3′-cyclic phosphate. The physical characteristics of the enzymes and their glycoprotein nature seem typical of such ribonucleases, but to our knowledge they are the first nonspecific ribonucleases reported to show a preference for pyrimidines (see Barnard, 1969; Egami and Nakamura, 1969).

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Structural Properties of 5-Fluorouracil-Containing Transfer Ribonucleic Acids from *Escherichia coli**

Ivan I. Kaiser

ABSTRACT: The structural properties of unfractionated, 5-fluorouracil-containing tRNAs (FU-tRNAs) from *Escherichia coli* B, having 84% of the uracil residues replaced by 5-fluorouracil, have been compared with unfractionated, normal tRNAs. The content of the minor pyrimidines, pseudouridine, ribothymidine, 4-thiouridine, and 5,6-dihydrouridine, have previously been shown to be reduced to essentially the same extent as uridine in the analog-containing tRNA. The effect of these massive replacements on the structure of FU-tRNAs have been examined by disc gel electrophoresis, ultraviolet absorbance–temperature and denaturation spectra, ribonuclease digestions, cochromatography on Sephadex, and cosedimentation on sucrose gradients. The results indicate that fluorouridine replacement of uridine and uridine-related minor base components has only minor effects on the secondary and tertiary properties of tRNA, particularly in the presence of Mg^{2+}. It is suggested that these minor bases are not essential in the formation or stabilization of the three-dimensional structures found in normal tRNAs.

When the uracil analog FU[1] is added to a rapidly growing culture of *Escherichia coli* B, extensive replacement of uracil and structurally related minor components in tRNA occurs. The odd nucleosides replaced include pseudouridine (Andoh and Chargaff, 1965; Lowrie and Bergquist, 1968; Johnson *et al.*, 1969), ribothymidine (Lowrie and

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[1] The following abbreviations are used: FU, 5-fluorouracil; FU-tRNA, tRNA containing FU; N-tRNA, tRNA from normal cells; A_{260} unit, a unit of material which in a volume of 1 ml will have an absorbance of one at 260 mμ when measured in a cell of 1-cm path length.

Bergquist, 1968; Johnson *et al.*, 1969; Baliga *et al.*, 1969), 4-thiouridine (Kaiser, 1969b), and 5,6-dihydrouridine (Kaiser *et al.*, 1969). FU-tRNA from yeast is also deficient is pseudouridine and ribothymidine, but not in 5,6-dihydrouridine (Giegé *et al.*, 1969). The extent of substitution of these minor components by FU appears to be at least as extensive as uracil replacement.

The role played by these and other minor base components in determining the structural and functional properties of the tRNAs remains largely unknown (see recent reviews by Starr and Sells, 1969; Zachau, 1969). To gain possible insights into their role, in addition to other structure–function relationships, the properties of FU-tRNAs are being examined. This paper compares the structure of normal and FU-tRNAs having 84% of the uracils replaced by FU. Presumably, the four minor pyrimidines are replaced to *at least* this extent. Unfractionated N- and FU-tRNAs were compared, since accumulating evidence indicates that the conformation of *all* tRNAs is similar (Levitt, 1969; Englander *et al.*, 1970; Blake *et al.*, 1970). Additionally, since all tRNA molecules whose primary structure is now known can be described by the cloverleaf model of Holley and coworkers (1965), this configuration is assumed in discussing the results in this paper.

The FU-tRNA used in this study has been shown to be free of contaminating N-tRNA and is about 90% active in accepting amino acids (Kaiser, 1969a). The results presented here indicate that in the presence of Mg^{2+}, FU-tRNA has secondary and tertiary structural properties very similar to N-tRNA.

