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I am submitting herewith a dissertation written by Cynthia K. Warner entitled "Tyrosyl tRNA Synthetase in Drosophila melanogaster." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Biomedical Sciences.

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TYROSYL tRNA SYNTHETASE IN *DROSOPHILA MELANOGASTER*

A Dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee

Cynthia K. Warner

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ABSTRACT

Previous studies have shown that two forms of the tyrosyl tRNA synthetase (Syn 1 and Syn 2) can be isolated from wild type Drosophila melanogaster. Further work showed that a third synthetase form, Syn 3, could also be isolated. Although the relationship, if any, between these three forms is unclear, one of the forms, Syn 1, possesses a specificity for one of the two $tRNA^{Tyr}$ isoacceptors in the suppressed vermilion strain of flies. Syn 1 and Syn 2 are fractionated on DEAE-cellulose, but the fractionation is not entirely reproducible. Sometimes only a single enzyme activity, Syn 3, is obtained. Syn 3 has been examined under conditions whereby it might acquire the isoacceptor specificity of Syn 1: heat inactivation, substrate concentration and inhibitor studies all failed to reveal such specificity. The molecular weight of Syn 3 as determined by Agarose column chromatography is 100,000. Although Syn 3 is unstable to gradient elution from DEAE-cellulose, rechromatography of this form on DEAE-cellulose results in a temperature-sensitive enzyme. Apparent K_Ms were determined for each substrate. This synthetase is inhibited by higher than optimal concentrations of both Mg^{++} and ATP. Spermine is not able to substitute for Mg^{++} in the Syn 3 reaction. In addition to the usual synthetase substrates

and cofactors (Mg^{++}, ATP, amino acid and tRNA), this form of the Drosophila tyrosyl tRNA synthetase requires K^{+} for activity.

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CHAPTER I

INTRODUCTION

The existence of isoaccepting forms of tRNA within an organism has led to the idea that multiple forms of the corresponding aminoacyl tRNA synthetases (E.C.6.1.1.X.) might also exist. In 1967 Novelli [1] reviewed the evidence to date regarding synthetase multiplicity. For prokaryotic systems, he concluded that available data indicated only one synthetase per amino acid. For eukaryotic systems, in spite of several reports, he felt that the only really convincing evidence for multiplicity was that of Barnett and Epler [2] for the phenylalanyl tRNA synthetase of Neurospora. Even at that time the minor synthetase activity appeared to be of mitochondrial origin and later work demonstrated that the mitochondria of Neurospora contained tRNA synthetases for all fifteen amino acids assayed [3].

Since Novelli's review of the area, numerous reports of synthetase multiplicity have appeared. What follows is not intended to be a comprehensive review of these reports, but rather to be a selected group including representatives of both the plant and animal kingdoms. In higher plants, two peaks of tyrosyl tRNA synthetase activity have been found upon DEAE-cellulose fractionation of pea root extracts [4].

The main differences between the two peaks of activity were their storage stabilities and their molecular weights as determined from G-200 Sephadex chromatography. The authors concluded that the two forms were stable to rechromatography on DEAE-cellulose, although one cannot completely rule out some interconversion. The authors do not rule out the possibility that one of the activities is of mitochondrial origin, but they feel that to be unlikely because the "minor" activity is 20-25% of the total activity or approximately 4-5 fold greater than one would expect from a mitochondrial synthetase. Although the two fractionated forms have the same isoacceptor specificity, crude enzyme can aminoacylate a third tyrosyl isoacceptor which the purified forms cannot. The authors do not discuss the possibility that this loss of activity upon purification may be due to a partial degradation of a single synthetase activity thus yielding two forms, each capable of acylating two out of three possible substrates, but neither any longer able to acylate the third.

Fractionation of soybean seedling extract on hydroxylapatite yields three leucyl tRNA synthetase activities [5]. Two of the activities are present in the seedling hypocotyls, while all three are present in the cotelydons. In addition to this organ specificity, each of the activities has a characteristic isoacceptor specificity for a subset of the six leucyl tRNAs present in the plant. The authors report that of the three forms one is chloroplastic, two are

cytoplasmic and that each is stable to rechromatography on hydroxylapatite [6]. Since the three forms are not completely resolved from each other one cannot dismiss the idea that the second form is a hybrid of the first and third forms. Consistent with this idea is the observation that the isoacceptor specificity of the second form is also hybrid between that of the first and third forms.

In a study of the chloroplastic phenylalanyl tRNA synthetase in wheat seedlings [7], the authors concluded that two forms of this synthetase exist within the chloroplast. Both the original separation and the rechromatography are inconclusive, but potassium ions stimulate one of the forms 2-3 fold while the other form is unaffected. One cannot conclude that these two enzymes are any more than different conformational forms of the same molecule, but if that is case, then potassium ions specifically affect only one of them.

The aforementioned Neurospora synthetases [3] are rather clear cases of mitochondrial vs. cytoplasmic type multiplicity. In addition to their intracellular localization, these synthetases (at least the ones so far analyzed) possess isoacceptor specificity. The mitochondrial synthetases can charge mitochondrial or E. coli tRNA while the cytoplasmic synthetases charge these tRNAs as well as Neurospora tRNA.

Yeast lysyl tRNA synthetase when purified to apparent homogeneity gave two distinct protein bands in polyacrylamide

gel electrophoresis [8]. These enzyme forms could also be resolved by Bio-rex 70 column chromatography, but when the two Bio-rex forms were mixed and then electrophoresed a single protein band resulted. The authors considered several possible explanations of their observations, but were unable to provide a completely satisfactory one. Since they ". . . found that isolation of the minor enzyme component was dependent on the manner in which fractions were selected for further purification in the early stages of the preparation," one must conclude that the minor enzyme activity is in some way an artifact of the method of preparation. Although they assayed for kinetic differences between the two forms, the only difference found was that the minor form was generally slightly less active than the major form--perhaps implying that the minor form is a partially degraded version of the major one.

Euglena possess separate chloroplastic and cytoplasmic synthetases for nine out of ten synthetases studied [9]. The authors of this study chose to characterize the pair of leucyl tRNA synthetases in greatest detail. Their study revealed that the enzymes differed from each other (1) in their elution from hydroxylapatite, (2) in the effect of tRNA on their exchange reactions, (3) in their sensitivity to monovalent cations, and (4) in their sensitivity to heat inactivation among other characteristics. While the enzymes

can be shown to be significantly different from each other, this is yet another clear case of intracellular localization of synthetase activities. Still further experimentation with Euglena synthetases [10] has indicated that both the chloroplastic and cytoplasmic enzymes are coded in the nuclear genome and translated on cytoplasmic ribosomes. When functional within the organism, however, the enzymes are localized.

Chui and Suyama [11] have demonstrated immunological and chromatographic differences between leucyl tRNA synthetases found in the mitochondria and the cytoplasma of Tetrahymena. As in the Neurospora and Euglena studies, these authors leave no doubt that this synthetase exists in two different forms, but the different forms reside within separate intracellular compartments.

Turning from plants and protozoans to animals, one realizes immediately that there are many fewer reports of multiple synthetases. In a study of the glycyl tRNA synthetase in Bombyx, Kurosawa and Onodera [12] reported multiple forms of the enzyme. The DEAE-cellulose chromatographic separation of the forms was far from convincing, however. The "second activity" was a shoulder of the first and no attempt was made to demonstrate that the two forms were any more than interconvertible forms of the same enzyme. The authors do attempt to establish isoacceptor specificity

for each of the two enzymes, but the data are not especially convincing.

Using female rats which had been injected intraperitoneally with hydrocortisone, Acs and colleagues were able to demonstrate the appearance of a new leucine tRNA in the liver cytoplasm [13]. This tRNA could only be aminoacylated by enzyme made from the same kind of cytoplasm implying that both new tRNA and new synthetase were produced in the liver in response to hydrocortisone. The synthetase could aminoacylate all of the original leucyl tRNAs as well as the new leucyl tRNA. This observation of a "multiple" synthetase is rather different from those previously described. While the authors demonstrated that protein synthesis was required for the new synthetase activity, one cannot rule out modification of the "old" synthetase as a possible explanation of its "new" activity.

From the preceding selected review there arise three general explanations for synthetase multiplicity. First, the possibility exists that a single synthetase may be partially degraded to yield a less active form. This may occur during the initial tissue homogenization or elsewhere during preparation. Secondly, a single enzyme may exist in two conformational forms which, under appropriate conditions, may be resolved. If separated forms are stable to rechromatography, one may be fairly confident in ruling out this

possibility. In many of the studies described however, the synthetase was either not subjected to rechromatography or its stability during rechromatography was questionable. The third and most frequent source of multiple synthetase activity arises because of intracellular compartmentalization. Since both the chloroplasts and mitochondria contain their own complement of tRNAs, the usual situation appears to be that they contain the appropriate synthetases for aminoacylating the tRNAs.

During a study of the mechanism of genetic suppression in Drosophila melanogaster, two forms of tyrosyl tRNA synthetase activity were observed [14]. One of the forms eluted from DEAE-cellulose in 0.1 M NaCl and could aminoacylate both tyrosine tRNA isoacceptors of wild type Drosophila ($tRNA_1^{Tyr}$ and $tRNA_2^{Tyr}$) but only one of the tyrosine tRNA isoacceptors of suppressed vermilion (su(s)^2,v) Drosophila ($tRNA_A^{Tyr}$). The second synthetase form eluted from DEAE-cellulose in 0.3 M NaCl and could aminoacylate the three isoacceptors mentioned above as well as the second tyrosine tRNA isoacceptor of su(s)^2,v flies, $tRNA_B^{Tyr}$. Since the evidence for genuine synthetase multiplicity in animal systems is quite limited, I undertook an investigation of the apparent multiplicity in Drosophila.

CHAPTER II

MATERIALS AND METHODS

Materials

The suppliers of the various materials used in this research are summarized in Table I.

TABLE I

SOURCES OF MATERIALS

Compound	Company
[^{3}H]tyrosine and [^{14}C]tyrosine	Schwartz/Mann Bioresearch Inc. and Amersham Searle Co.
ATP (di K^+ and di Na^+ salts)	Sigma Chem. Co.
DEAE-cellulose (DE-23)	H. Reeve Angel Co.
Sephadex	Pharmacia Fine Chemicals, Inc.
Agarose (A .5m)	BioRad Laboratories
BBOT (2,5-bis-[2-(5-tertbutyl-benzoxazolyl)]thiophene)	Packard Inst. Co.
Triton X-100	Rohm and Haas
3MM filter paper discs	W & R. Balston, Ltd.
Adogen 464	Ashland Chem. Co.
Plaskon CTFE (2300 powder)	Allied Chem. Corp.

RPC-5 column packing was prepared by coating the Plaskon CTFE beads with Adogen 464 as described by Pearson et al. [15]. All other chemicals were of reagent grade.

Drosophila Strains

Samarkand wild type and homozygous suppressed vermilion (su(s)^2,v;bw) Drosophila melanogaster were used.

Growth of Drosophila

Flies were grown in half-pint milk bottles or in plastic cages (7"x13"x15") with glass tops and ventilation ports on a modification of the medium by Lewis [16] described in Table II. Adult flies of unknown age were harvested with a modified portable vacuum cleaner and stored until needed at -85°C.

TABLE II

DROSOPHILA FOOD FORMULA[a]

Ingredients	Composition	Amounts (for 300-Bottle Batch)
Water	81.8%	13,000 ml (for dissolving agar) 4,000 ml (for wetting other components)
Agar	0.44%	93 gms
Dextrose	5.0%	1033 gms
Sucrose	2.5%	517
Corn Meal	8.3%	1716
Dried Yeast	1.5%	310
Phosphoric Acid	0.06%	200 mls of "Acid Mix"[b]
Propionic Acid	0.4%	

[a]After the food has been prepared and has cooled, it is seeded with "Y-2 original," a live yeast strain which is quite insensitive to the level of propionic acid present in the food.

[b]To prepared "Acid Mix": (1) make 836 ml of propionic acid to 1 liter with distilled water, (2) make 83 ml of phosphoric acid to 1 liter with distilled water, (3) combine the 2 liters of acid to give "Acid Mix."

Isolation of tRNA

Transfer RNA was prepared by a slight modification of the method described by Twardzik et al. [17]. Adult flies were homogenized in four volumes each of 88% phenol and buffer A (50 mM Tris-Cl pH 7.5, 10 mM $MgCl_2$ and 5 mM β-mercaptoethanol) containing 100 mM NaCl at 4°C. Centrifugation of this homogenate for 30 minutes at 10,000 x g resulted in the separation of three phases. The upper aqueous phase, containing the nucleic acids, was removed and precipitated by the addition of 2-1/2 volumes of 95% ethanol. After storage overnight at -20°C, the precipate was collected by centrifugation and suspended in buffer A. The solution was loaded on a DEAE-cellulose column which had been equilibrated with buffer A. The column was eluted sequentially with buffer A containing 0.35 M NaCl and then 0.75 M NaCl. Although some tRNAs elute from DEAE-cellulose in 0.35 M NaCl, $tRNA^{Tyr}$ of Drosophila does not do so. The RNA in the 0.75 M NaCl was therefore somewhat enriched for $tRNA^{Tyr}$. This tRNA was precipitated upon addition of 2-1/2 volumes of 95% ethanol. This precipitate was stored overnight at -20°C, collected by centrifugation and suspended in 50 mM Tris-Cl pH 7.5 and 10 mM $MgCl_2$. The tRNA was stored at -20°C. The second isoacceptor of $su(s)^2,v;bw$ flies, $tRNA_B^{Tyr}$, was separated from the first isoacceptor of this strain, $tRNA_A^{Tyr}$, by chromatography on RPC-5 by Mr. J. B. Murphy.

