discussions, and to Dr. Grubb for a careful reviewing of the text.

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Enzymatic Synthesis and Crystallographic Characterization of an Isomorphous Derivative of Yeast Formylatable Methionine Transfer Ribonucleic Acid Containing Iodocytidine†

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ABSTRACT: Isomorphous derivatives of yeast $tRNA^{fMet}$, yeast $tRNA^{fMet}$-A_{72}-ioC-C-A, and yeast $tRNA^{fMet}$-A_{72}-C-C-A were synthesized, crystallized, and characterized by X-ray diffraction. The synthetic procedure was as follows. Yeast $tRNA^{fMet}$-A_{72} was prepared by controlled snake venom phosphodiesterase digestion of yeast $tRNA^{fMet}$ and the terminal trinucleotide was subsequently rebuilt with the appropriate radioactive nucleoside triphosphates and yeast nucleotidyltransferase. The terminal sequences were established by analysis of the radioactive products of a combined pancreatic RNase and bacterial alkaline phosphatase digest. Both products were readily aminoacylated. The crystallographic coordinates of the iodine atom were established by a three-dimensional difference Fourier synthesis at 6 Å using phases obtained from three other isomorphous derivatives. The position is consistent with the three-dimensional difference Patterson synthesis.

This report describes the enzymatic synthesis and crystallographic characterization of yeast $tRNA^{fMet}$-A_{72}-ioC-C-A.[1] This derivative serves three functions. First, the covalently linked heavy atom can be used to correlate the site of attachment with a specific region in the electron density map. Second, this derivative provides an isomorphous heavy-atom substitution with which to improve the phase angles used in the crystallographic structure determination. Third, this type of derivative, in contrast to less chemically defined derivatives, has the virtue of a limited number of heavy-atom sites, thus constraining the analysis of their location in the unit cell of the crystal.

The syntheses of yeast $tRNA^{Phe}$-A_{73}-C-ioC-A, yeast $tRNA^{Phe}$-A_{73}-ioC-ioC-A (Sprinzl *et al.*, 1972), and yeast $tRNA^{Phe}$-A_{73}-C-C^sA (Schlimme *et al.*, 1970) have been reported. The synthesis of yeast $tRNA^{Phe}$-A_{73}-C-s^2C-A (Sprinzl

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[1] Abbreviations used are: A_{ox-red} and C_{ox-red}, nucleoside trialcohols of adenosine and cytidine, respectively, obtained by periodate oxidation of the 2′,3′-*cis*-diol and by subsequent reduction of the dialdehyde with borohydride; A_{258} unit, the amount of tRNA which, when dissolved in 1 ml of H_2O and measured with a 1-cm light path, has an absorbance of 1 at 258 mμ; PEI-cellulose, poly(ethylenimine)-cellulose; BD-cellulose, benzoylated DEAE-cellulose; yeast $tRNA^{fMet}$-A_{72}-C-C-A is equivalent to the parent molecule, yeast $tRNA^{fMet}$, and the subscript designates the sequence position (Simsek and RajBhandary, 1972) of the 5′-nucleotide to the left; yeast $tRNA^{Phe}$-A_{73}-C-C^sA is a phosphorothioate derivative of yeast $tRNA^{Phe}$; yeast $tRNA^{Phe}$-A_{73}-C-s^2C-A is a derivative of yeast $tRNA^{Phe}$ with 2-thiocytidine at the penultimate position; ioC, 5-iodocytidine.

et al., 1973) and the crystallization of its *p*-hydroxymercuribenzoate adduct have been reported without crystallographic characterization.

Materials and Methods

a. Isolation and Purification of Yeast tRNA^{fMet}. Bulk yeast tRNA^{fMet} was prepared according to Holley (1967). Lots of 10 g were subjected to three successive chromatographic steps (Gillam *et al.*, 1967; RajBhandary and Ghosh, 1969): first, BD-cellulose (5.3 × 135 cm column; 0.01 M $MgCl_2$, 12-l. linear NaCl gradient from 0.45 to 0.75 M); second, BD-cellulose (2.8 × 150 cm column; 0.001 M EDTA–0.005 M formate (pH 4.0), 12-l. linear NaCl gradient from 0.4 to 1.2 M); and third, DEAE-Sephadex A-50 (Pharmacia, 2.5 × 90 cm column; 0.01 M $MgCl_2$–0.2 M Tris-Cl (pH 7.3), 4-l. linear NaCl gradient from 0.400 to 0.425 M). Typical yields of the initiator were 600 A_{258} units accepting 1.8 nmol of methionine/A_{258} unit.

b. Enzymes. Methionine acceptance was assayed (RajBhandary and Ghosh, 1969) with a mixed-synthetase preparation from *Escherichia coli* (Muench and Berg, 1966). Nucleotidyltransferase from baker's yeast was purified essentially according to the procedure of Sternback *et al.* (1971). A partially purified preparation was used throughout which added 33 nmol of ATP/min per mg of enzyme to unfractionated baker's yeast tRNA at 0°. Pancreatic ribonuclease, alkaline phosphatase, and snake venom phosphodiesterase were from Worthington. T1 RNase, from Calbiochem, was heat treated prior to use (Reeves *et al.*, 1968).

