Chem. 241, 1819.
Hunter, F. R. (1961), *J. Cellular Comp. Physiol. 58*, 203.
Klipstein, F. A., and Ranney, H. M. (1960), *J. Clin. Invest. 39*, 1894.
Kruh, J., and Borsook, H. (1956), *J. Biol. Chem. 220*, 905.
Marks, P. A., Rifkind, R. A., and Danon, D. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 336.
Marks, P. A., Willson, C., Kruh, J., and Gros, F. (1962), *Biochem. Biophys. Res. Commun. 8*, 9.
Mitchell, C. D., and Hanahan, D. J. (1966), *Biochemistry 5*, 51.
Palade, G. E., and Siekevitz, P. (1956), *J. Biophys. Biochem. Cytol. 2*, 671.
Rimington, C. (1942), *Brit. Med. J. 1*, 177.
Scherrer, K., and Darnell, J. E. (1962), *Biochem. Biophys. Res. Commun. 7*, 486.
Schlessinger, D. (1963), *J. Mol. Biol. 7*, 569.
Schneider, W. C. (1957), *Methods Enzymol. 3*, 680.
Seeman, P. (1967), *J. Cell Biol. 32*, 55.
Starr, J. L., and Ramberg, B. (1966), *Nature 211*, 414.
Ts'o, P. O. P. (1962), *Ann. Rev. Plant Physiol. 13*, 45.

Inhibition of Aminoacyl Transfer Ribonucleic Acid Synthetases by Modified Transfer Ribonucleic Acids*

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ABSTRACT: A series of transfer ribonucleic acids (tRNAs) which have been subjected to various modifications at their acceptor ends was prepared. The rate of esterification of lysine, valine, and phenylalanine to tRNA in the presence and absence of each of the modified tRNAs was determined. The pattern of inhibition was different in each case, suggesting that each enzyme had different requirements for tRNA binding. The rate of valine and phenylalanine esterification to tRNA was measured over a range of magnesium ion concentrations and the response to free magnesium was different in each case. Optical rotatory dispersion studies of degraded tRNAs suggested that the conformations of all of them were very similar to native tRNA. Treatment of tRNA with sodium borohydride did not affect acceptor activity for lysine, valine, or phenylalanine. Despite only very slight inhibition of phenylalanine esterification to tRNA by periodate-oxidized tRNA it was shown that periodate causes no detectable alteration of phenylalanyl-tRNA. The findings are discussed in relation to other studies employing tRNAs with modified acceptor ends.

The specificity of interactions between tRNA and aminoacyl-tRNA synthetases is of the greatest importance in maintaining accuracy in translation of the genetic message. The challenge of elucidating the role of various structural features of tRNA in achieving this high degree of specificity has attracted many investigators, and a number of approaches have been used by them in attempts to answer this challenge. Because all functional species of tRNA are assumed to terminate at the 3′ (acceptor) end in the sequence cytidylyl(3′→5′)cytidylyl(3′→5′)adenosine (CpCpA), several studies have focused on the role, if any, of this end group in recognition by aminoacyl-tRNA synthetases. Hayashi and Miura (1964) and Torres-Gallardo and Kern (1965) independently showed inhibition of valine esterification to tRNA by periodate-oxidized tRNA. The latter workers established by kinetic data that this inhibitor was competitive with tRNA and that for the esterification of both valine and tyrosine in *Escherichia coli* the inhibitory species was the oxidized homologous tRNA and not some other species. Snake venom phosphodiesterase treatment of tRNA was also reported to produce a competitive inhibitor but with a higher K_i value.

Korzhov and Sandakhchiev (1966) have reported inhibition of valine, lysine, and alanine esterification to tRNA by $tRNA_{ox}$. They further showed that the

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[1] Abbreviations used: $tRNA_{ox}$, periodate-oxidized tRNA; $tRNA_{ox-red}$, tRNA which has been oxidized with periodate and then reduced with sodium borohydride; tRNA(-A), tRNA with the terminal adenosine removed; tRNA(-pA), tRNA(-CpA), tRNA(-pCpA), tRNA(-CpCpA), and tRNA(-pCpCpA), tRNAs with the group in parentheses removed; $tRNA^{Val}$, tRNA which accepts valine; valyl-tRNA, tRNA with valine esterified to the terminal adenosine; E_{Val}, the valyl-tRNA synthetase; TCA, trichloroacetic acid; ATP, adenosine triphosphate.

degree of inhibition of lysine esterification was decreased by sodium borohydride reduction of the oxidized end group and by treatment of the $tRNA_{ox}$ with amines or thiosemicarbazide.