Preparation of Tyrosyl tRNA Synthetase

Crude tyrosyl tRNA synthetase was prepared by homogenizing wild type adult flies in four volumes of buffer B (10 mM Tris-Cl pH 7.5, 10 mM $Mg(OAc)_2$, 10 mM β-mercapto-ethanol, and 10% glycerol) at 4°C. The initial supernatant of this homogenate (10,000 x g, 15 minutes) was recentrifuged at 65,000 x g for 90 minutes. The second supernatant could be stored at -85°C or applied directly to a DEAE-cellulose column which had been equilibrated with buffer B. The column was sequentially eluted with buffer B containing 0 M NaCl, 0.1 M NaCl, 0.2 M NaCl and 0.3 M NaCl. The fractions from each stepwise eluant were assayed for their capacity to tyrosylate tRNA. Those fractions with such activity were divided into 2 ml aliquots and stored at -20°C. Synthetase prepared in this fashion was stable for at least 4 months.

Aminoacylation of tRNA

Standard conditions for the formation of $Tyr\text{-}tRNA^{Tyr}$ were such that each ml of reaction contained: 70 μmoles Tris-Cl pH 8.0, 20 μmoles $Mg(OAc)_2$, 10 μmoles ATP as the di K^+ salt (or 10 μmoles ATP as the di Na^+ salt and 20 μmoles KCl), 5 μmoles [^{3}H]tyrosine, 2 to 10 A_{260} units of crude tRNA, and enough synthetase to aminoacylate all the tRNA. Reactions were usually for 30 minutes at either 21°C or 37°C in a final volume of .2 ml. For kinetic studies, 25 μl aliquots were pipetted onto Whatman 3MM filter paper discs

and precipitated in 10% TCA at 0°C. These discs were washed two times (10 minutes each) in 5% TCA, rinsed in 95% ethanol and then in ether and finally air dried. Initially these filter paper disc aliquots were precipitated and washed in TCA at room temperature (19-22°C), but a sequence of spurious kinetics led to the observation that the product of the amino-acylation reaction is unstable in 10% TCA at room temperature (Figure 1). The solution to this problem of hydrolysis was to wash the discs containing individual aliquots in TCA at 0°C (Figure 2). The amount of [^{3}H]Tyr-tRNA^{Tyr} precipitated on each disc was determined by liquid scintillation counting in a fluor of 0.4% BBOT in toluene. For assay of synthetase activity following chromatography the conditions were similar to those described above except the final reaction volume was only 85 µl. For preparation of tyrosyl-tRNA^{Tyr} prior to fractionation of isoacceptors on RPC-5 the conditions were identical except the final reaction volume was .4 ml. These reactions were stopped by the addition of .4 volumes of 2 M NaOAc pH 4.6. The tRNA was precipitated by the addition of 2-1/2 volumes of 95% ethanol and the precipitate was collected on a Millipore filter. The precipitate was suspended in buffer C (10 mM NaOAc pH 4.6, 10 mM $Mg(OAc)_2$, 1 mM EDTA, and 5 mM β-mercaptoethanol) containing .55 M NaCl and loaded on the RPC-5 column.

Figure 1. Comparison of a 30-minute charging assay (O——O) with a 120-minute charging assay (●——●) of wild type tRNA.

Both reactions were identical and substrate concentrations were as described in the text. The filter paper discs containing the aliquots for each time point from both reactions were washed in baths of 10% TCA at room temperature. All of the discs in the 30-minute reaction were processed for scintillation counting after 30 minutes, whereas those at the 120-minute reaction were processed after 120 minutes.

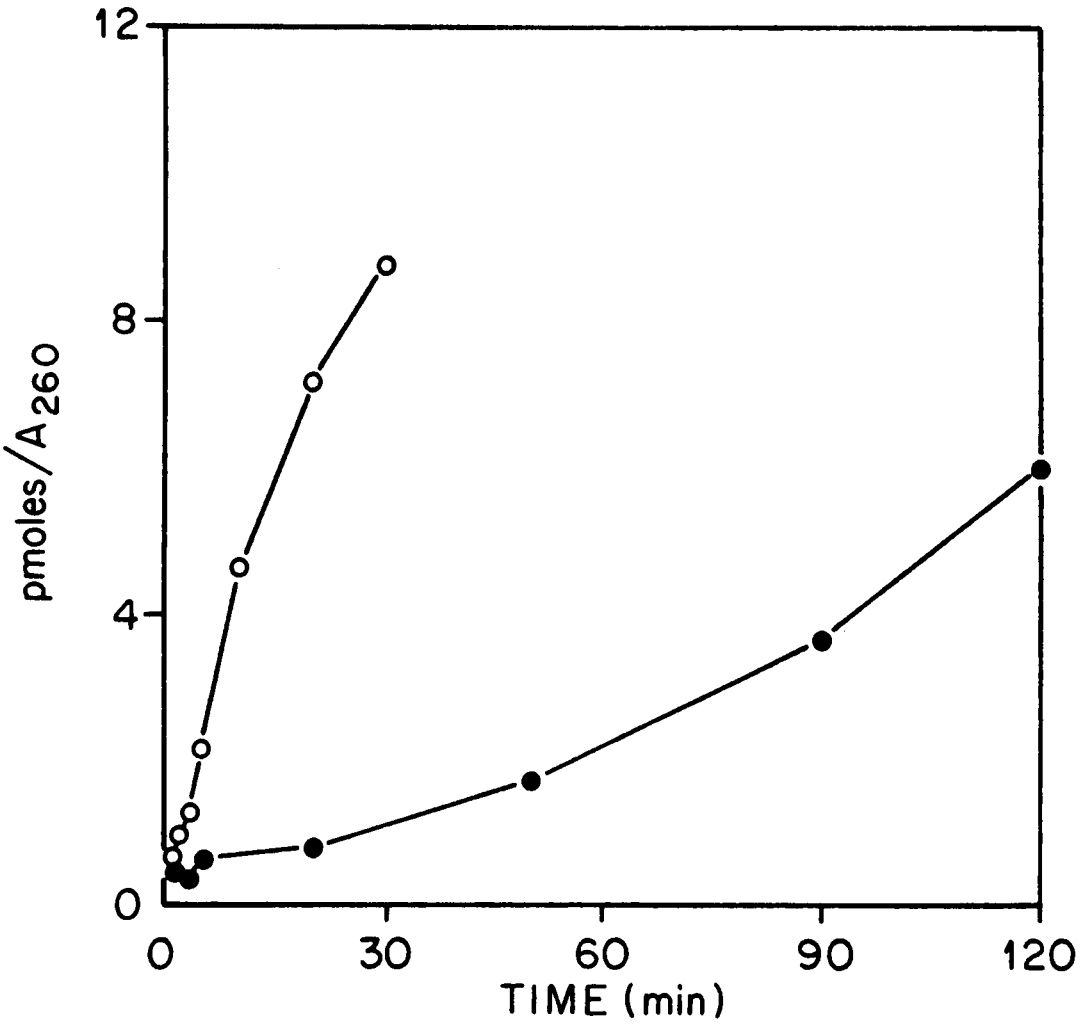


Figure 1

Figure 2. Comparison of the stability of tyrosyl-tRNA^{Tyr} in 10% TCA at 0°C (●——●) and 19°C (○——○) (room temperature).

Each reaction was run in duplicate as described in the text except that the discs were washed independently. Each point on the graph represents an aliquot after 30 minutes of the charging reaction which has been held in 10% TCA for the length of time indicated on the abscissa.

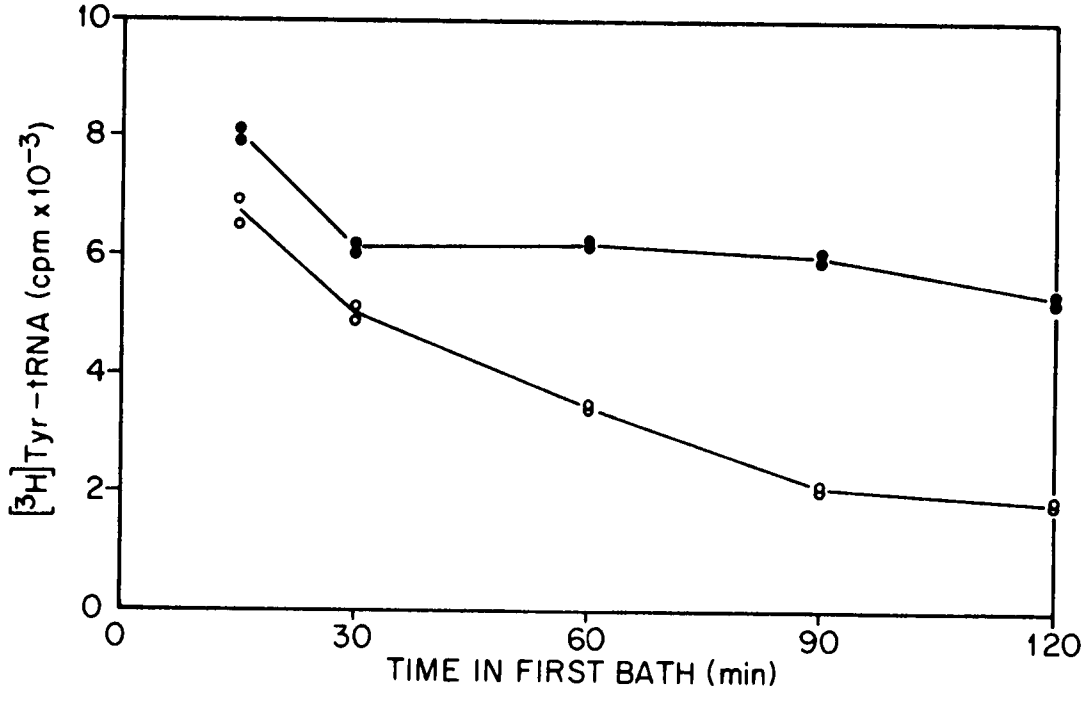

10
8
6
4
2
0
0
30
60
90
120
[3H]Tyr-tRNA (cpm x10-3)
TIME IN FIRST BATH (min)

Figure 2

Reversed Phase Chromatography

The RPC-5 system of Pearson et al. [15] was used for isoacceptor resolution. The dry column packing was mixed with buffer C containing .35 M NaCl and homogenized in a Waring blender to assure uniform particle size. Without further treatment, such packing was used directly to pour 20 cm column beds in jacketed, precision-bore, high pressure .63 x 33 cm columns (Chromatronix Corp.). Columns were operated at 40°C and 200-400 psi using a high rpm piston pump (Milton Roy No. 196-89). Samples were fractionated using a 400 ml NaCl gradient from .55 M-.75 M NaCl in buffer C. Tyrosyl-tRNA isoacceptors eluted in the first quarter of the gradient. One ml samples were collected and mixed with 10 ml of a 1:2 mixture of Triton X-100: 0.4% BBOT in toluene for counting in a liquid scintillation system. All experimental samples were cochromatogramed with standard samples of wild type [^{14}C]Tyr-tRNA^{Tyr}. After each isoacceptor fractionation, the column was eluted with 1.5 M NaCl in buffer C and then reequilibrated with .55 M NaCl in buffer C. Columns so treated could be used for 20-30 fractionations before they began to show evidences of poor isoacceptor resolution.

CHAPTER III

RESULTS

Enzyme Preparation

Initially tyrosyl tRNA synthetase was prepared as described under Materials and Methods. The results of such a preparation, as shown in Figure 3, clearly indicated a single peak of synthetase activity eluting in .1 M NaCl. This synthetase activity aminoacylated both tyrosine tRNA isoacceptors of both wild type and $su(s)^2,v$ flies. This observation of a single, nonisoacceptor specific form of Drosophila tyrosyl tRNA synthetase was in contrast to the two forms (Figure 4) observed by others using a similar protocol for preparation [14]. Figure 5 shows the specificity differences of these three enzyme forms. In an effort to establish the relationship between these three synthetase forms (which are listed in Table III), a series of enzyme preparations varying only slightly in their technical aspects was undertaken. Some of the possible sources of technical differences are diagrammed.

The first possible location of technical variability was in the process of homogenization. Perhaps two synthetase forms (Syn 1 and Syn 2) arose because the conditions of homogenization had been more extensive than when Syn 3 alone

Figure 3. Stepwise elution of a 3.5 x 13 cm DEAE-cellulose column yielding Syn 3.

The column and homogenate were prepared as described in the text. The column was eluted sequentially with 1300 mls 0 M NaCl, 1300 mls .1 M NaCl, 680 mls .2 M NaCl and 1000 mls .3 M NaCl, all in buffer B. Both absorbance at 280 nm (------) and enzyme activity (————) were monitored. Assay of enzyme activity is described in the text.

