Effect of Cleaving the Dihydrouridine Loop and the Ribothymidine Loop on the Amino Acid Acceptor Activity of Yeast Phenylalanine Transfer Ribonucleic Acid*

JAKOB SCHMIDT, BIRGIT BUCHARDT, AND BRIAN R. REID

From the Biochemistry Department, University of California, Riverside, California 92502

SUMMARY

Incubation of pure yeast phenylalanine transfer RNA with high levels of ribonuclease T1 at 37° in 20 mM MgCl2 leads initially to the production of a 3' three-quarter-molecule and a 5' quarter-molecule as a result of rapid cleavage in the dihydrouridine loop. Subsequently, scission in the ribothymidine loop occurs, generating 3' quarter-molecules and half-molecules; the latter comprise the central half of the molecule extending from the dihydrouridine loop to the ribothymidine loop. The identity of these fragments was established by column "fingerprinting" of the oligonucleotides produced by complete digestion with ribonuclease T1.

The central half-molecule, the 5' quarter-molecule, and the 3' quarter-molecule can be reannealed to form an aggregate the size of intact tRNA as judged by Sephadex G-100 chromatography. This reconstituted tRNA_Phe can be charged to approximately 20% with phenylalanine by partially purified phenylalanyl-tRNA synthetase despite the cleavages in the dihydrouridine and ribothymidine loops, thus indicating that these loops need not be intact for enzyme recognition. Neither individual fragments nor aggregates with large sections of the tRNA missing could be aminocacylated.

The relative ease of purification of yeast tRNA_Phe and the elucidation of its primary structure by RajBhandary and Chang (11) prompted us to attempt fragmentation and reconstitution experiments with this tRNA. The anticodon loop of this molecule is resistant to ribonuclease T1 since it contains only 1 guanosine that is 2'-O-methylated. We have studied the degradation of yeast tRNA_Phe by ribonuclease T1 and demonstrated cleavage into large fragments the size of half- and quarter-molecules; these fragments recombine upon mixing to produce an aggregate that accepts phenylalanine and is the size of intact tRNA (12). Further studies reported here have led to the identification of these fragments as being a 3' quarter extending into the dihydrouridine loop, a 3' quarter extending into the ribothymidine loop, and a half molecule comprising the central region from the dihydrouridine loop to the ribothymidine loop. More detailed experiments on the reconstitution of this "horizontally bisected" tRNA are now reported, and their significance with regard to enzyme recognition is discussed.

MATERIALS AND METHODS

Purification of Yeast tRNA_Phe—Brewers' yeast tRNA (Boehringer Mannheim) was fractionated on benzoylated DEAE-cellulose columns according to the procedure of Wimmer, Maxwell, and Tener (13) to yield partially purified tRNA_Phe. This material was preparatively aminocylated with 14C-phenylalanine and rechromatographed on benzoylated DEAE-cellulose according to the method of Litt (14), to yield a symmetrical peak of pure tRNA_Phe. The stripped tRNA_Phe which was used for fragmentation studies, was approximately 95% homogeneous as judged by quantitative 14C-phenylalanine acceptance and complete ribonuclease T1 digestion patterns.

Purification of Phe-tRNA Synthetase from Yeast—Aliquots, 450 g, of Fleischmann's pressed bakers' yeast (purchased frozen from Standard Brands, Inc., New York) were thawed in 250 ml of 50 mM Tris-Cl, pH 7.6, 10 mM MgCl2, and 10 mM mercaptoethanol buffer, and the suspension was homogenized with glass beads in a Bronwill MSK homogenizer. The homogenate was centrifuged twice at 15,000 x g at 2° for 15 min, and the supernatant was centrifuged at 200,000 x g for 1 hour to remove ribosomes. The post-ribosomal supernatant: proteins that precipitated between 40 and 70% saturation with ammonium sulfate at 0° were centrifuged, redissolved in 10 mM potassium phosphate, 1 mM...
EDTA, pH 7.2, to a concentration of 20 mg per ml, and dialyzed overnight into this buffer. The dialyzed material was applied to a column of DEAE-Sephadex A25 (6 × 10 cm) equilibrated with the same buffer. Elution with this buffer removed most of the protein, and the Phe-tRNA synthetase was subsequently eluted with the same buffer containing 0.2 M NaCl. The eluted enzyme was concentrated by ammonium sulfate precipitation, redissolved in 10 mM potassium phosphate, pH 7.2, and stored at -20°C in 50% glycerol. At this stage of purification, the enzyme was stable for several months, and the specific activity was routinely greater than 100 enzyme units per mg of protein (1 enzyme unit catalyzes the esterification of 1 nmole of 34C-phenylalanine to tRNA in 10 min at 37°C under the standard assay conditions; see below under "Assay for 34C-Phenylalanine Acceptance"). Although specific activities of 2,000 to 3,000 units per mg have been obtained by further purification steps, the highly purified enzyme is unstable upon storage, and such preparations were not used in these experiments.

