Human Tryptophanyl Transfer Ribonucleic Acid Synthetase. Comparison of the Kinetic Mechanism to that of the *Escherichia coli* Tryptophanyl Transfer Ribonucleic Acid Synthetase†

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ABSTRACT: Human and *Escherichia coli* tryptophanyl-tRNA synthetases have bi-uni-uni-bi ping-pong mechanisms. Both enzymes have ordered binding of substrates, ATP first. These conclusions from steady-state kinetics were supported by data obtained with the inhibitor L-tryptophanamide for both enzymes and with the inhibitor adenosine for the human enzyme. With product inhibition experiments we determined that the *E. coli* enzyme has ordered product release, AMP first, whereas the human enzyme has random product release. The rate of the L-tryptophan-dependent ATP-[^{32}P]PP$_i$ exchange reaction as catalyzed by either enzyme is not altered by the presence of either brewers' yeast tRNA or *E. coli* tRNA. For both enzymes high concentrations of ATP produced competitive inhibition patterns on Lineweaver–Burk plots derived from experiments with tRNA^{Trp} as the variable substrate. The human enzyme synthesizes stoichiometric amounts of tryptophanyl-ATP ester, whereas the enzyme from *E. coli* synthesizes more than stoichiometric amounts of this unusual product.

A functional homology exists between aminoacyl-tRNA synthetases from different species but specific for the same amino acid. Whether the kinetic mechanisms of catalysis are the same in enzymes from different species is unknown. We have partially purified the human epidermal tryptophanyl-tRNA synthetase (Penneys and Muench, 1971) and have purified to apparent homogeneity the human placental tryptophanyl-tRNA synthetase (Penneys and Muench, 1974). Those enzymes and that from leukemic lymphocytes (Tchou *et al.*, 1971) have the same chromatographic behavior, isoelectric point, and molecular weight. The same criteria demonstrate the expected differences between the human enzyme and that purified from *Escherichia coli* (Joseph and Muench, 1971a,b). In this paper, we present data derived from steady-state kinetic experiments (Cleland, 1963a–c) and from dead-end inhibition experiments that indicate that the functionally homologous human and *E. coli* tryptophanyl-tRNA synthetases have similar but not identical kinetic mechanisms.

Experimental Procedure

Materials. Brewers' yeast tRNA (40 pmol of tRNA^{Trp}/A_{260} unit) and *E. coli* tRNA (32 pmol of tRNA^{Trp}/A_{260} unit) were purchased from Boehringer-Mannheim and Schwarz-Mann, respectively. *E. coli* tRNA was activated by heating at 60° for 5 min in the presence of 10 mM Tris-HCl buffer (pH 8.0) containing 20 mM $MgCl_2$ and 1 mM EDTA (Muench, 1969b). L-[3-^{14}C]Tryptophan was from New England Nuclear Corporation and purified as previously described (Penneys and Muench, 1974). L-Tryptophanamide and adenosine were from Sigma. All other materials used were the highest grades available from sources previously named (Joseph and Muench, 1971a; Penneys and Muench, 1974).

Methods. The activity of tryptophanyl-tRNA synthetase was assayed by measuring the incorporation of L-[3-^{14}C]-tryptophan into tRNA as previously described (Joseph and Muench, 1971a). For the human tryptophanyl-tRNA synthetase, the 0.5-ml reaction mixture containing nearly saturating levels of substrates was: 100 mM potassium bicine buffer (pH 8.0), 20 mM $MgCl_2$, 2 mM ATP, 20 A_{260} units of brewers' yeast tRNA, and 0.1 mM L-[3-^{14}C]tryptophan. Human epidermal tryptophanyl-tRNA synthetase was an enzyme preparation 400-fold (1200 units/mg) purified (Penneys and Muench, 1971), human placental tryptophanyl-tRNA synthetase was an enzyme preparation 330-fold purified (6150 units/mg) (Penneys and Muench, 1974), and the *E. coli* tryptophanyl-tRNA synthetase was a fraction 700-fold (16,500 units/mg) purified (Joseph and Muench, 1971a). Assay conditions and the contents of the reaction mixture for the *E. coli* tryptophanyl-tRNA synthetase were as previously described (Joseph and Muench, 1971a). Kinetic experiments were continued for 2 min at 30° for the human tryptophanyl-tRNA synthetase and at 37° for the *E. coli* tryptophanyl-tRNA synthetase. The reactions were initiated by the addition of 5 μl of enzyme preparation containing 0.7–1.1 units. The kinetics of the human tryptophanyl-tRNA synthetase were studied primarily with the epidermal enzyme preparation. Product inhibition and dead-end inhibitor experiments were in part repeated and confirmed by studies using the human placental tryptophanyl-tRNA synethetase. Steady-state kinetic experiments were designed as described by Cleland (1963c) for a bi-uni-uni-bi ping-pong mechanism. The amounts of the various substrates, products, and dead-end inhibitors used in each experiment are listed in Table I. The rest of the assay procedure was as previously described (Tchou *et al.*, 1971; Joseph and Muench, 1971a), one enzyme unit synthesizing 1 nmol of tryptophanyl-tRNA in 10 min.