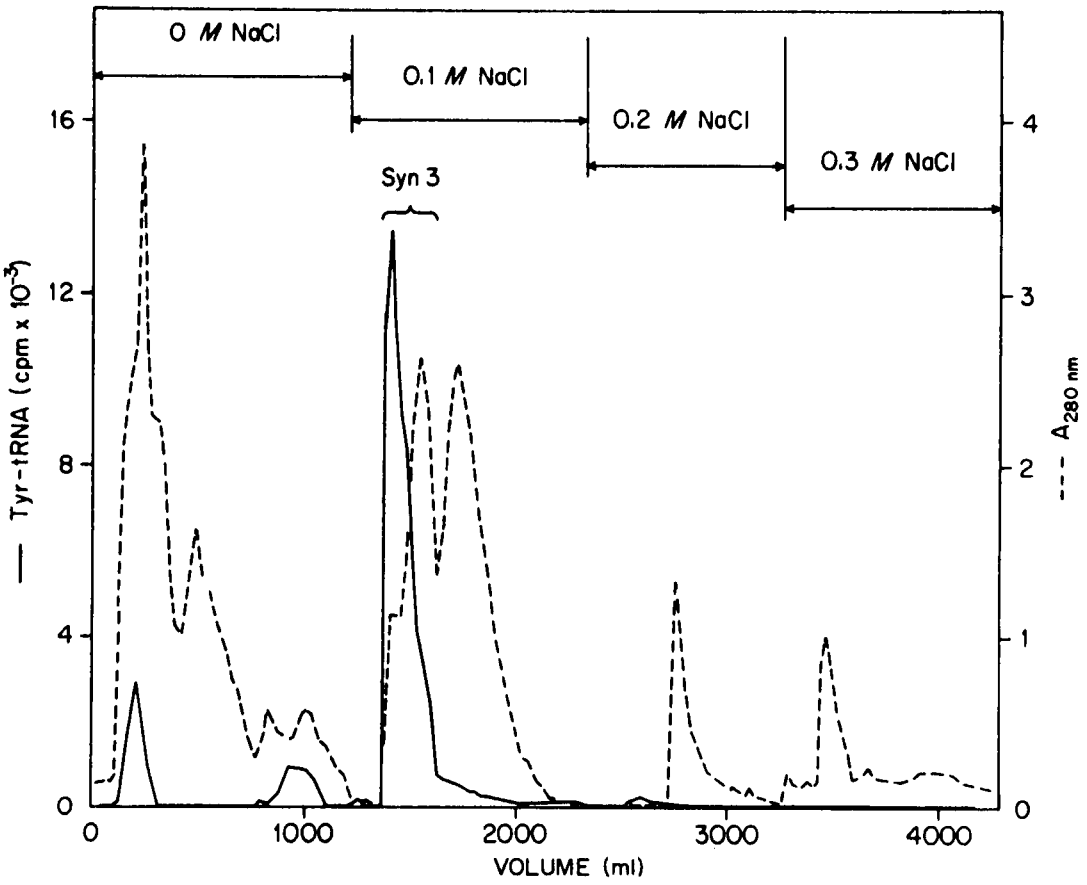

0 M NaCl
0.1 M NaCl
0.2 M NaCl
0.3 M NaCl
Syn 3
— Tyr-tRNA (cpm x 10⁻³)
--- A280 nm
VOLUME (ml)
0
1000
2000
3000
4000
4
8
12
16
1
2
3

Figure 3

Figure 4. Stepwise elution of a 3.5 x 13 cm DEAE-cellulose columns yielding Syns 1 and 2.

Column and homogenate preparation are virtually the same as for the results depicted in Figure 3. The elution sequence was 350 mls 0 M NaCl, 550 mls .1 M NaCl, 380 mls .2 M NaCl and 350 mls .3 M NaCl, all in buffer B. Two peaks of synthetase activity were obtained in this fractionation. See Figure 6 for possible differences between the results described in Figures 3 and 4.

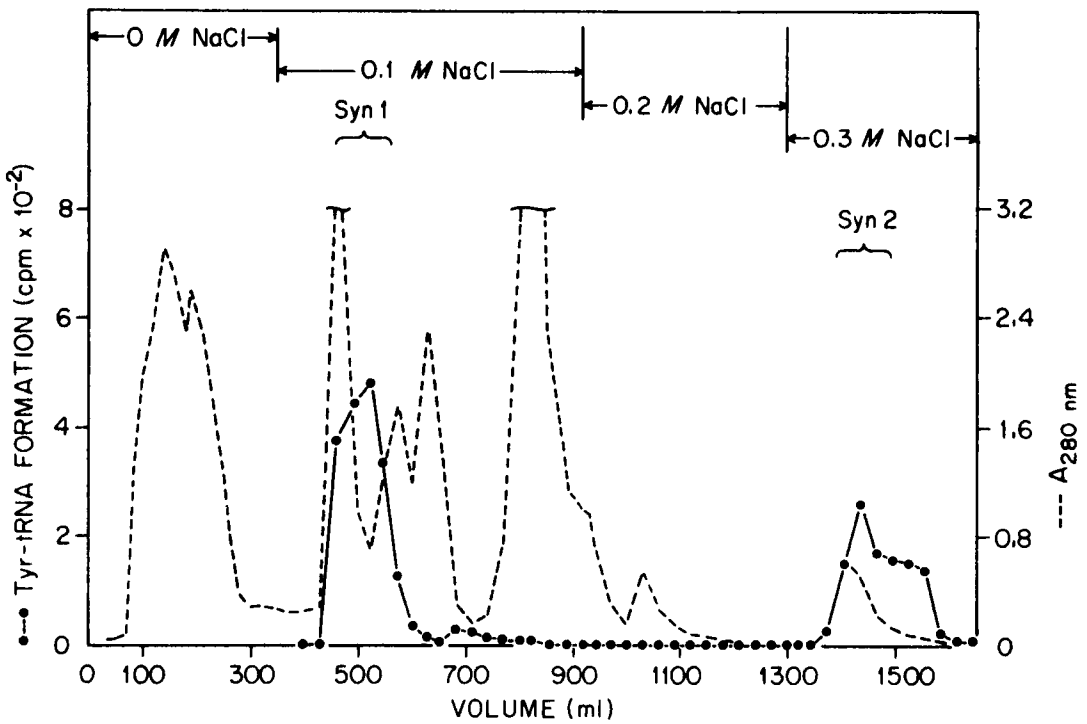

0 M NaCl
0.1 M NaCl
0.2 M NaCl
0.3 M NaCl
Syn 1
Syn 2
•—• Tyr-tRNA FORMATION (cpm x 10⁻²)
--- A280 nm
VOLUME (ml)
0
100
300
500
700
900
1100
1300
1500
2
4
6
8
0.8
1.6
2.4
3.2

Figure 4

Figure 5. Specificity of Syn 1, Syn 2 and Syn 3 for wild type (------) and <u>su(s)^2,v</u> (————) tyrosyl-tRNA.

Each enzyme was used to form [^{3}H]Tyr-tRNA$^{Tyr}_{s(su)^2,v}$ and [^{14}C]Tyr-tRNA$^{Tyr}_{wild\ type}$ in charging reactions as described in the text. The [^{3}H] and [^{14}C] products formed by each synthetase were mixed and co-chromatogramed on RPC-5.

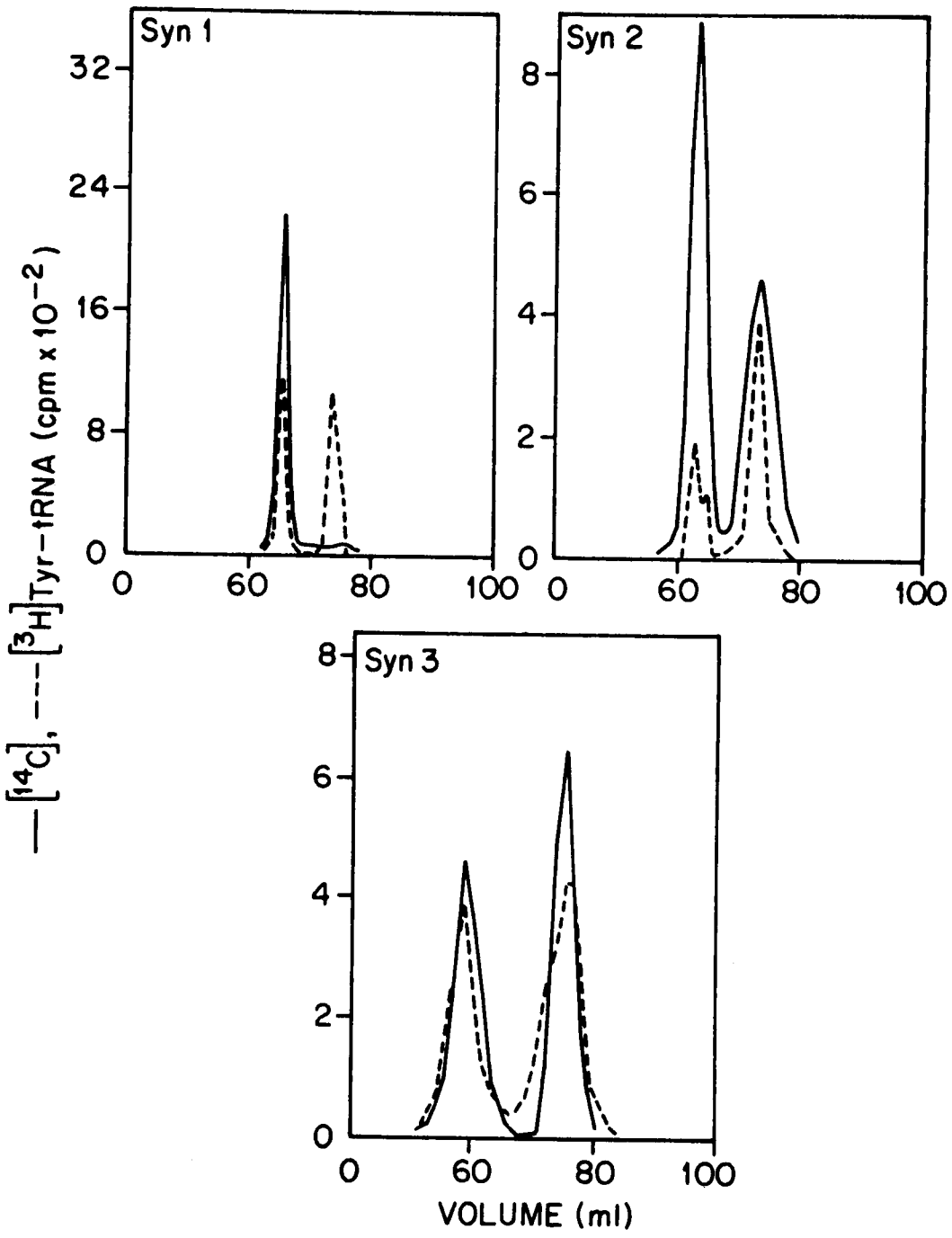

Syn 1
Syn 2
Syn 3
32
24
16
8
0
8
6
4
2
0
0
60
80
100
VOLUME (ml)
—[14C], ---[3H]Tyr-tRNA (cpm x 10-2)

Figure 5

TABLE III

SYNTHETASE CHARACTERISTICS

Enzyme	Designation	Position of Elution from DEAE-Cellulose	Aminoacylation Specificity
Synthetase 1	Syn 1	.1 M NaCl	$tRNA_1^{Tyr}$, $tRNA_2^{TYr}$, $tRNA_A^{Tyr}$
Synthetase 2	Syn 2	.3 M NaCl	$tRNA_1^{Tyr}$, $tRNA_2^{Tyr}$, $tRNA_A^{Tyr}$, $tRNA_B^{Tyr}$
Synthetase 3	Syn 3	.1 M NaCl	$tRNA_1^{Tyr}$, $tRNA_2^{Tyr}$, $tRNA_A^{Tyr}$, $tRNA_B^{Tyr}$

was observed. Variations in both the mechanics (Waring blender vs. ground glass homogenizer) and the duration (1 minute vs. 2 minutes vs. 5 minutes) of homogenization failed to reveal the relationship between the synthetase forms (see Figure 6). In all preparations, a single form, Syn 3, as shown in Figure 3, page 19, was observed.

A second point of technical variation concerned whether or not the 65,000 x g supernatant was frozen (-85°C) before being fractionated on DEAE-cellulose. If freezing resulted in the breakdown of a single synthetase form to yield two synthetase activities, the relationship between Syns 1, 2 and 3 could be resolved. Fractionation of frozen-thawed and never-frozen 65,000 x g supernatants was compared, but the activities

Figure 6. Sources of technical variability which could give rise to the different synthetase activities described in the text.

The differences are detailed in the text, but as diagrammed here they include: (1) mode of homogenization, (2) storage of homogenate before fractionation, (3) manner of washing DEAE-cellulose which forms column bed, and (4) means of elution from the column.

