the helix or other long-range cooperative motions of nucleotides cannot be responsible for the DNA motions monitored by NMR.

Loss of DNA Internal Motions May Be Accompanied by DNA Conformational Changes. The most important conclusion to be drawn from the work presented here is that the B-DNA helix does not present itself as a single well-defined structure to a ligand molecule but is instead an averaged structure which is "blurred" by fast internal motions. If, when bound, multiple contacts are made between a ligand and the helix, then internal motions may be obstructed, making the binding site more nearly rigid. Regardless of the details of binding or of the detailed mechanism of internal motion, the process of obstructing DNA motions will necessarily alter the time-averaged conformation of DNA at the binding site; i.e., ligand binding can "freeze" DNA into a rigid conformation substantially different from its averaged conformation in solution.

References

Abragam, A. (1961) *The Principles of Nuclear Magnetism*, pp 308–312, Oxford University Press, London.

Andrew, E. R., Hinshaw, W. S., & Hutchins, M. G. (1974) *J. Magn. Reson. 15*, 196–200.

Arnott, S., Dover, S. D., & Winocott, A. J. (1969) *Acta Crystallogr., Sect. B 25*, 2192–2206.

Bauer, W., & Vinograd, J. (1970) *J. Mol. Biol. 47*, 419–435.

Bolton, P. H., & James, T. L. (1980) *J. Am. Chem. Soc. 102*, 25–31.

Bresloff, J. L., & Crothers, D. M. (1975) *J. Mol. Biol. 95*, 103–123.

Early T. A., & Kearns, D. R. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 4165–4169.

Hogan, M. E., & Jardetzky, O. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 6341–6345.

Hogan, M. E., Dattagupta, N., & Crothers, D. M. (1978) *Proc. Natl. Acad. Sci. U.S.A. 75*, 195–199.

King, R., & Jardetzky, O. (1978) *Chem. Phys. Lett. 55*, 15–23.

Klevan, L., Armitage, I. M., & Crothers, D. M. (1979) *Nucleic Acids Res. 6*, 1607–1616.

LePecq, J. B., & Paoletti, C. (1967) *J. Mol. Biol. 27*, 87–106.

Marmur, J., & Doty, P. (1962) *J. Mol. Biol. 5*, 109–118.

Patel, D. J., & Canuel, L. L. (1976) *Proc. Natl. Acad. Sci. U.S.A. 73*, 3343–3347.

Redfield, A. G., & Kunz, S. D. (1975) *J. Magn. Reson. 19*, 114–117.

Schweizer, M. P., Chan, S. I., Helmkamp, G. K., & T'so, P. O. P. (1964) *J. Am. Chem. Soc. 86*, 696.

Tsai, C. C., Jain, S. C., & Sobell, H. M. (1975) *Proc Natl. Acad. Sci. U.S.A. 72*, 628–632.

Tsai, C., Jain, S. C., & Sobell, H. M. (1977) *J. Mol. Biol. 114*, 301–315.

Wahl, P. L., Paoletti, C., & LePecq, J. B. (1970) *Proc. Natl. Acad. Sci. U.S.A. 65*, 417.

Woessner, E. D. (1962) *J. Chem. Phys. 37*, 647–654.

Primary Structure of AUA-Specific Isoleucine Transfer Ribonucleic Acid from *Escherichia coli*†

Yoshiyuki Kuchino, Shigeaki Watanabe, Fumio Harada, and Susumu Nishimura*

ABSTRACT: The nucleotide sequence of an *E. coli* isoleucine tRNA ($tRNA^{Ile}_{minor}$) specific for the codon AUA was determined by postlabeling procedures using only 2.5 μg (0.05 A_{260} unit) of the material. The sequence was pG-G-C-C-C-C-U-s^4U-A-G-C-U-C-A-G-U-Gm-G-D-D-A-G-A-G-C-A-A-G-C-G-A-C-U-N^+-A-U-t^6A-A-Ψ-C-G-C-U-U-G-m^7G-acp^{3}U-C-G-C-U-G-G-T-Ψ-C-A-A-G-U-C-C-A-G-C-A-G-G-G-G-C-C-A-C-C-A_{OH}. The nucleotide sequences in the regions of the D arm and TΨC arm of $tRNA^{Ile}_{minor}$ were quite similar to the corresponding regions of $tRNA^{Ile}_{major}$. However, the sequences in the CCA stem and anticodon stem of $tRNA^{Ile}_{minor}$ were different from those of $tRNA^{Ile}_{major}$. The overall homology between the two isoleucine tRNAs was 68%. *E. coli* $tRNA^{Lys}$, $tRNA^{Met}$, $tRNA^{Val}_{IIA}$ and $tRNA^{Arg}$ also have relatively high sequence homology with $tRNA^{Ile}_{minor}$.