On the other hand, Lagerkvist *et al.* (1966) showed a specific interaction of yeast $tRNA^{Val}$ with highly purified yeast E_{Val} which is not inhibited by yeast $tRNA_{ox}$ or by yeast tRNA(-A). Makman and Cantoni (1966) have shown that yeast $tRNA^{Ser}$(-pA) does not inhibit serine esterification to adenylate-terminated yeast $tRNA^{Ser}$ when a crystalline E_{Ser} (yeast) is used. Baldwin and Berg (1966) reported that a purified *E. coli* E_{Ile} could form an enzyme-bound valyladenylate in the presence of valine, ATP, and magnesium ions and that this broke down rapidly on addition of *E. coli* $tRNA^{Ile}$. They found that periodate oxidation of the $tRNA^{Ile}$ abolished its capacity to cause this breakdown, as did elimination of the terminal adenosine or the terminal -pCpA sequence. They reported that this latter material (tRNA(-pCpA)) and also $tRNA_{ox}$ weakly inhibited isoleucine esterification to $tRNA^{Ile}$ while tRNA(-A) did not.

It is the purpose of the present report to show that despite the probable similarity of mechanism, inhibition data obtained with one amino acid are not necessarily applicable to tRNA aminoacylation studies using other amino acids, insofar as modifications of the acceptor end of the tRNA molecules are concerned. Our results indicate that very different degrees of inhibition are obtained with valyl-, lysyl-, and phenylalanyl-tRNA modified at the acceptor end. In addition it is shown that to obtain meaningful results in inhibition studies a careful control over the concentration of free magnesium ion is required.

Materials and Methods

The tRNA used in these experiments was isolated from brewer's yeast and purchased from C. F. Boehringer & Soehn. It was found by terminal nucleoside analysis to have over 90% of chains terminated at the 3′ end by adenosine. The amino acids used were uniformly labeled with ^{14}C and of the following specific activities: L-valine (194 mc/mmole), L-lysine 259 (mc/mmole), and L-phenylalanine (424 mc/mmole). They were purchased from New England Nuclear Corp.

The preparation of $tRNA_{ox}$ involved incubation of 30–60 mg of tRNA in 1 ml of 0.02 M periodate (made by dissolving sodium metaperiodate in distilled water to the appropriate concentration) at 22–24° for 1 hr in the dark. The reaction was terminated by the addition of excess ethylene glycol followed by incubation for a further 15 min, or by the addition of 0.2 ml of 0.1 M sodium arsenite. The solution in either case was chromatographed on a Sephadex G-25 column (1.2 × 60 cm) which had been preequilibrated with 0.5 M sodium chloride. Elution was with the same molarity of sodium chloride and 2–2.5-ml fractions were collected. Two volumes of ethanol was added to tubes containing $tRNA_{ox}$ and the precipitates which formed were collected by centrifugation after chilling in an ice bath. Precipitated $tRNA_{ox}$ was dissolved in distilled water and lyophilized. It was finally redissolved in distilled water to a concentration of 400 A_{260} units/ml.

The $tRNA_{ox-red}$ was prepared by making a solution of $tRNA_{ox}$ 0.1 M in potassium phosphate buffer (pH 7.5) and adding solid sodium borohydride (2 mg/20 mg of $tRNA_{ox}$). The pH of the solution was measured throughout the reaction and maintained between 8.4 and 8.8 by the addition of 1 M potassium phosphate (pH 7.5). After 1 hr at room temperature (22–24°) the reaction mixture was chromatographed on a Sephadex G-25 column and isolated exactly as described above. This sample and all other samples of modified tRNAs were dissolved in distilled water to a concentration of 400 A_{260} units/ml.