Materials and Methods

Stock tRNA Solutions. Normal tRNA from *E. coli* B was purchased from Schwarz BioResearch, stripped (Sarin and Zamecnik, 1964), and further purified by Sephadex G-75 chromatography. The peak corresponding to tRNA was pooled, precipitated with two volumes of 95% ethanol at −20° overnight, and collected by centrifugation. The purified tRNA was dissolved in a small volume of water, divided into several aliquots, and stored at −20°. No differences have been observed between purified, commercial N-tRNA from *E. coli* B and that prepared from cells grown in the laboratory.

FU-tRNA was prepared from *E. coli* B as previously described (Kaiser, 1969a) and had *ca.* 84% of the uracil residues replaced by FU. After DEAE-cellulose column chromatography (Kaiser, 1969a), the pooled samples were precipitated with ethanol and collected by centrifugation. The FU-tRNA was redissolved in water and stored as described for N-tRNA.

Absorbance Measurements. All absorbance measurements were made with a Beckman DU monochromator and a Gilford Model 222 photometer. Silica cells with 1-cm path length were used in all cases. When 1200-μl cuvets were used, a microaperature plate was installed and properly positioned.

Buffers and ε(P) Values. All work in this paper was carried out in a buffer composed of 0.01 M potassium cacodylate (pH 7.0), 0.15 M KCl, 0.0005 M NaEDTA, and ± 0.005 M $MgCl_2$ (standard buffer). In the absence of Mg^{2+}, N- and FU-tRNA had $E_{260}^{0.1\%}$ values of 23.1 and 23.4, respectively. In the presence of Mg^{2+}, both tRNA samples had an $E_{260}^{0.1\%}$ 22.3 at 25°. These values were determined by using 9.5% phosphorus for both samples as calculated from the major nucleotide composition of the tRNAs (Kaiser, 1969a). Total phosphorus was determined as previously described (Kaiser, 1969a).

Ultraviolet Absorbance–Temperature Measurements and Thermal-Denaturation Spectra. The Beckman-Gilford spectrophotometer was adapted for thermal-denaturation studies essentially as described by Mandel and Marmur (1968).

Stock tRNA samples were thawed and diluted up to about 150-fold with standard buffer to give starting absorbances of 1.730 A_{260} units (*plus* Mg^{2+}) and 1.929 A_{260} units (*minus* Mg^{2+}) at 20°. The N- and FU-tRNA profiles were determined in the same run, using the appropriate buffer as a blank. After equilibration at 20° for at least 30 min, the temperature was raised in *ca.* 5° increments. The temperature was stabilized at each recording point for at least 10 min, or until no further increase in absorbance could be detected, before recording the reading. All measurements were corrected for dilution as a result of solvent expansion at the higher temperatures.

At 20° and 90° the wavelength from 230 to 300 mμ was scanned. After correction for solvent expansion the ε(P) values at these temperatures were calculated and the Δε(P) values [$\epsilon(P)_{90°}$ *minus* $\epsilon(P)_{20°}$] determined.

Ribonuclease Digestion. Stock solutions of N- and FU-tRNAs were diluted about 150-fold with standard buffer $\pm Mg^{2+}$, to give similar absorbancies at 260 mμ. Crystalline bovine albumin was then added to give a final concentration of 1 mg/ml. Each sample (10 ml) was equilibrated at 25° for 20 min, a 1-ml aliquot was removed (zero-time control), and 40 μl of pancreatic ribonuclease (EC 2.7.7.16) was added to each remaining 9 ml. In the absence of Mg^{2+}, the final RNase concentration was 9 ng/ml, while in its presence it was twice that or 18 ng/ml. The zero-time aliquots and subsequent samples (1 ml), were pipetted directly into 1 ml of ice-cold 1 M $HClO_4$. After standing in ice for 10–20 min, the samples were centrifuged at 1500*g* (av) for 10 min at 4°, the supernatant was removed with a disposable pipet, and the absorbance at 260 mμ was determined.

The absorbance values for the diluted tRNA solutions before the addition of bovine albumin, in the absence of Mg^{2+} were: N-tRNA, A_{260} 2.109; FU-tRNA, A_{260} 2.105. In the presence of Mg^{2+} values were: N-tRNA, A_{260} 2.083; FU-tRNA, A_{260} 2.094. The acid-soluble, zero-time absorbancies were all less than 0.010 A_{260} unit.