In *Eschericia coli*, there are two isoleucine tRNA species, $tRNA^{Ile}_{major}$ and $tRNA^{Ile}_{minor}$ (Yarus & Barrell, 1971; Harada & Nishimura, 1974). The major species of $tRNA^{Ile}$ ($tRNA^{Ile}_{major}$) with guanosine in the first position of the anticodon recognizes A-U-U and A-U-C, while the minor species of $tRNA^{Ile}$ ($tRNA^{Ile}_{minor}$) recognizes only A-U-A (Harada & Nishimura, 1974). This $tRNA^{Ile}_{minor}$ contains an unknown modified nucleoside N^+ in the first position of the anticodon, which is responsible for specific recognition of the AUA codon (Harada & Nishimura, 1974).

Although the partial primary structure of $tRNA^{Ile}_{minor}$ is known (Harada & Nishimura, 1974), elucidation of the total primary structure was hampered by the difficulty of obtaining

† From the Biology Division, National Cancer Center Research Institute, Tsukiji 5-1, Chuo-ku, Tokyo, Japan. *Received October 3, 1979.*

0006-2960/80/0419-2085$01.00/0

a sufficient amount of material for analysis. The amount of $tRNA^{Ile}_{minor}$ in wild type *E. coli* cells was estimated to be less than 5% of that of $tRNA^{Ile}_{major}$ (Harada & Nishimura, 1974). It has been suggested that $tRNA^{Ile}_{minor}$ has a specific role as a modulator in regulation of protein synthesis (Jou et al., 1976). Information on the total structure of $tRNA^{Ile}_{minor}$ is necessary for studies on the tRNA–aminoacyl-tRNA synthetase interaction, location of the tRNA gene, and evolution of tRNA.

Recently sequencing of tRNA has been greatly simplified by introduction of postlabeling techniques (Donis-Keller et al., 1977; Simoncsits et al., 1977; Lockard et al., 1978; Stanley & Vassilenko, 1978; Peattie, 1979). The procedure of Stanley & Vassilenko (1978) consists of partial alkaline hydrolysis of tRNA with formamide followed by phosphorylation with polynucleotide kinase of the 5′ end of oligonucleotides, fractionation of the oligonucleotides labeled at the 5′ end by polyacrylamide gel electrophoresis, and analysis of the nucleoside 3′,5′-diphosphate as the 5′ terminal in each oligonucleotide by paper electrophoresis after alkaline hydrolysis. Using a modification of this procedure (Kuchino et al., 1979) we recently determined the total primary structure of starfish initiator tRNA. This paper reports the total nucleotide sequence of *E. coli* $tRNA^{Ile}_{minor}$, determined by this modification of Stanley and Vassilenko's procedure, combined with other postlabeling techniques. Only 2.5 μg (0.05 A_{260} unit) of $tRNA^{Ile}_{minor}$ was required for determination of its sequence. Results showed that $tRNA^{Ile}_{minor}$ consists of 76 nucleotide residues, one nucleotide less than $tRNA^{Ile}_{major}$. Moreover, the sequences in the regions of the D arm and TψC arm of $tRNA^{Ile}_{minor}$ are similar to those in the corresponding regions of $tRNA^{Ile}_{major}$.

Materials and Methods

E. coli $tRNA^{Ile}_{minor}$. *E. coli* $tRNA^{Ile}_{minor}$ used for sequence analysis was obtained by successive application of several column chromatographies as described previously (Harada & Nishimura, 1974). Final purification was achieved by two-dimensional polyacrylamide gel electrophoresis as described by Ikemura & Dahlberg (1973). Approximately 0.5 A_{260} unit of $tRNA^{Ile}_{minor}$ was applied to the gel. After electrophoresis, the main spot detected with a UV lamp and fluorescent thin-layer sheet (Hassur & Whitlock, 1974) was cut out and homogenized with 1.5 mL of a solution of 0.5 M ammonium acetate–0.01 M magnesium acetate–0.1% sodium dodecyl sulfate–0.1 mM EDTA. The extracted tRNA was precipitated by addition of 2.5 volumes of ethanol.