Whitfeld degradations were performed under the conditions described by Neu and Heppel (1964) except that a solution of 1 M lysine (pH 8.5) was used for amine cleavage. All reactions of this type were carried out on a 10–40 times larger scale than they described. Dephosphorylation of degraded chains was also carried out using their conditions but with 1 unit (Garen and Levinthal, 1960) of *E. coli* alkaline phosphatase (Worthington)/20 mg of tRNA. This enzyme was removed after incubation by phenol extraction (three times with an equal volume) and residual phenol was removed by ether extraction. After each step of degradation and dephosphorylation the degraded tRNA was freed of low molecular weight reactants and products by chromatography on Sephadex G-25 in 0.5 M sodium chloride, as described above.

Amino acid acceptor assays were performed by a modification of the method of Mans and Novelli (1961). Reaction mixtures contained 3 μmoles of magnesium chloride, 1.6 μmoles of ATP, 12 μmoles of pH 7.5 sodium cacodylate (or 12 μmoles of Tris-HCl (pH 7.8) for the valine assays), 12 A_{260} units of tRNA, 12 A_{260} units of a compound to be tested as an inhibitor, and distilled water to a volume of 110 μl, except as otherwise noted. The assay was begun by addition of 20 μl of enzyme, followed in exactly 1 min by a 10-μl aliquot of [^{14}C]amino acid (0.1 μc). Incubation was in a water bath maintained at 25°. Samples of 25 μl of reaction mixture were removed and spread evenly over 2.4-cm circles of Whatman 3MM filter paper at 1–5 min after addition of the amino acid. The reaction was stopped at an exact time by immersing the disk in a bath of cold 10% TCA. Each disk was washed for at least 8 min with occasional swirling in each of four baths of 10% TCA. Next the disks were washed in ethanol and twice in ether, then dried under a heat lamp. Radioactivity was measured by scintillation counting using a toluene base scintillation fluid. Initial rates of reaction were measured by plotting the data directly and taking the best straight line.

Enzyme was prepared by grinding freshly pressed baker's yeast with an equal weight of alumina (Fisher) by hand in a mortar and pestle, precooled to −18°. Grinding for 15–20 min was sufficient to cause extensive cell breakage. The resulting paste was taken up in a small volume (30 ml for 20 g of yeast) of a buffer which

TABLE I: Percentage of Control Reaction Rate for Esterification of Amino Acids to tRNA in the Presence of Eight Potential Inhibitors of Aminoacylation.

Inhibitor	Phenylalanine	Phe[a] + Inhibitor + Mg^{2+}	Lysine	Valine	Val[b] + Mg^{2+}	Val[c] + Inhibitor + Mg^{2+}
$tRNA_{ox}$	105	80	43	61	76	38
$tRNA_{ox-red}$	107	105	84	59	72	50
tRNA(-A)	114	105	62	35	44	
tRNA(-pA)	119	120	100	28	49	26
tRNA(-CpA)	105	93	89	57	77	57
tRNA(-pCpA)	105	102	91	70		
tRNA(-CpCpA)	104	107	78	57	71	54
tRNA(-pCpCpA)	105	102	87	57		
$+Mg^{2+}$ control		72				

[a] Inhibitor (48 A_{260} units) was used rather than the conventional 12 units, and all incubations contained an additional 2 μmoles of $MgCl_2$. [b] An additional 2 μmoles of $MgCl_2$ was included in all incubations in this column. [c] Inhibitor (24 A_{260} units) was used rather than the conventional 12 units and all incubations contained an additional 2 μmoles of $MgCl_2$.

was 0.05 M in Tris-HCl (pH 7.8), 0.005 M in magnesium chloride, and 0.001 M in EDTA. The resulting suspension was centrifuged for 10 min at 15,000g in a Sorvall SS-1 centrifuge and the supernatant was removed. This was centrifuged for 2 hr at 136,000g in a Spinco Model L ultracentrifuge (40 rotor). Solid ammonium sulfate was added to the high-speed supernatant to 80% saturation. The precipitate which formed within 30 min was collected by centrifugation at 15,000g for 10 min. It was redissolved in the previously described buffer and loaded onto a column (2.5 × 40 cm) of Bio-Gel P-4 (Bio-Rad Laboratories) preequilibrated with this buffer. This was followed by elution with the same buffer to remove ammonium sulfate. The enzyme solution from the Bio-Gel column was made 40% in glycerol and stored at −18°. Before use it was usually diluted with an equal volume of distilled water.

