Comparison of Isotope Labeling Patterns of Purines in Three Specific Transfer RNAs†

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ABSTRACT: Purine C-8 tritium-labeling rates have been measured at specific sites in *Escherichia coli* $tRNA^{Ile}$ and $tRNA_2^{Tyr}$. The results are compared with those obtained for yeast $tRNA^{Phe}$ (preceding paper (Gamble et al., 1976)). The $tRNA^{Ile}$ and $tRNA^{Phe}$ fall into the same general class of tRNA structures, while $tRNA_2^{Tyr}$ is in a different class; in particular, the latter is characterized by a large extra loop. In each of the three tRNAs the 3′-terminal A has the same labeling rate and, on a relative basis, appears to be the most rapidly labeled site. Bases in cloverleaf helical sections have markedly retarded labeling rates that collectively fall within an approximately threefold range of time constants. At some of the common purines, believed to be essential for the construction of a general system of tertiary interactions, exchange rates for yeast $tRNA^{Phe}$ are significantly different than those for the two *Escherichia coli* tRNAs. These differences may arise from variations among the tRNAs in the relative stabilities of specific tertiary interactions, or from other factors as well. In the case of $tRNA_2^{Tyr}$, labeling rates for bases in the large variable region are sufficiently retarded to suggest some structural organization for this part of the molecule. In addition, since exchange rates are similar for some of the bases common to *Escherichia coli* $tRNA^{Ile}$ and $tRNA_2^{Tyr}$, it is likely that the large variable loop of $tRNA_2^{Tyr}$ does not interact with or perturb these common sites. Finally, for all three tRNAs, structure formation (e.g., base pairing, base stacking) invariably decreases the labeling rate, even though the variety of base environments in the three-dimensional structures of these tRNAs might be expected to affect the acidity of C-8 and other chemical properties in diverse ways. Although these chemical effects no doubt bear influence, in these studies the dominant influence on exchange may be the effect of structure on the accessibility of solvent molecules, i.e., water.

In the preceding paper, the kinetics of exchange of the C-8 hydrogens of purine units in yeast $tRNA^{Phe}$ and in other nucleic acids was examined in some detail (Gamble et al., 1976). In the present paper, the data obtained on yeast $tRNA^{Phe}$ are compared with those obtained for two *Escherichia coli* tRNAs—$tRNA_2^{Tyr}$ and $tRNA^{Ile}$.

Sequences and cloverleaf structures of these tRNAs are shown in Figures 1a–c. T1 fragments of each of the tRNAs are also indicated. In addition, certain of the common bases for which exchange data have been obtained are accented by shading.

In making these comparisons, several questions are pursued. First, it is of interest to determine whether the conclusions drawn for yeast $tRNA^{Phe}$ are general and, therefore, valid for other tRNAs as well. For example, we can ask whether exchange rates are similar within general regions such as the cloverleaf helical segments, or whether these regions show considerable variation from structure to structure. Second, we wish to examine exchange rates of specific common bases that presumably participate in an important system of tertiary interactions shared by most or all tRNAs (Kim et al., 1974a,b; Robertus et al., 1974; Klug et al., 1974). Thus, identical bases occur at corresponding positions in the three tRNAs and presumably perform similar functions in each. If similar roles are filled by the common bases, this might well be reflected in their respective exchange rates. In addition, two of the tRNAs chosen for study—yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Ile}$—have extensive sequence homologies in the dihydrouridine region (see Figure 1). This situation affords an opportunity to compare exchange rates at loci that are flanked by neighboring bases that are similar or identical in the two tRNAs. Finally, although two of the tRNAs chosen for study fall into the same general class of structures (class 1 or D4V5), the third tRNA ($tRNA_2^{Tyr}$) is in a different class (class 3 or D3VN). (See Levitt (1969) and Kim et al. (1974a) for a discussion of classes of structures.) In particular, $tRNA_2^{Tyr}$ is distinguished by its unusually large extra loop. This situation enables us to compare features of the exchange rates that are characteristic of tRNAs in different structural classes, and to explore the microenvironment of a large extra loop.

Materials and Methods

The $tRNA_2^{Tyr}$ was purified from unfractionated *E. coli* B tRNA (Schwarz) by the method of Walker and RajBhandary (1972); $tRNA^{Ile}$ was purified by the method of Gillam et al. (1968). According to amino acid acceptance assays and T1 fingerprint analyses, each tRNA was 90–100% homogeneous.