Partial Ribonuclease T1 Digestion of tRNA^phe—Pure tRNA^phe, 1,320 A260 units, was incubated in 30 ml of 0.05 M Tris-Cl, pH 7.5, 0.02 M MgCl2, with 300,000 enzyme units (15) of ribonuclease T1 (Worthington) at 37°C for 10 min. At the end of the incubation period, the enzyme was removed by five consecutive extractions with 30-ml aliquots of phenol saturated with 0.05 M Tris-Cl, pH 7.5. The pooled phenolic phases were back-extracted with 30 ml of 0.05 M Tris-Cl, pH 7.5. Finally, the combined aqueous phases were extracted 10 times with 30-ml aliquots of ether (to remove residual phenol); the ether-extracted solution was then concentrated by flash evaporation to approximately 3 ml and processed immediately by chromatography on Sephadex G-100 at 57°C. For analytical experiments involving the investigation of the time course of production of various fragment sizes, the above quantities were scaled down by a factor of 100.

Separation of Large Fragments Produced from tRNA^phe—The ribonuclease T1-treated tRNA^phe was applied to a column of Sephadex G-100 (2.2 × 105 cm) equilibrated with 10 mM potassium phosphate, pH 7.5, containing 0.1 M NaCl, following the procedure of Imura, Schwan, and Chambers (6). The tRNA fragments were chromatographed under denaturing conditions by maintaining the column at 57°C. The column was eluted with boiled buffer from a reservoir which was maintained at approximately 50°C during the chromatography. Fractions, 5 ml, of the eluate were collected. Rechromatography of the individual fragments obtained by this procedure was performed under identical conditions. The columns were calibrated with the use of intact tRNA, tRNA^phe halves (4), the tRNA^phe anticodon dodecanucleotide (17), and GMP.

Identification of Large Fragments by Complete Ribonuclease T1 Digestion—The large fragments obtained from tRNA^phe and purified by hot Sephadex G-100 chromatography were identified by means of their complete ribonuclease T1 fingerprints obtained by following the digestion procedure described by RajBhandary, Stuart, and Chang (16). Solid urea was added to the complete digest to give a final concentration of 7 M, and the sample was applied to a column of DEAE-cellulose (0.9 × 95 cm) equilibrated with the starting Tris-urea buffer. Elution was carried out with a linear gradient between 500 ml of 0.02 M Tris-Cl, pH 7.5, 7 M urea, and 500 ml of the same buffer containing 0.3 M NaCl. A constant flow rate of 70 ml per hour was maintained; the effluent was passed through a flow cell assembly, and the A260 was continuously recorded. The reference fingerprint pattern of intact tRNA^phe was obtained in the same way.

Identification of Complete Ribonuclease T1 Digestion Product—The identity of the various oligonucleotides obtained upon complete ribonuclease T1 digestion was based on the DEAE-cellulose elution profile of RajBhandary et al. (16) with the two exceptions mentioned under "Results."

Base Composition of Oligonucleotides—The oligonucleotides were hydrolyzed at 37°C in 0.25 M KOH for 16 hours, and the hydrolysate was adjusted to pH 10 by the addition of Dowex 50 (H+ form). An aliquot was placed on a Dowex 1-formate column (0.0 × 25 cm) equilibrated with water and eluted with the double exponential formic acid gradient system described by Carbon (17). The eluted mononucleotides were identified by elution positions and ultraviolet spectra. Characterization of 7-methyl-GMP was obtained by the ultraviolet spectrum of the alkaline breakdown product (2-amino-4-hydroxy-5-N-methylformamido-6-(N-β-phosphoribofuranosylamino)pyrimidine), which eluted from the column in front of GMP.

