SUMMARY

The formation of a stable complex between valyl transfer ribonucleic acid synthetase from yeast and transfer RNA specific for valine (tRNAval) from the same source has been used as a model for the recognition between enzyme and tRNA. With the use of sucrose gradient centrifugation to isolate the complex, the major fraction of tRNAval (tRNAval) was found to retain its ability to form a stable complex with the enzyme even after a digestion with snake venom phosphodiesterase that had removed as much as an average of 7 nucleotide residues from the 3'-hydroxyl end. The digested tRNAs also showed competitive inhibition of complex formation with native tRNAval and the relative affinities of the enzyme for the different tRNAval preparations that could be calculated from the inhibition experiments showed that this extensive modification at the 3'-hydroxyl end had not lowered the affinity of the enzyme for the modified tRNAval compared to native tRNAval by more than a factor of 2.

The structural basis for the specificity of the aminoacyl transfer RNA synthetases constitutes a problem of considerable biological importance. Experiments with structurally modified tRNAs or oligonucleotides of known composition as competitive inhibitors of these enzymes have given ambiguous results (1-7). As part of an attempt to obtain a model system for the recognition mechanism of the enzymes, we have described the formation of a stable enzyme-substrate complex between valyl ribonucleic acid synthetase and tRNAVal which involves the first three base pairs at the 3'-hydroxyl end of the clover leaf model. As part of this investigation, we have attempted to obtain a model system for the recognition between valyl-tRNA synthetase and tRNAVal which involves the first three base pairs at the 3'-hydroxyl end of the clover leaf model of this tRNA as the sole recognition site for the enzyme.

EXPERIMENTAL PROCEDURE

Materials

Enzymes—Valyl-tRNA synthetase from yeast (Saccharomyces cerevisiae C 836) was prepared as described previously (10). The calculation of the molar concentration of the enzyme is detailed in an earlier communication (9). Snake venom phosphodiesterase was isolated from the venom of Crotalus adamanteus according to the method of Koerner and Sinsheimer (11). One unit of phosphodiesterase activity equals 1 µmole of phosphodiester bond split per hour at 37° in a DNAase limit digest of DNA (12). tRNA Preparations—Crude yeast tRNA was obtained from Boehringer and Soehne, Mannheim, Germany. The concentration of tRNA was expressed in terms of its optical density. The unit used (A260) was defined as the amount of material that, when dissolved in 1.0 ml, gave an absorbance of 1.0 at 260 mµ.
periodate oxidation followed by amine-catalyzed hydrolysis (15).

tRNAp' that had lost an average of 6 nucleotide residues from its 3'-terminal end (tRNAVa'-UCACCA) was obtained by diges-

tion with snake venom phosphodiesterase. tRNAp' (117 A260 units) was incubated with 10 mmoles of its 3'-terminal adenosine (tRNAVal CCp) was obtained by

treatment with the enzyme on Sephadex (9). The complex could be obtained as a complex with the enzyme (see below). Nevertheless better than 90% of the A260 present in the prepara-
tion, expressed as percentage of the A260 of native tRNAval, was corrected for the known ultraviolet absorbance of the enzyme. The complex was isolated by centrifugation through a sucrose gradient. For further experimental details see “Methods” and references therein. In similar series, 2.6 A168 of tRNAVa-CCp (O), 2.4 A168 of tRNAVal-UCACCA (■), and 2.1 A168 of tRNAVal-AUCACCA (△) were incubated in the

same way.

FIG. 1. Formation of enzyme-tRNA complexes with tRNAVal preparations structurally modified at the 3'-hydroxyl end. In one series of experiments (O) 3.56 A168 of native tRNAVal was incubated with increasing amounts of enzyme and complex formation, expressed as percentage of the A168 incubated obtained as a complex with the enzyme, was plotted against the molar ratio of enzyme to tRNAVal in the incubation mixture. The A168 of the tRNA in the complex was obtained after correction for the known ultraviolet absorbance of the enzyme. The complex was isolated by centrifugation through a sucrose gradient. For further experimental details see “Methods” and references therein. In similar series, 2.6 A168 of tRNAVa-CCp (O), 2.4 A168 of tRNAVal-UCACCA (■), and 2.1 A168 of tRNAVal-AUCACCA (△) were incubated in the same way.

with a light path of 1.0 cm. When the molar concentration of tRNA was calculated from its optical density, 1 A260 unit was taken to equal 1.8 nmoles of tRNA (13).