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TABLE I

Enzyme	Expt	[ATP] (mM)	[Trp] (μM)	[$tRNA^{Trp}$] (μM)	Other	Slope (M × min/nmol of Product)[b]	Intercept (min/nmol of Product)
Human	1a	0.8	Var[a]	1.6		180 ± 30[c]	44 ± 2
		1.6	Var	1.6		130 ± 20	36 ± 2
	1b	Var	20	1.6		10 ± 2	110 ± 10
		Var	60	1.6		7.6 ± 0.8	68 ± 2
		Var	100	1.6		5.2 ± 1.2	30 ± 4
	1c	2.0	Var	0.25		42 ± 6	22 ± 1
		2.0	Var	0.50		42 ± 4	17 ± <0.5
		2.0	Var	0.75		42 ± 4	14 ± <0.5
	1d	Var	100	0.25		3.0 ± 0.6	54 ± 2
		Var	100	0.50		2.8 ± 0.8	40 ± 2
		Var	100	0.75		2.6 ± 0.6	28 ± 2
	2a	Var	100	1.6	0 mM AMP	3.0 ± 11	52 ± 24
		Var	100	1.6	0.16 mM AMP	15 ± 28	50 ± 36
		Var	100	1.6	1.6 mM AMP	58 ± 36	50 ± 30
	2b	2.0	Var	1.6	0 mM AMP	220 ± 50	30 ± 4
		2.0	Var	1.6	0.16 mM AMP	220 ± 50	34 ± 4
		2.0	Var	1.6	1.6 mM AMP	170 ± 20	54 ± 2
	2c	2.0	100	Var	0 mM AMP	140 ± 20	48 ± 4
		2.0	100	Var	0.16 mM AMP	460 ± 100	86 ± 12
		2.0	100	Var	1.6 mM AMP	860 ± 360	130 ± 30
	3a	Var	100	0.25	50 μM PP_i	36 ± 22	38 ± 14
		Var	100	0.50	50 μM PP_i	32 ± 12	24 ± 6
		Var	100	0.75	50 μM PP_i	34 ± 12	20 ± 2
	3b	Var	40	0.25	50 μM PP_i	70 ± 26	48 ± 12
		Var	40	0.50	50 μM PP_i	52 ± 24	44 ± 14
		Var	40	0.75	50 μM PP_i	36 ± 18	24 ± 6
	3c	2.0	Var	0.25	50 μM PP_i	62 ± 18	6.4 ± 5.6
		2.0	Var	0.50	50 μM PP_i	54 ± 16	5.0 ± 4.8
		2.0	Var	0.75	50 μM PP_i	42 ± 8	4.2 ± 4.6
	4a	0.5	100	Var		54 ± 4	32 ± 6
		1.0	100	Var		52 ± 22	22 ± 1
		2.0	100	Var		50 ± 4	20 ± 1
	4b	2.0	100	Var		56 ± 20	38 ± 4
		6.0	100	Var		76 ± 28	30 ± 4
		10.0	100	Var		220 ± 160	30 ± 8
	5a	2.0	Var	1.6	0 mM $TrpNH_2$	17 ± 2	5.2 ± 0.2
		2.0	Var	1.6	2.0 mM $TrpNH_2$	240 ± <5	5.0 ± 0.6
		2.0	Var	1.6	5.0 mM $TrpNH_2$	1700 ± 200	8.4 ± 14
	5b	Var	40	1.6	0 mM $TrpNH_2$	220 ± 40	5.0 ± 0.6
		Var	40	1.6	2.0 mM $TrpNH_2$	280 ± 40	10 ± 1
		Var	40	1.6	5.0 mM $TrpNH_2$	260 ± 80	16 ± 2
	5c	1.0	Var	1.6	0 mM Ado	260 ± 60	14 ± 2
		1.0	Var	1.6	17 mM Ado	380 ± 80	19 ± 3
	5d	Var	100	1.6	0 mM Ado	260 ± 40	14 ± 3
		Var	100	1.6	3.5 mM Ado	500 ± 80	15 ± 2
		Var	100	1.6	17 mM Ado	1500 ± 140	16 ± 3
E. coli	6a	1.0	Var	0.7		130 ± 20	26 ± 2
		1.0	Var	1.2		140 ± 20	19 ± 1
		1.0	Var	1.8		110 ± 20	15 ± 1
	6b	0.67	Var	1.8		190 ± 20	10 ± 1
		1.2	Var	1.8		130 ± 20	10 ± 1
		2.0	Var	1.8		90 ± 10	8.4 ± 0.4
	7a	Var	100	0.7	450 μM PP_i	3.2 ± 0.4	2.4 ± 0.2
		Var	100	1.2	450 μM PP_i	3.2 ± 0.6	1.6 ± 0.1
		Var	100	1.8	450 μM PP_i	2.6 ± 0.6	1.0 ± 0.1
	7b	Var	40	1.2	450 μM PP_i	38 ± 2	150 ± 10
		Var	40	1.8	450 μM PP_i	30 ± 1	120 ± 30
	7c	1.0	Var	0.7	450 μM PP_i	440 ± 180	30 ± 2
		1.0	Var	1.2	450 μM PP_i	400 ± 60	20 ± 2
		1.0	Var	1.8	450 μM PP_i	260 ± 80	20 ± 2

