it easier for TN-I to interact with tropomyosin. TN-C changes the state of TN-I. This change is Ca^{2+} sensitive making it possible for TN-I to interact with tropomyosin in the minus-Ca^{2+} state only.

Acknowledgments

The authors thank Professor F. Oosawa, Professor S. Asakura, and Dr. K. Mihashi for their encouragement and stimulating discussions.

References

Arai, K., and Watanabe, S. (1968), *J. Biol. Chem. 243*, 5670.

Bremel, R. D., and Weber, A. (1972), *Nature (London), New Biol. 238*, 97.

Davis, D. J. (1964), *Ann. N. Y. Acad. Sci. 121*, 404.

Drabikowski, W., Dabrowska, R., and Barylko, B. (1971a), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 12*, 148.

Drabikowski, W., Rafalowska, U., Dabrowska, R., Szpacenko, A., and Barylko, B. (1971b), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 19*, 259.

Ebashi, S., and Endo, M. (1968), *Progr. Biophys. Mol. Biol. 18*, 123.

Ebashi, S., Kodama, A., and Ebashi, F. (1968), *J. Biochem. 64*, 465.

Ebashi, S., Ohtsuki, I., and Mihashi, K. (1972), *Cold Spring Harbor Symp. Quant. Biol. 37*, 235.

Ebashi, S., Wakabayashi, T., and Ebashi, F. (1971), *J. Biochem. 69*, 441.

Gornall, A. G., Bardawill, C. J., and David, M. M. (1949), *J. Biol. Chem. 177*, 751.

Greaser, M., and Gergely, J. (1970), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 29*, 463.

Greaser, M., and Gergely, J. (1971), *J. Biol. Chem. 246*, 4226.

Greaser, M., and Gergely, J. (1973), *J. Biol. Chem. 248*, 2125.

Hartshorne, D. J., and Pyun, H. Y. (1971), *Biochim. Biophys. Acta 229*, 698.

Hirs, C. H. W. (1967), *Methods Enzymol. 11*, 199.

Kawasaki, Y., and van Eerd, J. P. (1972), *Biochem. Biophys. Res. Commun. 49*, 898.

Lee, L. W., and Watanabe, S. (1970), *J. Biol. Chem. 245*, 3004.

Murray, A. C., and Kay, C. M. (1972), *Biochemistry 11*, 2622.

Perry, S. V. (1971), *Biochem. J. 125*, 83p.

Schwartzenbach, G., Senn, H., and Anderegg, G. (1957), *Helv. Chim. Acta 40*, 1886.

Staprans, I., and Watanabe, S. (1970), *J. Biol. Chem. 245*, 5962.

Tonomura, Y., Watanabe, S., and Morales, M. F. (1969), *Biochemistry 8*, 2171.

van Eerd, J. P., and Kawasaki, Y. (1972), *Biochem. Biophys. Res. Commun. 47*, 859.

Weber, K., and Osborn, M. (1969), *J. Biol. Chem. 244*, 4406.

Wilkinson, J. M., Perry, S. V., Cole, H., and Trayer, I. P. (1971), *Biochem. J. 124*, 55p.

Chemical Modification of Transfer Ribonucleic Acid Species. Thallium(III)-Mediated Iodination of Yeast Formylatable Methionine Transfer Ribonucleic Acid†

Francis J. Schmidt,‡ Daniel R. Omilianowski,§ and Robert M. Bock*

ABSTRACT: When yeast $tRNA_f^{Met}$ was treated with $TlCl_3$ and NaI in acetate buffer containing $MgCl_2$, 3.2 ± 0.2 mol of iodine was incorporated/mol of tRNA. The stably incorporated iodine was primarily localized in two of the oligonucleotides obtained by T1 ribonuclease digestion of the iodinated $tRNA_f^{Met}$, those containing the amino acid acceptor end and the anticodon sequence of the molecule. In contrast, previously digested tRNA molecules incorporated ^{125}I into seven additional peaks, including the sequence CpGp, which is unmodified in native $tRNA_f^{Met}$ and is located in the double-stranded regions exclusively. The $tRNA_f^{Met}$ iodinated by this procedure accepted amino acid to the same extent as native $tRNA_f^{Met}$, but with a higher K_m and lower V_{max}.