Highly purified tRNAVal was prepared from crude yeast tRNA by the method of Gillam et al. (14). The product obtained in the final purification step had a ratio of esterified valine to A260 that was close to the value expected for pure tRNAVal (1.8 nmoles per A260). However, after removal of the valine, the preparation gave only 1.2 to 1.3 nmoles per A260 in the standard assay. Nevertheless better than 90% of the A260 present in the preparation could be obtained as a complex with the enzyme (see below). This would be consistent with the assumption that the purified material contained only tRNAVal although some of the chains had been sufficiently altered in the purification procedure so as to make them inactive in the standard assay. The product was free of tRNAVal as indicated by its inability to form a stable complex with the enzyme on Sephadex (9).

Terminal Modification—tRNAVal that had been stripped of its 3'-terminal adenosine (tRNAVal-CCp) was obtained by periodic acid oxidation followed by amine-catalyzed hydrolysis (15), tRNAVal that had lost an average of 6 nucleotide residues from its 3'-terminal end (tRNAVal-UCACCA) was obtained by digestion with snake venom phosphodiesterase. tRNAVal (117 A260 units) was incubated with 10 mM MgCl2 and 380 units of phosphodiesterase in 10 mM glycine buffer, pH 8.6, at 37°C in a final volume of 1.0 ml. After 4 hours of incubation, the reaction was terminated by heat inactivation for 2 min at 100°C. After removal of the denatured protein by centrifugation and washing of the pellet with a small amount of water, the supernatant was filtered over Sephadex G-25, 1.3 cm2 × 60 cm, with water as eluent. The mononucleotide fraction was further separated by two-dimensional paper chromatography (16). The nucleotide spots were cut out together with the appropriate blank areas and eluted with 0.2 mM potassium phosphate buffer, pH 7.0, and the content of the different nucleotides was determined from their ultraviolet absorbance. The average composition of the digested 3'-terminal sequence was deduced from the molar proportion of the mononucleotides obtained from the digest and from the known sequence of the tRNAVal (17). tRNAVal minus an average of 7 nucleotide residues from the 3'-hydroxyl end (tRNAVal-AUCACCA) was obtained using a procedure similar to that described above except that the digestion time was 12 hours.

All of the terminally modified tRNAVal preparations had completely lost their ability to be esterified with valine in the standard assay. When tRNAVal-UCACCA and tRNAVal-AUCACCA were tested for their ability to accept AMP and CMP residues from ATP and CTP in the presence of ATP pyrophosphorylase from Escherichia coli (18), they were unable to do so. This is consistent with a digestion that has proceeded further than the terminal triphosphate.

Methods

Assay of tRNA for Ability to Form Aminoacyl-tRNA—Assays for tRNA were performed as previously described (8). Radioactivity Measurements—Determinations of 14C activity were performed in a Packard Tri-Carb liquid scintillation spectrometer, model 314 EX-2, with Bray’s scintillator solution (19).

Formation and Isolation of Complex—The previously described sucrose gradient centrifugation technique was used throughout this investigation (9).
RESULTS

Complex Formation with Modified tRNA$^{Val}_{Val}$—tRNA$^{Val}_{Val}$ modified at the 3'-hydroxyl end (tRNA$^{Val}_{Val}$CCp, tRNA$^{Val}_{Val}$-UCACCA, and tRNA$^{Val}_{Val}$-UCACCA) were prepared as described above. When these modified tRNA$^{Val}_{Val}$ preparations were incubated with the enzyme followed by centrifugation through a sucrose gradient the results obtained were identical with those already described for the native tRNA$^{Val}_{Val}$ (9). The centrifugation thus gave a good separation of tRNA$^{Val}_{Val}$ complexed to the enzyme from free tRNA$^{Val}_{Val}$ and a partial separation of the complex and the free enzyme. The complex formation was completely specific as indicated by the inability of highly purified tRNA$^{Val}_{Val}$, either native or structurally modified, to form a complex with the enzyme. This is also consistent with the specificity that we have previously reported (9) for complex formation with partially purified or crude tRNA preparations.

When enough enzyme was used, 70 to 80% of the A$_{260}$ of the modified tRNA$^{Val}_{Val}$ preparations could be obtained as a complex with the enzyme. This should be compared with a transfer of more than 90% of the A$_{260}$ of native tRNA$^{Val}_{Val}$ into the complex position in similar experiments (Fig. 1).