TABLE I (*Continued*)

Enzyme	Expt	[ATP] (mM)	[Trp] (μM)	[$tRNA^{Trp}$] (μM)	Other	Slope (M × min/ nmol of Product)[b]	Intercept (min/ nmol of Product)
	8a	1.0	Var	1.8	0 mM AMP	76 ± 24	10 ± 1
		1.0	Var	1.8	4 mM AMP	92 ± 8	15 ± <0 5
		1.0	Var	1.8	8 mM AMP	100 ± 10	17 ± <0.5
	8b	Var	100	1.8	0 mM AMP	8.4 ± 1.4	13 ± 1
		Var	100	1.8	4 mM AMP	12 ± 1	20 ± 2
		Var	100	1.8	8 mM AMP	13 ± 4	32 ± 4
	9a	0.5	100	Var		22 ± 2	5.8 ± 0.4
		1.0	100	Var		20 ± 2	9.0 ± 0.6
		1.5	100	Var		22 ± 2	5.2 ± 0.4
	9b	0.5	100	Var		14 ± 1	8.6 ± 1.0
		1.0	100	Var		15 ± 2	10 ± 1
		5.0	100	Var		22 ± 2	8.2 ± 0.8
	10a	1.0	Var	1.8	0 mM $TrpNH_2$	120 ± 20	22 ± 2
		1.0	Var	1.8	0.06 mM $TrpNH_2$	300 ± 60	18 ± 1
		1.0	Var	1.8	0.1 mM $TrpNH_2$	1500 ± 1100	9.0 ± 5.2
	10b	Var	40	1.8	0 mM $TrpNH_2$	1900 ± 500	6.2 ± 2.6
		Var	40	1.8	0.06 mM $TrpNH_2$	2000 ± 400	11 ± 4
		Var	40	1.8	0.1 mM $TrpNH_2$	2200 ± 600	16 ± 4

[a] Variable substrates. [b] Slope units when L-tryptophan is the variable substrate should be multiplied by a factor of 10^{-6}, ATP, by 10^{-3}, and $tRNA^{Trp}$, by 10^{-7}. [c] Standard error.

All plots and kinetic constants were determined on an IBM 370 computer with the program of Hanson *et al.* (1967) based on the theory of Bliss and James (1966). All data were examined on Lineweaver–Burk plots; we recognize that such double reciprocal plots may be unreliable to express unweighted data (Dowd and Riggs, 1965). However, the computer program weights each point in proportion to the fourth power of the velocity, and, therefore, points taken at low substrate concentrations and low velocities are relatively insignificant in the construction of the best line. In the figures we plotted low velocity points even though a low precision was evident in the measurement of the reaction velocities occurring at low substrate concentration. In five of the 27 experiments (3b, 3c, 4b, 5c, and 7c) intersecting lines could have been drawn parallel yet remain within the limits imposed by the standard errors of the slopes. However, data from the other experiments were consistent and supported the interpretation of these as intersecting patterns. Each figure represents the data from one experiment. The terminology is that of Cleland (1963a).

