Species Specificity of Amino Acid Acceptor Ribonucleic Acid and Aminoacyl Soluble Ribonucleic Acid Synthetases

B. P. Doctor and John A. Mudd

From the Division of Biochemistry, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington 12, D. C.

(Received for publication, May 20, 1963)

Several investigators have reported that the activation of amino acids for protein synthesis, i.e. the incorporation of amino acids into soluble ribonucleic acids, is dependent upon the source of both the aminoacyl S-RNA synthetases and the S-RNA itself. For example, Rendi and Oehoe (1) reported that aminoacyl S-RNA synthetases from Escherichia coli could not incorporate leucine into yeast or rat liver S-RNA, and vice versa. Benzer and Weinblum (2) compared the specificities of S-RNA and aminoacyl S-RNA synthetases in E. coli, yeast, and rabbit liver. They found striking differences; however, the pattern depended upon the particular amino acid in question. Clark and Eyzaguirre (3) and Berg et al. (4) demonstrated species specificities in the cases of tyrosine and methionine incorporation, respectively.

The species differences observed in these investigations suggest that amino acid incorporation is dependent not only upon the aminoacyl S-RNA synthetases and S-RNA, but also upon the amino acid in question. Although the species differences observed in many instances are quite striking, it is possible that certain homologies may exist (2). The purpose of this investigation is to show the existence of such homologies and further investigate the species differences that exist in cases heretofore uninvestigated. The observations presented here further show that the amino acid acceptor activity of at least one RNA is altered by the presence of a small amount of spermine. The principles of laboratory animal care as promulgated by the National Society for Medical Research were observed.

EXPERIMENTAL PROCEDURE

Preparation of Amino Acid Acceptor RNAs—Yeast and rat liver amino acid-acceptor RNA’s were prepared according to the procedures of Holley et al. (5, 6), and E. coli S-RNA was prepared according to the procedures of Zubay (7). It was assumed that a 1 mg per ml solution of all three RNAs in water had an absorbance at 260 mμ of 24.0. The assay for the presence of “nuclease” contaminants according to the procedures of Holley, Apgar, and Merrill (8) showed that the RNA preparations used in these investigations were devoid of any “nuclease” activity.

Preparation of Aminoacyl S-RNA Synthetases—The “pH 5.0 fraction” enzyme preparation from rat liver homogenates was prepared according to the procedures of Holley and Goldstein (5). The aminoacyl S-RNA synthetase from E. coli was prepared according to Matthaei and Nirenberg (9). The 105,000 X g supernatant fluid (10 ml) was put through a 500-g DEAE-cellulose (Selectachi-DEAE, Brown Company, Corvallis, Oregon) column which was previously equilibrated in the cold with 0.1 M Tris-HCl buffer, pH 7.5. The eluate and the wash (same buffer) were dialyzed against the same buffer for 1 to 2 hours. The enzyme preparation thus obtained was used as E. coli aminoacyl S-RNA synthetases.

The yeast aminoacyl S-RNA synthetase was prepared as follows: 20 g of fresh Fleischmann’s bakers’ yeast were combined with an equal amount of alumina (Alcoa A-305) and ground for 10 to 15 minutes in the cold until a soft paste was obtained. The paste was suspended in a buffer described by Matthaei and Nirenberg (9) and stirred for 1 hour. The preparation was then centrifuged for 10 minutes at 10,000 r.p.m. in a Servall centrifuge. The supernatant fraction was recentrifuged at 30,000 r.p.m. for 1 hour in a Spinco model L preparative ultracentrifuge (No. 30 rotor). The supernatant fluid was passed through a 2-g DEAE-cellulose column previously equilibrated with 0.05 M phosphate buffer, pH 6.8. The eluate plus wash was dialyzed as described for E. coli and used as yeast aminoacyl S-RNA synthetases.

Procedure for Testing Amino Acid Incorporation Activity of Various S-RNAs—The procedure of Holley et al. (6) was used with the following changes. The incubation mixture consisted of 100 μmoles of Tris-HCl, pH 7.5; 10 μmoles of potassium EDTA; 10 μmoles of MgCl₂; 10 μmoles of KCl; 2.5 μmoles of sodium ATP, pH 6.8; 0.05 μ of ¹⁴C amino acid; 25 to 50 μg of S-RNA; and enzyme plus water to make a 1-ml volume. Incubation time was 20 minutes at 37°.