Sephadex Chromatography. All Sephadex G-75 chromatography was carried out at room temperature on columns (0.9 × 230 cm) equilibrated with standard buffer ± Mg^{2+}. The Sephadex bed volume remained constant in the absence or presence of Mg^{2+}. Flow rates were 15–16 ml per hr. Fractions (3 ml) were collected.

Radioactivity in column effluents were determined by precipitating 2.5-ml aliquots from each fraction in the presence of 0.5 mg of bovine albumin with an equal volume of cold, 10% trichloroacetic acid. Following centrifugation, resuspension, and plating, the samples were counted in a Nuclear-Chicago (Model 4338) gas-flow counter, equipped with a Micromil window.

Sucrose Gradients. Transfer RNA samples, having a N- to [^{14}C]FU-tRNA ratio of 6 to 1, were prepared in standard buffer in the absence and presence of Mg^{2+}. A 0.20-ml aliquot, containing about 12 A_{260} units of tRNA, was layered on 12 ml of a 5–20% linear sucrose gradient (Britten and Roberts, 1960) prepared at 4° in the appropriate buffer. Centrifugations were carried out in the SB-283 rotor of the International B-60 preparative ultracentrifuge at 41,000 rpm (195,000*g*, av) for 47 hr at a temperature of 0–3°. Ten-drop

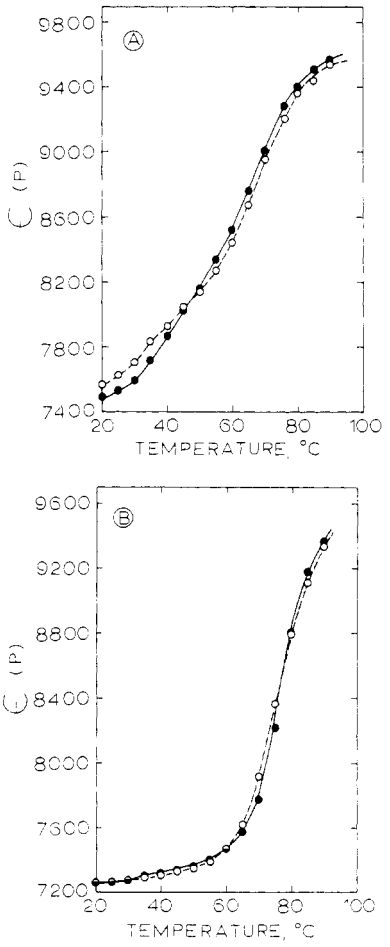

FIGURE 1: Ultraviolet absorbance–temperature profile of N- and FU-tRNA: (A) standard buffer *minus* Mg^{2+}; (B) standard buffer *plus* Mg^{2+}; N-tRNA, ●——●; Fu-tRNA, O------O.

fractions (*ca.* 0.75 ml) were collected manually and diluted by addition of 0.5 ml of standard buffer, and absorbancies were determined. The entire contents of each tube were then precipitated with trichloroacetic acid and collected on (HA WP 24) Millipore filters. The filters were dried and counted as previously described (Kaiser, 1969a).

Materials. Cacodylic acid (Ultra Pure); EDTA, disodium dihydrate (highly purified); and sucrose (Ultra Pure, RNase free) were purchased from Mann Research Laboratories. Crystallized pancreatic ribonuclease (EC 2.7.7.16) was a product of Worthington Biochemical Corp. All solutions were prepared using distilled water which had been passed through a Barnstead deionizer containing a standard cartridge. All other materials were as previously described (Kaiser, 1969a).