The crystallographic analysis of tRNA demands the introduction of heavy atoms into specific sites in the tRNA crystal. Several approaches may be utilized for this search, either soaking crystals in solutions containing heavy atom ions or organometallic compounds (Schevitz *et al.*, 1972; Kim *et al.*, 1972), treating the α-amino moiety of aminoacyl-tRNA with heavy-atom containing compounds (Schmidt *et al.*, 1972a), treating sites in the native tRNA molecule with heavy atoms (Omilianowski, 1971), or introducing such sites chemically (Hecht and Bock, 1971) or enzymatically (Schlimme *et al.*, 1970). Enzymatic introduction of 5-iodo-CMP into yeast $tRNA^{Phe}$ has been reported (Sprinzl *et al.*, 1972). In addition, Faulkner and Uziel (1971) added iodine to *Escherichia coli* $tRNA^{Phe}$, $tRNA^{Val}$, and $tRNA^{Tyr}$ using I_3^-. Iodine addition was localized at the 2-methylthio-N^6-(Δ^2)-isopentenyl-

† From the Department of Biochemistry, College of Agriculture and Life Sciences, and the Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin 53706. *Received May 29, 1973.* Portions of this work have been reported previously (Schmidt *et al.*, 1972b). This work was supported in part by National Institutes of Health Grant GM 12395.

‡ National Institutes of Health Trainee, Grant 5 T01-GM01874.

§ Present address: Biophysics Laboratory, University of Wisconsin, Madison, Wis. 53706.

adenosine (Robins *et al.*, 1967) and 4-thiouridine (Lipsett and Doctor, 1967) bases in these tRNAs and could be reversed by the addition of thiosulfate. Both these bases are absent in yeast tRNAs (Hall, 1971) in general, and in tRNA$_f^{Met}$ in particular (Simsek and RajBhandary, 1972). The procedure of Commerford (1971), utilizing Tl^{3+} to generate reactive iodine species from I^-, has been reported to lead to formation of stably bound iodine in nucleic acids containing cytidine nucleotides and has been used here to introduce a limited number of iodine atoms to the tRNA$_f^{Met}$ molecule. tRNA$_f^{Met}$ iodinated by this procedure accepted [^{14}C]methionine from *E. coli* methionyl-tRNA synthetase to the same extent as unmodified tRNA$_f^{Met}$, but with an altered K_m and V_{max}.

Materials

tRNA$_f^{Met}$ was prepared from *Saccharomyces cerevisiae*, strain Y-185, by Drs. L. Kirkegaard and M. Miyazaki in our laboratory (unpublished results). It was judged to be approximately 60% pure by amino acid acceptance. $TlCl_3$ was purchased from K&K Laboratories, P-2 polyacrylamide gel from Bio-Rad Laboratories, Sephadex A25 and Sephadex G-10 from Pharmacia, Inc., and Whatman DE23 DEAE-cellulose[1] from Reeve-Angel, Inc. All other chemicals used were of reagent grade. *E. coli* methionyl-tRNA synthetase was prepared by ammonium sulfate fractionation and chromatography on Sephadex A25 and Sephadex G-100 (T. Johnson and F. Schmidt, unpublished results). Ribonuclease T1 was a gift of Dr. J. Kelley.