Demonstration of Specific Reaggregation of Fragments—The various fragments used in reconstitution experiments were combined at room temperature in the concentrations indicated in the text in 10 mM MgCl2. The mixture was immediately applied to a column of Sephadex G-100 equilibrated with 0.05 M Tris-Cl, pH 7.5, 0.01 M MgCl2, which was eluted at room temperature with the same buffer. Fractions, 5 ml, of the eluate were collected and monitored for absorbance at 260 nm. 34C-Phenylalanine acceptance was determined on eluted aliquots with the use of partially purified phenylalanyl-tRNA synthetase under the standard tRNA-charging conditions.

Assay of 34C-Phenylalanine Acceptance—For assay, 0.01 to 2.0 A260 units of tRNA or recombined fragments were incubated with 008 M 34C-phenylalanine (18 μCi per μmole, or 90 μCi per μmole in the case of small amounts of tRNA when greater sensitivity is required) and 10 enzyme units of partially purified phenylalanyl-tRNA synthetase (100 to 200 units per mg) in a medium containing 10 mM ATP, 50 mM MgCl2, 100 mM Tris-Cl (pH 7.5), and 10 mM reduced glutathione. The reaction mixture (usually 0.2 ml) was incubated at 37°C, and 40-μl aliquots were removed at various time intervals onto filter paper dises (Schleicher and Schuell, 593-A) which were immediately dropped into ice-cold 10% trichloracetic acid. The dises were washed, dried, and counted in scintillation fluid according to previously published procedures (18).

RESULTS

RNase T1 cleavage of tRNA^phe—Fig. 1 shows the results of a small scale experiment designed to find, at a given substrate concentration, magnesium ion concentration, temperature, and incubation time, the optimal RNase T1 concentration for the breakdown of tRNA^phe into fragments that can reanneal to form functional aggregates. Following a suggestion by Oda et al. (3), tRNA^phe was incubated with increasing amounts of RNase T1 under the specified conditions; the reaction was stopped by 40-fold dilution of the incubation medium with water, and aliquots were assayed, immediately or after a heating-quick cooling step, for phenylalanine acceptor activity. As can be seen, the proportion of functional, albeit cleaved, molecules, i.e. molecules that retain chargeability when kept cold after the nuclease
treatment but lose it upon disruption by heating-quick cooling, reaches a maximum at 10,000 enzyme units per ml. The usefulness of this result was tested by an independent procedure. At the high enzyme level indicated, tRNA\textsuperscript{Phe} was incubated for various lengths of time, and the production of large fragments followed directly by chromatography of the partial digest on hot Sephadex G-100 columns. As can be seen in Figs. 2 and 3, initially three-quarter- and quarter-sized fragments are formed in approximately equal molar quantities. Subsequently, halves are generated while the amount of three-quarter-molecules decreases and the percentage of quarters keeps rising. Ultimately, all large fragments are degraded to the complete digest size, which ranges from GMP to the dodecanucleotide containing the anticodon loop. This experiment not only demonstrates that, at the previously determined enzyme concentration, the amount of large fragments reaches a maximum in a conveniently short time, but it also allows one to make statements as to the probable identity of the fragments produced. Assuming that the base-paired regions of the molecule ("stems") are less susceptible to the enzyme than are the single stranded sections ("loops"), the most plausible sites for enzyme attack appear to be in the dihydrouridine- and ribothymidine-containing loops and perhaps in the minor loop since, as a result of the absence of RNase T\textsubscript{1} susceptible residues in the anticodon loop, a vertical bisection of the molecule as reported for a number of other tRNAs (2, 3, 5-7, 19), is very unlikely. Random attack, therefore, should lead to the formation of three-quarter- and quarter-sized molecules in the initial stages of digestion, with subsequent attack giving rise to half-molecules and more quarters. This is indeed observed. The half-molecule would therefore be expected to extend from the dihydrouridine loop to the ribothymidine loop, while the quarter population should consist of 5' and 3' quarter fragments from the top half of the molecule. Cleavage of the extra loop apparently does not occur, as intermediate-size fragments are not observed.

For preparative scale fragment production, 70 mg of pure tRNA\textsuperscript{Phe} were digested under the conditions described above. Fig. 4 shows the elution pattern of the partial digest after chromatography on a hot Sephadex G-100 column. After double rechromatography of the individual peaks, the fragment populations appear homogeneous and free of cross-contamination, as can be seen from Fig. 5.