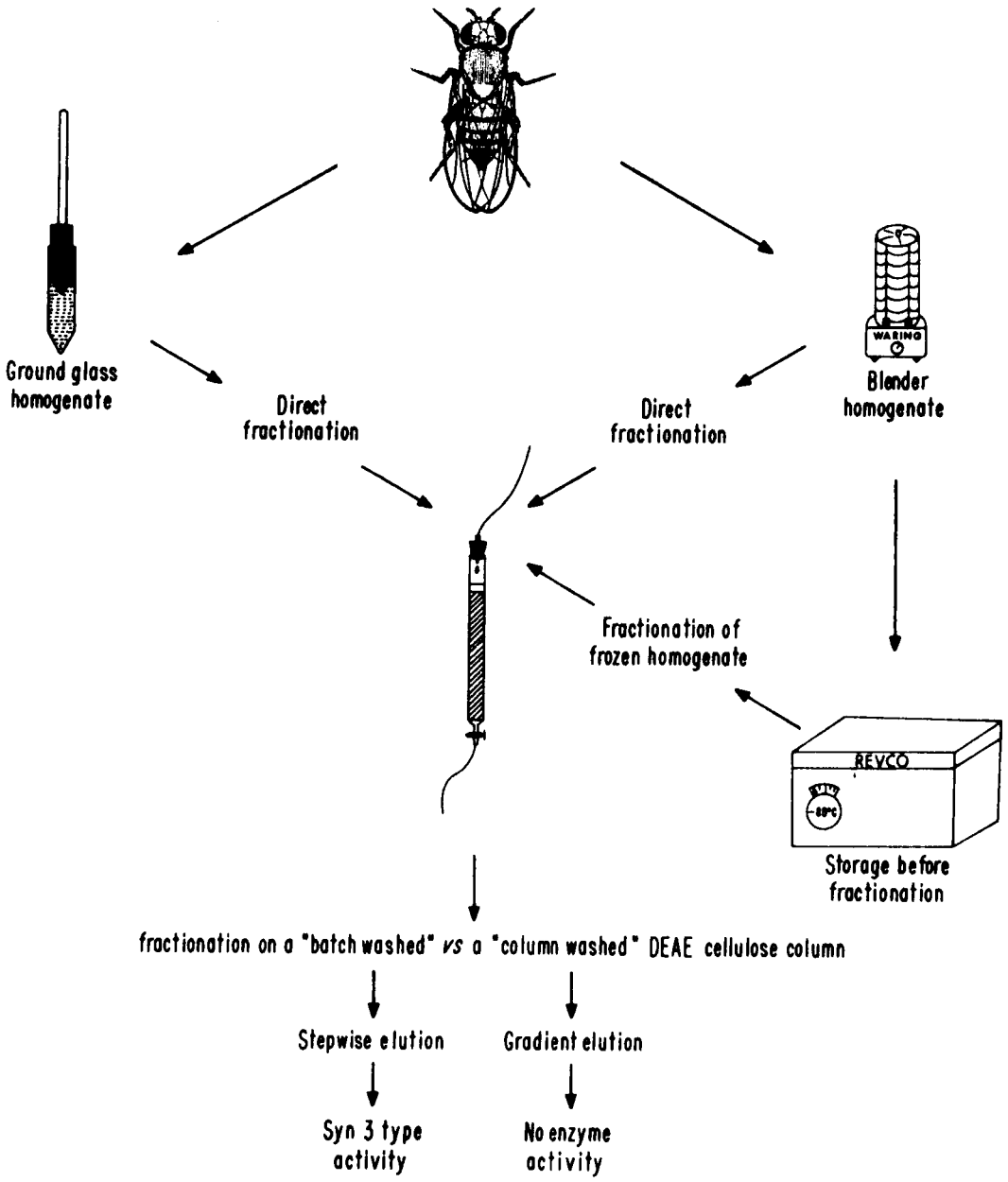

Ground glass homogenate
Direct fractionation
Direct fractionation
Blender homogenate
WARING
Fractionation of frozen homogenate
REVCO
-80°C
Storage before fractionation
fractionation on a "batch washed" *vs* a "column washed" DEAE cellulose column
Stepwise elution
Gradient elution
Syn 3 type activity
No enzyme activity

Figure 6

eluted from both chromatograms were the Syn 3 type and identical to that shown in Figure 3, page 19.

The method of DEAE-cellulose column preparation provided a third source of technical difference. Routine procedure for column preparation consisted of batchwise washing of the cellulose first with .1 N NaOH and then with .1 N HCl before the column was poured. A more extensively washed column could be obtained by first pouring the bed and then washing it with .1 N NaOH followed by .1 N HCl. When these two methods of column preparation were compared, both columns yielded a Syn 3 type enzyme.

One final physical means was used to try to separate Syn 1 and Syn 2. Since the two forms, Syn 1 and Syn 2, eluted at different NaCl concentrations, gradient elution should have resolved them from each other. When a DEAE-cellulose column was eluted with a gradient however, no enzyme activity was recovered (Figure 7). The reason for the loss of activity is not clear. Perhaps some essential component of the enzyme is resolved from the main component under the conditions of gradient elution. Appropriate reconstitution experiments failed to support the suggestion, however. Although the loss of all activity was surprising, the result of gradient elution did not help elucidate the relationship between Syn 1, Syn 2 and Syn 3. Since attempts to physically determine the relationship of the three forms were not successful, another approach was tried.

Figure 7. Gradient elution of a 3.5 x 13 cm DEAE-cellulose column.

A 4 liter linear gradient (0 M-.4 M NaCl in buffer B) was used to elute the column. Comparable amounts of crude homogenate were loaded onto this column and the column described in Figure 3; however, gradient elution resulted in the complete loss of all enzyme activity (————). The profile of absorbance at 280 nm (------) is rather similar to that of the column which was eluted stepwise (Figure 3).

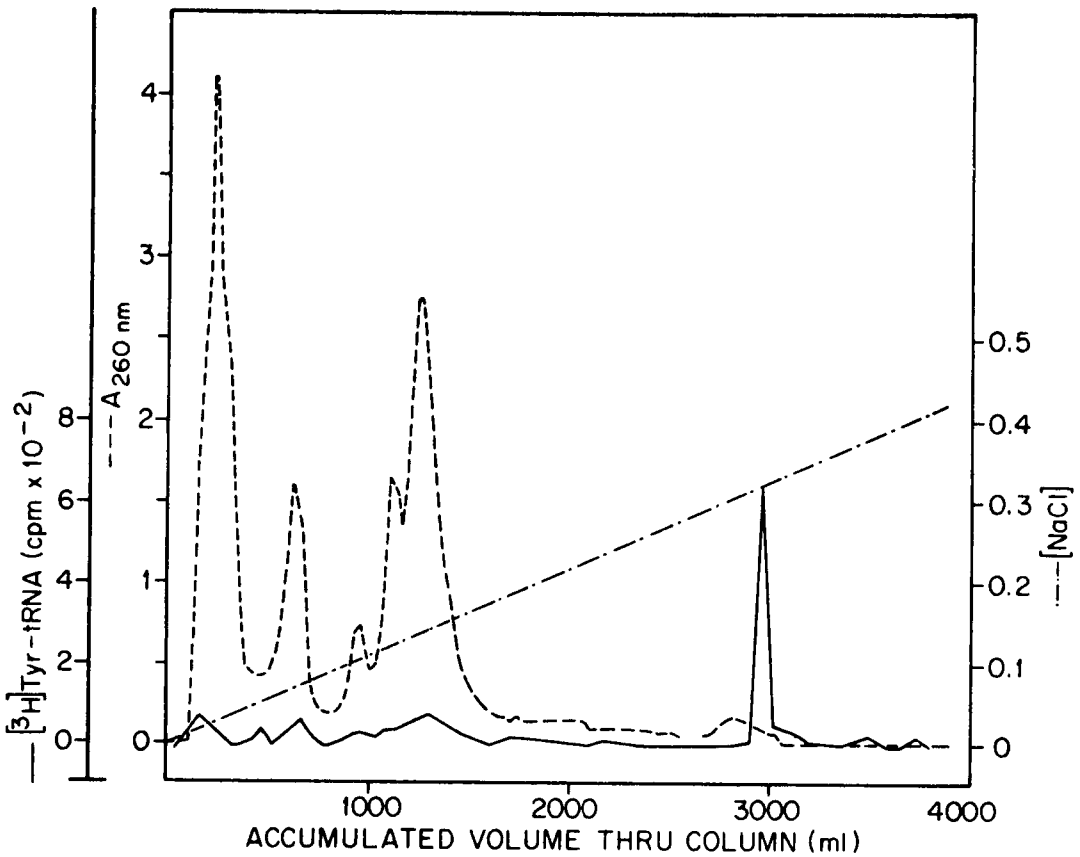

—[3H]Tyr-tRNA (cpm x 10-2)
--A260 nm
--[NaCl]
ACCUMULATED VOLUME THRU COLUMN (ml)
1000
2000
3000
4000
0
2
4
6
8
1
2
3
4
0
0.1
0.2
0.3
0.4
0.5

Figure 7

Enzyme Characterization

As Syn 3 was the synthetase form routinely available, a characterization of this form was begun. Several characteristics which could be useful in the purification of Syn 3 were observed. First, ammonium sulfate fractionation of Syn 3 showed that the enzyme precipitated between 60-70% of saturation of $(NH_4)_2SO_4$. Syn 3 activity proved to be very sensitive to $(NH_4)_2SO_4$ concentration: 2% $(NH_4)_2SO_4$ caused 20% inhibition of the extent of Syn 3 aminoacylating activity. Second, Syn 3 is stable to extensive dialysis and/or chromatography at pH 5. Third, rechromatography of Syn 3 yielded an apparently temperature sensitive enzyme. When Syn 3 was rechromatographed on a DEAE-cellulose column and eluted with the stepwise sequence of 0 M, .05 M and .1 M NaCl in buffer B, no enzyme activity was detected when assayed at 37°C (Figure 8A). If a comparable column was eluted with 0 M, .1 M NaCl in buffer B, then synthetase activity eluted as expected in the .1 M NaCl fraction when assayed at 37°C (Figure 8B). If the protein eluting in .05 M NaCl were assayed for aminoacylating activity at 20°C however, approximately 50% of the initial activity was found. Figure 9 compares the activities of a .05 M NaCl fraction at 20°C and 37°C. The reason for this apparent "temperature sensitivity" of the enzyme is not clear.

Since the temperature sensitivity of rechromatographed Syn 3 was rather unusual, a heat stability study was done.

Figure 8. DEAE-cellulose rechromatography of Syn 3 activity.

A. A 1 x 20 cm column was eluted with 100 ml 0 M NaCl, 100 ml .05 M NaCl and 50 mls .1 M NaCl, all in buffer B. No enzyme activity (———) was detected when the column fractions were assayed at 37°C. B. A 1 x 20 cm column was eluted with 200 ml 0 M NaCl and 50 mls .1 M NaCl in buffer B. The synthetase activity (———)reeluted in the .1 M NaCl when assayed at 37°C.

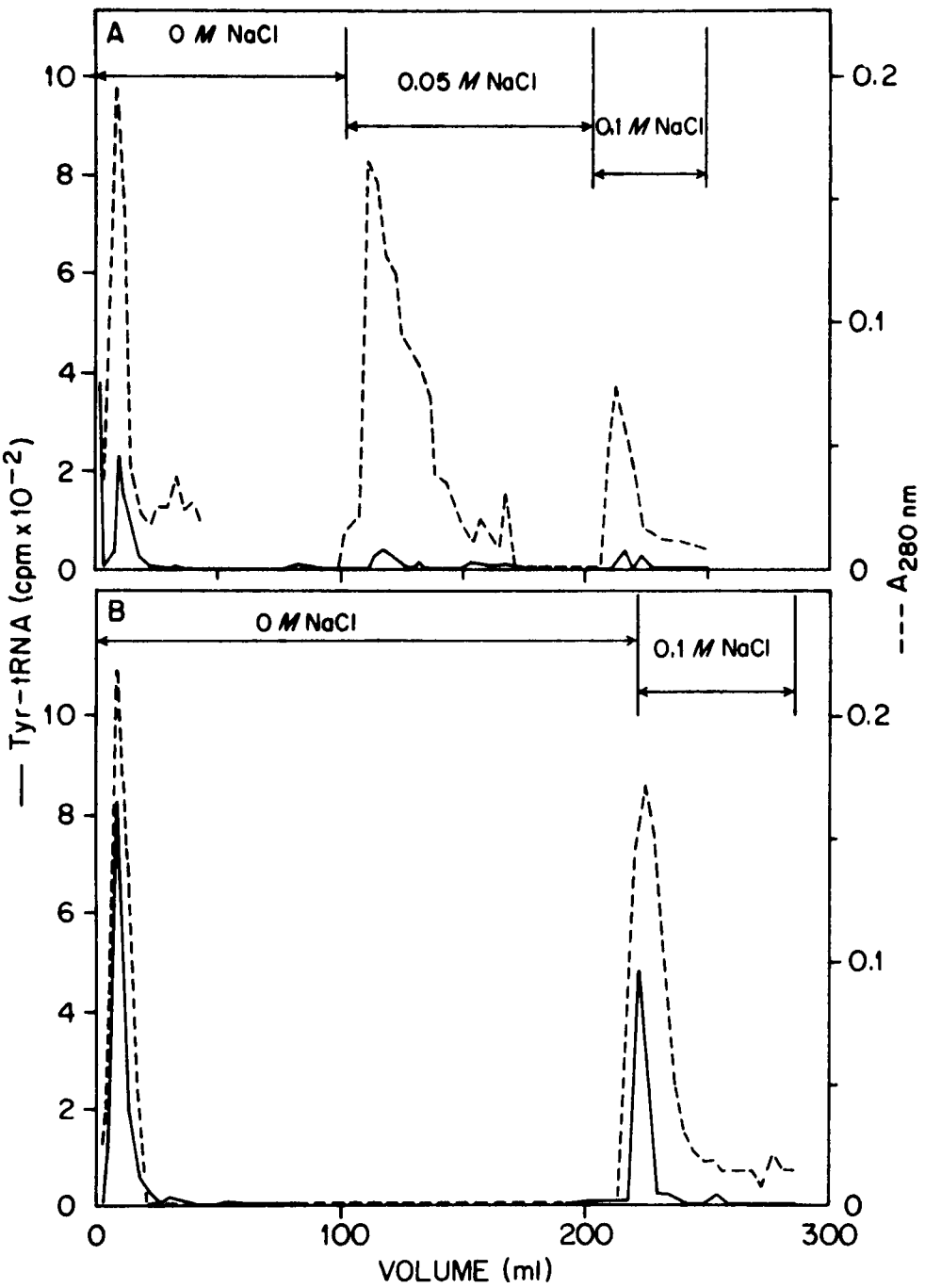

A
0 M NaCl
0.05 M NaCl
0.1 M NaCl
B
0 M NaCl
0.1 M NaCl
— Tyr-tRNA (cpm x 10⁻²)
--- A280 nm
VOLUME (ml)
0
100
200
300
2
4
6
8
10
0.1
0.2

Figure 8

Figure 9. Activity at the protein eluting in the .05 M NaCl step of the column described in Figure 8A.