Results

Noninhibition of Phenylalanine Esterification to tRNA. Several preliminary experiments showed that under the conditions previously described for measurement of inhibition, phenylalanine esterification to tRNA was not significantly inhibited by equal quantities of $tRNA_{ox}$, $tRNA_{ox-red}$, tRNA(-A), -(-pA), -(-CpA), -(-pCpA), -(-CpCpA), or -(-pCpCpA) as shown in Table I. Under these conditions all rates were about 5% greater than the control except those for the reactions containing tRNA(-A) and tRNA(-pA). This latter compound would be expected to be a substrate for the tRNA-CCA pyrophosphorylase which is present in our enzyme. It is well established that tRNA binds magnesium ions (Felsenfeld and Huang, 1961) so a study of the effect of varying the magnesium ion concentration on phenylalanine esterification was undertaken. Figure 1 shows that with 1.6 μmoles of ATP present the rate of esterification of phenylalanine reaches a maximum at about 2.25 μmoles of $MgCl_2$ and decreases at higher concentrations. Makman and Cantoni (1965) reported that maximal reaction rates for E_{Ser} were reached at ratios of Mg:ATP of 1.5–2.5 The increased rates of phenylalanine esterification in the presence of the degraded tRNAs might therefore be attributed to the decrease in free magnesium ion concentration toward

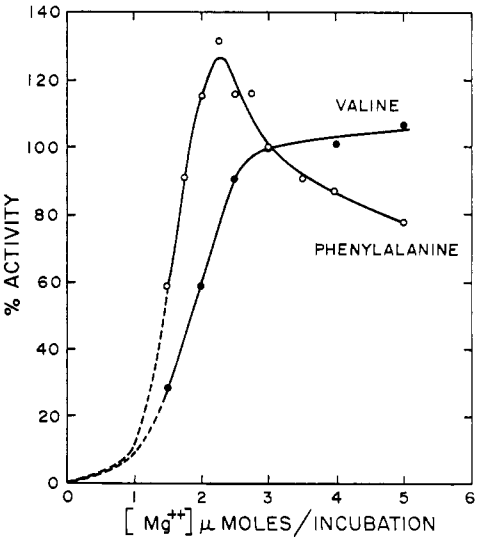

FIGURE 1: Variation in rate of phenylalanine and valine esterification to tRNA using the assay described in Materials and Methods but with varying quantities of magnesium ion.

its optimum level resulting from the addition of more polynucleotide than in the control.

Attempts were made to measure or detect inhibition of phenylalanine esterification by levels of degraded or modified tRNA four times the level of native tRNA. It was necessary in this case to add magnesium ion since the amount of polynucleotide added would bind a considerable portion of that present. Accordingly, 2 μmoles of magnesium chloride was added to all incubation mixtures. Controls were run with and without the additional magnesium. Table I shows that $tRNA_{ox-red}$, tRNA(-A), tRNA(-pCpA), tRNA(-CpCpA), and tRNA(-pCpCpA) are all noninhibitory using the control without additional magnesium chloride as the 100% value. The slight (7%) inhibition seen with tRNA(-CpA) may be real but is rather insignificant when compared with inhibitions seen in experiments with lysine and valine where equal quantities of tRNA and inhibitor were used. The 20% inhibition seen when the $tRNA_{ox}$ to tRNA ratio is four is significant and indicates a probable interaction between the $tRNA_{ox}^{Phe}$ and the E_{Phe}.

As would be predicted from Figure 1 the rate of phenylalanine esterification is inhibited by about 30% on addition of an extra 2 μmoles of magnesium chloride. The rate of phenylalanine esterification to tRNA in the presence of tRNA(-pA) is higher than control, again probably as a result of repair by the tRNA-CCA pyrophosphorylase. This enhancement is not seen with the other two amino acids studied because the tRNA(-pA) is an inhibitor of the reaction and masks repair.