c. Synthesis of [^{125}I]ioCTP. CTP was radioiodinated by a modification of the iodination procedure of Voytek *et al.* (1971). Carrier-free $^{125}I^-$ (Amersham) (1 mCi) was incubated with 18 mg of iodic acid, 30 mg of iodine, 0.8 ml of acetic acid, 0.2 ml of CCl_4, and 0.15 ml of H_2O for 10 min at 45° to allow thermal exchange. CTP (100 mg) was added in 0.15 ml of H_2O and stirred for 4 hr at 45° and then for 12 hr at 25°. After CCl_4 extraction, 267 mg of BaI in 20 ml of chilled methanol was added to the aqueous phase and the barium salt of [^{125}I]-ioCTP was isolated by filtration. The product was dissolved in 20 ml of a dense slurry of Bio-Rad AG-50 (Na form), and the filtrate was collected and lyophilized. The yield was approximately 60%. A single uv-absorbing spot containing 98.7% of the applied radioactivity was observed on a PEI-cellulose thin-layer chromatogram (Randerath and Randerath, 1967); 99.4% of the radioactivity of an alkaline phosphatase digest migrated with an authentic sample of 5-iodocytidine (Sigma) on cellulose thin layer (Massaglia *et al.*, 1965).

The stoichiometry of enzymatic incorporation of [^{125}I]nucleotide into tRNA was determined by comparing the activity of a purified reaction product with that of a known amount of [^{125}I]ioCTP counted with identical geometry in a Nuclear-Chicago well counter.

d. Preparation of Yeast tRNA^{fMet}-A_{72}. A reaction mixture having the proportions of 1 μg of snake venom phosphodiesterase/ml and 5 A_{258} units of yeast tRNA^{fMet}/ml was incubated in 10 mM Tris-Cl (pH 8.7)–10 mM $MgCl_2$ for 60 min at 25°. These conditions accumulate yeast tRNA^{fMet}-A_{72} as the main product (see Results).

e. Synthesis of Yeast tRNA^{fMet}-A_{72}-ioC-C-A. Figure 1 outlines the two routes employed for the insertion of ioC at position 73 (step 1, routes A and B) and the subsequent completion of the 3′ terminus with C at position 74 and A at position 75 (step 2).

In step 1, route A, the product of the snake venom digest

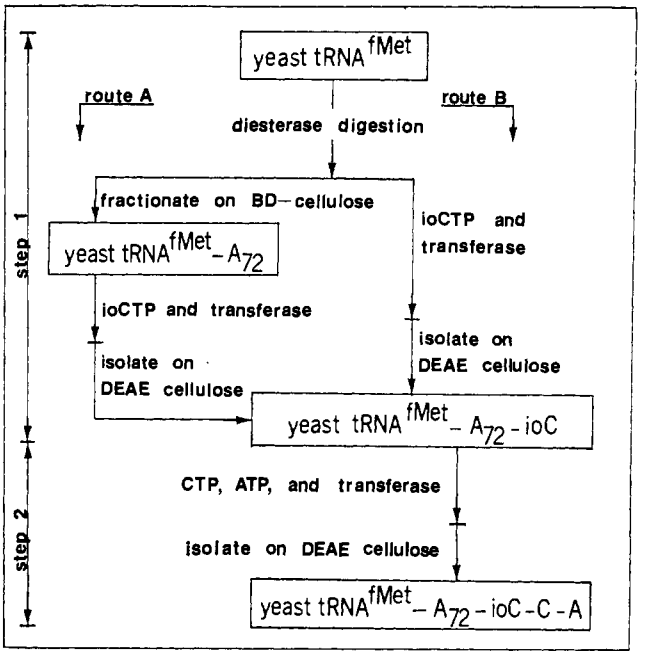

FIGURE 1: Schematic outline of the two step synthesis of yeast tRNA^{fMet}-A_{72}-ioC-C-A. The synthesis of yeast tRNA^{fMet}-A_{72}-ioC from native yeast tRNA^{fMet} constitutes the first step, and synthesis of yeast tRNA^{fMet}-A_{72}-ioC-C-A from yeast tRNA^{fMet}-A_{72}-ioC is accomplished in the second step.

described in (d) was isolated by bringing the concentration of NaCl in the reaction mixture to 0.45 M and fractionating the components on BD-cellulose as described in the legend to Figure 3. Fraction A (Figure 3) was precipitated with three volumes of ethanol and dried with acetone. ioC was inserted at position 73 by incubating 10 μmol of [^{125}I]ioCTP with 100 A_{258} units of yeast tRNA^{fMet}-A_{72} at 25° for 60 min in a 2.0-ml reaction mixture containing 1 mg of nucleotidyltransferase, 20 μmol of $MgCl_2$, and 20 μmol of Tris-Cl (pH 8.7). The product, presumed to be yeast tRNA^{fMet}-A_{72}-ioC, was isolated by applying the reaction mixture to a 1 × 3 cm column of DEAE-cellulose equilibrated with 0.3 M NaCl–10 mM $MgCl_2$, washing with five column volumes at the same buffer, and eluting the tRNA derivative with three column volumes of 1.0 M NaCl–10 mM $MgCl_2$. The product was precipitated with three volumes of ethanol and dried with acetone.

In step 1, route B, ioCTP was incorporated at the 3′ terminus of yeast tRNA^{fMet}-A_{72} by adding directly to the 60-min diesterase reaction mixture 10 μmol of [^{125}I]ioCTP and 1 mg of nucleotidyltransferase for each 100 A_{258} units of yeast tRNA^{fMet} and incubating at 25° for an additional 30 min. Yeast tRNA^{fMet}-A_{72}-ioC was isolated as in step 1, route A.