Procedures associated with the exchange kinetics measurements are described in detail by Gamble et al. (1976). The tRNA concentration was about 10 mg/ml during tritium incorporation. Experiments with $tRNA_2^{Tyr}$ were also done at 2.5 mg/ml and at 50 mg/ml; R values for individual T1 fragments at the three concentrations agreed within the experimental error of ±15% associated with a R value measurement.

Results and Discussion

General Profile of Labeling Rates. Figures 2a,b give bar graph representations of R vs. fragment number for $tRNA^{Ile}$ and $tRNA_2^{Tyr}$, respectively. The data were obtained at 37 °C in the presence of Mg^{2+}. The numbers on the abscissas refer to the T1 oligonucleotides that are marked in Figures 1a,b. The

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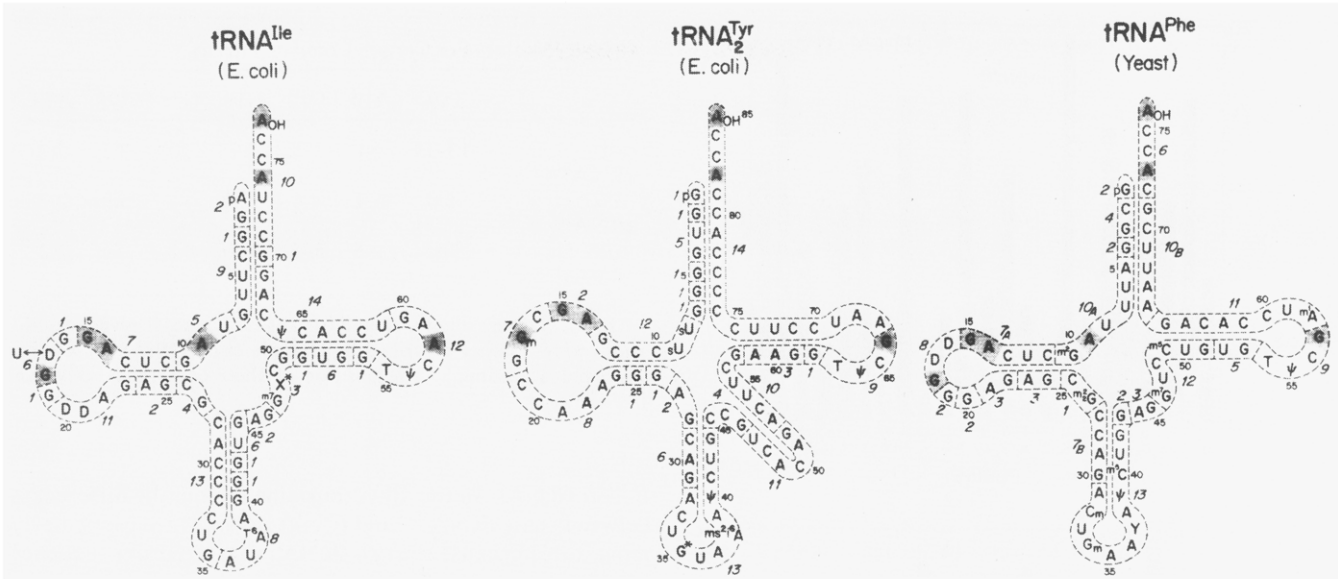

FIGURE 1: Sequence and cloverleaf structure (a) of *E. coli* $tRNA^{Ile}$ (Yarus and Barrell, 1971), (b) of *E. coli* $tRNA_2^{Tyr}$ (Goodman et al., 1968; RajBhandary et al., 1969), and (c) of yeast $tRNA^{Phe}$ (RajBhandary and Chang, 1968). T1 oligonucleotides are indicated and are numbered according to position of occurrence in a two dimensional chromatogram. Lower case numbers designate numerical positions of bases in the sequences. Purines that are shaded are common nucleotides, which are given special consideration in the text.

letter beneath each number in Figures 2a,b refers to a particular base (A or G) from the fragment. Tick marks across the tops of the figures designate positions in the sequences, in cases where unique positions can be specified. The parameter R is the ratio of time constants for exchange of a particular base in the tRNA to that of the corresponding mononucleotide (Gamble et al., 1976). R values in excess of unity indicate a retardation of the exchange. The figures show clearly that the exchange rates vary greatly for the different bases in each of the two molecules.