Sequencing of E. coli $tRNA^{Ile}_{minor}$ by Limited Alkaline Hydrolysis with Formamide. The procedure described by Stanley & Vassilenko (1978) was followed except that two-dimensional thin-layer cellulose chromatography was adopted instead of one-dimensional paper electrophoresis for identification of the 5′ end of the nucleotide residue in each oligonucleotide and nuclease P_1 digestion was performed instead of alkaline hydrolysis for characterization of the 5′-terminal nucleotides as nucleoside 5′-monophosphates rather than as nucleoside 3′,5′-diphosphates. The detailed reaction conditions were described previously (Kuchino et al., 1979).

Preparation of 5′-Terminal Labeled $tRNA^{Ile}_{minor}$ and Rapid Read-off Sequencing of tRNA by RNase Digestion. 5′-Labeling of tRNA with ^{32}P was carried out essentially as described by Silberklang et al. (1977). Partial digestion of the 5′-terminal labeled tRNA with RNases and alkali was performed as described by Simoncsits et al. (1977) with some modifications. Polyacrylamide gel electrophoresis of the partial digest of the tRNA was carried out as described by Lockard et al. (1978), using a spacer of 0.06-cm thickness. The exact conditions for these procedures were described previously (Kuchino et al., 1979).

3′-Labeling of $tRNA^{Ile}_{minor}$ and Rapid Read-off Sequencing by Chemical Degradation. [5′-^{32}P]Cytidine 3′,5′-diphosphate was prepared as described by Cranston et al. (1974). The reaction conditions were as follows: The reaction mixture (10 μL), containing 50 mM Tris-HCl (pH 8.2), 10 mM $MgCl_2$, 2 mM spermine, 10 mM dithiothreitol, 0.1 M KCl, 2 mM cytidine 3′-phosphate, 2.4 units of T_4 polynucleotide kinase, and 5 μL of [γ-^{32}P]ATP (specific activity 3.3×10^9 cpm/nmol, Cerenkov, 1 mM), was incubated at 37 °C for 1 h. Then the mixture was heated at 100 °C for 2 min and used directly for the kination reaction with *E. coli* $tRNA^{Ile}_{minor}$ as acceptor. The ligase reaction was carried out as described by Bruce & Uhlenbeck (1978). The solution (10 μL) containing 0.02 A_{260} unit of *E. coli* $tRNA^{Ile}_{minor}$, 50 mM Tris-HCl (pH 8.2), 10 mM $MgCl_2$, 10 mM dithiothreitol, 10 μg/mL bovine serum albumin, 10% (v/v) dimethyl sulfoxide, 50 μM ATP, and 0.7 unit of T_4 RNA ligase was mixed with 5 μL of [5′-^{32}P]cytidine 3′,5′-diphosphate solution as described above and incubated at 4 °C for 14 h. The 3′-labeled *E. coli* $tRNA^{Ile}_{minor}$ thus formed was separated by electrophoresis on 15% polyacrylamide gel (0.06-cm thickness) containing 7 M urea. Chemical degradations of 3′-labeled tRNA for rapid read-off sequencing were performed under exactly the conditions described by Peattie (1979).

Materials. The chemicals, enzymes, and thin-layer cellulose plates used for sequencing by postlabeling procedures were almost the same as in previous work (Kuchino et al., 1979). Carrier-free [^{32}P]phosphate dissolved in water was obtained from New England Nuclear. [γ-^{32}P]ATP (specific activity 3.3×10^9 cpm/nmol, Cerenkov) was prepared as described by Glynn & Chappell (1964). T_4 RNA ligase was kindly provided by Dr. E. Ohtsuka of Osaka University. Cytidine 3′-phosphate was purchased from Sigma.