Results

Ultraviolet Absorbance–Temperature Measurements. In the absence of Mg^{2+}, both N- and FU-tRNAs show broad, noncooperative thermal-denaturation profiles (Figure 1A). FU-tRNA actually shows a slight bimodality in the 20–45° region not seen in the N-tRNA. The extent of thermal hyperchromicity (20–90°) in N-tRNA is 27.5%, compared with 25.9% in FU-tRNA, or a difference of 1.6%. About 1% of this difference is due to the higher ϵ(P) value of FU-tRNA at 20° (see Figure 1A). The remaining difference is due to a decrease in the hyperchromicity of FU-tRNA in the 20–90° range. Denaturation spectra of N- and FU-tRNA (not shown) suggest that this 1.6% difference is primarily the result of the loss of A·FU base pairs in FU-tRNA. From the shape of the denaturation spectrum of N-tRNA it was estimated that about 70% of the base pairs disrupted were of the G·C type (Fresco *et al.*, 1963). For FU-tRNA the percentage of G·C base pairs appeared to increase slightly, but only by about 5%.

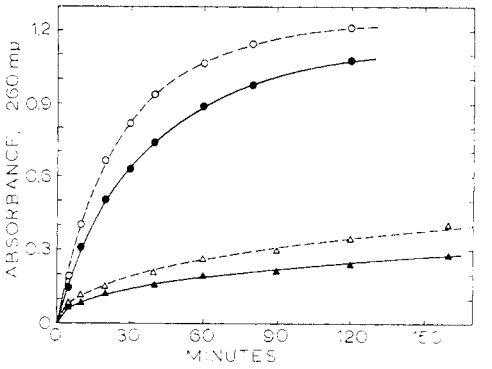

FIGURE 2: Rate of release of acid-soluble material from N- and FU-tRNA in the absence and presence of Mg^{2+} by pancreatic ribonuclease. Zero-time incubation blanks were subtracted from all readings (see Methods): *minus* Mg^{2+}: N-tRNA, ●——●; FU-tRNA, O------O; *plus* Mg^{2+}: N-tRNA, ▲——▲; FU-tRNA, △------△.

The T_m value for the N-tRNA was 60.5°, whereas for FU-tRNA this value was 62.5°. The increase in the T_m value for FU-tRNA is consistent with a greater percentage of G·C bonds remaining in the FU- as compared with the N-tRNA (see Mandel and Marmur, 1968).

When thermal denaturation studies were carried out at pH 8.8 [0.01 M *N*-tris(hydroxymethyl)methylglycine, 0.30 M KCl], the hyperchromicity of FU-tRNA was nearly 30% less than N-tRNA. The T_m value for FU-tRNA was also less than that of N-tRNA, 57° *vs.* 60°. At this high pH, the extensive ionization of FU (poly (FU), pK_a = 8.1; poly (U), pK_a = 9.81 in 0.15 M NaCl) not only decreases the A·FU interactions, but also decreases or weakens the G·C base pairing. Much of this hyperchromicity is lost at low temperatures, since $\epsilon(P)_{260}$ values at 25° for FU-tRNA increase about 6% in going from pH 6 to 9.5 in 0.30 M KCl. N-tRNA $\epsilon(P)_{260}$ values increase only a little over 2% under the same conditions (I. I. Kaiser, unpublished data).

When Mg^{2+} is added to the standard buffer, both tRNA samples show a greatly increased cooperative melting profile (Figure 1B). Addition of Mg^{2+} to the buffer increased the T_m value for N-tRNA 16.5° to 77°, whereas the T_m value for FU-tRNA was increased only 13° to 75.5°. Magnesium apparently stabilizes the helical structure of A·U- and A·FU-rich portions of the tRNA molecules, and merges their thermal denaturation with that of the G·C-rich regions. This merging results in a lower T_m for FU-tRNA relative to N-tRNA when measured at 260 mμ. The extent of hyperchromicity was similar in both cases, being 28.9% for normal and 28.4% for FU-tRNA.

The denaturation spectra of N- and FU-tRNA also showed nearly identical curves in the presence of Mg^{2+} (not shown).

