Three Photo-Cross-Linked Complexes of Yeast Phenylalanine Specific Transfer Ribonucleic Acid with Aminoacyl Transfer Ribonucleic Acid Synthetases

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SUMMARY

Yeast tRNA^Phe has been cross-linked photochemically to three aminoacyl-tRNA synthetases, yeast phenylalanyl-tRNA synthetase, Escherichia coli isoleucyl-tRNA synthetase, and E. coli valyl-tRNA synthetase. The two non-cognate enzymes are known to interact with tRNA^Phe. In each complex, three regions on the tRNA are found to cross-link. Two of these are common to all of the complexes, while the third is unique to each. Thus, the cognate and non-cognate complexes bear considerable similarity to each other in the way in which the respective enzyme orients on tRNA^Phe, a result which was also established for the complexes of E. coli tRNA^1re (Budzik, G. P., Lam, S. M., Schoemaker, H. J. P., and Schimmel, P. R. (1975) J. Biol. Chem. 250, 4433-4439). The common regions include a piece extending from the 5' side of the acceptor stem to the beginning of the dihydrouridine helix, and a segment running from the 3' side of the extra loop into the TψC helix. These two regions overlap with and include some of the homologous bases found in eight tRNAs aminoacylated by yeast phenylalanyl-tRNA synthetase (Rob, B., Sirover, M., and Dudock, B. (1973) Biochemistry 12, 4146-4153). Although well separated in the primary and secondary structure, these two segments are in close proximity in the crystallographic tertiary structure. In two of the complexes, the third cross-linked fragment is near to the two common ones. The picture which emerges is that the enzymes all interact with the general area in which the two helical branches of the L-shaped tertiary structure fuse together, with additional interactions on other parts of the tRNA as well.

Much work has been carried out on the yeast phenylalanine tRNA system. This includes not only the high resolution x-ray crystallographic studies of tRNA^Phe (1-3), but strong attempts to pin down the phenylalanyl-tRNA synthetase recognition site (4-7). Because of the large reservoir of structural and related data, it is an ideal system with which to extend investigations of anatomical features of synthetase-tRNA complexes.

In the present investigation, yeast tRNA^Phe has been photochemically joined to three aminoacyl-tRNA synthetases, yeast phenylalanyl-tRNA synthetase, Escherichia coli isoleucyl-tRNA synthetase, and E. coli valyl-tRNA synthetase. It is known from fluorescence titration studies that E. coli isoleucyl-tRNA synthetase forms a fairly strong complex with tRNA^Phe (8), and that attempts to aminoacylate tRNA^Phe with E. coli isoleucyl-tRNA synthetase have failed under a variety of conditions.† On the other hand, E. coli valyl-tRNA synthetase is known to aminoacylate tRNA^Phe with valine (9, 10). Thus, both non-cognate enzymes show evidence for interaction with tRNA^Phe, and make good candidates with which to explore structure-function aspects of non-cognate complexes and their relationship to the cognate one. In addition, the analysis of the structures of the three complexes gives considerably more input into the attempt to deduce a general picture, if any, for the way in which a synthetase orients on a tRNA.

EXPERIMENTAL PROCEDURES

Phenylalanyl-tRNA synthetase was purified from baker's yeast by the method of Fasiolo et al. (11). Valyl-tRNA synthetase and isoleucyl-tRNA synthetase were isolated from Escherichia coli by the procedures of Yaniv and Gros (12) and Eldred and Schimmel (13), respectively. Enzyme activities were generally at least 70% of that expected for the fully purified species. In one instance, cross-linking was carried out with two samples of an enzyme which were about 50 and 75% pure, respectively. The cross-linking results obtained with both preparations were the same, suggesting that effects of the impurities are inconsequential. Yeast tRNA^Phe was obtained from Boehringer and had an acceptor activity of about 1000 to 1500 (pmol)/(A260 unit in 0.1 N NaOH) as determined by us. T1 fingerprints revealed neither defects in the structure nor the presence of significant contaminants. Other details and methods are given in previous articles (14, 15).

RESULTS

The three enzymes, yeast phenylalanyl-tRNA synthetase, Escherichia coli isoleucyl-tRNA synthetase, and E. coli valyl-

the respective fragment number, for all of the three complexes. With all three enzymes, good and similar yields were obtained (generally 15 min or longer). Reactions were done in 10 mM MgCl₂ and 50 mM cacodylate at pH 5.2 or pH 5.5.