Results

Sequence Analysis of $tRNA^{Ile}_{minor}$ by Limited Alkaline Hydrolysis with Formamide. Most data on the primary structure of $tRNA^{Ile}_{minor}$ were obtained by a modification of the limited formamide hydrolysis method described by Stanley & Vassilenko (1978). Figure 1 shows the autoradiogram of partially digested and labeled $tRNA^{Ile}_{minor}$ separated by polyacrylamide gel electrophoresis. The bands were all clearly separated, indicating that the tRNA sample used in the experiment was pure. The bands of each oligonucleotide were cut out from the gel and immersed in 0.5 mL of buffer consisting of 0.5M ammonium acetate–0.01M magnesium acetate–0.1% sodium dodecyl sulfate–0.1 mM EDTA at 37 °C for 5 h in a 1.5-mL Eppendorf centrifuge tube. Since we used a spacer of 0.06-cm thickness in polyacrylamide gel electrophoresis, oligonucleotides were almost completely recovered from the gel without homogenization. The labeled oligonucleotides with carrier tRNA were precipitated with 2.5 volumes of ethanol, and the resulting material was hydrolyzed with nuclease P_1 to obtain ^{32}P-labeled 5′-terminal nucleotide. The digest was then analyzed by two-dimensional thin-layer cellulose chromatography by using small thin-layer plates (5 × 5 cm). Figure 2 shows the chromatograms of the 5′-terminal nucleotides in each band from nucleotide residue 3 to 71. By reading of the main spot in each chromatogram the nucleotide sequence from residue 3 to residue 71 was easily determined as C-C-C-C-U-s^4U-A-G-C-U-C-A-G-U-Gm-G-D-D-A-G-A-G-C-A-A-G-C-G-A-C-U-N^+-A-U-t^6A-A-Ψ-C-G-C-U-U-G-m^7G-acp^3U-C-G-C-U-G-G-T-Ψ-C-A-A-G-U-C-C-A-G-C-A-

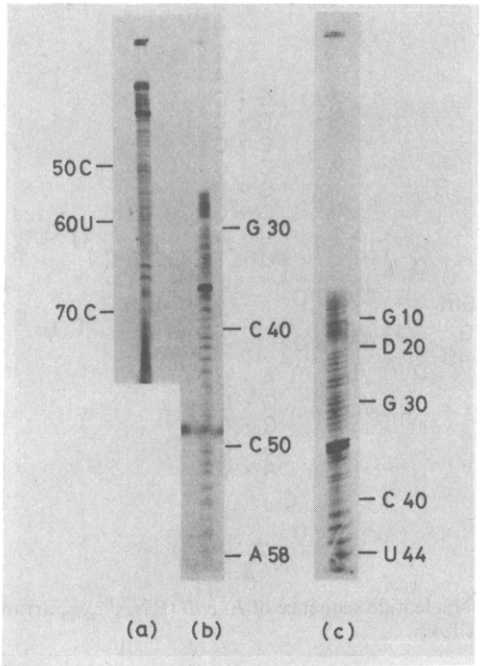

FIGURE 1: Autoradiograms of 5′-^{32}P-labeled oligonucleotides fractionated on 20% polyacrylamide–7 M urea gel. Electrophoresis was carried out for (a) 9 h, (b) 24 h, and (c) 35 h at 700 V (gel dimension: 40 × 20 × 0.06 cm).

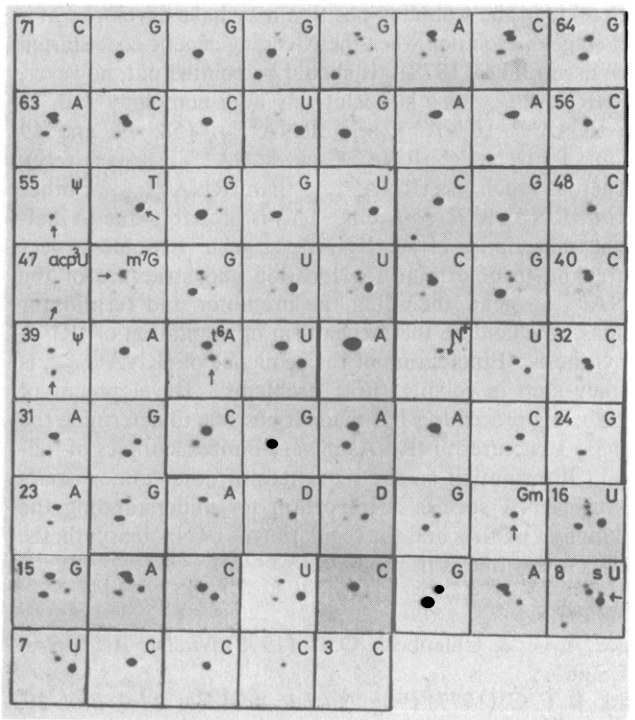

FIGURE 2: Analysis of the 5′-terminal nucleotides of the fragments of $tRNA^{Ile}_{minor}$ by two-dimensional cellulose thin-layer chromatography. Arrows indicate modified nucleotides.

