The aminoacyl RNA fraction was dialyzed *vs.* water and the dialysate was made alkaline by addition of a drop of concentrated ammonia, and then incubated for 1 hr at 37° to remove bound amino acids. The t-RNA was precipitated by the addition of cold HCl to a concentration of 1 M, and removed by centrifugation. The supernatant fluid, containing 90% of the radioactivity, was lyophilized. Aliquots of this material were mixed with 0.4 μmole each of tyrosine and F-tyrosine markers and were chromatographed on Whatman No. 3MM paper for 24 hr using solvent 1. Strips (0.25 in.) were cut horizontally in the chromatogram and eluted with water. Aliquots of the eluate were used to determine amino acid and radioactivity content. (a) L-Tyrosine marker recovery 88%; radioactivity recovery 91%. (b) F-Tyrosine marker recovery 80%; radioactivity recovery 91%. Similar results were obtained when the *B. subtilis* enzyme was used to esterify F-tyrosine to *E. coli* t-RNA.

B. AMINOACYL RNA SYNTHESIS. ^3H-F-Tyrosine (DL mixture) is esterified to t-RNA with the *E. coli* or *B. subtilis* tyrosyl RNA synthetases to the same extent as that with tyrosine as substrate (Table III). This is true whether the t-RNA is from *E. coli* or *B. subtilis*. If ^3H-labeled tyrosine and ^3H-F-tyrosine at the same specific radioactivity are incubated together, the amount of ^3H linked to t-RNA is the same as that found with each substrate alone. Thus it appears that tyrosine and F-tyrosine are esterified to the same chains and to the same extent (Table III). To eliminate the possibility that the esterified ^3H-label was tyrosine and not F-tyrosine, the esterified amino acid was recovered from the aminoacyl RNA and shown to cochromatograph with F-tyrosine and not tyrosine (Figure 2).

Although a rigorous demonstration of DOPA–RNA formation has not yet been achieved, the fact that prior incubation of t-RNA with ATP, purified DOPA, and the *E. coli* enzyme prevents the subsequent esterification of tyrosine, suggests that DOPA is linked to $t\text{-}RNA_{tyr}$.

Experiments with ^{14}C-D-tyrosine (K. Barron, R. Calendar, and P. Berg, unpublished data) indicate that D-tyrosyl RNA can be formed to the same extent as L-tyrosyl RNA using either tyrosyl RNA synthetase.

Specificity of Tyrosyl RNA Synthetases for Nucleotides. ADP, GTP, CTP, UTP, dATP, dGTP, dCTP, dTTP, and adenyl-5-methylenediphosphonate (2

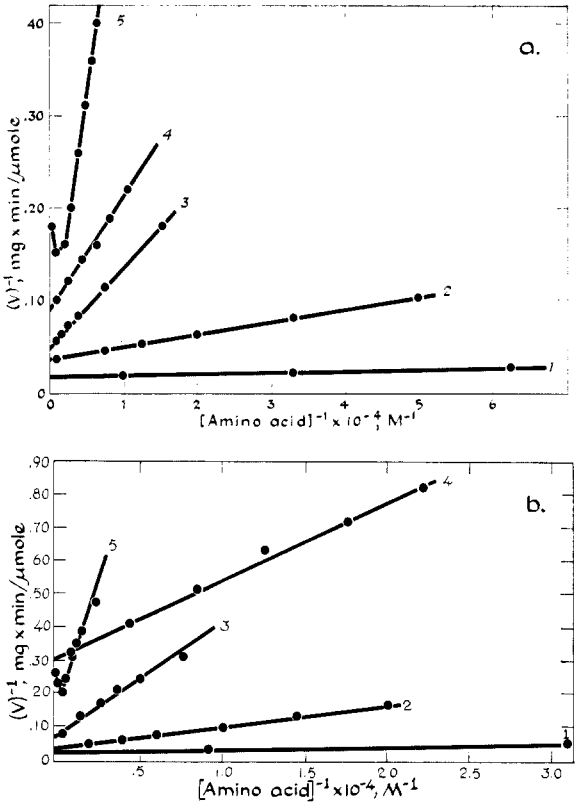

FIGURE 3: Lineweaver–Burk (1934) plots for activators of the ATP–$^{32}PP_i$ exchange reaction. Standard assay conditions were employed, varying the amino acid concentration. (A) *E. coli* enzyme; (B) *B. subtilis* enzyme; lines 1, L-tyrosine; lines 2, L-F-tyrosine; lines 3, DL-HPA; lines 4, D-tyrosine; lines 5, L-DOPA.

mM each) were inactive in the standard assay in place of ATP for activating tyrosine with either enzyme. dATP inhibited the ATP–$^{32}PP_i$ exchange reaction.

Specificity of Tyrosyl RNA Synthetases for t-RNA. The tyrosyl RNA synthetases of *E. coli* and *B. subtilis* were each capable of transferring tyrosine to their heterologous t-RNA's. The extent of transfer was determined by the t-RNA, not the enzyme (Table III). We conclude from this experiment that both enzymes cross-react completely with their heterologous t-RNA's and that this cross-reaction occurs with retention of specificity. Neither of these enzymes transferred tyrosine to yeast t-RNA.

Kinetic Constants for the Tyrosyl RNA Synthetases. A. FOR AMINO ACIDS. V_{max} and K_m values for tyrosine and several tyrosine analogs in the ATP–$^{32}PP_i$ exchange reaction or tyrosyl RNA synthesis were determined from Lineweaver and Burk (1934) plots (Figure 3) and are summarized in Table IV. With the *E. coli* enzyme the K_m values for L-tyrosine and L-F-tyrosine in aminoacyl RNA formation (*i.e.*, in the presence of t-RNA) are four and two times higher, respectively, than the corresponding values for the exchange reaction. This contrasts with the decreased K_m for glutamine and glutamic acid in the presence of t-RNA reported by Ravel *et al.* (1965).