The terminal dinucleotide was added (step 2) by incubating for 60 min at 25° a 2.0-ml reaction volume containing 100 A_{258} units of yeast tRNA^{fMet}-A_{72}-ioC, 1 mg of nucleotidyltransferase, 1 μmol of [^3H]CTP (25 Ci/mol), 5 μmol of [^{14}C]-ATP (1 Ci/mol), 20 μmol of $MgCl_2$, and 20 μmol of Tris-Cl (pH 8.7). Yeast tRNA^{fMet}-A_{72}-ioC-C-A was recovered as in step 1, route A.

f. Synthesis of Yeast tRNA^{fMet}-A_{72}-C-C-A. This species represents a synthetic "light atom" analog of the heavy-atom derivative in which ioC is replaced by C. Route B of Figure 1 was followed, using either yeast tRNA^{fMet}-A_{72}-ioC-C-A or native yeast tRNA^{fMet} as starting material, and CTP in lieu of ioCTP.

g. Purification of Yeast tRNA^{fMet}-A_{72}-ioC-C-A and Yeast tRNA^{fMet}-A_{72}-C-C-A. The last step in the preparation of these

TABLE I: 3′ End-Group Assignments of the Fractionated Products of Snake Venom Phosphodiesterase Digestion of Yeast $tRNA^{fMet}$.[a]

tRNA	Incorporation of [^{3}H]C and [^{14}C]A with Nucleotidyltransferase[b] mol of [^{3}H]CTP/ mol of Yeast $tRNA^{fMet}$[d]	mol of [^{14}C]ATP/ mol of Yeast $tRNA^{fMet}$[d]	Periodate Oxidation–KB^3H_4 Reduction of Terminal Diol[c] Total cpm at Terminus	cpm Recovd as A_{ox-red}	cpm Recovd as C_{ox-red}	Sequence Assigned
Fraction A (Figure 3)	1.8	0.9	14,126	12,341 (88%)	1,695 (12%)	tRNA-A_{72}
Fraction B (Figure 3)	0.9	0.9	17,297	1,038 (6%)	16,259 (94%)	tRNA-A_{72}-C
Fraction C (Figure 3)	0.0	0.9	11,648	1,048 (9%)	10,600 (91%)	tRNA-A_{72}-C-C
Yeast $tRNA^{fMet}$	0.0	0.0	13,487	12,942 (96%)	545 (4%)	tRNA-A_{72}-C-C-A

[a] Fractions A, B, and C were made 1 M in NaCl, precipitated with three volumes of ethanol, and dried with acetone. [b] Radioactive nucleotides were added by incubation at 25° in a 100-μl mixture containing 1 A_{258} unit of yeast $tRNA^{fMet}$, 20 nmol of [^{3}H]CTP (100 Ci/mol), 50 nmol of [^{14}C]ATP (25 Ci/mol), 1 μg of nucleotidyltransferase, 1 μmol of $MgCl_2$, and 1 μmol of Tris-Cl (pH 8.70). After 15 min 75 μl was spotted onto Whatman 3MM filter disks which were quenched and washed five times with cold 5% CCl_3COOH, dried with ethanol and ether, and counted in toluene–2,5-diphenyloxazole–1,4-bis[2-(5-phenyloxazolyl)]benzene scintillation fluid. [c] To label the end group with ^{3}H, 18 nmol of $NaIO_4$ in 5 μl of H_2O was added to 1 A_{258} unit of yeast $tRNA^{fMet}$ in 10 μl of H_2O. After 100 min in the dark at 25°, freshly prepared KB^3H_4 in 10 μl of 0.01 N KOH was added and the reaction was continued at 25° for an additional 100 min. The reaction mixture was applied to a small DEAE-cellulose column, and washed with 0.3 M NaCl, and the tritiated tRNA was eluted with 1.0 M NaCl, precipitated with three volumes of ethanol, and dried with acetone. The pellet was dissolved in 30 μl of 10 mM Tris-Cl (pH 8.70)–10 mM $MgCl_2$ and digested for 12 hr at 37° according to the proportions 1 A_{258} unit of tritiated tRNA, 1 μg of pancreatic ribonuclease, 1 μg of snake venom phosphodiesterase, and 1 μg of alkaline phosphatase; 9 nmol each of cold carrier trialcohols, A_{ox-red} and C_{ox-red}, was added to the digest and one-dimensional thin-layer chromatography was carried out according to Randerath and Randerath (1971). Uv-absorbing material migrating as A_{ox-red} and C_{ox-red} was excised and eluted with agitation in centrifuge tubes containing 2 ml of 1 M NaCl. Cellulose was removed by centrifugation and 1-ml aliquots were added to 15 ml of Aquasol (New England Nuclear) and counted. [d] Based on a molecular weight of 24,600 (Simsek and RajBhandary, 1972) and an absorbance of 20 A_{258} units/mg of yeast $tRNA^{fMet}$.

TABLE II: Addition of Radioactive Nucleotides to the 3′ Terminus of Yeast $tRNA^{fMet}$-A_{72}.