**Identification of Fragments**—The half- and quarter molecules were subjected to complete ribonuclease T\textsubscript{1} digestion to allow comparison of their oligonucleotide elution patterns on DEAE-cellulose with that of a ribonuclease T\textsubscript{1} fingerprint of intact tRNA\textsuperscript{Phe}. The complete digest pattern of intact tRNA\textsuperscript{Phe}, shown at the top of Fig. 6, is slightly different from the one originally published by RajBhandary et al. (16) in that the amino acid acceptor oligonucleotide CpApCpCpA (Peak 7) re-
Side Loop Cleavage of tRNA\(^{\text{Ph}}\)

Vol. 245, No. 21

FIG. 3. Rate of appearance of various sized fragments of tRNA\(^{\text{Ph}}\) during partial digestion with ribonuclease T\(_1\). The quantity of material (expressed as a percentage of the total \(A_{260}\) units in the \(\frac{1}{4}, \frac{1}{2}, \frac{3}{4}\), and 1 peaks) in each of the peaks in Fig. 2 was divided by the fragment size (expressed as a fraction of intact tRNA) to give the relative molar amounts of fragments.

![Graph showing the rate of appearance of various sized fragments of tRNA\(^{\text{Ph}}\) during partial digestion with ribonuclease T\(_1\).](image)

FIG. 4. Preparative scale fragmentation of tRNA\(^{\text{Ph}}\). Pure tRNA\(^{\text{Ph}}\), 70 mg, in 30 ml of 50 mM Tris-Cl, pH 7.5, 20 mM MgCl\(_2\), was digested with 300,000 enzyme units of ribonuclease T\(_1\) at 37° for 10 min. The sample was prepared for chromatography and fractionated at 57° on a column of Sephadex G-100 as described under "Materials and Methods." Fractions of 4.8 ml were collected and monitored for absorbance at 260 nm after appropriate dilution.

![Graph showing the preparative scale fragmentation of tRNA\(^{\text{Ph}}\).](image)

FIG. 5. Second rechromatography of individual fragments of tRNA\(^{\text{Ph}}\). The individual fragments obtained in Fig. 4 were rechromatographed, pooled to eliminate minor contamination with other fragments, and rechromatographed again on Sephadex G-100 at 57°.

![Graph showing the second rechromatography of individual fragments of tRNA\(^{\text{Ph}}\).](image)
FIG. 6. Complete ribonuclease T1 digestion patterns of fragments derived from tRNA\textsuperscript{Phe}. The half-molecule and quarter-molecule fractions shown in Fig. 5, as well as intact tRNA\textsuperscript{Phe}, were subjected to complete digestion with ribonuclease T1; the resulting oligonucleotides were fractionated on DEAE-cellulose columns as described under “Materials and Methods.” On the right is shown the complete cloverleaf structure of yeast tRNA\textsuperscript{Phe}, as elucidated by RajBhandary and Chang (12), indicating the positions in the molecule from which the individual oligonucleotide peaks are derived.

| Peak | CMP | AMP | GMP | UMP | Adenosine | 7-methyl-GMP |
|------|-----|-----|-----|-----|-----------|-------------|
| 7    | 3.00| 1.11| 1.09| <0.1| 0.60      | 0.72\textsuperscript{b} |
| 11   | 1.93| 1.00| 1.88|      |           |             |
| 12   | 1.99| 0.95| 1.00| 1.07|           |             |

\textsuperscript{a} The presence of GMP in this peak is due to the complete overlap of Peak 7 and Peak 6 (DpDpGp) in the preparative fractionation from which the oligonucleotide was obtained.

\textsuperscript{b} Based on the \( E_{max} \) of the base-catalyzed, ring fission product of 7-methyl-GMP (70).

amounts of quarters are added. In a corresponding manner, the addition of increasing amounts of halves to a constant amount of quarters produces a linear response in chargeability only up to an \( A_{450} \) ratio of halves to quarters of approximately 0.3. These results suggested that the quarter-molecule fraction might contain unequal amounts of the 5' quarter and 3' quarter. A rough estimate of the relative proportion of the two quarters can be obtained from the observation that a 6-fold molar excess of quarters is required to titrate the half-molecules, which would suggest a value of approximately 5:1 for the ratio of nonlimiting to limiting quarters. This interpretation should be verifiable by analyzing the complete ribonuclease T1 digestion pattern obtained from the mixed quarter population. From the fingerprints shown in Fig. 6, one observes that the 2 moles of oligonucleotide in Peak 15 are both derived from the 3' quarter (14 nucleotides total), whereas the hexanucleotide comprising

Fig. 7. Restoration of function by adding increasing amounts of half-molecules to quarter fragments and vice versa. a, 0.11 \( A_{260} \) unit of quarter fragments were mixed with increasing amounts of half-molecules in the ratios indicated and assayed for phenylalanine acceptor activity as described in the text. b, 0.037 \( A_{260} \) unit of half-molecules were mixed with increasing amounts of quarter fragments in the ratios indicated and assayed for phenylalanine acceptor activity as described in the text.