Two of the .05 M NaCl fractions containing protein were assayed for synthetase activity at 20°C (—·—·— and ------) and at 37°C (————). Although the fractions had no detectable activity when assayed at 37°C, there was synthetase activity present when assayed at 20°C.

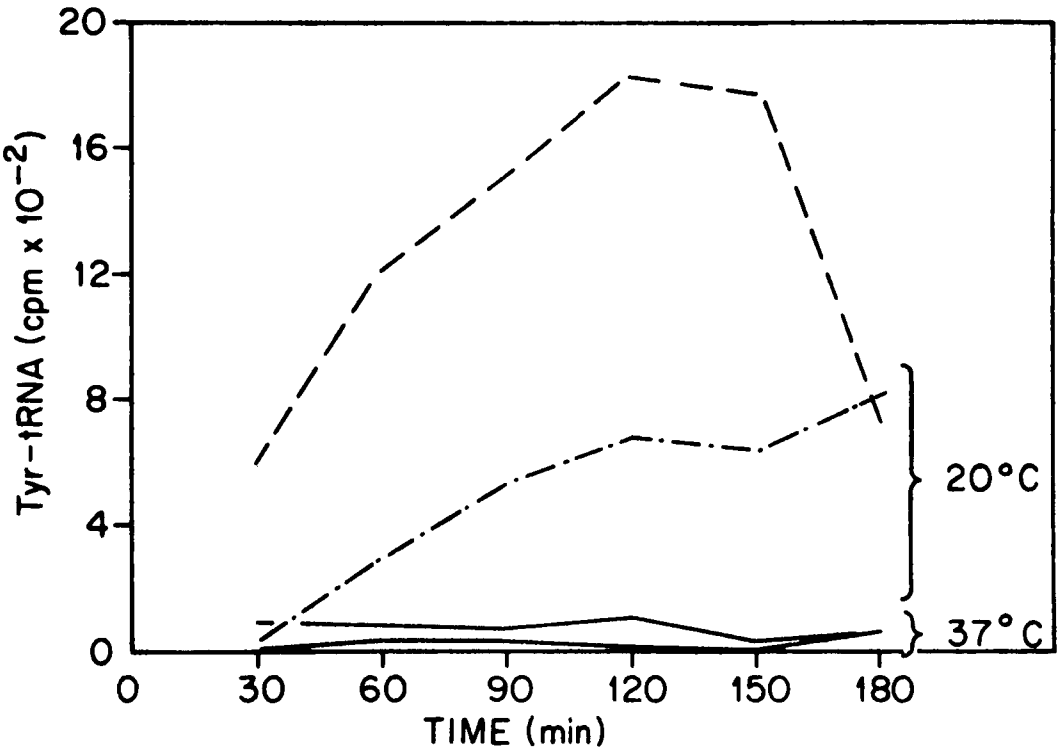

20
16
12
8
4
0
Tyr-tRNA (cpm x 10^{-2})
0
30
60
90
120
150
180
TIME (min)
20°C
37°C

Figure 9

Figure 10 shows the monophasic inactivation of Syn 3. Five minutes at 47°C destroys approximately one-third of the enzyme activity. If Syn 3 had contained any Syn 1 or Syn 2 activity, the inactivation might have occurred in a stepwise manner. The observed monophasic inactivation is indicative of a single enzyme activity.

The molecular weight of Syn 3 was determined to be approximately 100,000 by Agarose column chromatography (Figure 11). When synthetase activity was recovered (assay at 20°C) from the column, the elution position was always the same. Some preparations of enzyme, however, lost all activity during Agarose chromatography. On the other hand, all preparations of enzyme seemed especially sensitive to Sephadex chromatography. Syn 3 appeared in the void volume of a G-75 fractionation, but for those Sephadex systems which included Syn 3 activity (G-100, G-150 and G-200) the activity was either lost completely or reduced much more than would have been expected from dilution alone. As reported earlier, Syn 3 was very stable to extensive dialysis, even at pH 5. This observation rules out the idea that a small molecular weight component, essential for enzyme activity, is being separated from the synthetase during the Sephadex chromatography. The reason for the loss of activity in almost all Sephadex experiments and in some Agarose experiments is not clear.

Figure 10. Heat inactivation of Syn 3.

Equal aliquots of Syn 3 were preincubated for five minutes at 0°, 19°, 32°, 40°, 47°, 54° and 61°C. Each aliquot was then used in an aminoacylation reaction as described in the text. The extent of charging (pmoles/A_{260} formed after 60 minutes of reaction) is plotted vs. the temperature of preincubation. A five-minute preincubation at at 47°C results in approximately a one-third decrease in the extent of Syn 3 activity.

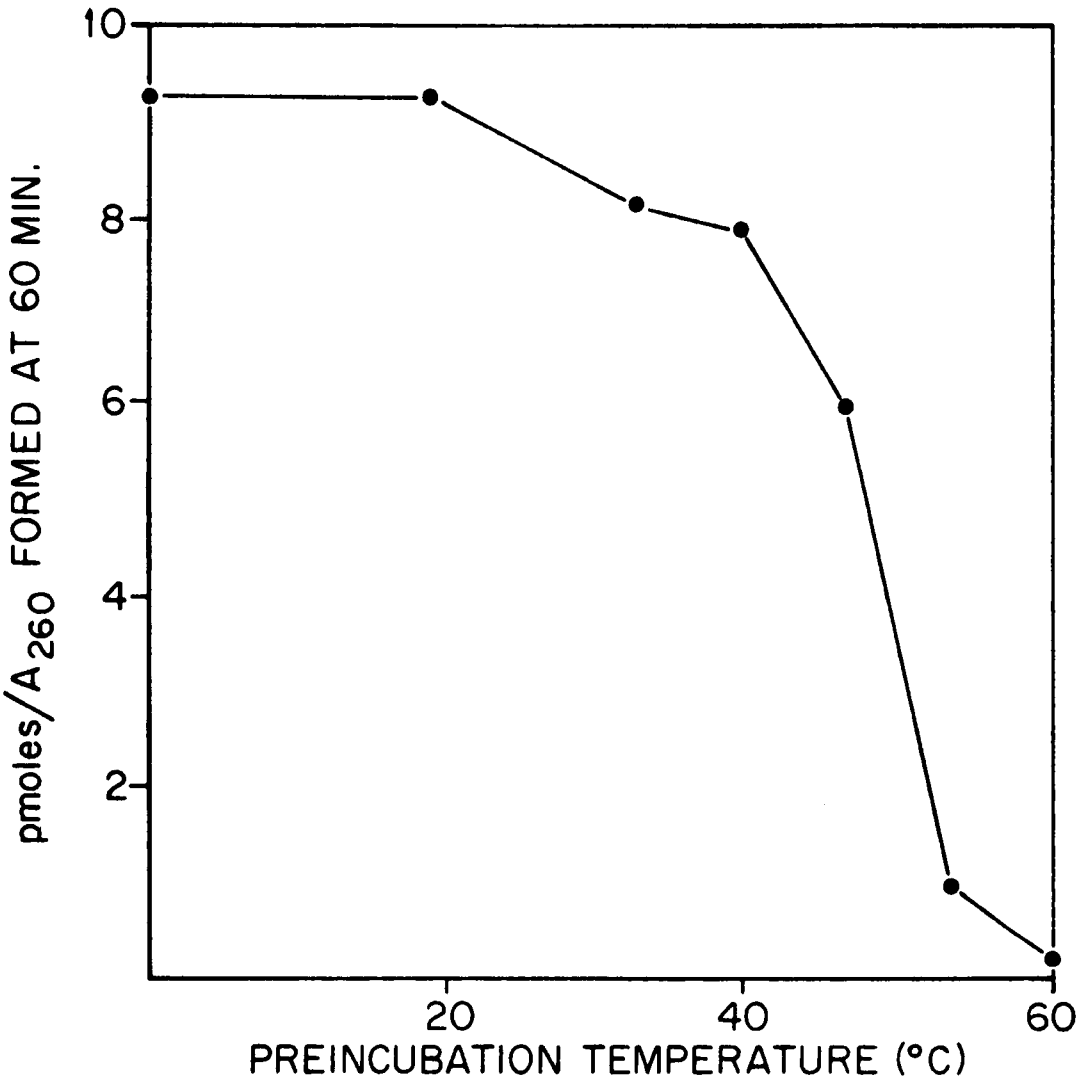

10
8
6
4
2
pmoles/A_{260} FORMED AT 60 MIN.
20
40
60
PREINCUBATION TEMPERATURE (°C)

Figure 10

Figure 11. Agarose column chromatography of Syn 3 activity.

One ml of Syn 3 was mixed with 5 mg catalase (250,000 daltons), 8 mg ovalbumin (43,500 daltons) and 4 mg myoglobin (17,800 daltons). The column was equilibrated with buffer B containing .1 M NaCl. Catalase was assayed for its ability to decrease the 240 nm absorbance of H_2O_2. Ovalbumin and myoglobin were detected by their 280 nm and 410 nm absorbances respectively. Synthetase activity was detected as described in the text. The marker elution positions and molecular weights are indicated on the chromatogram. The insert shows the molecular weights of the markers plotted vs. their elution positions. The molecular weight of Syn 3 was determined to be 100,000.

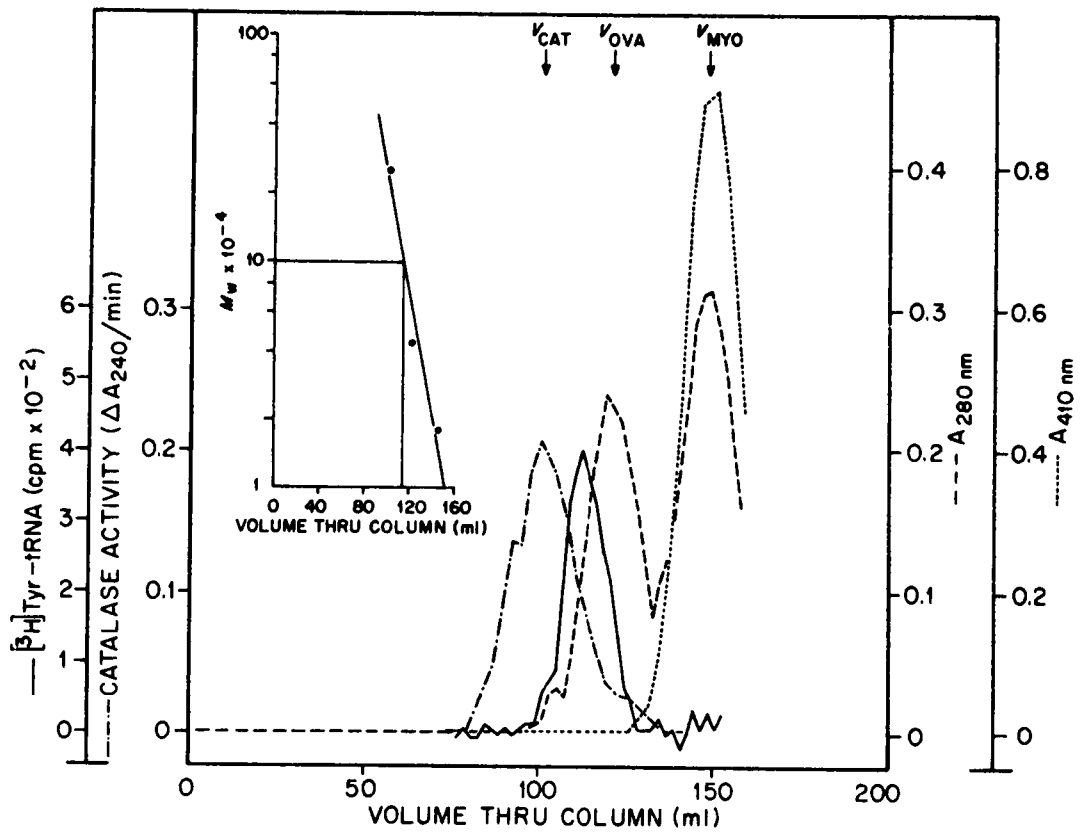

V_{CAT}
V_{OVA}
V_{MYO}
100
10
1
$M_w \times 10^{-4}$
0 40 80 120 160
VOLUME THRU COLUMN (ml)
— [^{3}H]Tyr-tRNA (cpm x 10^{-2})
---CATALASE ACTIVITY (ΔA_{240}/min)
--- $A_{280\ nm}$
..... $A_{410\ nm}$
0 50 100 150 200
VOLUME THRU COLUMN (ml)