Methods

Incorporation of Iodine into Transfer RNA. To 80 A_{260} units of tRNA$_f^{Met}$ in 1.6 ml of 0.10 N sodium acetate–0.01 N $MgCl_2$ buffer (pH 4.5) were added 0.2 ml of 1.6×10^{-2} N $TlCl_3$ solution and 0.2 ml of 2.5×10^{-3} N Na^{125}I solution in the same buffer. After incubation at 37°, 0.05-ml samples were taken and the reaction was stopped by the addition of 0.01 ml of 0.1 N Na_2SO_3 solution (Commerford, 1971). Aliquots (0.02 ml) were added to a 1.0-ml column of P-2 polyacrylamide gel and eluted with water. Three-drop fractions were taken and counted in 5 ml of scintillation solution containing toluene–ethoxyethanol (7:3, v/v) (Hall and Cocking, 1965). Iodine analysis of a sample of tRNA$_f^{Met}$ treated with KI and $TlCl_3$ was performed by instrumental neutron activation analysis. A 20-μl (9 A_{260} units) sample of iodinated tRNA$_f^{Met}$ was placed in the corner of a polyethylene bag and dried *in vacuo* over P_2O_5. The bag was sealed and placed in a polyethylene vial. The sample and a standard containing 5 μg of iodine were irradiated for 15 min in the pneumatic tube (thermal neutron flux = 4×10^{12} cm^{-2} sec^{-1}) of the 1000-kW University of Wisconsin nuclear reactor. After a 10-min decay time, samples were transferred to a new vial and counted for 300 sec on a 30 cm^3 Ge(Li) detector coupled to a multichannel analyzer. The area under the 442.7-keV ^{125}I γ-ray photopeak was used for the determinations, while the measured element was positively identified as iodine by half-life measurement and the 526.3-keV γ emission of the sample. The estimated uncertainty was 10%.

[1] Abbreviations used are: NH_4-Pipes, ammonium salt of piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); A_{260} units, the amount of tRNA such that a solution of 1 ml^{-1} has A_{260} = 1 (1-cm path-length cell); DEAE-cellulose, diethylaminoethylcellulose.

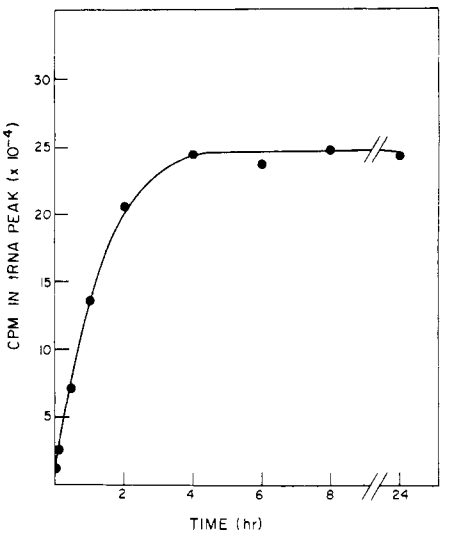

FIGURE 1: Iodination of tRNA$_f^{Met}$, measured as iodine in the first peak eluting on gel chromatography (Commerford, 1971). Total counts in the first (tRNA) and second ($^{125}I^-$) peaks were constant throughout the experiment. Each peak corresponds to 0.6 A_{260} unit of tRNA$_f^{Met}$.

Identification of the Reactive Nucleotides of the tRNA Molecule. tRNA (15 A_{260} units) labeled with ^{125}I for 10 hr was digested with 20 units of T1 ribonuclease for 24 hr at 37°. The solution was then applied to a 0.25 × 122 cm column of Whatman DE23 DEAE-cellulose in 0.02 N Tris-Cl (pH 7.5)–7 M urea and eluted with a linear gradient (80 ml + 80 ml) from 0.02 N Tris-Cl (pH 7.5)–7 M urea to 0.02 N Tris-Cl (pH 7.5)–7 M urea + 0.4 N NaCl (Tomlinson and Tener, 1963) at a flow rate of 2 ml hr^{-1}. The effluent was monitored at 260 nm, collected in vials, and counted directly in a Nuclear-Chicago well-type counter. T1 RNase digests of tRNA$_f^{Met}$ were iodinated as above and chromatographed after removal of free $^{125}I^-$ by chromatography on Sephadex G-10.

Amino Acid Acceptance of Iodinated tRNA. tRNA was iodinated using NaI and $TlCl_3$ as above. After 24 hr at 37°, tRNA was precipitated with ethanol and reprecipitated 3× from 0.05 N Tris-Cl (pH 7.5)–0.01 M $MgCl_2$–10^{-3} M EDTA. Amino acid acceptance was assayed in 1 mM ATP, 4 mM CTP, 0.01 N NH_4^+-Pipes (pH 7.0), 5×10^{-4} M EDTA, 0.01 N KCl, 0.01 N $MgCl_2$, and 0.5 mM [^{14}C]methionine, specific activity 10 μCi μmol^{-1}. Partially fractionated *E. coli* methionyl-tRNA synthetase was added and Met-tRNA precipitated on paper disks with 0.1 ml of 0.025 M cetyltrimethylammonium bromide in 1% acetic acid. After washing in 1% acetic acid, disks were dried and counted in toluene scintillator containing 5 g l.$^{-1}$ of 2,5-diphenyloxazole and 0.3 g l.$^{-1}$ of *p*-bis[2-(5-phenoxazolyl)]benzene.