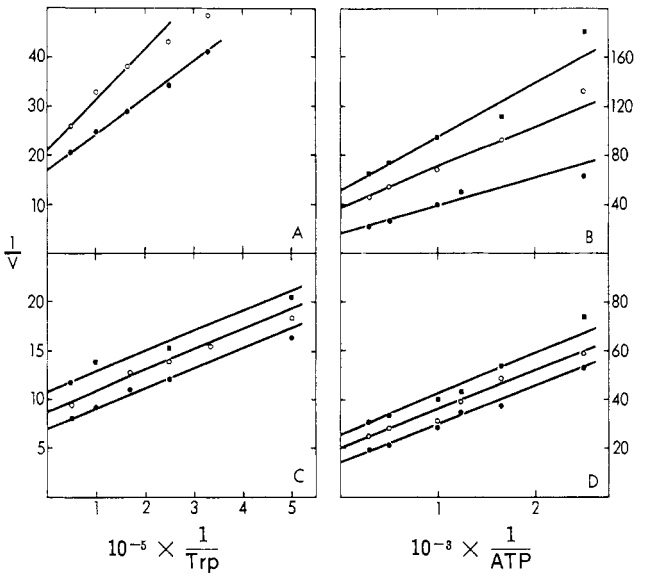

FIGURE 1: Initial velocity patterns for human tryptophanyl-tRNA synthetase. Plots of reciprocals of the initial reaction velocities *vs.* the reciprocals of the molar concentrations of either L-tryptophan (A, C) or ATP (B, D) at different fixed concentrations of either ATP (A), L-tryptophan (B), or $tRNA^{Trp}$ (C, D). In A and B, the concentration of $tRNA^{Trp}$ was 1.6 μM. In C, the concentration of ATP was 2 mM, and, in D, the concentration of L-tryptophan was 0.1 mM. Experimental conditions for this and all other figures are described under Experimental Procedure. The velocity units in this and all other figures are in terms of nmoles of product formed in the actual 2-min assay. (A) (○) [ATP], 0.8 mM; (●) 1.6 mM; (B) (■) [Trp], 20 μM; (○) 60 μM; (●) 100 μM; (C and D) (■) [$tRNA^{Trp}$], 0.25 μM; (○) 0.50 μM; (●) 0.75 μM (expts 1a–d).

Conditions for the *E. coli* tryptophanyl-tRNA synthetase ATP–[^{32}P]PP_i exchange assay were described by Muench (1971). Conditions for the ATP–[^{32}P]PP_i exchange assay with the human placental enzyme were previously described (Penneys and Muench, 1974).

Results

Kinetics of the Human Tryptophanyl-tRNA Synthetase. Slopes and intercepts for all experiments are presented in Table I. We observed an intersecting pattern when $1/v$ was plotted against 1/[Trp] for experiments done at different fixed levels of ATP and with nearly saturating levels of $tRNA^{Trp}$ (Figure 1A) (expt 1a). We noted a similar pattern when $1/v$ was plotted against 1/[ATP] for experiments done with different fixed concentrations of L-tryptophan and with nearly saturating levels of $tRNA^{Trp}$ (Figure 1B) (expt 1b). However, a parallel pattern resulted when $1/v$ was plotted against 1/[Trp] for experiments done with different fixed levels of $tRNA^{Trp}$ at nearly saturating levels of ATP (Figure 1C) (expt 1c), and this pattern occurred again in an experiment

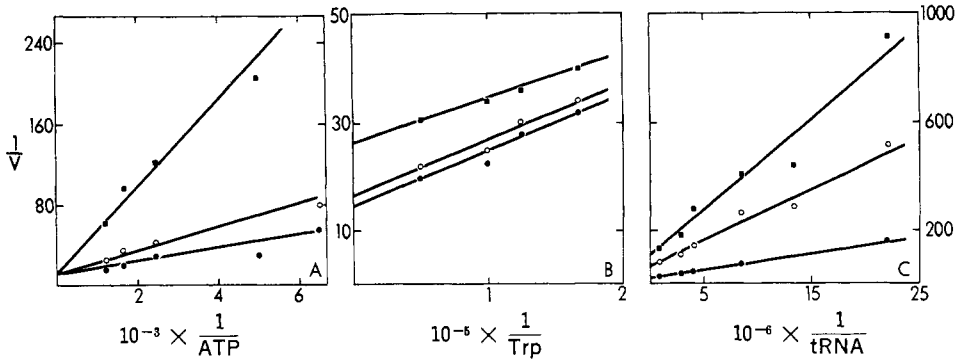

FIGURE 2: Initial velocity patterns for human tryptophanyl-tRNA synthetase. Plots of reciprocals of the initial reaction velocities *vs.* the reciprocals of the molar concentrations of either ATP (A) L-tryptophan (B), or $tRNA^{Trp}$ (C) in the absence (●) and the presence of either 160 μM (○) or 1.6 mM (■) AMP. In A and B, the concentration of $tRNA^{Trp}$ was 1.6 μM; in A and C, the concentration of L-tryptophan was 0.1 mM; and in B and C, the concentration of ATP was 2 mM (expts 2a–c).