Ribonuclease Degradation. At pH 7, in the absence and presence of Mg^{2+}, FU-tRNA is rendered acid soluble at both a *faster rate* and to a *greater extent* than is N-tRNA by pancreatic ribonuclease (Figure 2). These results suggest

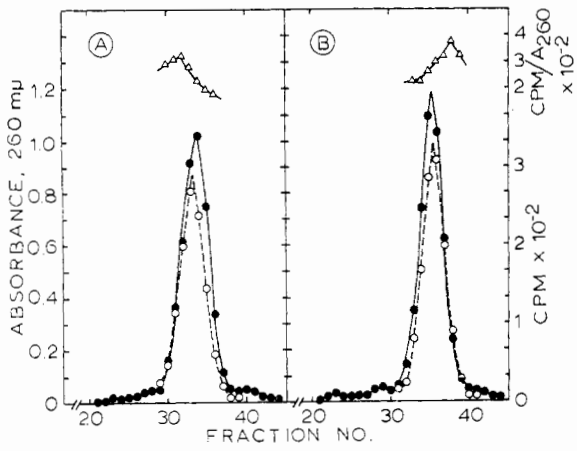

FIGURE 3: Gel-filtration on Sephadex G-75 of a mixture of N- and [^{14}C]FU-tRNAs. Transfer RNAs (13–14 A_{260} units) were passed over a column equilibrated with standard buffer in the absence (A) and presence (B) of Mg^{2+}. See Methods for details: absorbance, ●——●; radioactivity, O------O; specific activity (cpm/A_{260} units), △——△.

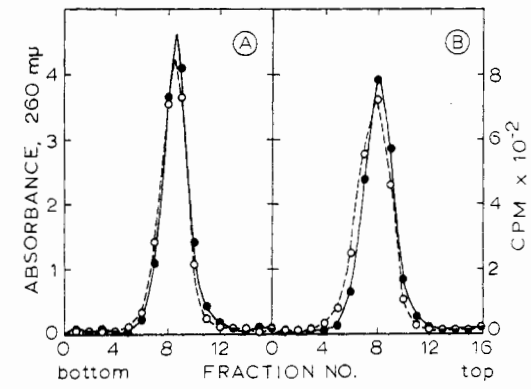

FIGURE 4: Sucrose gradient cocentrifugation of N- and [^{14}C]FU-tRNAs. Gradients were run in standard buffer *minus* Mg^{2+} (A) and *plus* Mg^{2+} (B): absorbance, ●——●; radioactivity, O------O.

that the secondary structure of N-tRNA may be slightly *more* ordered in both the absence and presence of Mg^{2+}, relative to FU-tRNA in the same buffer. Magnesium has a pronounced stabilizing effect on both tRNAs by rendering it more resistant to RNase attack (Figure 2).

Cochromatography of N- and [^{14}C]FU-tRNA on Sephadex. When passed over long columns of Sephadex G-75, a mixture of tRNAs having a normal to [^{14}C]FU-tRNA ratio of 6 to 1, gave the patterns shown in Figure 3. In the absence of Mg^{2+} (Figure 3A), the radioactive FU-tRNA eluted toward the leading edge of the N-tRNA peak. This is seen more clearly by the specific activity curve plotted above the peaks. In the presence of Mg^{2+} (Figure 3B), the [^{14}C]FU-tRNA elutes toward the trailing edge of the N-tRNA profile. These results suggest that in the absence of Mg^{2+}, FU-tRNA has slightly *larger* molecular dimensions than N-tRNA, whereas in the presence of Mg^{2+} these dimensions are smaller, allowing greater penetration into the gel beads. Both the N- and FU-tRNAs showed smaller dimensions in the presence of Mg^{2+} as judged by their retarded elution when compared with the run in the absence of Mg^{2+} (compare Figures 3A and B).