Because of the weak or complete lack of inhibition seen in these studies with phenylalanine it was considered possible that periodate was destroying some site in $tRNA^{Phe}$ which might be of great importance in recognition by the yeast E_{Phe}. To test this possibility the following experiment was performed. tRNA (60 A_{260} units) was esterified with phenylalanine by reaction with 100 mμmoles of [^{12}C]phenylalanine and 2.36 mμmoles of [^{14}C]phenylalanine. Five 20-μl aliquots (2.67 A_{260} units of tRNA each) were removed for determination of radioactivity incorporated at 5, 11, 21, 40, and 60 min. Maximum esterification was achieved within 11 min but the reaction was allowed to proceed for 60 min. Potassium acetate (one volume of 1 M, pH 4.6) was added and the tRNA was isolated by precipitation with two volumes of ethanol. The tRNA was redissolved in potassium acetate and precipitated twice more. Oxidation with a tenfold excess of periodate over terminal nucleoside was carried out at pH 4.6 for 60 min in the dark at 22°. The reaction was terminated by the addition of a large excess of ethylene glycol and the sample was chromatographed on Sephadex G-25 in 0.5 M sodium chloride, as described in Materials and Methods. The tRNA was precipitated as described and then redissolved in 1 M Tris-HCl (pH 7.8). After 60 min at 22° two volumes of ethanol was added, the tube was chilled, and tRNA was isolated by centrifugation. The tRNA was precipitated twice more to remove all remaining phenylalanine and then lyophilized. It was then tested and found to accept phenylalanine at the same rate as untreated tRNA.

Inhibition of Lysine Esterification by Degraded tRNA. Table I shows that lysine esterification to tRNA is inhibited strongly only by $tRNA_{ox}$ and tRNA(-A). Reduction of $tRNA_{ox}$ with sodium borohydride was sufficient to eliminate most of its inhibitory capacity, as was removal of more than the terminal adenosine. The loss of inhibitory capacity on reduction was shown to be due to the end-group reduction rather than some other reaction by the following experiment. tRNA was incubated with sodium borohydride under exactly the conditions described previously for conversion of $tRNA_{ox}$ to $tRNA_{ox-red}$. After the described purification it was found to accept lysine, valine, and phenylalanine as efficiently as untreated tRNA. Korzhov and Sandakhchiev (1966) also found that sodium borohydride treatment had no effect on the ability of tRNA to accept lysine. A sample of $tRNA_{ox-red}$ was shown to have the expected end group by complete digestion with pancreatic RNase followed by chromatography on DEAE-cellulose. The majority of the neutral (nonabsorbed) material had an adenosine-like spectrum, but moved on paper chromatography faster than adenosine (R_F 0.82, R_F adenosine 0.72 in propan-2-ol–water–concentrated ammonium hydroxide(7:2:1)). The higher R_F corresponded exactly with that of a compound prepared by oxidation and reduction of adenosine by the method described by Khym and Cohn (1960). This compound, a triprimary alcohol derivative of adenine, was isolated from $tRNA_{ox-red}$ in the same yield as was adenosine from unaltered tRNA.

In other experiments performed under conditions similar to those used in Table I even less inhibition than is shown there was found with tRNA(-CpA), -(-pCpA), -(-CpCpA), and -(-pCpCpA). It should be noted that inhibition of lysine esterification by $tRNA_{ox}$ is variable. The degree of inhibition is apparently a function of the age of the preparation of $tRNA_{ox}$ used.

Inhibition of Valine Esterification by Degraded tRNAs. Valine esterification was inhibited by all species tested (Table I). The most marked inhibitions are seen with tRNA(-A) and tRNA(-pA), while all others are less inhibitory. The difference in inhibition by tRNA(-pCpA) from that of tRNA(-CpA), tRNA(-CpCpA), and tRNA(-pCpCpA) is probably not real as other experiments have shown all four rates to be essentially the same. Little difference is seen in this case between the effects of $tRNA_{ox}$ and $tRNA_{ox-red}$. Other experiments have shown somewhat higher inhibition by $tRNA_{ox}$ but this is variable. As seen in Figure 1 the rate of valine esterification is dependent on the concentration of available magnesium ion. In contrast to phenylalanine esterification, valine esterification does not appear to be inhibited by higher levels of magnesium ion.