Cloverleaf Helical Sections. Consider first the cloverleaf helical sections. Bases arising solely from these sections in $tRNA^{Ile}$ are the G's of fragments 3, 5, 8, 9, 11, and 14, and the A's of fragments 13 and 14. In $tRNA_2^{Tyr}$, the appropriate helical G's are found in fragments 1, 3, 5, 6, 8, 10, and 13, while appropriate helical A's are found in fragments 3 and 6.[1] For the two tRNAs the R values for these bases vary from about 9 to 19. This compares with a range of $R = 17$–29 for the helical bases in yeast $tRNA^{Phe}$ (Gamble et al., 1976). Therefore, helical bases in the three tRNAs collectively show exchange rates that are 9–29-fold slower than those for the corresponding free mononucleotides. This wide range of exchange rates possible reflects a significant variation in local microenvironments and helix parameters in the duplex regions of the three cloverleafs (cf. Gamble et al., 1976). Certainly the various helical bases are flanked by a wide number of combinations of nearest neighbors, and these in turn occur in both bonded and single-stranded sections of the cloverleaf. It is also worth noting that, although there is a sizable range of exchange rates for helical units, there is no tendency for the retardation to be generally greater for the A's than for the G's or vice versa. This is not surprising; at neutral pH the dominant mechanism for exchange is the same for both bases (Tomasz et al., 1972), so the helix environment should perturb both in a similar fashion.

Variable Region of $tRNA_2^{Tyr}$. A special feature of $tRNA_2^{Tyr}$ is the large extra loop. The G's of fragments 4 and 11 and the A's of fragments 10 and 11 contain the purines from this section of the molecule. The exchange rates of these residues are depressed about 8–13-fold. This suggests some sort of shielding for these bases. Uhlenbeck (1972) found relatively weak specific binding of tetranucleotides to certain parts of the extra loop of $tRNA^{Tyr}$. His results, like the present ones, indicate some structural organization for the variable region.

Common Bases and Tertiary Interactions. Of perhaps most interest are the bases that are common to many tRNAs and which may participate in a common system of interactions in the native structure (Kim et al., 1974a,b; Robertus et al., 1974; Klug et al., 1974). Table I lists a number of these bases for which unambiguous exchange data were obtained. These bases are numbered in accordance with the position of their occurrence in yeast $tRNA^{Phe}$, the corresponding bases in the other two tRNAs having, in some cases, different numbers. Where possible, comparative data are given for all three tRNAs. Exceptions are: A9, which is common only to yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Ile}$; G57, which is replaced by an A in *E. coli* $tRNA^{Ile}$, and thus could be different in its R value owing to subtle effects arising from the chemical difference between an A and a G; A73, which could not be unambiguously appraised in *E. coli* $tRNA_2^{Tyr}$ because of the high redundancy of A's in fragment 14 of this tRNA.

The bases listed sample different parts of the tRNA cloverleaf structure, and in the crystal form of yeast $tRNA^{Phe}$ they represent many types of chemical environments (Kim et al., 1974a,b; Robertus et al., 1974; Klug et al., 1974). For example, A9, A14, G15, and G18 participate in tertiary hydrogen bonding schemes; G57 has a special interaction with the dihydrouridine loop; A73 stacks onto the end of the acceptor helix; and A76 is at the 3′-terminus, where it is relatively free of structural interactions with the rest of the molecule.

It should first be noted that A76 has the same low R value for each of the tRNAs. This is not surprising, since we expect the 3′-terminus to have a similar environment in each case and to be free of structural interactions. Likewise, A73, which is undoubtedly stacked onto the tail end of the acceptor helix, has about the same R value in yeast $tRNA^{Phe}$ as in *E. coli* $tRNA^{Ile}$. Not only are these results consistent with our expectations for these sites, but they also serve as a type of in-

[1] From this list we have omitted, somewhat arbitrarily, the presumably helical bases from the extra loop (from fragments 4, 10, and 11).