The amount of amino acid incorporated by each S-RNA was measured by assaying each of the three RNAs with various amounts of each enzyme. The data presented in Fig. 1 were calculated from such experiments in which maximal incorporation of each amino acid was observed. The experiments in which the effect of spermine on amino acid incorporation was measured were performed as follows. First, the amount of enzyme required for the maximal amino acid incorporation into a certain amount of S-RNA was measured. Then, with this enzyme and S-RNA concentration, the effect of various concentrations of spermine on amino acid incorporation was determined. Finally, in the presence of the enzyme and spermine levels thus found, the extent of amino acid incorporation into various concentrations of S-RNA was measured. The highest level of S-RNA used in such experiments was approximately the same as that used in the previous experiments in which the levels of enzyme and spermine concentrations were determined.
Species Specificity of S-RNA and Activating Enzymes

Vol. 238, No. 11

RAT LIVER S-RNA
YEAST S-RNA
E. COLI S-RNA

FIG. 1. Amino acid incorporation into rat liver, yeast, and E. coli S-RNAs when assayed with rat liver, yeast, and E. coli aminoacyl S-RNA synthetases. Each set of three bars from left to right shows the millimicromoles of amino acid (listed below the base-line) incorporated into milligrams of S-RNAs (see key) when assayed with rat liver, yeast, and E. coli enzymes, respectively. These figures were calculated as described under "Experimental Procedure.”

amounts of amino acid incorporated were found to be directly proportional to the amounts of S-RNA added.

RESULTS

The amino acid acceptor activities of S-RNA isolated from the rat liver, yeast, and E. coli when assayed with aminoacyl S-RNA synthetases isolated from rat liver, yeast, and E. coli are shown in Fig. 1. The values shown are calculated as millimicromoles of amino acid incorporated per mg of S-RNA. These results are calculated from such experiments as described under "Experimental Procedure.” At least two different enzyme preparations from each species were tested. Fig. 2 shows the effect of spermine on phenylalanine incorporation into E. coli (A), yeast (B), and rat liver (C) S-RNA when assayed with E. coli (1), rat liver (2), and yeast (3) aminoacyl S-RNA synthetases, respectively. Table I summarizes the results from Fig. 2 in terms of millimicromoles of phenylalanine incorporated per mg of RNA.

Alanine—The results presented here show that a complete homology appears to exist between E. coli, yeast, and rat liver S-RNA and the respective enzymes. It should be pointed out, however, that the countercurrent distribution patterns of yeast and rat liver alanine RNA have been shown to be different (10).

Arginine—Rat liver enzyme incorporates arginine into homologous S-RNA, to a greater extent into E. coli S-RNA, and to a very small degree into yeast S-RNA. Yeast enzyme, on the other hand, incorporates arginine into yeast S-RNA, to a lesser extent into rat liver S-RNA, and to an even lesser extent into E. coli S-RNA. In the case of E. coli enzyme, arginine incorporation into E. coli S-RNA is the highest, very little is incorporated into rat liver S-RNA, and almost none into yeast S-RNA.

Aspartic Acid—Rat liver enzyme incorporates aspartic acid mainly into rat liver S-RNA, with almost no incorporation into yeast and very little into E. coli S-RNA. In the case of yeast enzyme, a complete homology appears to exist, in that this enzyme appears to be able to incorporate amino acid into all three RNAs. On the contrary, E. coli enzyme completely discriminates between E. coli S-RNA on one hand and rat liver and yeast S-RNA on the other.

Histidine—In the cases of rat liver enzyme, the extent of histidine incorporation into rat liver and yeast S-RNA is quite similar; whereas a little incorporation is observed into E. coli S-RNA. The incorporation of histidine into yeast S-RNA by yeast enzyme is higher than into rat or E. coli S-RNA, in which cases the extent of incorporation is about the same. However, E. coli enzyme is completely species-specific and does not incorporate at all into yeast or rat RNA.