In order to assess the contribution of magnesium binding to the inhibitions of valine esterification, some of the incubations were repeated with an additional 2

μmoles of magnesium chloride present. In some cases two levels of inhibitor were also tested. The results indicated that a small but significant proportion of the inhibitions seen with 3 μmoles of magnesium chloride was a consequence of insufficient magnesium ion.

A problem was encountered in the experiments with valine which was probably the result of not using a highly purified enzyme. The extent of esterification after 5 min was in all cases about equivalent to that of the 4th min. Occasionally the latter value was also lower than expected. We attribute this to the presence of an enzyme which destroys valine or deacylates valyl-tRNA. However, in all cases samples from the first 3 or 4 min gave good linear plots.

Optical Dispersion Studies. Samples of untreated tRNA and of tRNA(-A), -(-pA), -(-CpA), -(-pCpA), -(-CpCpA), and -(-pCpCpA) were dissolved at a concentration of about 2 A_{260} units/ml in 0.05 M sodium cacodylate (pH 7.5), 2 mM magnesium chloride, and 0.2 mM EDTA and their optical rotatory dispersion was measured over the range 320–220 mμ using a Jasco optical rotatory dispersion 5 spectropolarimeter. The differences in the optical rotatory dispersion curves were too slight to suggest any significant change in the secondary structure of these various tRNA molecules.

A sample of 27-fold-purified $tRNA^{Phe}$ (yeast) was obtained from Dr. I. H. Maxwell of this laboratory and its optical rotatory dispersion was measured in 0.05 M cacodylate (pH 7.5) and 5 mM magnesium chloride. Immediately afterward it was oxidized with periodate, lyophilized, chromatographed on Sephadex G-25 in distilled water, lyophilized again, and redissolved in the cacodylate buffer. Another optical rotatory dispersion measurement was taken of the $tRNA_{ox}^{Phe}$ which proved to be very similar to that of $tRNA^{Phe}$.

Discussion

Because of the apparent similarities in the secondary structure of those tRNAs whose sequences are known and the similarity of function of aminoacyl-tRNA synthetases it might seem reasonable to suppose that each of these enzymes would recognize certain structural features of its tRNA substrate in a similar manner. That this is not the case with the acceptor end of tRNA molecules is evident from the findings reported here. Our data confirm previous observations of inhibition of valine esterification by $tRNA_{ox}$ (Hayashi and Miura, 1964; Torres-Gallardo and Kern, 1965; Korzhov and Sandakhchiev, 1966). We also confirm the report by these latter authors of inhibition of lysine esterification to tRNA by $tRNA_{ox}$ and the partial loss of this inhibition which occurs on borohydride reduction of $tRNA_{ox}$.

Our findings indicate that of the eight modified tRNA species tested, only $tRNA_{ox}$ is weakly inhibitory to phenylalanine esterification. This suggests that any modification of the acceptor end of yeast $tRNA^{Phe}$ makes it unrecognizable to E_{Phe} of yeast. That oxidation of the terminal nucleoside by periodate does not cause major disruption of the secondary structure of $tRNA^{Phe}$ is shown by optical rotatory dispersion measurements. Disruption of hydrogen bonding and base stacking has been shown to cause considerable decrease in the rotation of tRNA in the 320–220-mμ range, and also a shift in the center of the Cotton effect. In our work both $tRNA^{Phe}$ and $tRNA_{ox}^{Phe}$ had similar optical rotatory dispersion curves and therefore probably very similar secondary structures. The optical rotatory dispersion measurements with mixed tRNA and its degraded derivatives indicated that the chemical and enzymic procedures to which they were subjected caused little change in the secondary structure of the component molecules. The possibility that some additional site or conformation in $tRNA^{Phe}$, important for recognition by E_{Phe}, was being altered by periodate seems unlikely since phenylalanyl acceptor activity was not lost when phenylalanyl-$tRNA^{Phe}$, was treated with periodate, deacylated, and reassayed.