Synthetic Product	mol of [^{14}C]Met/ mol[a] of Yeast $tRNA^{fMet}$	mol of [^{125}I]ioCTP/ mol[a] of Yeast $tRNA^{fMet}$	mol of [^{3}H]CTP/ mol[a] of Yeast $tRNA^{fMet}$	mol of [^{14}C]ATP/ mol[a] of Yeast $tRNA^{fMet}$
A. Yeast $tRNA^{fMet}$-A_{72}-[^{125}I]ioC-[^{3}H]C-[^{14}C]A by synthetic route A	0.74	0.72	0.82	0.94
B. Yeast $tRNA^{fMet}$-A_{72}-[^{125}I]ioC-[^{3}H]C-[^{14}C]A by synthetic route B	0.83	0.96	1.14	0.88
C. Yeast $tRNA^{fMet}$-A_{72}-[^{3}H]C-[^{3}H]C-[^{14}C]A by synthetic route B	0.92	0.0	2.02	0.94

[a] Based on a molecular weight of 24,600 (Simsek and RajBhandary, 1972) and an absorbance of 20 A_{258} units/mg of yeast $tRNA^{fMet}$.

species was chromatography on BD-cellulose and selection of those fractions having the highest capacity for enzymatic aminoacylation (Figure 2).

Results

a. Preparation and Characterization of Yeast $tRNA^{fMet}$-A_{72}. Ten A_{258} units of native yeast $tRNA^{fMet}$ was digested with snake venom phosphodiesterase as in Materials and Methods and the product was fractionated on a 1.2 × 50 cm column of BD-cellulose (Figure 3). The terminal nucleotides of fractions A, B, and C were established both by regeneration with nucleotidyltransferase, [^{3}H]CTP, and [^{14}C]ATP as well as by periodate oxidation–KB^3H_4 reduction of the terminal diols (Table I). Under the conditions specified above, the dominant product of the diesterase digest was yeast $tRNA^{fMet}$-A_{72} which could be isolated in greater than 80% yield in a single chromatographic step.

b. Characterization of the Synthetic Products; Yeast $tRNA^{fMet}$-A_{72}-ioC-C-A and Yeast $tRNA^{fMet}$-A_{72}-C-C-A. Chromatographic analysis (Figure 4) of the products of a T1 RNase digest of the [^{125}I]iodo derivative localized the iodine site to the 3′-terminal hexanucleotide (RajBhandary and Ghosh, 1969).

The 3′ terminus of yeast $tRNA^{fMet}$-A_{72} was rebuilt with a different radioactive isotope at each position in order to simplify the analysis of the products. The stoichiometry of addition is shown in Table II. One molecule of the heavy-atom derivative contains approximately 1 residue each of [^{125}I]ioC, [^{3}H]C, and [^{14}C]A at the 3′-terminal trinucleotide, and, as ex-

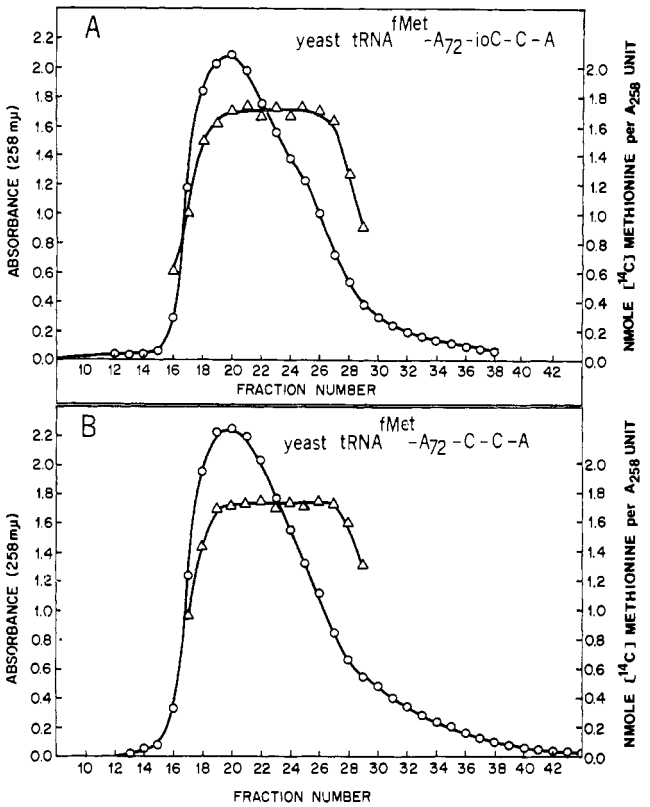

FIGURE 2: BD-cellulose chromatography of yeast tRNA^{fMet}-A$_{72}$-ioC-C-A and yeast tRNA^{fMet}-A$_{72}$-C-C-A. (A) 100 A_{258} units of yeast tRNA^{fMet}-A$_{72}$-ioC-C-A was dissolved in 2 ml of 0.45 M NaCl–10 mM $MgCl_2$ and applied to a 1.2 × 50 cm column of BD-cellulose previously equilibrated with 0.45 M NaCl–10 mM $MgCl_2$. The derivative was then eluted at 0.3 ml/min (20-min fractions) with a 300-ml linear gradient of NaCl (0.45–0.75 M, 10 mM $MgCl_2$). The methionine acceptance of each fraction was subsequently determined. (○) A_{258}; (△) nmole of [^{14}C]methionine esterified per A_{258} unit of iodo derivative. (B) 100 A_{258} units of yeast tRNA^{fMet}-A$_{72}$-C-C-A was dissolved in 2 ml of 0.45 M NaCl–10 mM $MgCl_2$, applied to a 1.2 × 50 cm column of BD-cellulose preequilibrated with 0.45 M NaCl–10 mM $MgCl_2$, and chromatographed as in (A). Each fraction was assayed for methionine acceptance. (○) A_{258}; (△) nmole of [^{14}C]methionine esterified per A_{258} unit of noniodo derivative.