The regions on tRNA<sup>Phe</sup> cross-linked to each of the respective enzymes were found to couple to yeast tRNA<sup>Phe</sup> under the action of ultraviolet light, using methods described elsewhere (14, 15). The results obtained are summarized in Table I which gives values for the per cent joining for the three different complexes. With all three enzymes, good and similar yields were obtained.

The regions on tRNA<sup>Phe</sup> cross-linked to each of the respective enzymes were worked out by the previously described techniques (14, 15). The results are summarized in Fig. 1 which compares in bar graph form the per cent joining of each Tl fragment versus the respective fragment number, for all of the three complexes.

The error in each bar is generally less than ±10% joining. It is clear that in all cases only three regions are found to cross-link, with the percentage coupled varying from less than 30% to almost 90%. Moreover, in all three complexes two of the linked fragments are identical, TlA and Tl2 (see below), while the third is unique to each complex.

Fig. 2, a to c, give the sequence, cloverleaf structure, and numbered Tl fragments of yeast tRNA<sup>Phe</sup>. The sequence is based on that determined by RajBhandary and Chang (10). Shaded regions on each of these figures give the areas cross-linked to phenylalanyl-tRNA synthetase (Fig. 2a), isoleucyl-tRNA synthetase (Fig. 2b), and valyl-tRNA synthetase (Fig. 2c). The cross-linked regions common to all of the complexes are seen to involve a piece extending from the 3' half of the 5' side of the acceptor stem to the beginning of the dihydrouridine helix, and a segment running from the 3' side of the extra loop into the ΨC helix. The former (Fragment 1A) is particularly interesting since its 3' side overlaps with the region proposed by Dudock and co-workers (5, 7) as the yeast phenylalanyl-tRNA synthetase recognition site. This proposal was based on the finding that the sequence A-G-C-U-C (and the base-paired complement G-A-C-C) in the dihydrouridine area is uniquely common to eight different tRNAs that can be aminoacylated by phenylalanyl-tRNA synthetase, which include tRNA<sup>Leu<sub>E.coli</sub></sup> and tRNA<sup>Val<sub>E.coli</sub></sup> (7). Moreover, the same authors noted that m<sup>7</sup>G and C, the first and third bases of the other commonly linked fragment (No. 12), are also homologous bases in all of the eight tRNAs aminoacylated by phenylalanyl-tRNA synthetase (7). Thus, parts of those regions implicated as possibly significant for phenylalanyl-tRNA synthetase recognition are found to cross-link to this enzyme. In addition, these same areas are found to cross-link to two E. coli synthetases, isoleucyl-tRNA synthetase and valyl-tRNA synthetase, which bind to yeast tRNA<sup>Phe</sup>. This suggests a significant degree of similarity in the mode of binding of these enzymes to that of the cognate one.

The areas which are unique to each complex include the 3' side of the acceptor stem (valyl-tRNA synthetase), the 3' side of the ΨC region (phenylalanyl-tRNA synthetase), and the anticodon region (isoleucyl-tRNA synthetase). The possible significance of these segments is more apparent when their location on the three dimensional structure is viewed (see "Discussion").

### Table I

Cross linking of synthetases to yeast tRNA<sup>Phe</sup>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Per cent joining</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast phenylalanyl-tRNA synthetase</td>
<td>40&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Escherichia coli isoleucyl-tRNA synthetase</td>
<td>32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>E. coli valyl-tRNA synthetase</td>
<td>40&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> 2 μM phenylalanyl-tRNA synthetase and 1 μM tRNA<sup>Phe</sup>, pH 5.2.

<sup>b</sup> 3 μM isoleucyl-tRNA synthetase and 1.5 μM tRNA<sup>Phe</sup>, pH 5.5.

<sup>c</sup> 4 μM valyl-tRNA synthetase and 1 μM tRNA<sup>Phe</sup>, pH 5.2.