It was reported (Torres-Gallardo and Kern, 1965) that partial digestion of tRNA with snake venom diesterase, sufficient to remove an average of 2.25 nucleotides/chain according to Zubay and Takanami (1964), caused that tRNA in *E. coli* to become an inhibitor of valine esterification, although a less effective one than the $tRNA_{ox}$. Our data indicate that yeast tRNA which was chemically degraded to the same extent (and probably much less heterogeneous) causes about as much inhibition of valine esterification as yeast $tRNA_{ox}$. The extent of inhibition of valine esterification by $tRNA_{ox}$ varies with the investigator doing the experiment. Thus Hayashi and Miura (1964) report 50% inhibition by a quantity of $tRNA_{ox}$ equal to tRNA. (They used equal quantities of ATP and magnesium chloride, however, and our findings would suggest that this would cause them to see anomalously high inhibitions.) Torres-Gallardo and Kern (1965) report about 30% inhibition after a 20-min incubation period with equal quantities of tRNA and $tRNA_{ox}$, while Korzhov and Sandakhchiev (1966) report nearly 50% inhibition with only half as much $tRNA_{ox}$ as tRNA. Our own findings indicate 25–35% inhibition of the rate of tRNA esterification by an equal quantity of $tRNA_{ox}$, but the results were somewhat variable. The possibility exists that the configuration of the hydroxyl groups of the hydrated dialdehyde end group can change, and in changing cause greater or less inhibition.

It is interesting to compare the response to tRNA-(-pA) of the yeast E_{Val} with that of the yeast E_{Ser} reported by Makman and Cantoni (1966). These authors found a complete lack of effect of $tRNA^{Ser}$-(-pA) on serine esterification to $tRNA^{Ser}$, whereas with valine esterification we find that both tRNA(-A) and tRNA(-pA) inhibit the reaction by 55 and 50% when present at concentrations equal to that of the tRNA. Such high degrees of inhibition suggest that these two degraded species should be bound to E_{Val} as firmly as the intact tRNA. Binding of $tRNA^{Va}$ to yeast E_{Val} has recently been reported by Lagerkvist *et al.* (1966) to occur in the absence of ATP, magnesium ion, and valine. Formation of this E_{Val}–$tRNA^{Val}$

complex was not inhibited by even a tenfold excess of $tRNA_{ox}$ or tRNA(-A). They noted that this complex was relatively stable but that even at very high levels of tRNA the E_{Val} was not more than 55% complexed with $tRNA^{Val}$. This binding might therefore be interpreted as being a different mode of binding than that involved in valine esterification of tRNA.

In contrast to the almost complete lack of inhibition by modifiedt RNAs shown by E_{Phe} and the high sensitivity to these of E_{Val}, the E_{Lys} shows an intermediate sensitivity. It is quite variable with respect to inhibition by $tRNA_{ox}$ and this again may reflect changes in configuration of the terminal hydroxyls of $tRNA_{ox}$ as mentioned previously. In no case was inhibition of lysine esterification as high as that reported by Korzhov and Sandakhchiev (1966). They found 60% inhibition of rate at a ratio of tRNA:$tRNA_{ox}$ of 4:1 and 70% inhibition at a ratio of 2:1.

The conditions of their treatment of $tRNA_{ox}$ with lysine or cyclohexylamine to form the putative Schiff's bases might be expected actually to cause considerable elimination of the oxidized terminal nucleoside to form tRNA(-A). The reduced inhibition observed with these derivatives would fit our data for inhibition by tRNA(-A). Treatment of tRNA(-A) with alkaline phosphatase is sufficient to convert an effective inhibitor into a noninhibitory species. This suggests a specific binding site on E_{Lys} for that particular phosphate (assuming that the 5′-terminal phosphate is unnecessary (Harkness and Hilmoe, 1962)). The low levels of inhibition shown for tRNA(-CpA), -(-pCpA), -(-CpCpA), and -(-pCpCpA) may nevertheless be real and reflect a weak recognition of some part of the tRNA or may possibly be an effect of binding of magnesium ion to the added polynucleotide since the effect of magnesium chloride concentration on lysine esterification to tRNA has not yet been studied.