In experiments not shown, no separation of N- and [^{14}C]-FU-tRNAs could be detected on either Sephadex G-75 or G-100 in a solvent of 0.01 M potassium cacodylate (pH 7) with 0.30 M KCl. Similar results have been reported on Sephadex G-100 in 1 M NaCl (Kaiser, 1969a; Johnson *et al.*, 1969). Apparently in buffers where no Mg^{2+} chelating agent (*e.g.*, EDTA, citrate, etc.) is present, a residual amount of the divalent cation remains with the FU-tRNA during isolation and is effective in *not allowing* the molecules to fully expand. Addition of EDTA at neutral pH values probably removes this residual Mg^{2+}. This permits the FU-tRNA to become more expanded than N-tRNA as a result of the electrostatic interactions and enolization of FU residues (see Discussion).

Increasing the pH to 8.9, in either 0.15 or 0.30 M KCl (no Mg^{2+}), resulted in an even greater expansion of the FU-tRNA relative to N-tRNA. The separation achieved (not shown) by cochromatography on Sephadex G-75 was considerably greater than that shown in Figure 3A. Addition of Mg^{2+} to these buffers (pH 8.9) allowed FU-tRNA to decrease in molecular size, but not to the extent of becoming as compact as N-tRNA (as judged by Sephadex chromatography).

Sucrose Gradients. Sucrose gradient sedimentation velocity experiments of a mixture of N- and [^{14}C]FU-tRNAs, in the absence and presence of Mg^{2+}, gave the results shown in Figure 4. In the absence of Mg^{2+}, FU-tRNA sedimented at essentially the same velocity or slightly faster than N-tRNA (Figure 4A) at 4°. When run at 25° (not shown), no appreciable separation was observed. The specific activity was constant across the main peak.

In the presence of Mg^{2+} considerably more separation between N- and FU-tRNAs was observed (Figure 4B). Similar results were obtained at 25°. The peaks of both samples were shifted one tube closer toward the bottom of the gradient in the presence of Mg^{2+} (compare Figures 4A and B).

Discussion

In the absence of Mg^{2+}, FU-tRNA showed both an altered absorbance–temperature profile and a reduction in the hyperchromicity relative to N-tRNA (Figure 1). The bimodality seen in the low temperature region for FU-tRNA probably resulted from A·FU base pairs melting out at a lower temperature than the corresponding A·U base pairs in N-tRNA (Massoulié *et al.*, 1966). The decrease in total hyperchromicity suggests a slight loss of Watson–Crick-type base pairing (*i.e.*, helical content) in FU-tRNA. The shape differences in the thermal-denaturation profiles (not shown) were consistent with the idea that A FU base pairs were disrupted.

Absorbance–temperature profiles for FU-tRNA, having 82% of the uracils replaced by FU, have been reported by Lowrie and Bergquist (1968). These experiments were carried out in a saline–citrate buffer (no Mg^{2+}) and should be comparable to those reported here in standard buffer *minus* Mg^{2+}. The shapes of the N- and FU-tRNA curves in both studies are in fact, very similar. The hyperchromicity they observed for FU-tRNA however, was only two-thirds that of N-tRNA. This may indicate that partial degradation of their FU-tRNA occurred during its isolation, or some residual methylated albumin contaminated their samples during chromatography on albumin–kieselguhr columns. Free methylated albumin has been shown to have great affinity for tRNA (Goldin and Kaiser, 1969).

From the shape of the melting curves (Figure 1B) and denaturation spectra (not shown) in the presence of Mg^{2+}, which presumably most closely represent the predominant

molecular species of tRNA *in vivo* (Fresco *et al.*, 1966), there appear to be no essential secondary structural differences between N- and FU-tRNA.

