Effects of Amino Acid Structure, Ionic Strength, and Magnesium Ion Concentration on Rates of Nonenzymic Hydrolysis of Aminoacyl Transfer Ribonucleic Acid†

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ABSTRACT: We have used the results of studies of heterologous aminoacylation reactions to obtain purified *Escherichia coli* valine tRNA and *Neurospora crassa* phenylalanine tRNA, each charged with [^{14}C]valine and [^{14}C]phenylalanine. With these preparations it was possible to study the rates of nonenzymic hydrolysis of aminoacyl-tRNAs under conditions in which any contributions of tRNA structure in the vicinity of the ester bond would be identical for both amino acids, and consequently differences in hydrolysis rates could be attributed solely to amino acid structure. The two valyl-tRNAs deacylated at similar rates, as did the two phenylalanyl-tRNAs. The valyl-tRNAs, however, were about four times more stable than the phenylalanyl-tRNAs. Increases in KCl or magnesium acetate levels increased the rates of nonenzymic hydrolysis of both valyl-tRNA and phenylalanyl-tRNA, though the effect was greater with valyl-tRNA. The increase in hydrolysis rate caused by 1 mM magnesium acetate was duplicated by KCl at 100–200 mM.

Nonenzymic hydrolysis of the aminoacyl-tRNA ester bond is strongly dependent on hydroxide ion concentration at pH greater than 6. Recent studies of heterologous aminoacylation reactions (Ritter *et al.*, 1970; Strickland and Jacobson, 1972) have made it possible to obtain a single species of tRNA charged with two different amino acids. We have prepared purified *Escherichia coli* valine tRNA ($tRNA^{Val}$) and *Neurospora crassa* phenylalanine tRNA ($tRNA^{Phe}$), each charged with [^{14}C]valine and [^{14}C]phenylalanine. With these preparations it was possible to determine the rates of nonenzymic hydrolysis of aminoacyl-tRNAs under conditions in which any contributions of tRNA structure in the vicinity of the ester bond would be identical for both amino acids. We also examined the effects of ionic strength and magnesium ion concentration on the rate of hydrolysis.

Materials and Methods

Materials. L-[^{14}C]Phenylalanine (566 dpm/pmole) was obtained from Schwarz BioResearch. L-[^{14}C]Valine (457 dpm/pmole) was a product of New England Nuclear Corp.

E. coli $tRNA^{Val}$ was purified by reversed-phase chromatography according to Weiss *et al.* (1968) and was generously provided by them. Acceptance was 1100 pmoles/A_{260}. *N. crassa* $tRNA^{Phe}$ (acceptance 570 pmoles/A_{260}) was purified on DEAE-cellulose and benzoylated DEAE-cellulose essentially according to Gillam *et al.* (1967). Valine acceptance of this preparation was less than 1 pmole/A_{260}.

Val-tRNA synthetase (L-valine:tRNA ligase (AMP), EC 6.1.1.9) was purified from *Escherichia coli* by a modification of the method of George and Meister (1967) and was homogenous as judged by polyacrylamide gel electrophoresis. Specific activity was 2.1×10^5 units/mg of protein, where a unit is that amount of enzyme which produces 1 pmole of aminoacyl-tRNA/ml per min. Phe-tRNA synthetase (L-phenylalanine:tRNA ligase (AMP), EC 6.1.1.4) was purified from *N. crassa* by the method of Kull and Jacobson (1971). Specific activity was 2.0×10^4 units/mg of protein.

Magnesium acetate, dimethyl sulfoxide, and potassium chloride were Baker analyzed reagents. Tris-HCl was prepared from Trizma base (Sigma Chemical Co.). Cacodylic acid was obtained from Fisher Scientific Co. ATP was a product of P-L Biochemicals. All solutions were prepared with glass-distilled water.