Peak 14 is derived from the 5' quarter. Hence, in an equal mixture of 3' and 5' quarters, the ratio (in \( A_{450} \) units) of Peak 15 to Peak 14 should be approximately 2.4:1, which is in fact observed in the fingerprint of intact tRNA\textsuperscript{Phe}. In the fingerprint derived from the mixed quarter population, however, the ratio of Peak 15 to Peak 14 is only approximately 0.6:1, indicating a 4-fold lower amount of the Peak 15-containing (i.e. 3') quarter. Similarly, fingerprints of the three-quarter-molecule fraction were found to contain negligible amounts of Peaks 12 and 14 indicating a marked predominance of the 3' three-quarter-molecule resulting from preferential cleavage of the dihydrouridine-containing loop. As a corollary, an excess of 3' quarter frag-
ments is expected to be formed. Thus, our interpretation involving the presence of a limited quantity of the 3' quarter fragment appears to be corroborated by direct chemical evidence.

A further experiment designed to show the dependence on the half-molecule as well as the two types of quarters for phenylalanine acceptance was carried out by the continuous variation mixing method in which halves and quarters are reciprocally varied in the mixture. Such an experiment is shown in Fig. 8. It should be noted in this experiment that the stock solution of quarters used had 4 times the A_{600} of the half-molecule solution. At the optimum of this curve, acceptor activity is obviously limited both by the half and by the limiting 3' quarter; i.e. their molar concentrations should be equal. At this point, the A_{600} ratio of quarters to halves is 3:1, indicating a molar ratio of quarters to halves of 6:1 under conditions in which the limiting 3' quarter and the half-molecule are present in equimolar amounts. This again indicates a 5-fold molar excess of 5' quarters over the limiting 3' quarter. Thus we conclude that the mixed quarter population comprises 16 to 20% 3' quarters and 80 to 84% 5' quarters. These experiments prove that isolated quarter and half fragments can be recombined to form a functional complex that can be recognized by phenylalanyl-tRNA synthetase despite the scission of both the dihydrouridine and ribothymidine-containing loops. It was interesting to note in these experiments that preliminary incubation of the mixed fragments for various periods at 37° in buffer (50 mM Tris-Cl, pH 7.5, 20 mM MgCl₂), had no effect on the level of charging, indicating that the functional reconstitution between fragments is quite rapid. In all of the foregoing experiments, the molar acceptance of ^{14}C-phenylalanine was found to be 20%, based on the molar concentration of the limiting fragment.

Since the synthetase recognition site may be located exclusively in the "upper half" of the molecule, an attempt was made to change the mixed quarter fraction in the absence of the "lower half" of the molecule under conditions favoring the reannealing of the two quarters. Results of these experiments are shown in Table II. As can be seen, the level of phenylalanine acceptance is barely detectable.

**Physical Demonstration of Reconstitution** — Although our previous experiments demonstrated the capacity of a mixture of fragments of tRNA^{Phe} to accept ^{14}C-phenylalanine, one can say very little from these purely functional assays about the structure of the functional aggregate. The presumed aggregate, although capable of phenylalanine acceptance, might be of only transient stability, and the possibility remains that some aggregate smaller than intact tRNA might contain the required...
recognition loci and be capable of phenylalanine acceptance. Therefore, the interaction between fragments was studied by means of Sephadex G-100 chromatography under conditions in which reannealing of the fragments is facilitated. The first two patterns in Fig. 9 show the elution profiles of tRNAΦhe halves and quarters chromatographed alone under these conditions. The halves show some reannealing into molecules the size of intact tRNA, which may be explained to some extent by the presence of some right and left half-molecules produced by nonspecific anticodon cleavage. The quarters show significant aggregation into apparent half-molecules, which we envisage as the reannealing of 3' quarters with 5' quarters to form the upper half of the tRNAΦhe molecule. Mixing of halves and quarters, however, produces an elution profile in which reconstituted molecules the size of whole tRNA are observed. The uncomplexed quarter molecules in this profile are probably due to the excess 5' quarters in the quarter population. Furthermore, the usually sharp separation between whole molecules and half-molecules is not apparent, which suggests the formation of some three-quarter-molecules from halves and single quarters. When aliquots of the effluent fractions were tested for charging by phenylalanine-tRNA synthetase, acceptor activity could be demonstrated in the reconstructed whole tRNA region only.