Figure 11

In addition to those properties of Syn 3 already described, namely purification properties, heat stability and molecular weight, the kinetic properties of the enzyme offered another means of characterization. For a kinetic analysis of an enzyme activity, one would like to have a direct proportionality between enzyme activity and enzyme concentration. Figure 12 shows, however, that the relationship between activity and Syn 3 concentration is sigmoidal. If the relatively high total protein concentration of the crude enzyme extract had provided a stabilizing environment for synthetase activity, then dilution of the extract could have lowered the protein concentration sufficiently to destroy whatever stabilizing influence it may have had. Under these circumstances addition of bovine serum albumin to each enzyme concentration used (to standardize protein concentrations at each enzyme dilution) might have resulted in a linear relationship between activity and enzyme concentration. When bovine serum albumin was added to each enzyme dilution to provide such a standardization, it had no effect.

One can speculate that this situation implies some sort of cooperativity among the enzyme molecules themselves, but the actual reason for this relationship is unclear. The kinetic studies which follow were done at enzyme levels which correspond to the 50 µl point on the concentration curve. The optimal concentrations for each of the substrates were

Figure 12. Enzyme concentration vs. velocity.

Increasing amounts of Syn 3 were added to a standard reaction volume and the initial velocities (v) of each reaction were determined by measuring the amount of Tyr-$tRNA^{Tyr}$ formed with time. The initial velocities (as pmoles/A_{260}/min) are plotted vs. the amount of enzyme in the reaction.

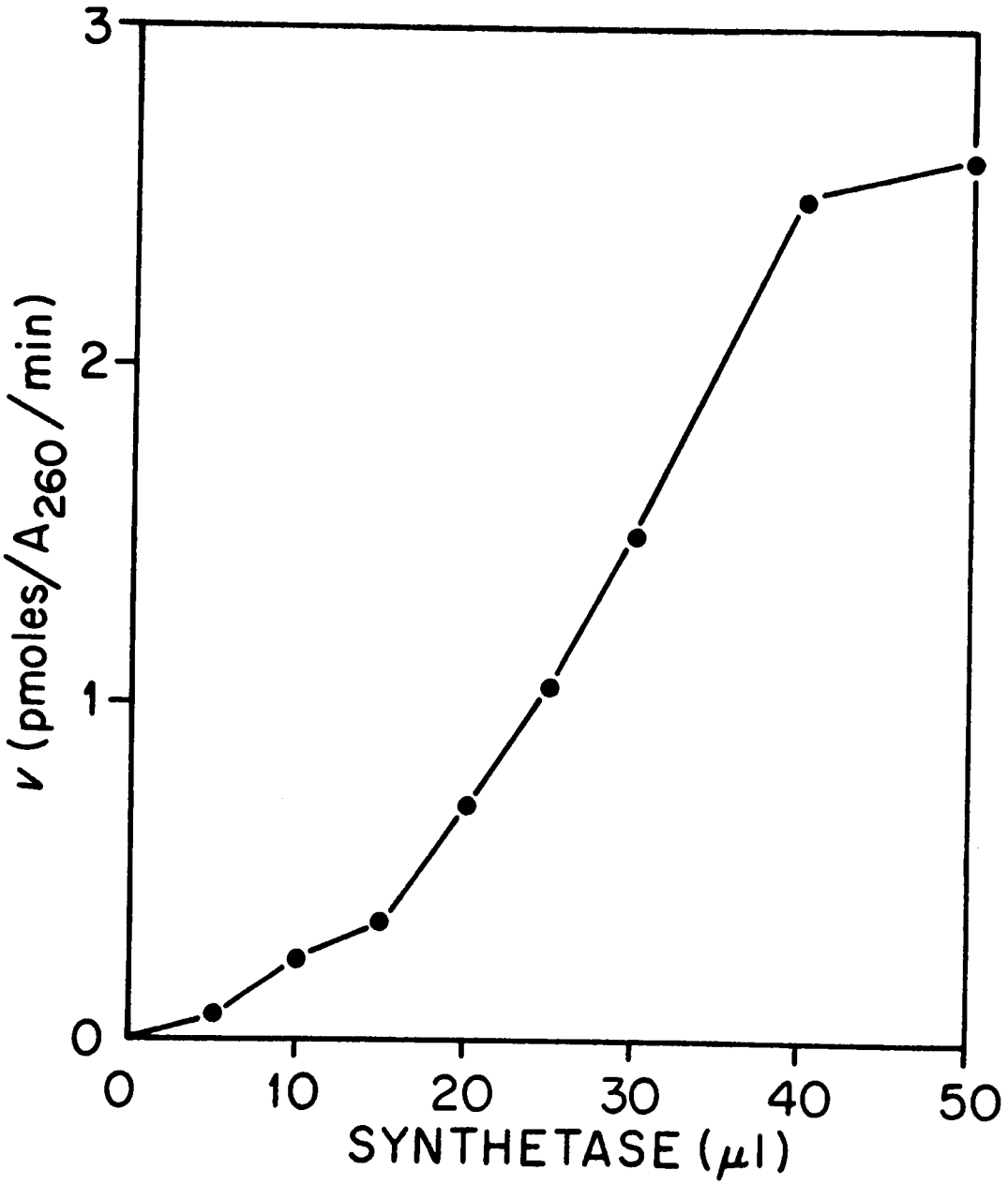


Figure 12

determined. Representative data are shown in Figures 13 and 14. The apparent K_ms for each substrate are summarized in Table IV.

As can be seen from the table, the effect of Mg^{++} concentration was examined at two different levels of ATP, and conversely the effect of ATP concentration was examined at two different levels of Mg^{++}. Historically, the Mg^{++}/ATP ratio has been deemed one of the key factors affecting the activity of an individual synthetase [1]. The Drosophila tyrosyl tRNA synthetase seems to be rather insensitive to the actual ratio of Mg^{++} to ATP. This synthetase requires at least .5 mM Mg^{++} to have any activity; this requirement is independent of ATP concentrations between .5 mM and 25 mM. Since Mg^{++} and ATP are complexed when they exist in the same solution, one might presume that the .5 mM Mg^{++} requires at least equal concentrations of ATP to be effective and these experiments support this suggestion. Further examination of these data shows that Syn 3 has an absolute requirement for K^+; Syn 1 and Syn 2 do not share this requirement, though they are stimulated three to five fold by K^+ [14]. This observation, in addition to the characteristics listed in Table III, page 25, strengthens the idea that Syn 3 really is a unique form of the Drosophila tyrosyl tRNA synthetase.

The Drosophila tyrosyl tRNA synthetase as originally isolated was found to be unstable in 10% glycerol [17].

Figure 13. Effect of K^+ and tRNA on Syn 3 activity.

A. Standard assay conditions were used except that the K^+ concentration was varied. The insert is a double-reciprocal plot which gives an apparent K_M of .01 M. B. Standard assay conditions were used except that the tRNA concentration was varied. The insert is a double-reciprocal plot which gives an apparent K_M of 3×10^{-7} M.

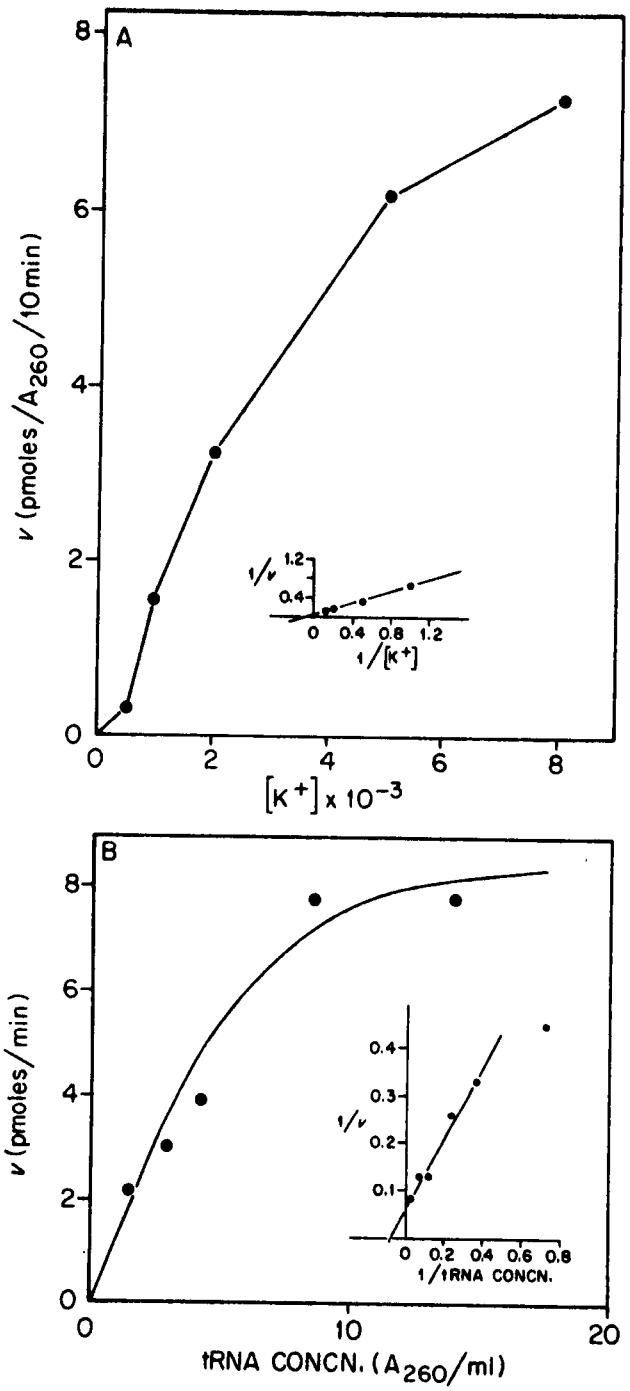

A
8
6
4
2
0
0
2
4
6
8
ν (pmoles/A260/10min)
[K+] x 10-3
1/ν
1.2
0.4
0 0.4 0.8 1.2
1/[K+]
B
8
6
4
2
0
0
10
20
ν (pmoles/min)
tRNA CONCN. (A260/ml)
0.4
0.3
0.2
0.1
1/ν
0 0.2 0.4 0.6 0.8
1/tRNA CONCN.

Figure 13

Figure 14. Effect of Mg^{++} and ATP on Syn 3 activity.

A. Standard assay conditions were used except that Mg^{++} concentration was varied. ATP concentration was 10 mM while Mg^{++} concentration varied from 1 mM to 50 mM. The Mg^{++}/ATP ratio therefore varied from .1 to 5. The insert is a double-reciprocal plot which reflects the inhibition of Syn 3 activity by high Mg^{++} concentrations. The apparent K_M for Mg^{++} was determined to be that Mg^{++} concentration which allowed one-half maximal activity in the v vs. Mg^{++} plot. B. Standard assay conditions were used except that ATP concentration was varied and Mg^{++} concentration was 10 mM. The insert is a double-reciprocal plot which gives an apparent K_M of .007 M.