Charging of tRNAs. All charging reactions contained 7.5 mM magnesium acetate and 0.5 mM ATP. In addition, the mixture for charging $tRNA^{Val}$ (*E. coli*) with valine contained 50 mM Tris-HCl (pH 8), 25 μM [^{14}C]valine, 2.6 A_{260}/ml of Val-tRNA, and 630 units/ml of Val-tRNA synthetase (*E. coli*). For charging $tRNA^{Val}$ (*E. coli*) with phenylalanine, the reaction mixture contained 50 mM potassium cacodylate (pH 6.3), 24 μM [^{14}C]phenylalanine, 2.6 A_{260}/ml of Val-tRNA, and 91 units/ml of Phe-tRNA synthetase (*N. crassa*). For charging $tRNA^{Phe}$ (*N. crassa*), each reaction mixture contained 3.5 A_{260}/ml of Phe-tRNA and 50 mM Tris-HCl (pH 8). For charging with valine, reaction mixtures contained in addition 20% dimethyl sulfoxide, 24 μM [^{14}C]valine, and 1260 units/ml of Val-tRNA synthetase (*E. coli*). For charging with phenylalanine, reactions also contained 19 μM [^{14}C]phenylalanine and 182 units/ml of tRNA (*N. crassa*).

Charging reaction mixtures were incubated at 37° for 1–2 hr and transferred to an ice bath. A half-volume of 1.0 M sodium acetate (pH 4.5) was added plus an equal volume of water-saturated phenol. Charged tRNAs were precipitated from the aqueous layer with ethanol, collected by filtration onto 0.45 μ Millipore disks, and redissolved in 1 mM sodium acetate (pH 4.5).

Measurement of Discharge Rates. Rates of deacylation were measured at 22° in 20 mM Tris-HCl (pH 8.5) containing charged tRNA and various concentrations of KCl and magnesium acetate, as indicated. Samples were precipitated on filter paper disks with 5% acetic acid–0.7% HCl (v/v), and after several acid washes disks were dried and counted in a liquid scintillation counter. Half-lives were determined from plots of time *vs.* logarithms of acid-insoluble radioactive

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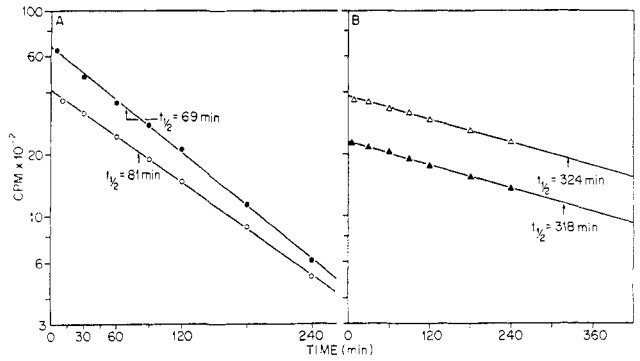

FIGURE 1: Nonenzymic hydrolysis of aminoacyl-tRNAs in 0.02 M Tris-HCl (pH 8.5) at 22°. (A) [^{14}C]Phe-tRNA^{Val} (*E. coli*), (○); [^{14}C]Phe-tRNA^{Phe} (*N. crassa*), (●). (B) [^{14}C]Val-tRNA^{Val} (*E. coli*) (Δ); [^{14}C]Val-tRNA^{Phe} (*N. crassa*), (▲).

amino acid at each time point. Each time point was the average of three or more determinations. Plots were linear throughout in every case, with at least six points per curve.

Results

Using the conditions for heterologous charging as described in Materials and Methods, we obtained [^{14}C]valyl-tRNA^{Val} (*E. coli*), [^{14}C]phenylalanyl-tRNA^{Val} (*E. coli*), [^{14}C]valyl-tRNA^{Phe} (*N. crassa*), and [^{14}C]phenylalanyl-tRNA^{Phe} (*N. crassa*). Rates of nonenzymic hydrolysis of each of these aminoacyl-tRNA species were determined at alkaline pH. In Figure 1A the deacylation rates of Phe-tRNA^{Val} and Phe-tRNA^{Phe} are seen to be quite similar, with half-lives of 81 and 69 min, respectively. A comparison of deacylation rates of Val-tRNA^{Val} and Val-tRNA^{Phe} in Figure 1B shows that the lines are nearly parallel, and half-lives are 324 and 318 min, respectively.