In Fig. 2, a to c, give the sequence, cloverleaf structure, and numbered Tl fragments of yeast tRNA<sup>Phe</sup>. The sequence is based on that determined by RajBhandary and Chang (10). Shaded regions on each of these figures give the areas cross-linked to phenylalanyl-tRNA synthetase (Fig. 2a), isoleucyl- tRNA synthetase (Fig. 2b), and valyl-tRNA synthetase (Fig. 2c). The cross-linked regions common to all of the complexes are seen to involve a piece extending from the 3' half of the 5' side of the acceptor stem to the beginning of the dihydrouridine helix, and a segment running from the 3' side of the extra loop into the ΨC helix. The former (Fragment 1A) is particularly interesting since its 3' side overlaps with the region proposed by Dudock and co-workers (5, 7) as the yeast phenylalanyl-tRNA synthetase recognition site. This proposal was based on the finding that the sequence A-G-C-U-C (and the base-paired complement G-A-C-C) in the dihydrouridine area is uniquely common to eight different tRNAs that can be aminoacylated by phenylalanyl-tRNA synthetase, which include tRNA<sup>Leu<sub>E.coli</sub></sup> and tRNA<sup>Val<sub>E.coli</sub></sup> (7). Moreover, the same authors noted that m<sup>7</sup>G and C, the first and third bases of the other commonly linked fragment (No. 12), are also homologous bases in all of the eight tRNAs aminoacylated by phenylalanyl-tRNA synthetase (7). Thus, parts of those regions implicated as possibly significant for phenylalanyl-tRNA synthetase recognition are found to cross-link to this enzyme. In addition, these same areas are found to cross-link to two E. coli synthetases, isoleucyl-tRNA synthetase and valyl-tRNA synthetase, which bind to yeast tRNA<sup>Phe</sup>. This suggests a significant degree of similarity in the mode of binding of these enzymes to that of the cognate one.

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Effect of pH on Cross-Linked Regions—As mentioned previously (14, 15), cross-linking was generally accomplished in the range of pH 5.2 to 5.5 where synthetase-tRNA interactions appear to be strongest. The objection can be raised, however, that such complexes may differ significantly from those at pH 7. Therefore, experiments were carried out to check on the effect of pH on the regions found to be cross-linked.

Reasonable joining of phenylalanyl-tRNA synthetase to tRNA<sup>Phe</sup> was accomplished at pH 7, although the yield of cross-linked complex, at a given enzyme and tRNA concentration, was less than that found at pH 5.2, as expected. The results of the analysis for linked regions are summarized in bar graph form in Fig. 3. The dashed bars give results at pH 5.2 while the solid ones apply to pH 7. It is clear that at both pH values exactly the same regions are found to link to the enzyme, although there may be small variations in the relative frequencies of cross-linking of the joined segments. These results thus indicate that the complexes are very similar in structure over the range pH 5.2 to 7. Moreover, it appears that the photochemistry per se does not give values for the per cent joining for the three different complexes.
A schematic model (1) of the yeast tRNA^Phe^ structure is shown in Fig. 4, a to c. Regions found to cross-link to phenylalanyl-tRNA synthetase (PheRS) indicated by shading, b, sequence and cloverleaf structure of yeast tRNA^Phe^ (16) with regions cross-linked to E. coli isoleucyl-tRNA synthetase (IleRS) indicated by shading, c, sequence and cloverleaf structure of yeast tRNA^Phe^ (16) with regions cross-linked to E. coli valyl-tRNA synthetase (ValRS) indicated by shading.

Fig. 2. a, sequence and cloverleaf structure of yeast tRNA^Phe^ (16) with regions cross-linked to yeast phenylalanyl-tRNA synthetase (PheRS) indicated by shading. b, sequence and cloverleaf structure of yeast tRNA^Phe^ (16) with regions cross-linked to E. coli isoleucyl-tRNA synthetase (IleRS) indicated by shading. c, sequence and cloverleaf structure of yeast tRNA^Phe^ (16) with regions cross-linked to E. coli valyl-tRNA synthetase (ValRS) indicated by shading.

The sketches in Fig. 4, a to c, are approximate low resolution representations of the chain backbone. At this resolution, they are generally consistent with results of two independent structure determinations (1-3).