G-G-G-G-C. Caution should be taken in the case of assignment of modified nucleosides because modified nucleosides such as N^+, t^6A, m^7G and acp^3U, expected as 5′-terminal residues of fragments, have low intensities which are sometimes much less than those of contaminating pA, pG, pC, and pU, owing to the low efficiency of phosphorylation of modified nucleoside 3′-phosphate. As discussed previously (Kuchino et al., 1979), two-dimensional thin-layer chromatography was necessary for assignment of the position of the modified nucleosides since modified nucleotides of low intensity cannot be detected by one-dimensional separation.

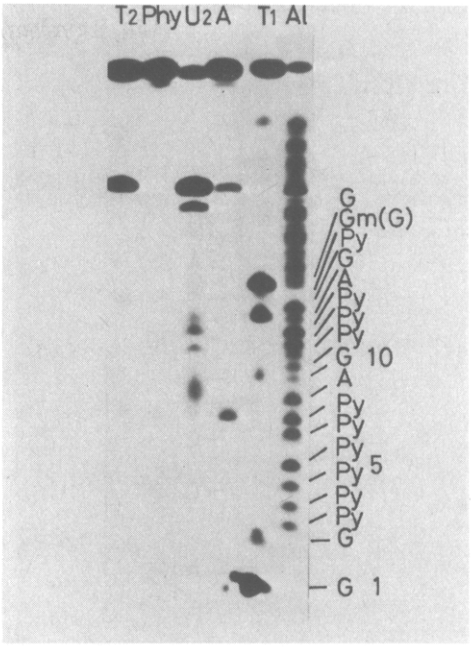

FIGURE 3: Autoradiogram of partial enzymatic and alkaline digests of 5′-^{32}P-labeled *E. coli* $tRNA^{Ile}_{minor}$. Al shows the ladder obtained by heating the tRNA in alkali. T_1, A, U_2, Phy, and T_2 show the ladders obtained by incubation with these RNases. Gel electrophoresis was carried out on a 20% polyacrylamide–7 M urea gel for 9 h at 700 V (gel dimension: 40 × 20 × 0.06 cm). Py shows the pyrimidine residue.

We generally overexpose thin-layer plates on X-ray film in order to locate the positions of four common nucleotides. By doing so we can more easily identify modified nucleotides by judging from their relative positions against four common nucleotides. X-ray film developed showed spots with more different contrast than the photograph shown. Therefore we can easily identify which is major spot in most cases. When two spots were seen with an equal density, the following consideration was taken. For example, pC and pA are seen equally in plate 65. This is due to contamination of pA of band 66 because (i) pA was seen as major spot in plate 66 and (ii) with the same tendency of overlapping, pC of band 65 was seen in plate 64. Assignment of nucleotides by using 5′- and 3′-terminal-labeled tRNA as described later was also helpful for the confirmation of the sequence.

It should be noted that part of residue 17 was replaced by G in place of Gm, so that it was possible to deduce that residue 18 was G. This assignment was confirmed by the rapid read-off sequencing procedure using RNase T_1 and 5′-terminal-labeled tRNA as described later.

Assignment of Nucleotide Sequences Near the 3′ and 5′ Ends of $tRNA^{Ile}_{minor}$. The nucleotide sequence near the 5′ end cannot be determined unambiguously by limited alkaline hydrolysis since long oligonucleotides were not well phosphorylated and were not well separated from each other by polyacrylamide gel electrophoresis. In addition the 5′ end of the tRNA cannot be determined because the 5′ end of the tRNA is naturally phosphorylated. However, the sequence in this region can be determined by the rapid read-off sequencing procedure using RNases and 5′-terminal labeled $tRNA^{Ile}_{minor}$. As shown in Figure 3, the sequence from nucleotide residue 1 to 18 was found to be pG-G-Py-Py-Py-Py-Py-Py-A-G-Py-Py-Py-A-G-Py-Gm(G)-G.