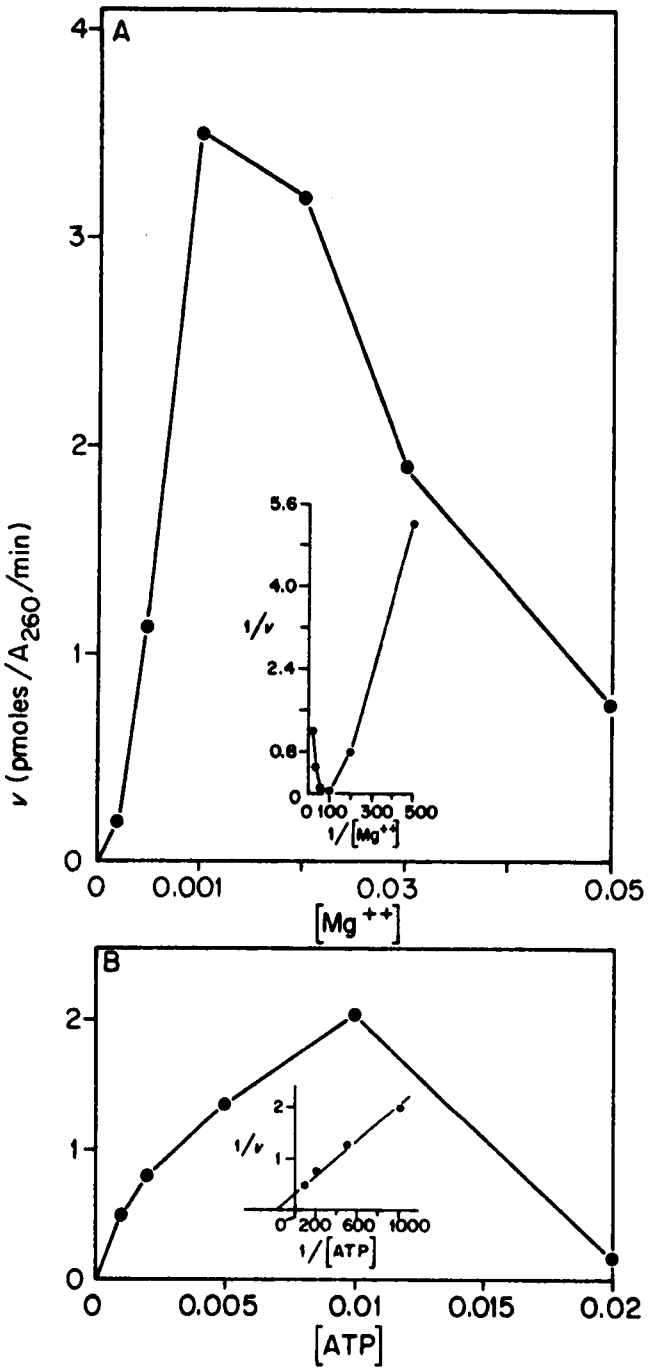

A
B
v (pmoles /A260/min)
[Mg++]
[ATP]
1/v
1/[Mg++]
1/[ATP]
0
0.001
0.03
0.05
0.005
0.01
0.015
0.02
100
300
500
200
600
1000
5.6
4.0
2.4
0.8
1
2
3
4

Figure 14

TABLE IV

APPARENT K_Ms FOR SYN 3 SUBSTRATES

Variable Substrate	Range of Concentration	Apparent K_M
K^+	.5-20 mM	.01 M
Mg^{++}	1-50 mM at 5 and 10 mM ATP	.001 M
ATP	1-50 mM at 10 and 50 mM Mg^{++}	.007 M
Tyr	.5-20 mM	2.5×10^{-6} M
$tRNA^+$	3-80 μM	3×10^{-7} M

Syn 3, however, was quite stable in 10% glycerol and could be stored at -20°C for at least four months. Because of this difference in stability in glycerol, the effect of glycerol on the kinetics of aminoacylation was investigated. No effect on either rate or extent of charging could be detected when glycerol concentration in the assay was varied between 0% and 25%.

In some synthetase reactions, spermine can replace Mg^{++} in the formation of aminoacyl tRNA [18]. Spermine could not replace Mg^{++} in the formation of Drosophila tyrosyl-$tRNA^{Tyr}$ however (Figure 15A), and at limiting Mg^{++} concentrations, high concentrations of spermine were inhibitory as shown in Figure 15B. The K_i of spermine inhibition was 1×10^{-3} M. Inhibition by p-chloromercuribenzoic acid (pClHgBzOH) is shown in Figure 16. The K_i of this inhibitor was 1×10^{-6} M.

Enzyme Specificity

The most intriguing difference between Syn 1 and Syn 2 is the isoacceptor specificity of Syn 1. Although Syn 2

Figure 15. Effect of spermine on Syn 3 activity.

A. Spermine concentrations were varied from .05 mM to 7.5 mM (————) but none was able to replace Mg^{++} (—·—·—·) in the aminoacylation reaction. B. Standard reactions conditions were used except Mg^{++} was 5 mM and spermine was varied from .5 mM to 16 mM. The insert is a plot of 1/v vs. spermine concentration from which the K_i was determined to be .001 M.

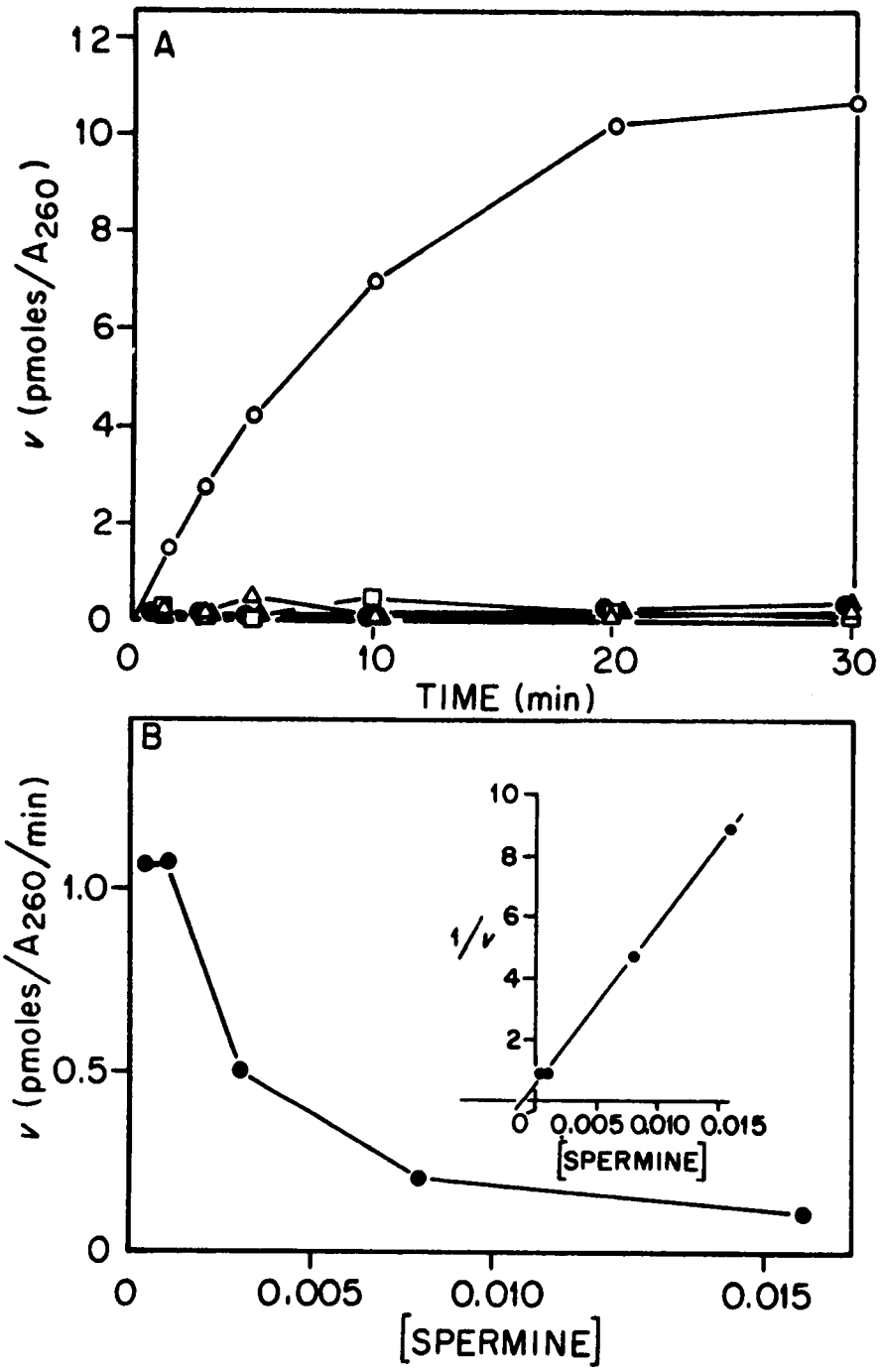

A
12
10
8
6
4
2
0
v (pmoles/A260)
0
10
20
30
TIME (min)
B
1.0
0.5
0
v (pmoles/A260/min)
0
0.005
0.010
0.015
[SPERMINE]
10
8
6
4
2
1/v
0
0.005
0.010
0.015
[SPERMINE]

Figure 15

Figure 16. Inhibition of Syn 3 activity by pClHgBzOH.

Standard assay conditions were used except pClHgBzOH was present. The insert shows a plot of 1/v vs. pClHgBzOH concentration from which the K_i was determined to be 1×10^{-6} M.

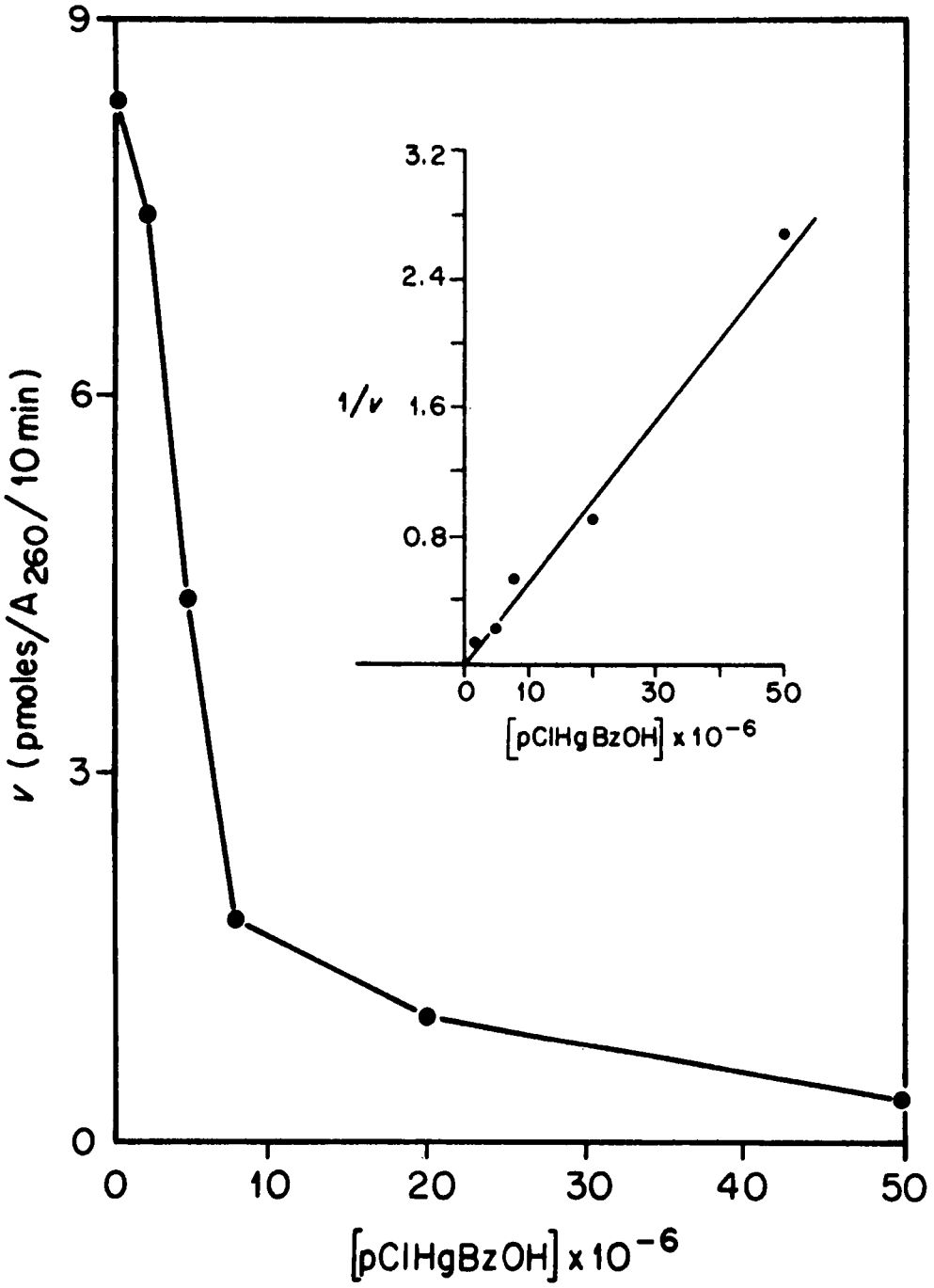

9
6
3
0
v (pmoles/A260/10min)
0
10
20
30
40
50
[pClHgBzOH] x 10⁻⁶
3.2
2.4
1.6
0.8
1/v
0
10
30
50
[pClHgBzOH] x 10⁻⁶

Figure 16

cannot distinguish $tRNA_A^{Tyr}$ from $tRNA_B^{Tyr}$ of $\underline{su(s)^2,v}$ flies, Syn 1 can recognize the difference between them. Since the initial attempts to show a physical relationship between Syns 1, 2 and 3 were unsuccessful, various substrate parameters were altered to determine if Syn 3 could be made to distinguish between $tRNA_A^{Tyr}$ and $tRNA_B^{Tyr}$. Two kinds of assays were used in these studies: (1) $\underline{su(s)^2,v}$ tRNA was charged under various sorts of conditions and chromatographed on RPC-5 to determine whether or not $tRNA_B^{Tyr}$ was present, and (2) purified $\underline{su(s)^2,v}$ $tRNA_B^{Tyr}$ was used as a substrate in various sorts of aminoacylation reactions to determine whether or not it would be charged.