**DISCUSSION**

One of the most interesting aspects of the present work is the demonstration of selective side loop cleavage of yeast tRNAΦhe by ribonuclease T1 at high temperature, with a marked preference seen for the dihydrouridine-containing loop. This finding may simply reflect the preponderance of guanosine residues in this loop, or else it could be interpreted as being due to the tertiary structure of the molecule. In the tRNAΦhe model proposed by Cramer et al. (21), and corroborated by Litt (22), the G46 in the dihydrouridine loop is the only unprotected guanosine in the polynucleotide chain; enzyme attack at this site might expose the other guanosine residues in this loop, resulting in further digestion and thus, in turn, perhaps affecting the stability of the ribothymidine loop and rendering its guanosine susceptible to ribonuclease T1. Susceptibility of side loops to enzymatic attack is expected to be similar in the model proposed by Levitt (23), the major difference, however, would be the greater accessibility of the extra loop in his proposed structure. Since ready cleavage at G46 is normally not seen, we interpret our data as supporting conformations in which the extra loop is protected, such a model has recently been presented by Ninio, Favre, and Yann (24) for tRNAΦhe34; however, in the structure they propose, the ribothymidine loop appears to be at least as accessible as the dihydrouridine loop.

The aim of the work reported here was to produce fragments of tRNAΦhe for a study of the requirements for synthetase recognition during aminoacylation. The fragments can be recombined to form aggregates differing from intact tRNA by polynucleotide chain cleavages or the absence of sequences. We have been able to extend to the yeast tRNAΦhe case the findings of Seno, Kobayashi, and Nishimura (8) and Mirzabekov et al. (9) that the intactness of neither the dihydrouridine loop nor the ribothymidine loop, respectively, is required for aminoacylation. Seen in conjunction with the reported amino acid acceptor activity of tRNAΦhe cleaved in the anticodon loop (4), this suggests that none of the major loops is crucial in specific synthetase recognition. Moreover, our experiments show, as has been demonstrated for yeast tRNAΦhe by Imura, Weiss, and Chambers (25) and for yeast tRNAΦhe by Mirzabekov et al. (9), that even multiple cleavages do not abolish acceptor activity. Attempts, however, to charge tRNAΦhe with all major loops ruptured, by combining the quarter-molecule fraction with the anticodon-scission products (4) of the center half-molecule, have so far been unsuccessful. Similarly, the one-third and one-sixth fragments (presumably the dihydrouridine to extra loop and extra loop to ribothymidine loop fragments) that are formed at the expense of center half-molecules when tRNAΦhe is carefully desalted before ribonuclease T1 treatment do not produce a functional entity when combined with the amino acid acceptor stem fragments. Thus, when both side loops are cleaved, the intactness of the anticodon half seems to be important for restoration of function.

In view of the claim by Imura et al. (25) that, in the case of yeast tRNAΦhe, the amino acid acceptor stem alone is capable of being specifically aminoacylated by alanyl-tRNA synthetase, an attempt was made to charge the amino acid acceptor stem of tRNAΦhe. Even at 0°C, the level of acceptance was at most 1.2 mol %, despite the readily demonstrable acceptor activity of reconstituted tRNAΦhe at this temperature. No other aggregate with large sections of the molecule missing was found to be functional.

Isolation of a tRNA center half-molecule in high yields has not, to our knowledge, been reported so far. It is quite obviously a fragment of many uses. With the 5' and 3' halves obtained by chemical anticodon loop scission (4), it allows an investigation, by inhibition studies, of how the three major loops participate in synthetase recognition; in a radioactive form, it can be used for anticodon site demonstration as described for tRNAΦhe by Clark, Dube, and Marecker (26); incorporation of a suitable fluorescent probe at its 3' end would afford a spectroscopic ruler (27) for estimating the distance between the ribothymidine loop and the anticodon region in the reannealed molecule; finally, hybridization experiments with heterologous 5' and 3' half combinations as recently described by Mirzabekov, Levin, and Bayev (7) are now feasible with heterologous amino acid acceptor half-anticodon half combinations, thus opening up a novel approach toward verification of the adaptor hypothesis.