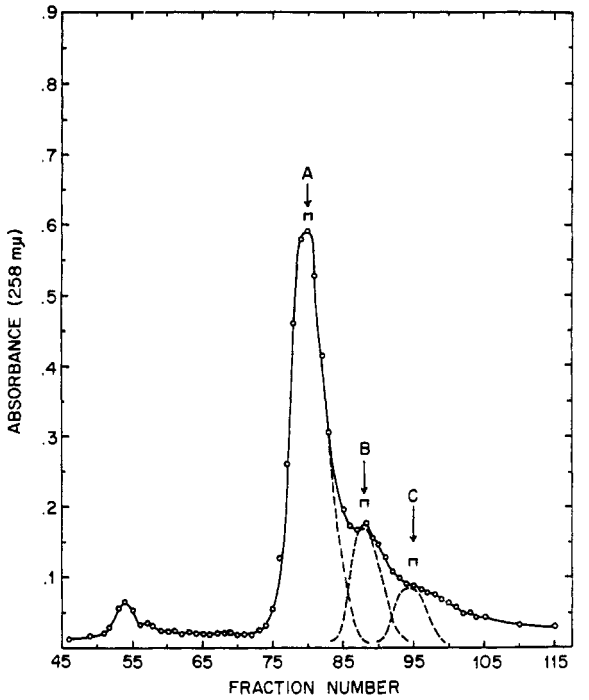

FIGURE 3: BD-cellulose chromatography of the snake venom phosphodiesterase digest products of native yeast tRNA^{fMet}. A diesterase digest mixture (see Materials and Methods) containing 10 A_{258} units of native yeast tRNA^{fMet} was brought to 0.45 M NaCl–10 mM $MgCl_2$ with 4.0 M NaCl–10 mM $MgCl_2$ and applied to a 1.2 × 50 cm column of BD-cellulose previously equilibrated with 0.45 M NaCl–10 mM $MgCl_2$. The column was then eluted at 0.3 ml/min (5-min fractions) with a 300-ml linear gradient of NaCl (0.45–0.75 M, 10 mM $MgCl_2$); (○) A_{258}. Dashed Gaussian curves have been superimposed on the A_{258} profile.

pected, the noniodinated synthetic analog contains two residues of [^{3}H]C and one of [^{14}C]A.

The sequence of the 3′-terminal trinucleotide is summarized in Table III. Based on the sequence of Simsek and RajBhandary (1972), Figure 5 shows that digestion of yeast tRNA^{fMet}-A$_{72}$-[^{125}I]ioC-[^{3}H]C-[^{14}C]A with pancreatic RNase and bacterial alkaline phosphatase should yield the dinucleoside phosphate, A$_{72}$-[^{125}I]ioC, and the nucleosides [^{3}H]C and [^{14}C]A. The dinucleoside phosphate bears a negative charge and should therefore be the only radioactive fragment retained on DEAE-cellulose at neutral pH. The nucleosides which bear no negative charge should not be retained. The chromatographic behavior of the radioactive fragments is shown in Table III. At low ionic strength, 92% of the ^{125}I and none of the ^{3}H was retained as oligonucleotide by DEAE-cellulose, indicating that position 73 is [^{125}I]ioC and not [^{3}H]C. Similarly, the fact that 97% of the ^{3}H and none of the ^{125}I passed through the column at low ionic strength indicates that [^{3}H]C and not [^{125}I]ioC is at position 74. Similar analysis of the putative product, yeast tRNA^{fMet}-A$_{72}$-[^{3}H]C-[^{3}H]C-[^{14}C]A, confirms the assigned sequence.

Having established the exact sequence of the 3′-terminal trinucleotide of yeast tRNA^{fMet}-A$_{72}$-[^{3}H]C-[^{3}H]C-[^{14}C]A, it was possible to more clearly characterize the progress of the diesterase digestion used to produce yeast tRNA^{fMet}-A$_{72}$. This was done because limited diesterase digestion of the 3′-terminal trinucleotide of yeast tRNA^{fMet} did not appear to