Previous work had indicated that while Syn 3 had an absolute requirement for K^+, Syn 1 and Syn 2 did not. Perhaps the difference in isoacceptor specificity was related to the monovalent cation specificity. RPC-5 cochromatography of $[^2H]$Tyr-$tRNA^{Tyr}_{su(s)2,v}$ charged by Syn 1 in the presence of K^+ and $[^{14}C]$Tyr-$tRNA^{Tyr}_{su(s)2,v}$ charged by Syn 1 in the absence of K^+ showed that the isoacceptor specificity of Syn 1 was not dependent on the monovalent cation present: in both cases only $tRNA_A^{Tyr}$ was charged.

During a developmental study of tRNAs in both wild type and $\underline{su(s)^2,v}$ $\underline{Drosophila}$ White et al. [19] found that $\underline{su(s)^2,v}$ adult flies had only one major tyrosyl tRNA isoacceptor whereas wild type flies had two such isoacceptors. Their observation is consistent with the idea that they were using a Syn 1 type

of enzyme to perform their charging reactions. While this is a possible interpretation of their results, two other differences between their conditions and our own must be considered. Both the reaction temperature and the Mg^{++}/ATP ratio used by these authors were different from our own, so these variables were tested independently. Cochromatography of [^{3}H]Tyr-$tRNA^{Tyr}_{su(s)2,v}$ charged at 20°C (White's condition) with [^{14}C]Tyr-$tRNA^{Tyr}_{su(s)2,v}$ charged at 37°C (our condition) showed both isoacceptors aminoacylated under both conditions as in Figure 5C, page 23. Comparison of the Mg^{++}/ATP ratios also gave results as in Figure 5C.

Although divers combinations of substrate concentrations which might affect the specificity of Syn 3 exist, the rate limiting step in assaying such combinations was the need for a chromatographic identification of $tRNA^{Tyr}_{B}$. When $tRNA^{Tyr}_{B}$ was prepared as a substrate, many more concentration effects could be tested. Substrate and inhibitor concentrations at which aminoacylation of wild type $tRNA^{Tyr}$ and $tRNA^{Tyr}_{B}$ were compared are summarized in Table V. For each of the concentrations listed in the table, a separate charging assay was run using $tRNA^{Tyr}_{B}$ as a substrate so that the kinetics of tyrosyl-$tRNA^{Tyr}_{B}$ formation could be compared with those obtained for tyrosyl-$tRNA^{Tyr}_{wild\ type}$ formation at identical substrate concentrations. In every case, as indicated in the table, both the rate and extent of Tyr-$tRNA^{Tyr}_{B}$ and

TABLE V

SUBSTRATE AND INHIBITOR CONCENTRATIONS FOR COMPARISON OF $Tyr\text{-}tRNA^{Tyr}_{1,2}$ AND $Tyr\text{-}tRNA^{Tyr}_{B}$ FORMATION

Substrate or Inhibitor	Concentration (mM)	$Tyr\text{-}tRNA^{Tyr}_{1,2}$		$Tyr\text{-}tRNA^{Tyr}_{B}$	
		Rate[a]	Extent[b]	Rate[a]	Extent[b]
K^+	2	.25	7.5	.75	15.0
	8	.75	12.0	2.75	35.0
	20	1.25	12.5	5.00	45.0
Mg^{++}	10:5	2.50	17.5	6.50	60.0
	10:10	3.75	17.5	9.50	67.5
	10:25	.50	10.0	.50	22.5
	50:5	.25	5.0	1.25	28.7
	50:10	.75	8.7	2.00	40.0
	50:50	1.25	13.7	3.25	52.5
Tris	65	2.25	15.0	5.00	48.2
	95	1.75	13.2	4.00	44.5
	215	.50	7.5	1.25	25.0
Spermine	.5	1.10	12.5	7.25	60.0
	1	1.10	12.5	5.25	60.0
	3	.50	9.0	4.00	50.0
	8	.21	6.2	1.75	28.7
	16	.11	2.2	.75	10.7
pClHgBzOH	.002	.75	12.5	6.75	50.0
	.005	.43	10.0	2.00	46.2
	.008	.18	6.5	.75	11.7
	.020	.10	2.5	.25	9.0
	.050	.02	2.5	.25	12.5

[a]Rates are expressed as pmoles/A_{260}/min.

[b]Extents are expressed as pmoles/A_{260}/30 min at the completion of charging.

Tyr-$tRNA^{Tyr}_{wild\ type}$ formation were identical. The K_is found for pClHgBzOH and spermine as inhibitors of Tyr-$tRNA^{Tyr}_{B}$ formation were the same as those found for these compounds as inhibitors of wild type Tyr-$tRNA^{Tyr}$ formation. Finally, as indicated earlier, Figure 10, page 37, shows the heat inactivation of Syn 3 using wild type $tRNA^{Tyr}$ as a substrate. Repeating this type of heat inactivation using $tRNA^{Tyr}_{B}$ as a substrate gave identical results.

CHAPTER IV

DISCUSSION

The biochemical mechanism of genetic suppression in Drosophila remains elusive. In a model for a mechanism of suppression of the vermilion locus in Drosophila, Jacobson [20] proposed that the restored activity of tryptophan pyrrolase in su(s)^2,v flies existed because the su(s) mutation resulted in the loss of the second of two tyrosyl-tRNA isoacceptors. In the wild type situation, this isoacceptor was normally bound to tryptophan pyrrolase and in su(s)^2,v flies, even though the tryptophan pyrrolase itself was defective, since the tyrosine isoacceptor normally bound to it was absent, the enzyme was partially active. Further work clearly demonstrating that two $tRNA^{Tyr}$ isoacceptors exist in both wild type and su(s)^2,v flies [21], required a modification of the original model. If both wild type and suppressed strains had two $tRNA^{Tyr}$ isoacceptors, then the second isoacceptor of the su(s)^2,v strain, $tRNA_B^{Tyr}$, must be different from the second isoacceptor of the wild type strain, $tRNA_2^{Tyr}$, in such a way that $tRNA_B^{Tyr}$ did not inhibit tryptophan pyrrolase activity. Either $tRNA_B^{Tyr}$ was not bound to tryptophan pyrrolase at all, or it was sufficiently different from $tRNA_2^{Tyr}$ so that it did not prevent all enzyme activity. In either case, the tryptophan pyrrolase of vermilion Drosophila could distinguish

between $tRNA_2^{Tyr}$ and $tRNA_B^{Tyr}$. Since these two isoacceptors are both recognized by Syn 3, and since they both elute from an RPC-5 column in exactly the same position, they can be presumed to be rather similar structurally. (In fact, one explanation for the suppression of the vermilion locus in Drosophila attributes to the gene product of the su(s)^2 locus in the function of converting $tRNA_1^{Tyr}$ to $tRNA_2^{Tyr}$ [21]. When this gene product is defective $tRNA_B^{Tyr}$ results.) Thus while these isoacceptors are quite similar, the vermilion tryptophan pyrrolase can "recognize" the difference between them and it is not unreasonable to postulate that the tyrosyl tRNA synthetase could "recognize" the difference as well. This prediction was fulfilled by the Syn 1 type of enzyme isolated from Drosophila [14]. Since Syn 2, a nonisoacceptor-specific synthetase was isolated concurrently with Syn 1, the inability of Syn 1 to aminoacylate $tRNA_B^{Tyr}$ must be an "inherent" specificity rather than a partial loss of activity.

Continued work on the Drosophila tyrosyl tRNA synthetase has revealed a third form of the enzyme, Syn 3. Although this form had the same chromatographic properties as Syn 1, it did not have the same isoacceptor specificity. Extensive work with Syn 3 has failed to yield a relationship between it and Syn 1 and/or Syn 2. Also, imposing various kinds of "kinetic stress" conditions on Syn 3 has failed to create the isoacceptor specificity of the sort possessed by Syn 1.

As indicated in the introduction to this dissertation, most of the evidence for multiple synthetase comes from systems in which one of the synthetase activities is organellar and the other is cytoplasmic. In the cases where such intracellular compartmentalization cannot provide the rationale for the multiplicity, the evidence itself is not particularly convincing. In Drosophila the evidence for multiplicity is based on chromatographic fractionation, but the separation is not regularly reproducible. None of the usual means of distinguishing two distinct forms of enzyme activity within a single chromatographic peak of activity have provided evidence of multiplicity. Thus Syn 3 probably represents the single aminoacyl synthetase activity for tyrosine present in Drosophila. Syn 1 and Syn 2 may be related in some way to the degradation of Syn 3 and perhaps a partially degraded form of Syn 3 could lose the ability to aminoacylate $tRNA_B^{Tyr}$.

The relationship of Syn 3 to Syn 1 and Syn 2 is of continued interest. The tryptophan pyrrolase of vermilion flies could differentiate between $tRNA_2^{Tyr}$ and $tRNA_B^{Tyr}$. As stated earlier, if one enzyme can distinguish between two very similar isoacceptors, one may presume that other enzymes might be able to make the same distinction. If Syns 1 and 2 are partially degraded forms of Syn 3, the degradation is of a very specific sort--resulting in only a partial loss of activity for Syn 1. No matter what the mechanism of the

loss of activity may be, the enzyme activity which persists could be quite useful in determining the portions of $tRNA_2^{Tyr}$ which are essential for synthetase recognition.

The partial characterization of Drosophila tyrosyl tRNA synthetase contained within this dissertation provides several points for comparison with tyrosyl tRNA synthetases studied in other organisms. Those synthetases most extensively characterized are from bacteria [22,23,24] and yeast [25,26, 27]. The characteristics of these enzymes are compared with those of the Drosophila enzyme in Table VI. The discrepancies between the molecular weights and numbers of subunits found for the two yeast enzymes have not been resolved. At least one author has offered the suggestion that the Baker's yeast enzyme is only half of a dimer-type synthetase (but still functional) whereas the enzyme from the pure strain is actually also a dimer but hidden breaks within the dimer result in an apparently tetrameric synthetase []8]. According to the data included in the table as well as other sources [29,30], polyamines are generally more successful Mg^{++} substitutes in prokaryotic systems than they are in eukaryotic systems. The mechanism of synthetase action is altered when polyamines substitute for Mg^{++} [29], and perhaps this mechanistic flexibility is characteristic only of prokaryotic synthetases.

TABLE VI

COMPARISON OF TYROSYL tRNA SYNTHETASES OF VARIOUS ORGANISMS

	E. coli	*B. subtilis*	Baker's Yeast	*Saccharomyces cerevisiae*	*Drosophila melanogaster*
Molecular weight	95,000[22,23]	88,000[23]	46,500[26]	116,000[27]	100,000
Subunit mol. wt.	48,000[22]	-	40,000[26]	31,500[27]	-
Proposed # subunits	2[22]	-	1[26]	4[27]	-
Effect of polyamines	replace Mg^{++}[24]	-	cannot replace Mg^{++}[26]	-	cannot replace Mg^{++}
Apparent K_Ms					
Tyr	27 μM[23] 5 M[22]	48 μM[23]	5 μM[25]	-	2 μM
ATP	.4 mM[23]	.3 mM[23]	1 mM[25]	-	7 mM
tRNA	.1 μM[23]	.1 μM[23]	.2 μM[25]	.35 μM[27]	.3 μM

Note: Superscripts refer to corresponding entries in the References section.

The apparent Michaelis constants listed in the table deserve brief comment. All of the apparent K_Ms for the E. coli and B. subtilis synthetases were determined using E. coli tRNA--making all of the B. subtilis constants those of a heterologous systems. The K_M for tRNA for the Saccharomyces enzyme was determined using Baker's yeast tRNA again making a heterologous aminoacylation system. The amount of kinetic data available for tyrosyl tRNA synthetases is limited and hence correlations and conclusions drawn from the comparison of such data must be limited.

One interesting kinetic effect not included in the table was reported by Beikirch et al. [26]. These authors found that K^+ caused a 12-fold stimulation of the extent of aminoacylation of purified $tRNA^{Tyr}_{yeast}$. They found only a 4-fold stimulation of the extent of aminoacylation of crude $tRNA^{Tyr}_{yeast}$ and were unable to explain the discrepancy. Their data are presented as percentages and hence are difficult to compare with data of other authors.

The only other Drosophila aminoacyl tRNA synthetase which has been characterized to date is that for phenylalamine [31]. This is the largest synthetase, 180,000 daltons, in single celled organisms and appeared to be at least as large in Drosophila [31]. This enzyme has a narrow temperature optimum (35°-40°C), but has broad optima for both pH and Mg^{++} concentration.

Although the comparable data are limited, it appears that the tyrosyl tRNA synthetase described in this dissertation is rather unique in several ways. First, unlike the other synthetases listed, the Drosophila synthetase activity is inhibited by higher than optimal concentrations of Mg^{++} and ATP. Second, the other tRNA synthetases listed here are stable to gradient elution, a characteristic not found in the Drosophila synthetase. Finally, rechromatography of Syn 3 on DEAE-cellulose results in an apparently temperature-sensitive enzyme.

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VITA

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