K_m Determination of Iodinated tRNA. Varying concentrations of tRNA$_f^{Met}$ or iodinated tRNA$_f^{Met}$ were assayed as described above. The Met-tRNA synthetase used above was diluted 1:10; 0.02 ml was added to 0.10 ml of the assay solution; after 5-min incubation, aliquots of 0.05 ml were precipitated on disks, washed, dried, and counted as above.

Results

Incorporation of Iodine into tRNA. Figure 1 shows the incorporation of ^{125}I into tRNA$_f^{Met}$; the final level of incorporation was determined from the ratio of counts at the void volume of the gel filtration column to the sum counts in both the tRNA

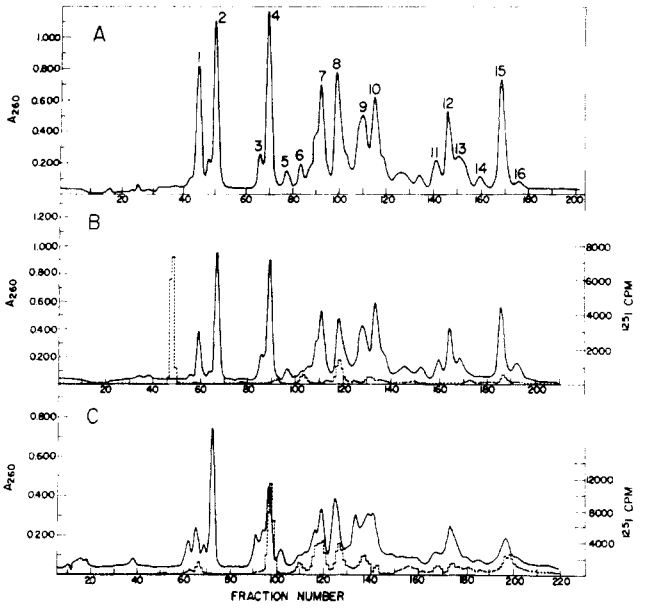

FIGURE 2: Identification of iodinated portions of the $tRNA_f^{Met}$ molecule by chromatography on DEAE-cellulose in 7 M urea: (A) digest of $tRNA_f^{Met}$; (B) digest of $tRNA_f^{Met}$ after iodination; (C) digest of $tRNA_f^{Met}$ iodinated after digestion; (—) A_{260}; (- - -) ^{125}I; counts per minute.

and iodine peaks; the final level of incorporation was 3.2 ± 0.2 equiv of iodine/$tRNA_f^{Met}$. At 24 hr, all the label was insensitive to heating at 90° in the presence of sulfite (data not shown). A sample of $tRNA_f^{Met}$ iodinated with nonradioactive iodide was analyzed by neutron activation analysis. Nine A_{260} units of iodinated tRNA contained 5.5 μg (4.3 × 10^{-8} mol) of iodine, corresponding to a molar ratio of 2.9 iodine/tRNA.

Localization of Incorporated Iodine. DEAE-cellulose chromatography in 7 M urea (Tomlinson and Tener, 1963) was used to separate T1 oligonucleotides of $tRNA_f^{Met}$. Figure 2A is the elution pattern of unmodified $tRNA_f^{Met}$. Figures 2B and 2C represent the elution of a T1 digest of iodinated $tRNA_f^{Met}$ and of $tRNA_f^{Met}$ iodinated after digestion. It is apparent that the incorporation into native $tRNA_f^{Met}$ was constrained in a manner dependent on secondary and tertiary structures of the tRNA molecule. Peaks 8 and 15 have been identified by comparison with the previously published T1 map of $tRNA_f^{Met}$ as those containing the 3′-terminal end of the tRNA and the anticodon sequence, respectively (RajBhandary and Ghosh, 1969; U. L. RajBhandary, personal communication).