with ATP as the variable substrate and with increasing fixed levels of $tRNA^{Trp}$ at nearly saturating levels of L-tryptophan (Figure 1D) (expt 1d). These data support a ping-pong mechanism with ATP and L-tryptophan binding to an enzyme form not reversibly connected to the enzyme form to which $tRNA^{Trp}$ binds, the release of PP_i at zero concentration being considered an irreversible step (Cleland, 1970).

Product inhibition studies indicated the order of addition of substrates and order of release of products. We observed a competitive inhibition pattern when $1/v$ was plotted against 1/[ATP] for experiments done with increasing fixed levels of AMP with nearly saturating levels of $tRNA^{Trp}$ and tryptophan (Figure 2A) (expt 2a). An uncompetitive pattern occurred when $1/v$ was plotted against 1/[Trp] for experiments done with increasing fixed levels of AMP with nearly saturating levels of ATP and $tRNA^{Trp}$ (Figure 2B) (expt 2b). These data suggest that AMP and ATP compete for the same enzyme form and that AMP and tryptophan do not. Therefore, ATP must be the first substrate to bind to the enzyme, and AMP could be the last product released; alternatively, product release could be random. A noncompetitive pattern resulted when $1/v$ was plotted against $1/[tRNA^{Trp}]$ for experiments done with increasing fixed levels of AMP and with nearly saturating levels of tryptophan and ATP (Figure 2C) (expt 2c). If AMP were released last from the enzyme surface, an uncompetitive pattern would have been seen on this plot, the release of Trp-tRNA at zero concentration being considered an irreversible step between the point of addition of the variable substrate, $tRNA^{Trp}$, and the point of release of the changing fixed product, AMP (Cleland, 1970). Therefore, release of products from the human tryptophanyl-tRNA synthetase must be random.

The order of addition of the substrates, ATP and then L-tryptophan, was confirmed by a series of experiments done in the presence of 50 μM $NaPP_i$ to make the release of that product reversible. The plot of $1/v$ against 1/[ATP] for experiments done with different fixed levels of $tRNA^{Trp}$ and with nearly saturating levels of L-tryptophan revealed a parallel pattern (Figure 1D) (expt 1d). The same pattern resulted in experiments done with $NaPP_i$ when the L-tryptophan concentration was nearly saturating (making L-tryptophan addition an irreversible step) (expt 3a), but an intersecting pattern occurred if the L-tryptophan concentration was not saturating (expt 3b). Similarly, an intersecting pattern was seen when $1/v$ was plotted against 1/[Trp] for experiments done with increasing fixed amounts of $tRNA^{Trp}$, a nearly saturating concentration of ATP and 50 μM $NaPP_i$ (expt 3c). In the absence of added PP_i to make its release reversible a parallel pattern was seen (Figure 1C) (expt 1c). Taken together, the above kinetic data are consistent with a bi-uni-uni-bi ping-pong mechanism with sequential addition of substrates and random release of products (Cleland, 1963a–c).

When $1/v$ was plotted against 1/[tRNA] for experiments done with increasing fixed levels of ATP but at levels of ATP less than 6 mM, we observed a parallel pattern (expt 4a). At ATP concentrations greater than 6 mM, an intersecting pattern resulted (expt 4b). For the aminoacyl-tRNA synthetases, competition between ATP and the 3′-terminal adenosine of $tRNA^{Trp}$ for the activated amino acid is not unexpected. Myers *et al.* (1971) reported the competitive effects of high concentrations of ATP in kinetic experiments with the seryl- and valyl-tRNA synthetases from *E. coli*. Weiss *et al.* (1959) showed tryptophanyl-ATP ester to be a product of beef pancreas tryptophanyl-tRNA synthetase, and Muench (1969-a) showed the *E. coli* enzyme to synthesize the same product in the presence of ATP at concentrations of 6 mM or higher. For the human enzyme the K_m for tRNA as determined from a replot was 1.1×10^{-7} M, in close agreement with the apparent K_m, 1.1×10^{-7} M reported by Tchou *et al.* (1971) for human leukemic lymphocytes. Replots for ATP and L-tryptophan were not done, but the apparent K_m values were 1.6×10^{-4} and 2.8×10^{-6} M, respectively (Table II), figures identical with those previously reported (Tchou *et al.*, 1971). A turnover number of 200 mol/mol per min was determined with homogeneous enzyme (Penneys and Muench, 1974).