Control experiments showed that intramolecular cross-links do not form within the tRNA to any significant degree, upon irradiation of the free tRNA. Thus, for example, Fragments 10A and 12 are not joined together by the irradiation.
FIG. 3. Per cent joining versus fragment number for the cross-
linked complex of phenylalanyl-tRNA synthetase and tRNA\textsubscript{Phe}. The dark bars represent results at pH 7.0, while the dashed bars give results for pH 5.2.

FIG. 4. a, three-dimensional model of tRNA\textsubscript{Phe} (1) with regions cross-linked to phenylalanyl-tRNA synthetase (PheRS) indicated by shading. b, three-dimensional model of tRNA\textsubscript{Phe} (1) with regions cross-linked to isoleucyl-tRNA synthetase (IleRS) indicated by shading. c, three-dimensional model of tRNA\textsubscript{Phe} (1) with regions cross-linked to valyl-tRNA synthetase (ValRS) indicated by shading.
the orientation on a tRNA of cognate and non-cognate enzymes is likely to be very similar, a conclusion also reached in the study with *E. coli* tRNA\textsuperscript{Phe}.

In the case of the phenylalanyl-tRNA synthetase and valyl-tRNA synthetase complexes, the third cross-linked fragment is close in the tertiary structure to the location of the two common ones. Isoeucyl-tRNA synthetase, on the other hand, joins to a piece of the anticodon region. However, this area may also be in close contact with the other synthetases, even though they do not cross-link to it. For example, Hörz and Zachau (6) have shown that nuclease attack at the anticodon of tRNA\textsuperscript{Phe} is greatly suppressed by phenylalanyl-tRNA synthetase.

It is of interest to compare the complexes investigated here with those of tRNA\textsuperscript{1Le} (15). However, caution must be exercised in making a close comparison of the tRNA\textsuperscript{Phe} and tRNA\textsuperscript{1Le} complexes. This is due to the fact that 11 ribonuclease cuts these two tRNAs in different ways, so that the locations of the Tl fragments in the structure are inherently different in each case and, therefore, cross-linked regions cannot be compared directly. In spite of this limitation, it is clear that the complexes of yeast tRNA\textsuperscript{Phe} bear similarities to the ones involving *E. coli* tRNA\textsuperscript{Phe} (15), particularly with regard to the fact that all of them show interactions with the general area in which the two helical branches of the L structure come together. Therefore, this may be one of the most crucial and common parts of tRNA involved in interactions with synthetases.

With any given complex, one might ask if the regions on the tRNA found cross-linked represent sites which have an unusually high intrinsic photoreactivity, due to local conformational effects or other factors (e.g. modified bases) present in that part of the tRNA. If this is true, the joining of a particular region would mean that although it must be in close contact with the protein, it is given undue emphasis because of its intrinsically enhanced photoreactivity. However, five different Tl fragments are collectively cross-linked to the three enzymes. This suggests that virtually any part of the tRNA structure is capable of undergoing ultraviolet cross-linking provided, of course, it is in close contact with the bound protein. Moreover, fragments which contain no modified bases are found to cross-link. The results are probably not biased, therefore, by cross-linking to special photoreactive "hot spots" on the tRNA.

The fact that two fragments are common to all three complexes suggest that each enzyme has photoreactive amino acids in closely similar positions. This is exactly analogous to the situation found with the two complexes of tRNA\textsuperscript{1Le} discussed in the previous article (15). Once again it leads to the speculation that the tRNA binding sites of the synthetases may be lined with many common amino acids.

It is interesting to note that ultraviolet cross-linking to tRNA has been easy to achieve while joining the lac repressor to DNA, for example, occurs with a negligible yield (17). Good linking in the latter case has only been achieved by substituting the more photocative 5 BrdU for the DT residues in the DNA (17). One might surmise that the occurrence of many single-stranded sections in tRNA accounts for its greater reactivity, since these sections are known to be more photo-active in certain reactions than double-stranded sections (18). It would then follow that the actual base involved in cross-linking within each Tl fragment of a tRNA most probably occurs in a single-stranded section of that fragment. Some doubt is cast on this interpretation, however, by the observation that Fragment 10B cross-links to valyl-tRNA synthetase (see Fig. 3c). This fragment appears to have each residue in the continuous helix formed by the acceptor and T\textsuperscript{Y}C arms. Therefore, the reason for the facile cross-linking to tRNA as opposed to DNA remains unclear.

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