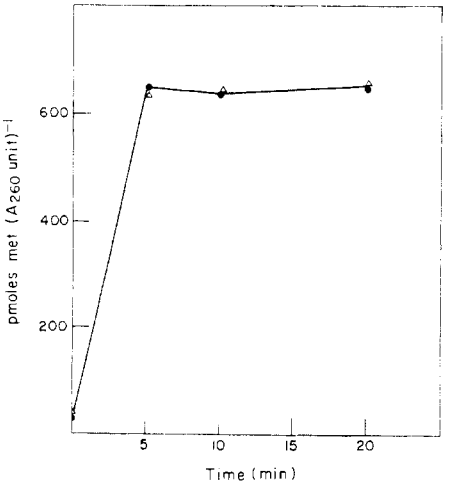

FIGURE 3: [^{14}C]Methionine acceptance of iodinated tRNA. Conditions are as described under Methods: (△) $tRNA_f^{Met}$; (●) iodinated $tRNA_f^{Met}$.

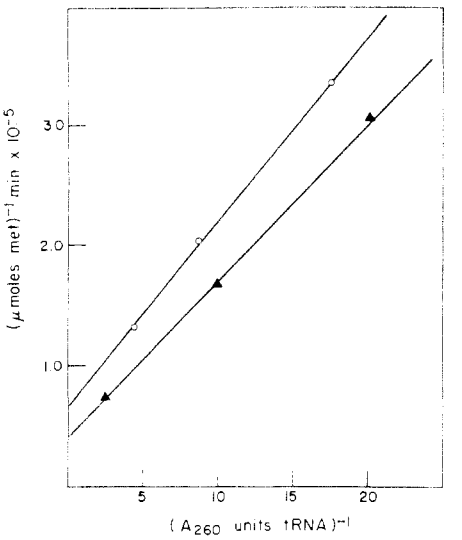

FIGURE 4: Lineweaver–Burk plot of reciprocal velocity of aminoacylation *vs.* reciprocal concentration of tRNA: (▲) $tRNA_f^{Met}$; (○) iodinated $tRNA_f^{Met}$.

Amino Acid Acceptance of $tRNA_f^{Met}$ after Iodination. $tRNA_f^{Met}$ and iodinated $tRNA_f^{Met}$ accepted [^{14}C]methionine to the same extent (Figure 3). However, the iodinated tRNA exhibited a higher K_m and lower V_{max} than the unmodified species (Figure 4).

Discussion

Figure 5 shows the sequence of yeast $tRNA_f^{Met}$ as reported by Simsek and RajBhandary (1972); the major sites of io-

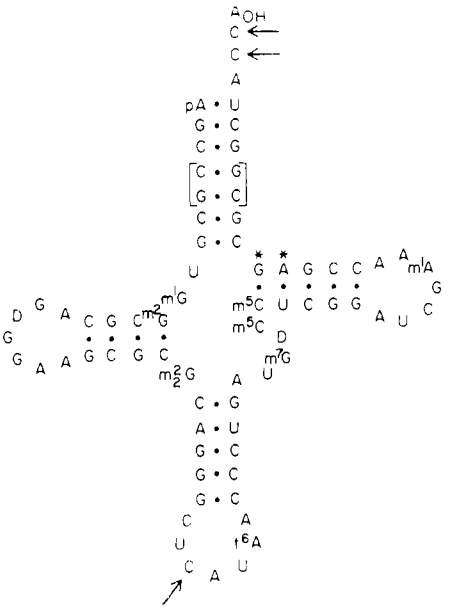

FIGURE 5: Sequence of yeast $tRNA_f^{Met}$ as determined by Simsek and RajBhandary (1972). Probable sites of iodination are indicated by arrows.

dination are indicated by arrows. The presence of iodine incorporated in peak 6 (Figure 2b) is somewhat puzzling, as the oligonucleotide has not been shown to contain cytidine (U. L. RajBhandary, personal communication). While no iodination of bases other than cytidine by this procedure has been reported, we cannot rule out the possibility that minor bases in tRNA may show reactivity to iodine under these conditions. Alternatively, the incorporation may be due to a highly reactive contaminant in the $tRNA_f^{Met}$ sample used. At present, we cannot distinguish between these alternatives. However, the less than molar stoichiometry of this peak as well as the absence of an increase in iodination when a T1 digest of $tRNA_f^{Met}$ was iodinated (Figure 2C) favor the latter interpretation.