Kinetics of the E. coli Tryptophanyl-tRNA Synthetase. Initial velocity kinetic experiments with L-tryptophan as the variable substrate, ATP or $tRNA^{Trp}$ as the fixed variable substrate, and nearly saturating levels of the third substrate yielded plots supporting a ping-pong mechanism (expts 6a and 6b).

When $1/v$ was plotted against $1/[tRNA^{Trp}]$ in experiments done with increasing fixed levels of ATP but with levels less

TABLE II: Kinetic Parameters for Human Tryptophanyl-tRNA Synthetase.

Substrate	K_m (M)	V_{max} (nmol/min per μg)
L-Tryptophan	2.8×10^{-6}	0.053
ATP	1.6×10^{-4}	0.050
$tRNA^{Trp}$	1.1×10^{-7}	0.041

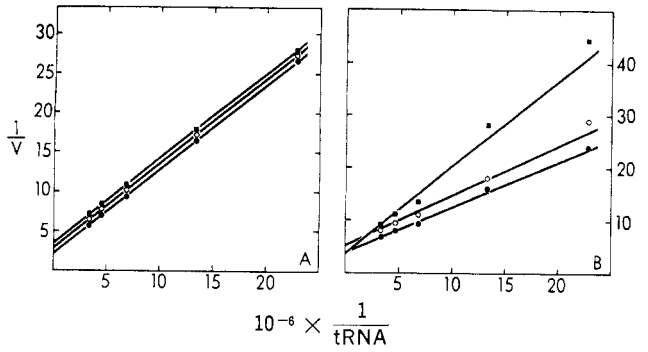

FIGURE 3: Initial velocity patterns for *E. coli* tryptophanyl-tRNA synthetase. Plots of reciprocals of the initial reaction velocities *vs.* the reciprocals of the molar concentrations of $tRNA^{Trp}$ at different fixed concentrations of ATP. The concentration of L-tryptophan was 0.1 mM: (A) (■) [ATP], 0.5 mM; (○) 1.0 mM; (●) 1.5 mM; (B) (■) [ATP], 5.0 mM; (○) 1.0 mM; (●) 0.5 mM (expts 9a–b).

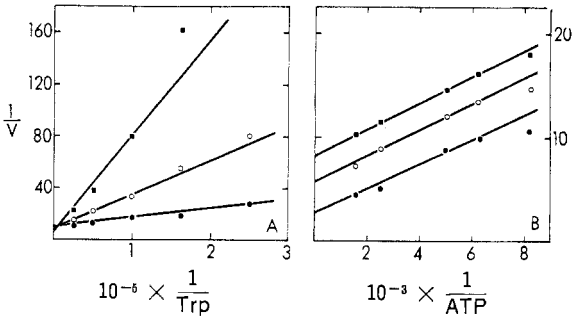

FIGURE 5: Initial velocity patterns of *E. coli* tryptophanyl-tRNA synthetase. Plots of reciprocals of the initial reaction velocities *vs.* the reciprocals of the molar concentrations of either L-tryptophan (A) or ATP (B) in the absence (●) or the presence of either 60 μM (○) or 100 μM (■) L-tryptophanamide. In A, the concentration of ATP was 2 mM; in B, the concentration of L-tryptophan was 0.04 mM. The concentration of $tRNA^{Trp}$ was 1.8 μM (expts 10a and b).

than 3 mM, a parallel pattern occurred (Figure 3A) (expt 9a). At ATP concentrations greater than 3 mM we observed an intersecting pattern (Figure 3B) (expt 9b).

We did product inhibition experiments to define the order of addition of substrates and order of release of products. We observed a parallel pattern when $1/v$ was plotted against 1/[ATP] in experiments done with 450 μM $NaPP_i$ and with increasing fixed levels of $tRNA^{Trp}$ in the presence of 100 μM L-tryptophan (expt 7a). An intersecting pattern occurred in the presence of 40 μM L-tryptophan (expt 7b). The plot of $1/v$ against 1/[Trp] in the presence of increasing fixed levels of $tRNA^{Trp}$, nearly saturating levels of ATP, and with 450 μM PP_i revealed an intersecting pattern (expt 7c) in contrast to that seen in the absence of added PP_i wherein release of PP_i was an irreversible step (Cleland, 1970). These experiments indicate that ATP is bound first to the enzyme and is followed by the addition of L-tryptophan.