**REFERENCES**

1. ZACHAU, H. G., Angew. Chem., 81, 717 (1969).
2. BAYEV, A. A., FOGRÉI, I., MIRZABEKOY, A. D., AXELROD, V. D., AND KAZARINOVA, L. Y., Mol. Biol., 1, 859 (1967).
3. ODA, K., KIMURA, F., HARADA, F., AND NISHIMURA, S., Biochim. Biophys. Acta, 170, 97 (1969).
4. PHILIPSSEN, P., TRIEBB, R., WINTERMEYER, W., AND ZACHAU, H. G., Biochem. Biophys. Res. Commun., 33, 922 (1968).
5. CHOUGUEY, I. I., AXELROD, V. D., AND BAYEV, A. A., Biochem. Biophys. Res. Commun., 34, 348 (1969).
6. IMURA, N., SCHWAM, H., AND CHAMBERS, R. W., Proc. Nat. Acad. Sci. U. S. A., 62, 1203 (1969).
7. MIRZABEKOY, A. D., LUTIWA. F. S., AND BAYEV, E. E., Fed. Eur. Biochem. Soc. Lett., 5, 218 (1969).
8. SENO, T., KOBAYASHI, M., AND NISHIMURA, S., Biochim. Biophys. Acta, 174, 406 (1969).
9. MIRZABEKOY, A. D., KAZARINOVA, L. Y., LASITTY, D., AND BAYEV, A. A., Fed. Eur. Biochem. Soc. Lett., 3, 288 (1969).
10. SENO, T., KOBAYASHI, M., AND NISHIMURA, S., Biochim. Biophys. Acta, 182, 280 (1969).
11. RAJBIHANDARY, U. L., AND CHANG, S. H., J. Biol. Chem., 243, 598 (1968).

\* Unpublished observations.
12. Schmidt, J., and Reid, B. R., Biochim. Biophys. Acta, **213**, 539 (1970).
13. Wimmer, E., Maxwell, I. H., and Tener, G. M., Biochemistry, **7**, 2623 (1968).
14. Litt, M., Biochim. Biophys. Res. Commun., **32**, 507 (1968).
15. Egami, F., Takahashi, K., and Uchida, T., Progr. Nucl. Acid Res. Mol. Biol., **3**, 50 (1964).
16. Rajbhandary, U. L., Stuart, A., and Chang, S. H., J. Biol. Chem., **243**, 584 (1968).
17. Carbon, J. A., Biochim. Biophys. Acta, **95**, 550 (1965).
18. Reid, B. R., Biochem. Biophys. Res. Commun., **33**, 627 (1968).
19. Penswick, J. R., and Holley, R. W., Proc. Nat. Acad. Sci. U. S. A., **53**, 543 (1965).
20. Brookes, P., and Lawley, P. D., J. Chem. Soc., 3923 (1961).
21. Cramer, F., Doppner, H., Haar, F. v. d., Schlimme, E., and Seidel, H., Proc. Nat. Acad. Sci. U. S. A., **61**, 1384 (1968).
22. Litt, M., Biochemistry, **8**, 3210 (1969).
23. Levitt, M., Nature, **224**, 759 (1969).
24. Ninio, J., Faye, A., and Yaniv, M., Nature, **223**, 1333 (1969).
25. Imura, N., Weiss, G. D., and Chambers, R. W., Nature, **222**, 1147 (1969).
26. Clark, B. F. C., Dube, S. K., and Marcker, K. A., Nature, **219**, 434 (1968).
27. Stryer, L., and Haugland, R. P., Proc. Nat. Acad. Sci. U. S. A., **58**, 719 (1967).
Effect of Cleaving the Dihydrouridine Loop and the Ribothymidine Loop on the Amino Acid Acceptor Activity of Yeast Phenylalanine Transfer Ribonucleic Acid

Jakob Schmidt, Birgit Buchardt and Brian R. Reid

J. Biol. Chem. 1970, 245:5743-5750.

Access the most updated version of this article at http://www.jbc.org/content/245/21/5743

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/245/21/5743.full.html#ref-list-1