The presence of full amino acid acceptor ability in the modified tRNA argues that neither the cytidines in the anticodon loop nor the terminal CCA stem were required to be unmodified for recognition by the heterologous synthetase; this is in agreement with the results of Sprinzl *et al.* (1972) using yeast $tRNA^{Phe}$, where the terminal CCA stem was substituted with 5-iodocytidine. The terminal CCA of yeast $tRNA^{Tyr}$ was also modified to UUA (Kúcan *et al.*, 1969), again without loss of amino acid acceptance. In apparent contrast to the results reported here, Schulman and Goddard (1973) have reported that the deamination of the cytidine residue immediately adjacent to the acceptor adenosine of *E. coli* $tRNA_f^{Met}$ results in the loss of amino acid acceptance, while the deamination of the other cytidine in the CCA stem has no effect.

The primary purpose of this study was to investigate the utility of the $TlCl_3$–NaI system for the generation of heavy-atom derivatives of tRNA species. These results demonstrate the introduction of a limited number of covalently bound iodine atoms into a tRNA molecule; their presence in defined bases of the tRNA molecule would be advantageous for these studies, while the activity of the iodinated tRNA indicates a conformation similar to that of native $tRNA_f^{Met}$ in solution.

Acknowledgments

We wish to thank Dr. U. L. RajBhandary for communicating results before publication as well as for helpful discussions, Drs. K. Clifton and M. B. Yatvin for use of counting facilities, and Mary Anne Helmes for excellent technical assistance throughout the course of this work.

References

Commerford, S. L. (1971), *Biochemistry 10*, 1993.
Faulkner, R. D., and Uziel, M. (1971), *Biochim. Biophys. Acta 238*, 464.
Hall, R. H. (1971), The Modified Nucleosides in Nucleic Acids, New York, N. Y., Columbia University Press.
Hall, T. C., and Cocking, E. C. (1965), *Biochem. J. 96*, 626.
Hecht, S. M., and Bock, R. M. (1971), *Biochem. Biophys. Res. Commun. 45*, 416.
Kim, S. H., Quigley, G., Suddath, F. L., McPherson, A., Kim, J. J., Weinzerl, J., Blattman, P., and Rich, A. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 3746.
Kúcan, Z., Freude, K. A., Kúcan, I., and Chambers, R. W. (1969), *Nature (London), New Biol. 232*, 177.
Lipsett, M. N., and Doctor, B. P. (1967), *J. Biol. Chem. 242*, 4072.
Omilianowski, D. R. (1971), Ph.D. Thesis, University of Wisconsin.
RajBhandary, U. L., and Ghosh, H. P. (1969), *J. Biol. Chem. 244*, 1104.
Robins, M. J., Hall, R. M., and Thedford, R. (1967), *Biochemistry 6*, 1837.
Schevitz, R. W., Navia, M. A., Bantz, D. A., Cornick, G., Rosa, J. J., Rosa, M. D. H., and Sigler, P. (1972), *Science 177*, 429.
Schlimme, E., von der Haar, F., Eckstein, F., and Cramer, F. (1970), *Eur. J. Biochem. 14*, 351.
Schmidt, F. J., Bock, R. M., and Hecht, S. M. (1972a), *Biochem. Biophys. Res. Commun. 48*, 451.
Schmidt, F. J., Omilianowski, D. R., and Bock, R. M. (1972b), *Fed. Proc., Fed. Amer. Soc. Exp. Biol. 31*, 903.
Schulman, L. H., and Goddard, J. P. (1973), *J. Biol. Chem. 248*, 1341.
Simsek, M., and RajBhandary, U. L. (1972), *Biochem. Biophys. Res. Commun. 49*, 508.
Sprinzl, M., von der Haar, F., Schlimme, E., Sternbach, H., and Cramer, F. (1972), *Eur. J. Biochem. 25*, 262.
Tomlinson, R. V., and Tener, G. M. (1963), *Biochemistry 2*, 697.