The nucleotide sequence near the 3′ end can also not be determined by limited alkaline hydrolysis because contami-

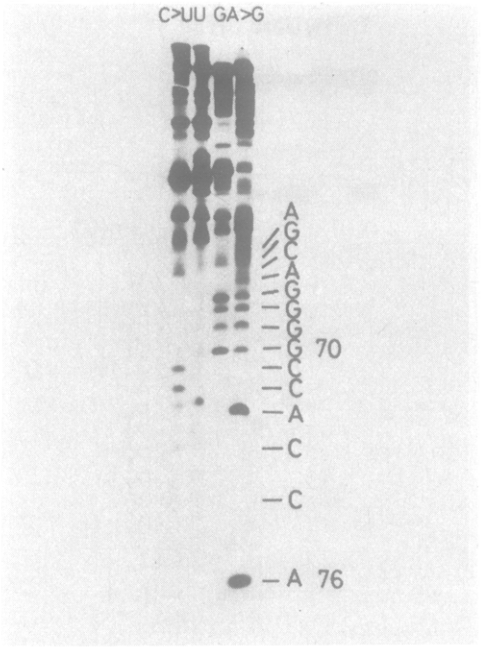

FIGURE 4: Autoradiogram of 3′-end labeled $tRNA^{Ile}_{minor}$ sequenced by chemical degradations. Gel electrophoresis was carried out on 20% polyacrylamide–7 M urea gel for 9 h at 700 V (gel dimension: 40 × 20 × 0.06 cm).

nants present in [γ-^{32}P]ATP complicate the autoradiogram. Thus the nucleotide sequence near the 3′ end was determined by adopting the rapid read-off sequencing procedure with chemical degradation of 3′-labeled $tRNA^{Ile}_{minor}$. As shown in Figure 4, the sequence from nucleotide residue 66 to 76 was clearly determined to be -A-G-G-G-G-C-C-A-C-C-A.

Combination of data obtained by limited alkaline hydrolysis with those obtained by the two rapid read-off sequencing techniques enabled us to obtain the total primary sequence of *E. coli* $tRNA^{Ile}_{minor}$. The sequence arranged in a cloverleaf form is shown in Figure 5.

Discussion

The primary structure of E. *coli* $tRNA^{Ile}_{minor}$ determined by the postlabeling procedures reported here is completely consistent with its partial sequence previously obtained by the conventional method (Harada & Nishimura, 1974). All modified nucleosides previously identified by Harada & Nishimura (1974) were found in the total sequence of $tRNA^{Ile}_{minor}$ (Figures 2 and 5).

E. coli $tRNA^{Ile}_{minor}$ possesses the common structural features of most tRNAs (Clark, 1977). Thus it can be concluded that its specific recognition of the AUA codon is due entirely to the presence of a unique unknown modified nucleoside located in the wobbling position. *E. coli* $tRNA^{Ile}_{minor}$ consists of 76 nucleotide residues. The chain length of $tRNA^{Ile}_{minor}$ is one nucleotide residue shorter than that of $tRNA^{Ile}_{major}$ (Yarus & Barrell, 1971). The sequence of $tRNA^{Ile}_{minor}$ is quite similar to that of $tRNA^{Ile}_{major}$: 52 of 76 or 77 nucleotide residues are identical in the two tRNAs, and the overall sequence homology is 68%. It should be noted that the nucleotide sequences in the regions of the D arm and TψC arm of $tRNA^{Ile}_{minor}$ are quite similar to those in the corresponding regions of $tRNA^{Ile}_{major}$, whereas the sequences in the CCA stem and anticodon stem are different from those of $tRNA^{Ile}_{major}$. A large extent of sequence homology (55 residues) is also seen between $tRNA^{Ile}_{minor}$ and $tRNA^{Met}$, but not between $tRNA^{Ile}_{minor}$ and initiator $tRNA^{Met}$. This suggests that $tRNA^{Ile}_{minor}$ and $tRNA^{Ile}_{major}$ are evolutionary related to

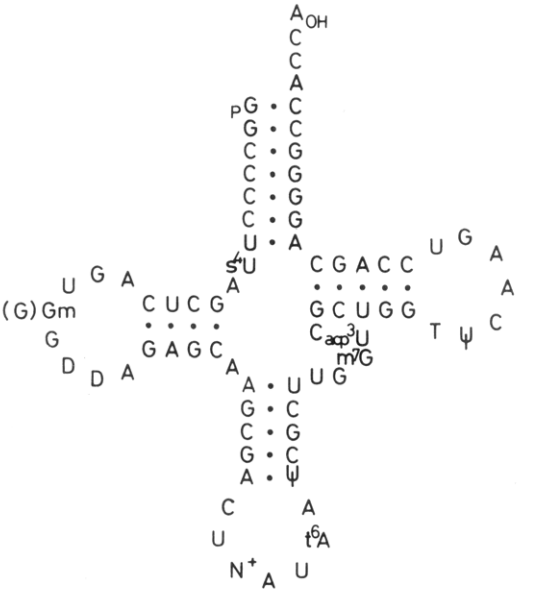

FIGURE 5: Nucleotide sequence of *E. coli* $tRNA^{Ile}_{minor}$ arranged in a cloverleaf form.