Competitive Inhibition between Native and Modified tRNA$^{Val}_{Val}$—tRNA$^{Val}_{Val}$CCp, tRNA$^{Val}_{Val}$-UCACCA, and tRNA$^{Val}_{Val}$-UCACCA acted as competitive inhibitors of complex formation with native tRNA$^{Val}_{Val}$. This finding is consistent with the data presented in the previous section and also makes it possible to compare the affinity of the enzyme for modified and native tRNA$^{Val}_{Val}$. In Fig. 2 the molar ratio of modified to native tRNA$^{Val}_{Val}$ in the complex has been plotted against the same ratio in the incubation mixture. The slope of the straight line obtained then gives the relative affinity of the enzyme for the modified tRNA$^{Val}_{Val}$, i.e., the ratio of the affinity for the modified preparation to that for the native one.

DISCUSSION

Although great progress has been made in the elucidation of tRNA structure we still have no real information regarding what parts of the molecule play a role in the recognition between an aminoacyl-tRNA synthetase and its tRNA substrate. Several hypotheses have been advanced that implicate, for example, the anticodon region (1) or the first three base pairs of the clover leaf model (20) in which this part of the tRNA synthetases.

In the clover leaf model of tRNA$^{Val}_{Val}$ cannot be the sole recognition site. The possibility that complex formation was caused by the presence of a small amount of undigested or only slightly digested chains would seem to be ruled out by the following considerations. (a) The complete loss of acceptor activity. (b) The inability of the modified preparations to accept AMP and CMP residues when incubated with tRNA pyrophosphorylase in the presence of ATP and CTP. (c) The fact that 70 to 80% of the modified tRNA$^{Val}_{Val}$ preparations (compared to 90 to 100% of native tRNA$^{Val}_{Val}$) could be obtained as a complex with the enzyme.

In view of their ability to form a stable complex the modified tRNA$^{Val}_{Val}$ preparations would be expected to act as competitive inhibitors of complex formation with native tRNA$^{Val}_{Val}$ and this was indeed found to be the case. The slope of the lines presented in Fig. 2 gives the relative affinity of the enzyme for the modified tRNAs (the ratio of the affinity for the modified preparation to that for the native one). The removal of the 3'-terminal adenosine from tRNA$^{Val}_{Val}$ has obviously not had much effect on the affinity of the enzyme for the tRNA. If a correction is made for the fact that only 75 to 80% of the tRNA$^{Val}_{Val}$CCp can form a complex with the enzyme (Fig. 1) the slope of the line for this tRNA comes very close to one. The relative affinities for the more extensively modified preparations, tRNA$^{Val}_{Val}$-UCACCA and tRNA$^{Val}_{Val}$-UCACCA, are still as high as 0.5 to 0.6 if the same correction is applied as in the case of tRNA$^{Val}_{Val}$CCp. The apparent contradiction of these results is probably caused by the fact that in the earlier experiments we used filtration on Sephadex to isolate the complex. We now know that the stability of the complex on Sephadex is very different from that in the sucrose gradient as illustrated by the inability of native tRNA$^{Val}_{Val}$ to form a complex with the enzyme that is stable on Sephadex (9). Results regarding the formation of complexes with modified tRNAs obtained with the sucrose gradient technique can, therefore, probably not be extrapolated to apply directly to the Sephadex method.

If the ability to form a stable complex with the enzyme is accepted as an indication of recognition between enzyme and tRNA the results of this investigation would suggest that the first three base pairs that form part of the double-stranded stem in the clover leaf model of tRNA$^{Val}_{Val}$ cannot be the sole recognition site of this molecule. This is in contrast to the recently proposed model by Schulman and Chambers (20) in which this part of the molecule is suggested as the recognition site for all aminoacyl-tRNA synthetases.

Acknowledgments—We want to thank Mr. Leif Jansson for the preparation of some of the tRNA$^{Val}_{Val}$ used in this investigation and Mrs. Anne-Marie von Essen for expert technical assistance.

REFERENCES

Structure and Function of Transfer Ribonucleic Acid: IV. COMPLEXES BETWEEN VALYL TRANSFER RIBONUCLEIC ACID SYNTHETASE AND STRUCTURALLY MODIFIED TRANSFER RIBONUCLEIC ACID SPECIFIC FOR VALINE

Ulf Lagerkvist and Lars Rymo


Access the most updated version of this article at http://www.jbc.org/content/245/2/435

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/245/2/435.full.html#ref-list-1