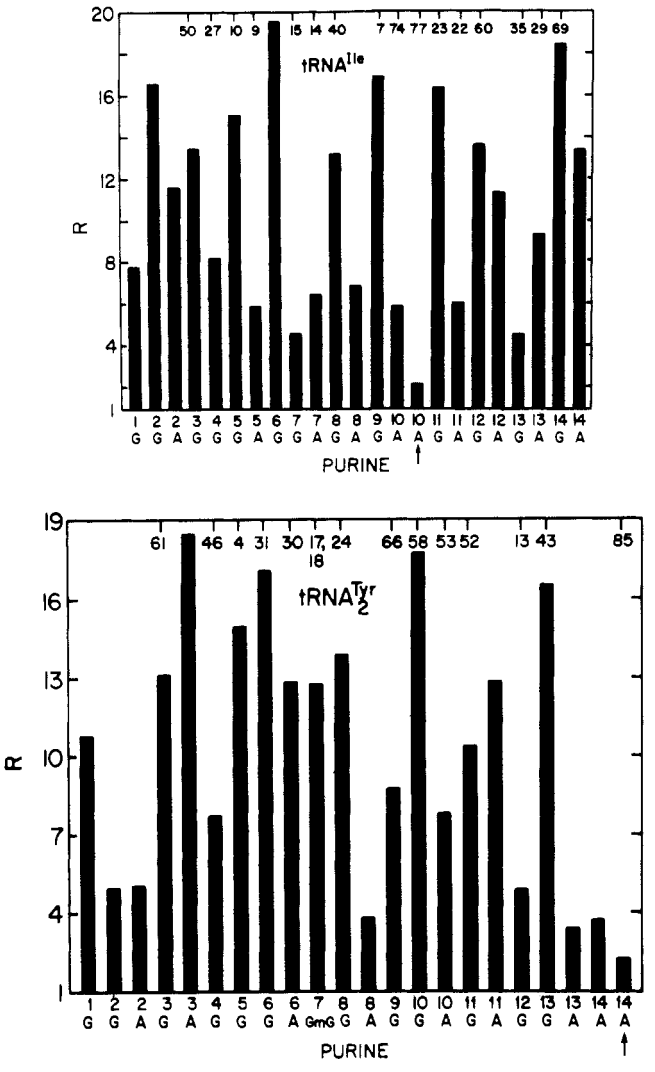

FIGURE 2: *R* vs. fragment number (a) for *E. coli* $tRNA^{Ile}$ and (b) for *E. coli* $tRNA_2^{Tyr}$. The fragment numbers refer to the T1 oligonucleotides defined in Figure 1. The letter beneath each fragment number refers to the A or G from that fragment; GmG denotes 2′-*O*-methyl-GpG. In the case of $tRNA_2^{Tyr}$, GmG is obtained instead of mononucleotides due to interference of the 2′-*O*-methyl group with T1 and T2 ribonuclease cleavage. *R* values are given only for purines that are unmodified in the ring moiety (see footnote 3 of Gamble et al. (1976) for further discussion). Tick marks across the tops of the figures designate the numerical positions of bases in the sequences, in cases where the bases originate from unique positions. The arrow in each bar graph designates the 3′-terminal A. Data were obtained from incubations carried out at 37 °C in 50 mM sodium cacodylate (pH 6.5) and 10 mM Mg^{2+}, with a tRNA concentration of about 10 mg/ml.

TABLE I: *R* Values For Certain Common Purines.[a]

	A9	A14	G15	G18	G57	A73	A76
Yeast $tRNA^{Phe}$	13–19	20	27	3.7	20	7.5	2.2
E. coli $tRNA^{Ile}$	6.0	6.4	4.6	≥20	—	5.9	2.2
E. coli $tRNA_2^{Tyr}$	—	3–5	3–5	≥10	8.8	—	2.2

[a] In cases where a lower bound or range of values is given, calculations were done as described by Gamble et al. (1976). Bases are numbered according to the position of their occurrence in yeast $tRNA^{Phe}$.

ternal standard. Thus, the close agreement between the tRNAs with respect to the 3′ ends gives some assurance that other comparisons have a valid basis.

The most striking feature of Table I is the significant differences between yeast $tRNA^{Phe}$ and the *E. coli* tRNAs in their exchange rates at several loci. These differences are particularly pronounced at A14 and G15, where the two *E. coli* tRNA exchange rates are in good agreement. At these two loci, the data indicate that the bases in yeast $tRNA^{Phe}$ have a markedly altered environment, presumably due to their participation in tertiary interactions, whereas those in the *E. coli* tRNAs appear free of such perturbations. On the other hand, the converse appears to hold true at site G18, where the *R* value of yeast $tRNA^{Phe}$ is substantially lower than the values for the *E. coli* tRNAs. In the dihydrouridine region, the differences between yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Ile}$ are particularly remarkable because the two tRNAs bear extensive sequence homologies between positions 8 and 26 (see Figure 1). Finally, it should be noted that the similarity in exchange rates at several of the common sites for the two *E. coli* tRNAs implies that the large extra loop of *E. coli* $tRNA_2^{Tyr}$ does not perturb exchange at these sites.

Another interesting comparison is G26 in yeast $tRNA^{Phe}$ with G27 in *E. coli* $tRNA^{Ile}$. In the crystal structure of $tRNA^{Phe}$ (Kim et al., 1974b) this base is intercalated between the adjacent cloverleaf base pairs and may also be hydrogen bonded to A44 of the variable loop (Kim et al., 1974b). This base has a *R* value of 9.8 in yeast $tRNA^{Phe}$ and 8.2 in *E. coli* $tRNA^{Ile}$. This close agreement is probably meaningful, although caution must be exercised, since G26 is a dimethylated guanine in $tRNA^{Phe}$ but G27 is unmodified in $tRNA^{Ile}$.[2] These chemical differences lend some ambiguity to the comparison.