$tRNA^{Met}$. In this connection, it should be mentioned that the gene for $tRNA^{Ile}_{minor}$ contains C in the wobbling position, indicating that N^+ is derived from C (J. Abelson, personal communication). The complex posttranscriptional modification process involved in the synthesis of the unknown modified nucleoside in the wobbling position may have developed at a later stage of evolution when the A-ending genetic code started to be used (Jukes, 1978). It should be pointed out, however, that $tRNA^{Ile}_{minor}$ also has relatively high homology with *E. coli* $tRNA^{Lys}$, $tRNA^{Arg}$, and $tRNA^{Val}_{IIA}$ (58, 49, and 49 residues for $tRNA^{Lys}$, $tRNA^{Arg}$, and $tRNA^{Val}_{IIA}$, respectively).

There is much less $tRNA^{Ile}_{minor}$ than $tRNA^{Ile}_{major}$ or other *E. coli* tRNAs in *E. coli* cells. This is probably due to inefficient transcription of the $tRNA^{Ile}_{minor}$ gene. It would be very interesting to determine the location and structure of the $tRNA^{Ile}_{minor}$ gene, including the promotor and terminator regions, to elucidate the mechanism of regulation of tRNA biosynthesis. Elucidation of the sequence of $tRNA^{Ile}_{minor}$ is the key step in solving these problems. Development of postlabeling procedures has made it possible to determine the primary structures of tRNAs in very limited samples of material. Information on the sequences of other minor isoaccepting tRNA species is important for understanding the evolution of tRNAs and the regulation of tRNA biosynthesis. Work on this line is in progress.

References

Bruce, A. G., & Uhlenbeck, O. C. (1978) *Nucleic Acids Res. 5*, 3665.

Clark, B. F. C. (1977) *Prog. Nucleic Acid Res. Mol. Biol. 20*, 1.

Cranston, J. W., Silber, R., Malathi, V. G., & Hurwitz, J. (1974) *J. Biol. Chem. 249*, 7447.

Donis-Keller, H., Maxam, A. M., & Gilbert, W. (1977) *Nucleic Acid Res. 4*, 2527.

Glynn, I. M., & Chappell, J. B. (1964) *Biochem. J. 90*, 147.

Harada, F., & Nishimura, S. (1974) *Biochemistry 13*, 300.

Hassur, S. M., & Whitlock, H. W., Jr. (1974) *Anal. Biochem. 59*, 162.

Ikemura, T., & Dahlberg, J. E. (1973) *J. Biol. Chem. 248*, 5024.

Jou, W. M., Montagu, M. V., & Fiers, W. (1976) *Biochem. Biophys. Res. Commun. 73*, 1083.

Jukes, T. H. (1978) *Adv. Enzymol. 47*, 375.
Kuchino, Y., Kato, M., Sugisaki, H., & Nishimura, S. (1979) *Nucleic Acids Res. 6*, 3459.
Lockard, R. E., Alzner-Deweerd, B., Heckman, J. E., MacGee, J., Tabor, M. W., & RajBhandary, U. L. (1978) *Nucleic Acids Res. 5*, 37.
Peattie, D. A. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1760.
Silberklang, M., Prochiantz, A., Haenni, A. L., & RajBhandary, U. L. (1977) *Eur. J. Biochem. 72*, 465.
Simoncsits, A., Brownlee, G. G., Brown, R. S., Rubin, J. R., & Guilley, H. (1977) *Nature (London) 269*, 833.
Stanley, J., & Vassilenko, S. (1978) *Nature (London) 274*, 87.
Yarus, M., & Barrell, B. G. (1971) *Biochem. Biophys. Res. Commun. 43*, 729.