TABLE III: Extent of Tyrosyl and F-Tyrosyl RNA Synthesis.

	E. coli t-RNA	*B. subtilis* t-RNA		
	Enzyme from: ($\mu\mu$moles of aminoacyl RNA/10A_{260})			
	E. coli	*B. subtilis*	*E. coli*	*B. subtilis*
L-Tyrosine	260	240	70	70
DL-F-Tyrosine	280	260	70	70
L-Tyrosine + DL-F-tyrosine	250	230	70	70

[a] Extent of formation of aminoacyl RNA using purified tyrosyl RNA synthetases in 100-fold excess, with limiting t-RNA (unfractionated material, 9–18A_{260}). [^{3}H]-L-Tyrosine and [^{3}H]-DL-F-tyrosine were used, both at a specific activity of 5×10^6 cpm/μmole.

Certain of the tyrosine analogs are good competitive inhibitors of tyrosine activation (Tables I and IV). With either enzyme, if the tyrosine carboxyl group is esterified, amidated, reduced, or absent, the compound can obviously not be converted to the adenylate, but such derivatives are strongly bound to the enzyme. The K_I values are quite low, and in some cases even lower than the K_m for tyrosine. Substitutions in the ring, which still permit activation (F, or OH, or a ring nitrogen in place of CH), produce an increase in the K_m and a decrease in V_{max}.

One distinctive difference between the *E. coli* and *B. subtilis* enzymes is their activity with D-tyrosine (Figure 3, Table IV). The ratio of the K_m values for D- and L-tyrosine with the *E. coli* enzyme is 23, while it is only 3 with the *B. subtilis* enzyme; yet the ratios of the V_{max} values with D- and L-tyrosine for the two enzymes are 0.2 and 0.09, respectively. Thus, although the *B. subtilis* enzyme has a lower relative V_{max} with the D isomer than does the *E. coli* enzyme, it has a higher affinity for D-tyrosine relative to L-tyrosine.

B. FOR NUCLEOTIDES. The K_m for ATP in the tyrosine-dependent ATP–$^{32}PP_i$ exchange reaction or for tyrosyl RNA synthesis with either enzyme was 0.3–0.4 mM (Table III). The K_I for inhibition of the ATP–$^{32}PP_i$ exchange reaction by dATP was less than 4 mM.

C. FOR PP_i AND t-RNA. These two components react with the enzyme–aminoacyl adenylate intermediate formed from the amino acid, ATP, and the enzyme. Although the reaction of aminoacyl transfer to t-RNA is slower than reaction with PP_i (Table IV), the K_m for $t\text{-RNA}_{tyr}$ (<0.0001 mM for either enzyme with *E. coli* t-RNA) is considerably less than that of PP_i (0.03 mM). Thus, one would expect that even if PP_i were not rapidly removed, aminoacyl transfer would still occur readily.

TABLE IV: Michaelis–Menton Constants for Substrates and Inhibitors for *E. coli* and *B. subtilis* Tyrosyl RNA Synthetases.

	E. coli Enzyme			*B. subtilis* Enzyme		
	K_m (mM)	V_{max} (μmoles), mg^{-1} min^{-1}	K_I (mM)	K_m (mM)	V_{max} (μmoles), mg^{-1} min^{-1}	K_I (mM)
A. ATP exchange reaction						
L-Tyrosine	0.0061	56		0.025	36	
D-Tyrosine	0.14	11		0.078	3.3	
L-F-Tyrosine	0.039	29		0.18	28	
DL-F-Tyrosine	0.13	38		0.33	21	
L-DOPA	1.4	25[a]		1.1	7[a]	
DL-HPA	0.19	21		0.65	18	
L-Tyrosinol			0.0041			0.01
Tyramine			0.0060			0.042
L-Tyrosine amide			0.0081			0.11
L-Tyrosine methyl ester			0.017			0.19
L-Tyrosine ethyl ester			0.020			...
ATP	0.25	70		0.19	42	
PP_i	0.032	62		0.035	38	
B. Tyrosyl RNA formation[b]						
L-Tyrosine	0.027	2.6		0.048	5.8	
DL-F-Tyrosine	0.48	2.2		0.24	2.0	
ATP	0.40	2.3		0.29	5.0	

[a] Extrapolated, ignoring substrate inhibition. [b] Purified t-RNA_{tyr} from *E. coli* was used throughout.

The ratio of V_{max} in the exchange reaction to V_{max} in the transfer reaction was 22 for the *E. coli* tyrosyl RNA synthetase and 6 for the *B. subtilis* enzyme. Similar differences between the V_{max} ratios for other aminoacyl RNA synthetases have been reported (Bergmann *et al.*, 1961). Table III shows that *B. subtilis* tyrosyl RNA synthetase transfers tyrosine to *E. coli* t-RNA faster than does the *E. coli* enzyme. Using the values given in Table III for V_{max} in the transfer reaction, plus the molecular weights of the enzymes, one can assign turnover numbers of 300 and 640 (per minute) to the *E. coli* and *B. subtilis* enzymes, respectively.

pH and Temperature Optima of the ATP–$^{32}PP_i$ Exchange Reaction. The tyrosine-dependent exchange of ATP and PP_i occurred optimally at pH 6–8; at pH 4.5 and 10 the rates were 40% of the maximum. Although all routine assays were carried out at 37°, the rate at 45° with the two enzymes was 1.5 times faster than at 37°; at 20° the rates with *E. coli* and *B. subtilis* enzymes were 39 and 28%, respectively, of those found at 37°.

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