The effect of various levels of KCl on the rates of deacylation of Val-tRNA^{Val} (*E. coli*) and Phe-tRNA^{Val} (*E. coli*) is shown in Table I. In each case, an increase in KCl concentration from 0 to 0.40 M accelerates the rate of hydrolysis; however, the effect appears to be more pronounced with Val-tRNA^{Val}. First-order kinetics was obtained in both cases. An overall decrease in half-life of 48% was obtained with Val-tRNA^{Val} in 0.4 M KCl. The corresponding decrease with Phe-tRNA^{Val} was 23%.

TABLE I: Effect of KCl and Magnesium Acetate on Nonenzymic Hydrolysis of Aminoacyl-tRNA.[a]

Addition	Half-Life (min)	
	Val-tRNA^{Val}	Phe-tRNA^{Val}
None	324	81
KCl		
0.05 M	264	77
0.10 M	204	75
0.20 M	183	66
0.40 M	168	62
$Mg(OAc)_2$		
0.10 mM	270	73
1.0 mM	198	69
10.0 mM	147	56
100.0 mM	114	41

[a] The aminoacyl-tRNA was dissolved in 0.02 M Tris-HCl (pH 8.5) and incubated at 22°. The above additions were employed as indicated.

Similar decreases in half-life of these aminoacyl-tRNAs were noted in the presence of increasing levels of magnesium acetate, although magnesium acetate gave much more pronounced effects than comparable levels of KCl. Again greater decreases in half-life were seen with Val-tRNA^{Val} than with Phe-tRNA^{Val} at equal magnesium acetate concentrations. The overall decrease in half-life is 65% with Val-tRNA^{Val} as compared to 52% with Phe-tRNA^{Val}.

Discussion

The discovery of conditions permitting incorrect aminoacylation of *E. coli* tRNAs enabled us to compare the nonenzymic hydrolysis rates of tRNA^{Val} (*E. coli*) and of tRNA^{Phe} (*N. crassa*), each charged with valine and phenylalanine, in order to determine how amino acid structure affects these rates. The effect of amino acid structure can be clearly segregated from the effect of tRNA structure. Our results indicate that amino acid structure is a major factor in determining the hydrolysis rate of an aminoacyl-tRNA. Deacylation rates of valyl-tRNA^{Val} and valyl-tRNA^{Phe} were similar, as were deacylation rates of phenylalanyl-tRNA^{Val} and phenylalanyl-tRNA^{Phe}; yet the deacylation rate of the valyl-tRNAs was much slower than that for phenylalanyl-tRNAs. Ishida and Miura (1965) also found that yeast and rat liver valyl-tRNAs were more stable than the corresponding Phe-tRNAs under neutral and mildly acid conditions.

Using slightly different conditions, *viz.*, 0.02 M Tris-HCl (pH 8.8) and 37°, Novogrodsky (1971) found identical rates of hydrolysis for valyl-tRNAs of *E. coli* and of rat liver, with half-lives of 75 min for each. We and Novogrodsky find very similar effects of salt concentration; a decrease of 56 and 52% in half-lives of Val-tRNA of *E. coli* and of rat liver, respectively, at 0.264 M KCl (Novogrodsky, 1971) and a decrease of 57% for valyl-tRNA^{Val} (*E. coli*) in 0.20 M KCl (this study). Agreement is also noted in that, the presence of magnesium decreases the half-life of valyl-tRNA though to somewhat different extents. An opposite effect of ionic strength was found by Wolfenden (1963) who reported an increase in the half-life of Leu-tRNA as ionic strength was increased from 0.25 to 1.0 by adding KCl at pH 8.0 but no change was observed at pH 10.5. The mechanism of the effect of KCl and magnesium acetate may be different. The high charge density around the ester bond in KCl solutions may affect the stability in a manner represented by the Debye–Hückel theory. The effect of magnesium acetate at 0.01 M (ionic strength 0.03) is probably different from that of KCl at 0.1–0.4 M and may be the result of alteration of the tRNA conformation.