A parallel pattern was seen when $1/v$ was plotted against either 1/[Trp] or 1/[ATP] in experiments done with increasing fixed amounts of AMP and with nearly saturating levels of the other substrates (expts 8a and 8b). These data indicate that the release of products is ordered and that AMP is released before Trp-tRNA.

Effects of the Inhibitors, L-Tryptophanamide and Adenosine. Additional data that confirmed the ordered addition of ATP and L-tryptophan to both enzymes were obtained by the use of the dead-end inhibitors, L-tryptophanamide and adenosine. L-Tryptophanamide was a competitive inhibitor with respect to the addition of L-tryptophan to both enzymes (Figures 4A and 5A) (expts 5a and 10a) but was an uncompetitive inhibitor with respect to the addition of ATP (Figures 4B, 5B) (expts 5b and 10b). Adenosine was a competitive inhibitor with respect to the addition of ATP to the human placental tryptophanyl-tRNA synthetase (Figure 4D) (expt 5d) but was a noncompetitive inhibitor when L-tryptophan was the variable substrate (Figure 4C) (expt 5c). We saw no inhibition of the *E. coli* tryptophanyl-tRNA synthetase in the presence of 17.5 mM adenosine, the highest feasible concentration that could be attained in the reaction mixture. These experiments confirm that substrate binding to both the human and *E. coli* enzymes is ordered with ATP first, followed by L-tryptophan.

ATP–[^{32}P]PP_i Exchange Assay. Both the human placental and *E. coli* tryptophanyl-tRNA synthetases perform the ATP–[^{32}P]PP_i exchange assay in the absence of $tRNA^{Trp}$. The ratio of ATP–PP_i exchange to tryptophanyl-tRNA formation under similar reaction conditions is 6.6 for the human placental enzyme and 17.4 for the *E. coli* enzyme. The optimal L-tryptophan concentration for the human placental enzyme for the exchange reaction was a plateau from 0.25 to 2.5 mM, with 45% inhibition of the exchange assay at a concentration of 5 mM L-tryptophan. The human placental enzyme differs in this respect from the tryptophanyl-tRNA synthetase purified from water buffalo brain, which performs the exchange reaction at very low levels of L-tryptophan (50 μM) and is inhibited completely in the presence of 2 mM L-tryptophan (Liu *et al.*, 1973).

The presence of 20 A_{260} units of either brewers' yeast tRNA or *E. coli* tRNA in the reaction mixtures did not alter the rate of ATP–[^{32}P]PP_i exchange for either enzyme. Aminoacyl-tRNA synthetases that have random mechanisms, such

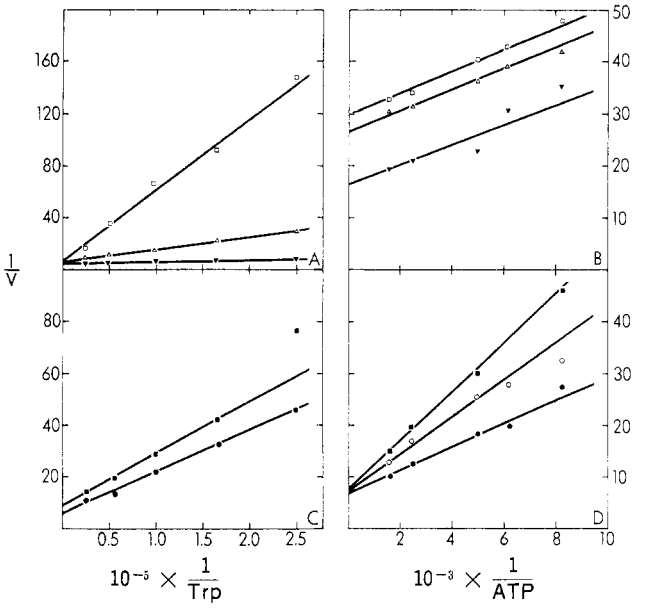

FIGURE 4: Initial velocity patterns of human tryptophanyl-tRNA synthetase. Plots of reciprocals of the initial reaction velocities *vs.* the reciprocals of the molar concentrations of either L-tryptophan (A, C) or ATP (B, D) in the absence (▼) or the presence of 2 mM (△) or 5 mM (□) L-tryptophanamide (A, B) and in the absence (●) or the presence of 3.5 mM (○) or 17 mM (■) adenosine (C, D). In C the concentration of ATP was 1 mM; in B, the concentration of L-tryptophan was 0.04 mM. The concentration of $tRNA^{Trp}$ was 1.6 μM (expts 5a–d). ADDED IN PROOF: The ordinate values in Figure 4B are incorrect. Refer to Table I for correct values.

as the arginyl-tRNA synthetase from *E. coli* (Papas and Peterkofsky, 1972), perform the ATP-[^{32}P]PP$_i$ exchange reaction poorly if at all in the absence of specific tRNA. That the tryptophanyl-tRNA synthetases from *E. coli* and human placenta perform this reaction in the absence of tRNA and that the rate of the exchange reaction is not affected by the presence of tRNA are evidence against the random addition of all three substrates.

