STRUCTURALLY MODIFIED TRANSFER RIBONUCLEIC ACID SPECIFIC FOR VALINE*

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SUMMARY

The formation of a stable complex between valyl transfer ribonucleic acid synthetase from yeast and tRNA specific for valine (tRNA^val) 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 tRNA^val (tRNA^val) 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 tRNA^val and the relative affinities of the enzyme for the different tRNA^val 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 tRNA^val compared to native tRNA^val 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 from yeast and tRNA^val from the same source (8, 9). Three different methods were used for the isolation of the complex: filtration on Sephadex, centrifugation through a sucrose gradient, and electrophoresis on a Pevikon column. Of the two major components of tRNA^val (tRNA^val and tRNA^val) only tRNA^val gave a complex that was stable on Sephadex while with the sucrose gradient centrifugation and electrophoretic techniques both tRNA^val and tRNA^val formed stable complexes with the enzyme.

It is the purpose of this communication to report the formation of stable complexes between the enzyme and highly purified tRNA^val structurally modified at the 3'-hydroxyl end, using sucrose gradient centrifugation to isolate the complex. With this technique the most extensively modified preparation of tRNA^val from which an average of 7 nucleotide residues had been removed from the 3'-hydroxyl terminus, still formed a stable complex with the enzyme as indicated by the transfer of 70 to 80% of the A260 of the preparation from the position characteristic of free tRNA to that of the complex. Furthermore, the structurally modified preparations of tRNA^val gave a competitive inhibition of complex formation with native tRNA^val. The inhibition experiments also showed that the affinity of the enzyme for the most extensively modified tRNA^val preparations was approximately half of that for native tRNA^val. On the basis of these findings a mechanism for the recognition between valyl-tRNA synthetase and tRNA^val 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 would seem unlikely.

EXPERIMENTAL PROCEDURE

Materials

Enzymes—Valyl-tRNA synthetase from yeast (Saccharomyces cerevisiae C 830) 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 µ mole of phosphodiester bond split per hour at 37°C in a D N Ase 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 nm.
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 I final purification step had a ratio of esterified valine to A260 that was close to the value expected for pure tRNAVal (1.8 nmols per A260). However, after removal of the valine, the preparation gave only 1.2 to 1.3 nmols 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 of tRNAs—**tRNAVal that had been stripped of its 3'-terminal adenosine (tRNAVal-CCp) was obtained by periodic oxidation followed by amino-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 8.5, 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 M 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 proportions 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 tRNA pyrophosphorylase from Escherichia coli (18), they were unable to do so. This is consistent with a digestion that has proceeded further than the terminal trimonucleotide.

**Methods**

**Assay of tRNA for Ability to Form Aminoacyl-tRNA—**Assays for tRNA were performed as previously described (8).

**Radioactivity Measurements—**Determination of 14C activity was 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 tRNAval—tRNAval modified at the 3’-hydroxyl end (tRNAval-UCCP, tRNAval-UCACCA, and tRNAval-AUCACCA) were prepared as described above. When these modified tRNAval 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 tRNAval (9). The centrifugation thus gave a good separation of tRNAval complexed to the enzyme from free tRNAval 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 tRNAVal, 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 A260 of the modified tRNAval preparations could be obtained as a complex with the enzyme. This should be compared with a transfer of more than 90% of the A260 of native tRNAval into the complex position in similar experiments (Fig. 1). Competitive Inhibition between Native and Modified tRNAval—tRNAval-UCCCP, tRNAval-UCACCA, and tRNAval-AUCACCA acted as competitive inhibitors of complex formation with native tRNAval. 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 the modified and native tRNAval. In Fig. 2 the molar ratio of modified to native tRNAval 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 tRNAval, 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) as the recognition site. The experimental data presented in support of these hypotheses are, however, not entirely convincing. The ability of the valyl-tRNA synthetase and its tRNA substrate. Several preparations (compared to 90 to 100% of native tRNAval) could be obtained as a complex with the enzyme.

In view of their ability to form a stable complex the modified tRNAval preparations would be expected to act as competitive inhibitors of complex formation with native tRNAval 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 tRNAval 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 tRNAval-CCCP 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, tRNAval-UCACCA and tRNAval-AUCACCA, are still as high as 0.5 to 0.6 if the same correction is applied as in the case of tRNAval-CCCP. The findings presented in this paper are in contrast to our previously reported inability to obtain competitive inhibition with a crude preparation of tRNA containing both tRNAval and tRNAval-UCCCP that had been stripped of its terminal adenosine (9). 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 tRNAval 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 tRNAval 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.

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REFERENCES


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