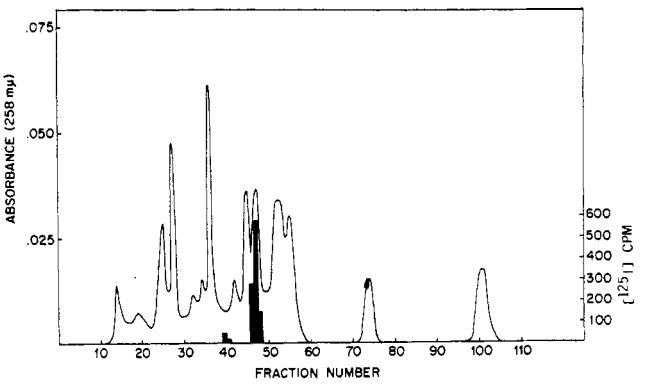

FIGURE 4: Elution from DEAE-cellulose of oligonucleotides produced by the T1 RNase digest of [^{125}I]iodo derivative. 1.5 A_{258} units of [^{125}I]iodo derivative was mixed with 1.5 A_{258} units of native yeast tRNA^{fMet} and applied to a 1.5 × 30 cm G-25 column. After elution with 0.02 M ammonium bicarbonate (pH 7.9), the sample was lyophilized and dissolved in 200 μl of 0.10 M Tris-Cl (pH 7.5). 150 units of heat-treated T1 ribonuclease was added in 30 μl of H_2O, and the mixture was incubated for 2.5 hr at 37°. The digest was applied to a .6 × 120 cm column of DEAE-cellulose preequilibrated with 0.02 M Tris-Cl (pH 7.5)–7 M urea and eluted in 0.02 M Tris-Cl (pH 7.5)–7 M urea with a 860-ml linear NaCl gradient from 0.0 to 0.45 M. The flow rate was 10 ml/hr. 5-ml fractions were collected and counted directly in a Nuclear-Chicago well detector. Solid line, A_{258}; histogram, ^{125}I cpm.

TABLE III: Analysis of the 3′-Terminal Trinucleotide by Combined Pancreatic Ribonuclease and Alkaline Phosphatase Digestion Followed by DEAE-cellulose Chromatography.[a]

Digest of	^{125}I (cpm)			^{3}H (cpm)			^{14}C (cpm)		
	Total Applied	Eluted with H_2O	Eluted with 0.3 M NaCl	Total Applied	Eluted with H_2O	Eluted with 0.3 M NaCl	Total Applied	Eluted with H_2O	Eluted with 0.3 M NaCl
Yeast tRNA^{fMet}-A_{72}-[^{125}I]ioC-[^{3}H]C-[^{14}C]A	4977	0	4603 (92%)	9,746	9469 (97%)	0	6543	6387 (98%)	0
Yeast tRNA^{fMet}-A_{72}-[^{3}H]C-[^{3}H]C-[^{14}C]A				20,905	9804 (47%)	10,924 (53%)	6718	6496 (97%)	0

[a] Both samples were digested at 37° under the following conditions: 50 μl of 20 A_{258} units of yeast tRNA^{fMet}/ml, 5 μl of 1 mg of pancreatic ribonuclease/ml, 5 μl of 1 mg of bacterial alkaline phosphatase/ml, and 50 mM Tris-Cl (pH 7.50). After 3 hr, 50 μl was withdrawn from the digest tube, diluted with 500 μl of H_2O, applied to a 100-μl plug of neutral DEAE-cellulose, previously equilibrated with H_2O, and washed with 2 ml of H_2O and then with 2 ml of 0.3 M NaCl. For yeast tRNA^{fMet}-A_{72}-[^{125}I]ioC-[^{3}H]C-[^{14}C]A, 1-ml aliquots from each fraction were first counted for ^{125}I and 1-ml samples were then added to 15 ml of Aquasol and counted for ^{3}H and ^{14}C in a Packard liquid scintillation spectrometer. These latter values have been corrected for ^{125}I.

proceed in the biphasic manner reported by Miller *et al.* (1970) who showed that the "second C" (here C_{73}) is removed appreciably slower than the "first C" (here C_{74}). Figure 6 plots the diesterase-catalyzed release of [^{3}H]C and [^{14}C]A from yeast tRNA^{fMet}-A_{72}-[^{3}H]C-[^{3}H]C-[^{14}C]A and clearly shows that the terminal [^{14}C]A is released the fastest. Were the release of [^{3}H]C_{73} 16 times slower than that of [^{3}H]C_{74}, as in the experiments of Miller *et al.* (1970), then a plot of the release of [^{3}H]C would have the form shown in the insert to Figure 6. There is no suggestion that the rate constant for the release of [^{3}H]C_{74} is larger than that for the release of [^{3}H]C_{73} under the conditions chosen here to optimize the production of yeast tRNA^{fMet}-A_{72}.

The capacity of the iodo derivative and its noniodo-analog to enzymatically accept methionine is given in Table II, which indicates that replacement of C_{73} in yeast tRNA^{fMet} with ioC_{73} does not interfere with the extent of enzymatic esterification of methionine. Preliminary experiments have demonstrated that V_{max} for the aminoacylation reaction is similarly not decreased.

c. Preparation and Characterization of Crystals of Yeast tRNA^{fMet}-A_{72}-ioC-C-A. Large single crystals (>0.3 mm in the smallest dimension), suitable for X-ray diffraction to 4 Å and apparently isomorphous with those of the parent (Young

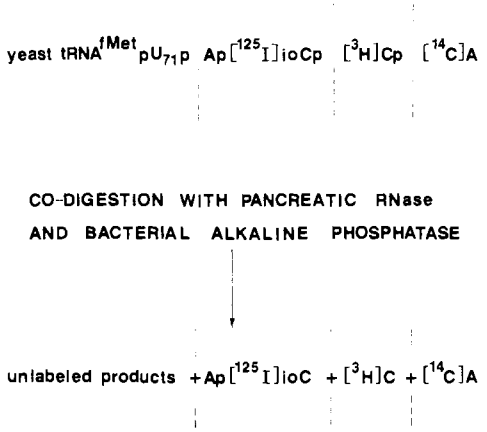

FIGURE 5: Radioactive fragments expected from digestion of yeast tRNA^{fMet}-A_{72}-[^{125}I]ioC-[^{3}H]C-[^{14}C]A with pancreatic RNase A and alkaline phosphatase.