Interpretation of Variable Rates among Common Bases in the Three tRNAs. The variations found in the exchange rates at common sites probably arise from some sorts of structural differences between the tRNAs, under the conditions of the experiments. These structural differences can be of two types. First, they might be due to the presence of certain specific interactions in one tRNA and not in another. This could be caused by structural fluctuations introduced by localized melting; if this is the case, adjustment of solution conditions (e.g., lowering the temperature) should eliminate such structural differences. Alternatively, the differences may persist under a variety of conditions and thus be intrinsic to the polymers themselves.

Second, under the conditions of the experiments each tRNA may possess identical interactions with respect to the common bases discussed above. The variations in exchange rates among the tRNAs at these common sites would then be due to higher order effects. For example, bases in cloverleaf helical sections show a significant range of *R* values (*R* = 9–29), and even larger differences are found for helical bases in DNA vs. those in RNA (Gamble et al., 1976). Thus, subtle variations in the atomic coordinates of a base participating in a given tertiary interaction and, or alternatively, of those of its neighboring bases conceivably could give rise to severalfold alterations in the observed labeling rate.

Although a clear-cut choice cannot be made between the two alternatives, the first is likely in at least certain instances. In

[2] The *R*-value of m_2^2G in yeast $tRNA^{Phe}$ was calculated with reference to free m_2^2G, which exchanges at a somewhat different rate than unmodified G (cf. Gamble et al., 1976).

yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Ile}$, the A9 and A14 tertiary hydrogen bonds occur within the region in which there are extensive sequence homologies between the two tRNAs. Thus, A9 and A14 can not only form the identical tertiary bonds in the two tRNAs, but can do so within a similar framework of neighboring bases. It is likely, therefore, that the marked differences in labeling rates at these sites, and possibly at the other sites as well, are due to the partial or complete lack of certain tertiary bonds in some instances and not in others.

Conclusions

The results obtained with *E. coli* $tRNA^{Ile}$ and $tRNA_2^{Tyr}$ reinforce many of the conclusions obtained with yeast $tRNA^{Phe}$ (Gamble et al., 1976). For example, in all three cases helical purines show substantially reduced labeling rates, even though a wide range of helical sequences is collectively represented. Moreover, the helical units in the tRNAs, though showing some variations in labeling rates among themselves, consistently label much slower than helical units in DNA (see Gamble et al., 1976). Also, in all three tRNAs the terminal adenosine has the same *R* value and the highest relative labeling rate; the latter observation checks with the presumably open, unbonded state of this residue.

It is particularly noteworthy that in no instance have we observed labeling rates greater than those achieved in the free mononucleotides or random coils. As a general rule, it appears that structure formation (e.g., base pairing, base stacking) invariably decreases the labeling rate, even though the wide variety of base environments in the three tRNA structures might be expected to affect the acidity of C-8 and other chemical properties in diverse ways. Although these chemical effects no doubt bear influence, it is possible that the dominant influence is the effect of structure on the accessibility of solvent molecules, i.e., water.

The isotope labeling data presented here show that many features of the three tRNAs are similar. At a few loci, involving common tertiary interactions, some differences are apparent. These differences stand out against a background of similarities, which in turn provide an internal standardization of the data. It is possible that, for at least some sites (e.g., A9 and A14 (see above)), the differences represent microscopic structural variations, which may arise from localized melting processes.

Some of these differences occur among common bases in the dihydrouridine loop of two tRNAs that fall in the same structural class—yeast $tRNA^{Phe}$ and *E. coli* $tRNA^{Ile}$. It is interesting to note, however, that the 3′ side of this loop contains an extra base in *E. coli* $tRNA^{Ile}$, as compared to yeast $tRNA^{Phe}$. Although the additional unit should not sterically interfere with the construction of the common system of tertiary bonds in this region (see Kim et al., 1974a), it conceivably may influence the rate of H-8 exchange; this could occur through altered spatial relationships that affect solvent accessibility to nearby sites in the structure and, additionally or alternatively, through effects on the stability (and thereby the H-8 exchange rate) of nearby tertiary interactions.

Microscopic structural fluctuations, such as those suggested by the data presented here, may have important consequences. For example, they provide a subtle mechanism by which enzymes (such as aminoacyl-tRNA synthetases) can distinguish between tRNA species. And further investigation might show that subtle structural fluctuations are easy to induce, and that they serve an important function as a tRNA molecule cycles through a round of protein synthesis.

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