Coles *et al.* (1962) found that at 37° in 0.1 M Tris-HCl (pH 7.25) the half-lives for glycyl-, arginyl-, glutamyl-, and aspartyl-tRNAs from yeast were about 8, 11, 12, and 17 min, respectively, while valyl-tRNA was considerably more stable, with a half-life of 70 min. Wolfenden (1963) reported that the rate of nonenzymic hydrolysis of leucyl-tRNA at pH 7.25 was twice that of valyl-tRNA reported by Coles *et al.* (1962). He attributed this to the presence in leucine of five additional hydrogen atoms attached to γ-carbon atoms in a position to interfere with nucleophilic attack on the ester linkage. Model studies of hydrolysis of amino acid esters were reviewed by Zachau and Feldman (1965).

Presumably the structures of $tRNA^{Val}$ (*E. coli*) and $tRNA^{Phe}$ (*N. crassa*) are quite similar; otherwise, the heterologous charging reactions that have been observed would not be expected to occur. Consequently, we cannot say to what extent different bases in the vicinity of the aminoacyl bond might affect stability. Since details of tRNA tertiary structure are not yet available, it is unclear which areas of the molecule might be in the vicinity of the -C-C-A end under various conditions. It is clear, however, from our data and that of others that factors affecting tRNA structure change the rates of nonenzymic hydrolysis of aminoacyl-tRNAs. Using various techniques, a number of studies have shown changes in tRNA conformation induced by low levels of magnesium (Eisinger *et al.*, 1970; Zimmerman *et al.*, 1970; Beardsley *et al.*, 1970; Robison and Zimmerman, 1971; Willick and Kay, 1971; Rosenfeld *et al.*, 1970; Römer *et al.*, 1970). The greatest changes appeared to take place between 0 and 10 mM Mg^{2+} (Willick and Kay, 1971). High concentrations of NaCl mimicked to a lesser degree the effects of lower Mg^{2+} concentrations in fluorescence studies with $tRNA^{Phe}$ of beef liver (Zimmerman *et al.*, 1970) and yeast (Robison and Zimmerman, 1971). This is consistent with our results that equivalent effects on rates of hydrolysis of aminoacyl-tRNAs were achieved by Mg^{2+} and KCl when the concentration of the latter was 100–200 times that of the former.

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A 5S Ribonucleic Acid–Protein Complex Extracted from Rat Liver Ribosomes by Formamide†

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ABSTRACT: When rat liver ribosomes were dialyzed against 30 mM KCl–0.2 mM $MgCl_2$–3 mM potassium phosphate, pH 7.3, and formamide was added to a final concentration of 8.9 M, in the cold, the 5S RNA was detached in the form of a nucleoprotein complex (RNP). This complex was isolated by differential centrifugation and by fractionation on G-200 Sephadex, where its elution volume corresponded to a molecular weight of about 80,000. Its protein had a molecular weight of 41,000, measured by gel electrophoresis in sodium dodecyl sulfate. The RNP complex was unstable, being easily dissociated by moderate concentrations of electrolyte. After formaldehyde fixation it had a sedimentation coefficient of 6.8 S; in a cesium chloride gradient it banded at a density of 1.568 g/cm^3, corresponding to an RNA content of 48.5%.

Although the 5S RNA, found in the large subunits of both 70S and 80S ribosomes (Attardi and Amaldi, 1970) appears to be essential for protein-synthetic activity, its function is still unknown. When it is extracted from the ribosome by EDTA treatment a fraction of it is found in a ribonucleoprotein complex (RNP)[1] (Mazelis, 1970; Lebleu *et al.*, 1971; Petermann *et al.*, 1971; Blobel, 1971). With rat liver ribosomes a higher ratio of complex to free RNA is found when the RNP is extracted with formamide (Petermann *et al.*, 1971). This paper describes the extraction of the RNP, its stability, its partial purification, and some of its physical properties.

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[1] Abbreviation used is: RNP, the 5S RNA–protein complex.