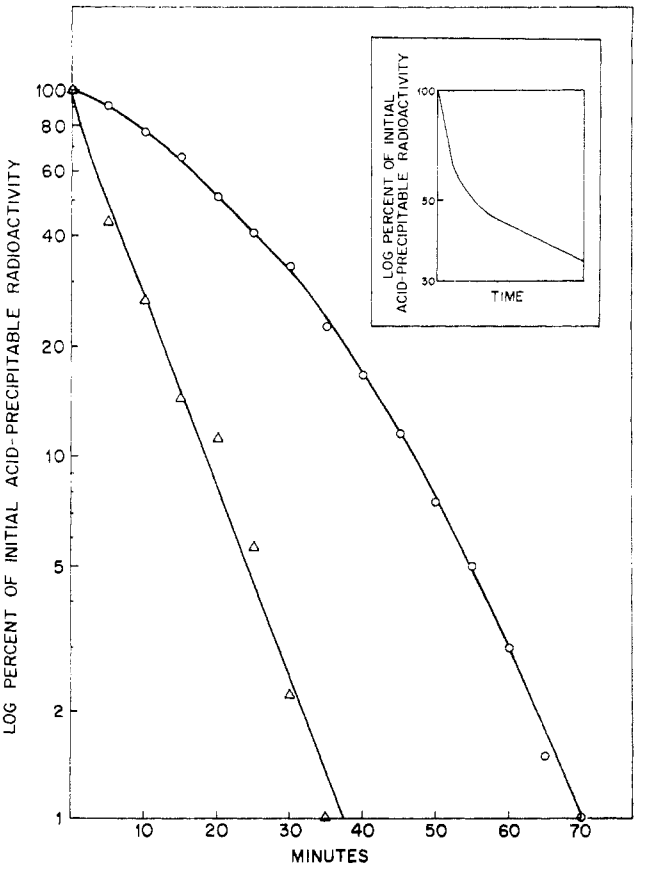

FIGURE 6: Diesterase digestion of yeast tRNA^{fMet}-A_{72}-[^{3}H]C-[^{3}H]C-[^{14}C]A. 10 A_{258} units of yeast tRNA^{fMet}-A_{72}-[^{3}H]C-[^{3}H]C-[^{14}C]A, synthesized as in Materials and Methods, part f, was digested as described in part d. At regular intervals over a 90-min period, 100-μl aliquots were withdrawn from the digest tube, spotted onto Whatman 3MM filter disks, quenched with 5% CCl_3COOH, and worked up and counted as described in the legend to Table I. (○) ^{3}H cpm; (△) ^{14}C cpm. Insert discussed in text.

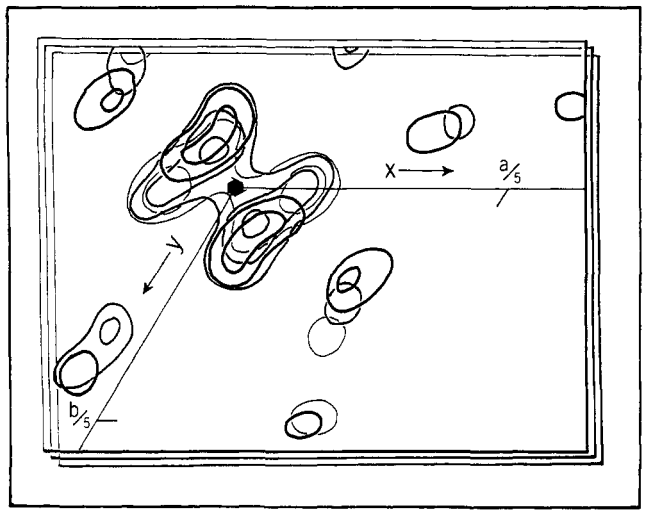

FIGURE 7: Dominant feature of the three-dimensional difference Fourier synthesis comparing the iodo derivative with the parent structure. Coefficients explained in the text. Three sections, at z = (5/72)c (top), (6/72)c, and (7/72)c (bottom), have been superimposed. The 6_2 screw axis through the origin is perpendicular to the planes and has the property of a twofold axis of rotation.

et al., 1969), were produced from both iodo and noniodo derivatives by procedures routinely used in this laboratory (Johnson *et al.*, 1970). Whereas the diffraction pattern of the iodo derivative showed noticeable differences in intensities, the diffraction pattern of the noniodo derivative was indistinguishable from that of the parent. Three-dimensional 6-Å data for this derivative have been collected as previously described (Schevitz *et al.*, 1972) and a three-dimensional difference map, comparing the iodo derivative to the parent structure, has been calculated using parent phase angles and figures of merit based on three other isomorphous derivatives (R. Schevitz, G. Cornick, M. Navia, W. Hewitt, and P. Sigler, unpublished results). The difference map shows a dominant feature (Figure 7) indicating a heavy-atom position consistent with the three-dimensional difference Patterson function. Refinement of the heavy-atom site, occupancy, and temperature factor are in progress.