With the exception of ψU, the minor pyrimidines replaced by FU do not appear to be involved in Watson–Crick-type base pairs, since most of these bases appear in non-hydrogen-bonded regions (Zachau, 1969). Their replacement by FU should not therefore *directly* effect the secondary structure in FU-tRNA. Giegé *et al.* (1969) examined the T_m profile of yeast FU-tRNA having up to 50% of the U replaced by FU in the presence of 0.01 M Mg^{2+}. They also found that the FU-tRNA had less hyperchromicity and a lower T_m than N-tRNA. Their absorbance–temperature curves appeared less cooperative than those reported here and the differences in the extent of hyperchromicity were greater. The reasons for these differences is not entirely clear.

The total hyperchromicity is slightly greater for both tRNA samples in the presence of Mg^{2+}. The melting profiles also indicate that they were not at their maximum when heating was terminated (90°), so an additional increase would most certainly have resulted if heating had been continued. The $\epsilon(P)_{260}$ values in the absence of Mg^{2+} were 3–4% higher than in its presence, at 20°. This probably accounts for the majority of the difference in hyperchromicity in the absence and presence of Mg^{2+}.

The greater susceptibility of FU-tRNA toward RNase *plus* or *minus* Mg^{2+}, is probably the result of FU's more acidic pK_a value (Results) and the replacement of the minor pyrimidines in the unpaired regions. The increased ionization would destabilize the helical region where the ionization occurred and render it more prone to nuclease attack.

The minor pyrimidines replaced by FU also serve as suitable substrates for pancreatic ribonuclease. On the basis of studies with polynucleotide analogs and model systems (Massoulié *et al.*, 1966; Witzel, 1963), of the minor bases replaced by FU, only ribothymidine should be hydrolyzed at a significantly greater rate than FU in unstructured polymers. The remaining three minor bases would probably be hydrolyzed at a similar or *slower* rate than FU. Since most, if not all, of the ribothymidine in tRNA is present in the G-rT-ψ-C sequence and since it has been shown to be unusually stable toward hydrolysis by RNase (Zachau *et al.*, 1966), it should not contribute a significant portion of acid-soluble material during hydrolysis of N-tRNA. Considering only the change in primary structure, the overall replacement of these minor bases by FU would make FU-tRNA *more* susceptible to RNase. The replacement of the odd bases by FU may in fact be the major reason for FU-tRNAs greater sensitivity to RNase. The primary locations of these minor pyrimidines are the looped or single-stranded regions of the cloverleaf model (Holley *et al.*, 1965), which are known to be more susceptible to endonucleolytic attack than the double-stranded ones (Zachau *et al.*, 1966).

In standard buffer in the presence of Mg^{2+}, FU-tRNA has both a slightly increased sedimentation velocity and K_d value on Sephadex than N-tRNA (see Figures 3B and 4B). These findings indicate that FU-tRNA is *more* compact in overall molecular dimensions than N-tRNA.

In the absence of Mg^{2+}, the results are less definitive. Unfractionated FU-tRNAs used in these studies should have an average molecular weight about 1% higher than N-tRNAs as a result of FU replacement of U. If this replacement occurs without any shape change in the molecules, no separation should be detected on Sephadex. Sephadex runs (Figure 3A) do show a smaller K_d value for FU- than N-tRNAs, however, suggesting *larger* molecular dimensions. Sucrose gradient runs, on the other hand (Figure 4A), *do not* indicate any appreciable separation (some at 0–3°, none at 25°). This increase is not detected on sucrose gradients, since it is offset by the increase in molecular weight. With tRNA, Sephadex chromatography is more sensitive to changes in Stokes' radii than molecular weight (see the changes observed in $s_{20,w}$, K_d values, Stokes' radii, and M in the paper by Adams *et al.*, 1967). The small changes in molecular dimensions (in the order of 1%) between N- and FU-tRNAs should be, and are, detectable on long columns (230 cm) of Sephadex G-75 by cochromatography.

The electrophoretic mobilities of both N- and FU-tRNAs were examined on 7.5, 10, 12, and 16% acrylamide gels. The extent of their migration relative to bromophenol blue did not reveal any differences, although variations of less than about 5% could probably not have been detected.