Baldwin and Berg (1966) reported that the valyl-adenylate formed by *E. coli* E_{Ile} was specifically and rapidly hydrolyzed on addition of *E. coli* $tRNA^{Ile}$ to the system, but not on addition of $tRNA_{ox}^{Ile}$, tRNA-(-A), or a tRNA(-pCpA) prepared by treating tRNA with snake venom phosphodiesterase. They did, however, mention that both $tRNA_{ox}$ and tRNA(-pCpA) weakly inhibited isoleucine esterification to tRNA while tRNA(-A) did not. The requirements of their E_{Ile} for inhibition by modified tRNAs might be reinvestigated with a more complete range of degraded tRNA molecules, thereby clarifying the acceptor end-group requirements for recognition.

The data presented here indicate a great variation in effects of tRNA molecules with modified acceptor ends on aminoacylation of intact tRNAs. They show that in at least these three cases each aminoacyl-tRNA synthetase must be treated as a unique entity, and that results obtained with one enzyme are not necessarily applicable to another. The remaining aminoacyl-tRNA synthetases of yeast will have to be examined for susceptibility to inhibition by these altered tRNAs to determine whether or not any patterns exist. Degradations further along the chain may not be as informative since the secondary structure of the tRNA might be altered. The cloverleaf conformations suggested by Holley *et al.* (1965), Madison *et al.* (1966), Zachau *et al.* (1966a,b), and RajBhandary *et al.* (1967) for alanine, tyrosine, and two serine and phenylalanine tRNAs show the probability of double-helix formation only one base past the terminal pCpCpA sequence in all five cases.

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References

Baldwin, A. N., and Berg, P. (1966), *J. Biol. Chem. 241*, 839.

Felsenfeld, G., and Huang, S. (1961), *Biochim. Biophys. Acta 51*, 19.

Garen, A., and Levinthal, C. (1960), *Biochim. Biophys. Acta 38*, 470.

Harkness, D. R., and Hilmoe, R. J. (1962), *Biochem. Biophys. Res. Commun. 9*, 393.

Hayashi, H., and Miura, K. (1964), *J. Mol. Biol. 10*, 345.

Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merill, S. H., Penswick, J. R., and Zamir, A. (1965), *Science 147*, 1462.

Khym, J. X., and Cohn, W. E. (1960), *J. Am. Chem. Soc. 82*, 6380.

Korzhov, V. A., and Sandakhchiev, L. S. (1966), *Biokhimiya 31*, 71.

Lagerkvist, U., Rymo, L., and Waldenström, J. (1966), *J. Biol. Chem. 241*, 5391.

Madison, J. T., Everett, G. A., and Kung, H. (1966), *Science 153*, 531.

Makman, M. H., and Cantoni, G. L. (1965), *Biochemistry 4*, 1434.

Makman, M. H., and Cantoni, G. L. (1966), *Biochemistry 5*, 2246.

Mans, R. J., and Novelli, G. D. (1961), *Arch. Biochem. Biophys. 94*, 48.

Neu, H., and Heppel, L. A. (1964), *J. Biol. Chem. 239*, 2927.

RajBhandary, U. L., Chang, S. H., Stuart, A., Faulkner, R. D., Hoskinson, R. M., and Khorana, H. G. (1967), *Proc. Natl. Acad. Sci. U. S. 57*, 751.

Torres-Gallardo, J., and Kern, M. (1965), *Proc. Natl. Acad. Sci. U. S. 53*, 91.

Zachau, H., Dütting, D., Feldmann, H., Melchers, F., and Karau, W. (1966a), *Cold Spring Harbor Symp. Quant. Biol.* (in press).

Zachau, H., Dütting, D., Feldmann, H., Melchers, F., and Karau, W. (1966b), *Angew. Chem. Intern. Ed. Engl. 5*, 422.

Zubay, G., and Takanami, M. (1964), *Biochem. Biophys. Res. Commun. 15*, 207.