Discussion

The synthetic scheme initially was designed to insert ioC at either position 73 or possibly both positions 73 and 74. Following the scheme outlined in Figure 1, only position 73 was substituted with ioC. The fact that ioC was not inserted in position 74 can be explained by the report of Deutscher (1973) who has discovered a nucleolytic activity associated with highly purified rabbit liver nucleotidyltransferase. This activity removes C_{74} from tRNA-X_{72}-C-C at pH 8.7 in the absence of ATP at a substantial rate; that is, under the conditions used in this laboratory to add ioCTP to yeast tRNA^{fMet}-A_{72}. A similar activity in partially purified yeast nucleotidyltransferase could explain the incorporation of ioCTP into yeast tRNA^{fMet}-A_{72} exclusively at position 73. Moreover, the fact that Sprinzl *et al.* (1972) successfully synthesized yeast tRNA^{Phe}-A_{73}-ioC-ioC-A can be explained by Deutscher's observation that at 0.1 M KCl, the salt concentration used by Sprinzl *et al.* (1972), this exonucleolytic activity is inhibited. Experiments to synthesize yeast tRNA^{fMet}-A-ioC-ioC-A are in progress.

There is strong precedent for the use of iodinated derivatives in crystal structure determination for the purposes specified in the introduction. The sulfonylation of the active serine with *p*-iodobenzenesulfonyl fluoride and direct iodination of a limited number of tyrosine residues served as markers for specific sites in the crystallographic study of both the γ and α forms of bovine chymotrypsin A (Sigler *et al.*, 1964, 1966; Cohen *et al.*, 1969; Sigler, 1970). Phase information was provided by iodo derivatives in the structure determination of γ- and α-chymotrypsin (Matthews *et al.*, 1967; Cohen *et al.*, 1969) as well as ribonuclease (Wyckoff *et al.*, 1967) and micrococcal nuclease (Arnone *et al.*, 1969).

The relative value of the iodo derivative of crystalline yeast tRNA^{fMet} will be fully evaluated within the context of a discussion of the structure of yeast tRNA^{fMet} (R. Schevitz, M. Navia, G. Cornick, M. Pasek, W. Hewitt, and P. Sigler, work in progress). It is clear at this stage that the dominant heavy-atom site in Figure 7 is sufficiently well defined to provide a marker for the -CCA terminus in the electron density map of yeast tRNA^{fMet}. On the other hand, the tubular shape of the heay-atom peak as well as preliminary refinement results suggest that this region of the molecule exhibits a degree of disorder that may preclude further improvement of the parent phase angles with this derivative.

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Studies on Polynucleotides Containing Hybrid Sequences. Synthesis of Oligonucleotides Containing Thymidine, Adenosine, and a Single Deoxyribonucleotidyl-(3′–5′)-ribonucleotide Linkage†

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ABSTRACT: The polymerization of thymidine 5′-phosphate in the presence of 2,4,6-triisopropylbenzenesulfonyl chloride and N^6,$O^{2'}$,$O^{3'}$-triacetyladenosine 5′-phosphate yields two series of oligonucleotides, pdT-dT_n-dT and pdT-dT_n-A, as the major products. The two sets of products may be readily separated by chromatography on a column of dihydroxyboryl-substituted cellulose, and the individual members of each series can be subsequently resolved by anion-exchange chromatography on polystyrene resins using aqueous ethanol solutions containing linear gradients of salt concentration as the eluting solvents. The series, pdT-dT_n-A, and the dephosphorylated series, dT-dT_n-A, are shown to serve both as acceptor molecules for single addition reactions catalyzed by polynucleotide phosphorylase in the presence of 2′-*O*-(α-methoxyethyl)adenosine 5′-diphosphate, and also as acceptors for polymerization reactions involving adenosine 5′-diphosphate and polynucleotide phosphorylase. The products, pdT-dT_n-A_m-A and dT-dT_n-A_m-A, are suitable substrates for the study of the mode of action of nucleases on polynucleotides containing hybrid sequences.

The recent discoveries of the occurrence, in biological systems, of nucleic acids containing covalently linked ribo- and deoxyribopolynucleotide chains have generated an interest in their chemical, biochemical, and physical properties as well as an interest in the relationship that these properties might have to the biological function of such hybrid molecules. For example, the involvement of RNA primers in a number of DNA-synthesizing systems raises questions concerning both the nature of the enzymatic processing of the hybrid product formed and the physical and chemical properties of the hybrid that might be responsible for directing the processing. Apart from their obvious biological importance covalent hybrid polynucleotides are of some biochemical interest in that, in many *in vitro* enzyme systems, they are capable of serving in place of normal ribo- or deoxyribopolynucleotides, and this substitution can provide distinct diagnostic advantages in the study of the system. Canellakis and Canellakis (1963) have pointed out that DNA molecules containing a ribonucleotide at their 3′ terminals can serve as primers for a variety of nucleotide-polymerizing enzymes. The use of unusual primers of this type not only provides some indication of the primer requirements of the particular enzyme system but also facilitates the identification and characterization of the reaction products.

Up to the present time, studies on hybrid oligonucleotides have been limited to the synthesis and the investigation of the properties of some small hybrid molecules containing a single type of nucleotide base. For example, Kondo *et al.* (1972) have prepared adenosine–deoxyadenosine dinucleoside phosphates for a comparative study of their conformations in solution, while Moon *et al.* (1966) have synthesized five trinucleoside diphosphates containing adenosine and deoxyadenosine moieties for the study of their ability to bind lysyl-tRNA to ribosomes. Certain covalent hybrid molecules have been prepared by the action of polynucleotide phosphorylase. This enzyme, in the presence of the appropriate nucleoside diphosphates, has been shown to (i) catalyze the addition of

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