A number of investigators have also examined the properties of FU-tRNAs on MAK or MASA columns (Sueoka and Yamane, 1963; Lowrie and Bergquist, 1968; Johnson *et al.*, 1969). In all cases, differences between total and amino acid specific tRNAs have been noted when the N- and FU-tRNA profiles were compared. The pH range in the chromatography studies has varied from pH 5 to 8. Greater separation is achieved at the higher pH values, probably as a result of FU's more acidic pK_a. At a pH of 5.2, where there should be no essential charge difference between N- and FU-tRNA, and in the absence of Mg^{2+} the FU-material *was still* more strongly bound to MASA columns (Johnson *et al.*, 1969). This may be interpreted as a decrease in secondary structure (Sueoka and Cheng, 1962), and is consistent with the results reported here.

The replacements of uridine and the minor nucelosides (ψU, rT, 4-thiouridine, and 5,6-dihydrouridine) by FU, do not appear to have any gross effects on either the secondary or tertiary properties of FU-tRNAs. This is particularly true in the presence of Mg^{2+}. These findings strongly suggest that these minor bases *do not* function in the formation or stabilization of specific conformations in normal tRNAs. The results also support the idea of Zachau (1969), that unmodified or virgin tRNAs already possess three-dimensional structures similar to that of modified tRNAs, and that the odd nucleotides are found predominantly in single-stranded regions because of easier access of modifying enzymes to these parts.

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Concerted Reduction of Yeast Uridine Diphosphate Galactose 4-Epimerase*

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ABSTRACT: A concerted reduction of yeast UDPGal 4-epimerase by 5′-UMP and specific carbohydrates results in a seven- to tenfold increase in the 450-mμ fluorescence emission, with a concomitant loss in catalytic activity. During the reduction approximately 1 mole of 5′-UMP is bound per mole of epimerase dimer. Sodium borohydride will bring about the same type of reduction, again provided that 5′-UMP is also present. The inactive reduced epimerase can be rendered active by dilution of the reduced epimerase and subsequent storage at 4°. The bound NAD group of epimerase or the NADH group formed after the concerted reduction of the epimerase can be isolated by titration of the available sulfhydryl groups in the epimerases, using *p*-chloromercuribenzoic acid and subsequent chromatography on Sephadex.

UDPGal 4-epimerase[1] from yeast and *Escherichia coli* can undergo a modification, which has been called concerted reductive inactivation, since the bound pyridine nucleotide is reduced by a specific sugar in the presence of 5′-UMP, and the catalytic activity decreases as a function of the degree of reduction (Kalckar *et al.*, 1970). Since the fluorescence characteristics (Bertland, 1970) and the alterations in the ordered backbone peptide structure (Bertland and Kalckar, 1968), which accompanies such a reductive inactivation, have largely been studied in the yeast epimerase the detailed description of the reductive modifications will mainly center around the operations carried out on the yeast enzyme.

The process seems to be a concerted reaction, even in the presence of specific deoxy sugars, since any extensive reduction of the prosthetic group of the intact epimerase requires not only the addition of specific sugars but also the presence of small amounts of 5′-UMP. The reduced inactive epimerase can be reactivated.

The present investigation describes the systematic investigation of the concerted reductive process.

Materials

UDPGlc dehydrogenase, NAD, NADH, 5′-UMP, galactose, and D- and L-fucose were purchased from Sigma Chemical Company; L-arabinose and UDPGal from Calbiochem; [2-^{14}C]5′-UMP from Schwarz BioResearch; *p*-chloromercuribenzoic acid was purchased from Mann Research Laboratories; sodium borohydride was obtained from Matheson Coleman and Bell; D-mannose, D-ribose, 2-deoxy-D-galactose, and 2-deoxy-D-ribose were obtained from Pfanstiehl; 3-

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[1] Abbreviations used are: UDPGal, uridine 5′-(α-D-galactopyranosyl pyrophosphate); UDPGal 4-epimerase (UDPGlc 4-epimerase, EC 5.1.3.2).