Synthesis of the Tryptophanyl-ATP Ester. Certain aminoacyl-tRNA synthetases synthesize an unusual product, an aminoacyl-ATP ester, in the presence of excess ATP and diminished or absent tRNA (Muench, 1969a; Weiss *et al.*, 1959). The tryptophanyl-tRNA synthetase from *E. coli* synthesizes this product (Muench, 1969a). We have not determined directly whether the tryptophanyl-ATP ester is synthesized during our standard assay. However, our kinetic data suggest that this is unlikely; the synthesis of an additional product would produce an intercept effect when $1/v$ is plotted against $1/[tRNA^{Trp}]$, and this effect was not seen.

With catalytic amounts of enzyme (30 units) present in the reaction mix the synthesis of tryptophanyl-ATP ester by the human tryptophanyl-tRNA synthetase was undetectable in 30 min. When more enzyme (1800 units) was used, roughly stoichiometric amounts of the tryptophanyl-ATP ester were detectable, 1.8 nmol of enzyme producing 1.5 nmol of tryptophanyl-ATP ester in 30 min. Under similar conditions, 0.04 nmol of the *E. coli* tryptophanyl-tRNA synthetase produced 1.4 nmol of tryptophanyl-ATP ester, in agreement with earlier work (Muench, 1969a).

Discussion

With the kinetic approach described by Cleland (1963a–c) three different types of kinetic mechanisms have now been described in the group of aminoacyl-tRNA synthetases: (1) ordered addition of substrates and release of products, as exemplified by the *E. coli* prolyl- (Papas and Mehler, 1971) and tryptophanyl-tRNA synthetases, (2) ordered addition of substrates and random release of products, as by the human tryptophanyl-tRNA synthetase, and (3) random addition of substrates and random release of products, as by the *E. coli* arginyl-tRNA synthetase (Papas and Peterkofsky, 1972). Other studies have shown that ATP binds first to leucyl- (Rouget and Chapeville, 1968), threonyl- (Allende *et al.*, 1970), seryl-, and valyl-tRNA synthetases (Myers *et al.*, 1971) of *E. coli*. A study of substrate addition with the ATP-PP$_i$ exchange assay indicated that addition of substrates to the tyrosyl-tRNA synthetase of *E. coli* was random (Santi and Peña, 1970). In those studies order of product release was not determined.

In the case of the *E. coli* arginyl-tRNA synthetase, random addition and random release were confirmed with isotope exchange studies. We attempted to confirm the mechanism of product release in the *E. coli* and human tryptophanyl-tRNA synthetases by measuring the exchange of radioactive L-tryptophan into Trp-tRNA in the presence of high concentrations of substrate–product pair, ATP–AMP. Two lines of kinetic evidence indicated that substrate addition to both enzymes was ordered, ATP first; therefore, the rate of exchange would depend solely on the concentration of AMP when the substrate–product pair, ATP–AMP, was varied. Unfortunately, we found that there was a complex relationship between the rate of isotope exchange and the Mg^{2+} concentration when ATP and AMP were present in high concentrations. For the *E. coli* tryptophanyl-tRNA synthetase, the rate of isotope exchange was inhibited at high concentrations of the substrate–product pair, ATP–AMP, with 5 mM Mg^{2+} but was not inhibited at concentrations of 20 mM Mg^{2+}.

There seems to be no required kinetic mechanism as defined by this system for the aminoacyl-tRNA synthetases as a group. We were interested in comparing two functionally homologous aminoacyl-tRNA synthetases with standard kinetic approaches to see how they differ. We have shown that the functionally homologous *E. coli* and human tryptophanyl-tRNA synthetases differ somewhat in their kinetic mechanisms as defined by these techniques, in their sensitivities to product and dead-end inhibitors, in the ease by which they are inhibited by high concentrations of ATP, and in the ability to synthesize tryptophanyl-ATP ester. A more detailed understanding of the similarities and differences between these enzymes will come from structural studies now in progress.

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