Mechanism of Inhibition of the Avian Myeloblastosis Virus Deoxyribonucleic Acid Polymerase by Adriamycin†

Steven W. Matson,‡ Philip J. Fay,‡ and Robert A. Bambara*

ABSTRACT: The avian myeloblastosis virus (AMV) DNA polymerase utilizes endonuclease-nicked DNA as a substrate for processive DNA synthesis, adding ~25 nucleotides each time it binds this template. This number can be lowered by increasing the KCl concentration of the reaction but is unaffected by shifts in pH which significantly alter reaction rate. Inhibition of the polymerization reaction by adriamycin has been probed with respect to the basic steps of DNA synthesis: binding of enzyme to DNA, catalysis, and enzyme translocation. Adriamycin has essentially no effect on initial enzyme–DNA interaction, as measured by a nitrocellulose filter binding assay. This assay measures total binding, not necessarily specific binding at 3′ termini. Kinetic assays demonstrate that inhibition of the reaction by adriamycin is competitive with respect to DNA terminus concentration. This suggests that inhibition involves specific binding to 3′ termini by the DNA polymerase or dissociation from these termini during synthesis. Inhibition is essentially noncompetitive with respect to deoxynucleoside triphosphate concentration, confirming that catalytic steps are not perturbed by the durg. The extent of processive synthesis falls to 22% of the uninhibited value at a drug concentration causing 96% inhibition of the rate of DNA synthesis. This indicates that a major effect of the drug is to cause premature dissociation of the DNA polymerase during synthesis. The component of inhibition not accounted for by lower processivity must result from a lowering of specific binding to 3′ termini by the DNA polymerase. The above information is incorporated into a comprehensive model for the inhibitory action of adriamycin.

The avian myeloblastosis virus (AMV)[1] DNA polymerase has been purified and extensively studied with respect to its role in the life cycle of the RNA tumor virus and its catalytic activity (Verma, 1977). It is a reverse transcriptase, responsible for transcription of the RNA genome of the virus into DNA (Verna et al., 1976). The purified enzyme consists of two polypeptides of apparent molecular weight 65 000 (α) and 95 000 (β) (Kacian et al., 1971; Grandgenett et al., 1973; Gibson & Verma, 1974). It is active for DNA synthesis on both RNA and DNA primer–templates with a 3′-OH terminus (Temin & Baltimore, 1972). In addition to its DNA polymerizing activity, the enzyme possesses an RNase H activity which degrades the RNA strand of a DNA–RNA hybrid in both 5′ → 3′ and 3′ → 5′ directions (Leis et al., 1973). The AMV DNA polymerase does not, however, possess DNA nuclease activities (Verma, 1977; Seal & Loeb, 1976; Battula & Loeb, 1976).

This report addresses two aspects of the enzymology of this DNA polymerase: (a) the basic steps of the DNA polymerization reaction in vitro and (b) the molecular mechanism by which these steps are affected by the inhibitor adriamycin. An initial requirement is an understanding of the way that the DNA polymerase and DNA interact during the synthetic process. This interaction has been studied in vitro in other laboratories by using homopolymer primer–templates with seemingly contradictory results (Leis, 1976; Dube & Loeb, 1976). It has been reported that if synthesis is begun on poly(A)·poly(dT), the enzyme requires several minutes to distribute to and synthesize on newly added poly(I)·poly(dC) (Leis, 1976). This result suggests a processive mechanism of synthesis, that is, the addition of more than one nucleotide each time the DNA polymerase binds a primer terminus, accompanied by translocation of the bound DNA polymerase along the template strand.

However, it has also been reported that the enzyme will distribute from oligo(dT)·poly(A) to oligo(dG)·poly(C) very rapidly (Dube & Loeb, 1976), which suggests that the enzyme may dissociate from the primer–template after addition of each nucleotide. The problem with the template competition technique employed for these measurements is that it yields the rate of distribution of enzyme molecules among DNA templates, a parameter not necessarily related to the number of nucleotides added each time a polymerase molecule binds the DNA template. In addition, when oligonucleotide primers are used on homopolymer templates, the primer molecules may

† From the Department of Biochemistry and Cancer Center, University of Rochester School of Medicine and Dentistry, Rochester, New York 14642. *Received November 14, 1979.* This work was supported by a grant-in-aid from the Jane Coffin Childs Fund for Medical Research, National Institutes of Health Grant GM-24441, and Cancer Center Core Grant 5-P30-CA11198-11.

‡ Predoctoral Trainee supported by National Institutes of Health Training Grant T32-GM07102.

[1] Abbreviations used: AMV, avian myeloblastosis virus; dNTPs, deoxynucleoside triphosphates; drug/DNA phosphate or drug/DNA-P, molar ratio of adriamycin molecules to DNA nucleotides in a solution.

0006-2960/80/0419-2089$01.00/0