Isoleucine—Rat liver enzyme appears to be able to incorporate isoleucine into rat S-RNA; however, the extent of isoleucine incorporation into yeast and E. coli S-RNA is much higher than in rat liver S-RNA. Yeast enzyme incorporates this amino acid readily into yeast and E. coli S-RNA and to a very small extent into rat liver S-RNA. Here again, however, E. coli enzyme discriminates between E. coli S-RNA on the one hand and yeast and rat liver S-RNA on the other.

Leucine—Rat liver enzyme incorporates leucine into rat liver and yeast S-RNA readily, and to a lesser extent into E. coli S-RNA. Yeast enzyme appears to incorporate leucine into all three RNAs to about the same degree, whereas E. coli enzyme appears to show an absolute specificity toward E. coli S-RNA.

Lysine—The yeast and rat liver enzymes appear to be active toward all three S-RNAs, whereas E. coli enzyme incorporates lysine into E. coli S-RNA and to a lesser extent into yeast and rat liver S-RNA.
Fig. 2. Effect of spermine on phenylalanine incorporation into S-RNAs from various species. Graphs 1A, 1B, and 1C represent the effect of 2.5 μmoles of spermine on phenylalanine incorporation into E. coli, yeast, and rat liver S-RNAs, respectively, when assayed with aminoacyl S-RNA synthetases from E. coli. Graphs 2A, 2B, and 2C represent results of similar experiments performed with rat liver enzymes, and Graphs 3A, 3B, and 3C represent the results of the experiments with yeast enzymes. The amounts of spermine added in 2A, 2B, and 2C were 1 μmole, and in 3A, 3B, and 3C, 0.2 μmole, respectively. The details of the experiments are described under “Experimental Procedure.” X—X, Phenylalanine incorporation in the presence of spermine; O—O, phenylalanine incorporation in the absence of spermine.

Methionine—Rat liver enzyme incorporates methionine into all three S-RNAs. Yeast enzyme incorporates this amino acid into yeast and E. coli S-RNA and about half as much into rat liver S-RNA. E. coli enzyme incorporates methionine into E. coli S-RNA, but to a lesser extent into yeast and rat liver S-RNA.

Phenylalanine—Rat liver and yeast enzymes incorporate phenylalanine into rat liver and yeast S-RNAs, but very little incorporation into E. coli S-RNA is observed. However, an absolute specificity toward E. coli S-RNA is shown by E. coli enzyme. This absolute specificity is altered in the presence of a small amount of spermine.

Serine—Rat liver and yeast enzymes incorporate serine into rat liver and yeast S-RNA and about half this amount into E. coli S-RNA. E. coli enzyme incorporates this amino acid into E. coli S-RNA and to a lesser extent into yeast and rat liver S-RNA.

Threonine—Rat liver enzyme incorporates threonine into rat liver and yeast S-RNA and to a lesser extent into E. coli S-RNA. Yeast enzyme incorporates threonine into all three S-RNAs; however, the extent of incorporation into rat liver and E. coli S-RNA is a little less than into yeast S-RNA. E. coli enzyme incorporates this amino acid into E. coli S-RNA, about half this amount into yeast S-RNA, and a smaller amount into rat liver S-RNA.

Tryptophan—Rat liver enzyme incorporates tryptophan into rat liver and yeast S-RNA and to a lesser extent into E. coli S-RNA. Yeast enzyme incorporates this amino acid into yeast S-RNA, about half as much into rat liver S-RNA, and less into E. coli S-RNA. E. coli enzyme readily incorporates tryptophan into all three S-RNAs.

Tyrosine—Incorporation of tyrosine into the three S-RNAs with the three enzymes in question appears to be unique in that the rat liver and yeast enzymes are homologous to the respective S-RNAs. However, with E. coli S-RNA, little or no incorpora-
Table I

Effect of spermine on phenylalanine incorporation into soluble ribonucleic acids

The values in this table are calculated from the experiments described in Fig. 2.

<table>
<thead>
<tr>
<th>Source of enzyme</th>
<th>Spermine</th>
<th>Phenylalanine incorporated into S-RNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μmoles</td>
<td>E. coli</td>
</tr>
<tr>
<td>E. coli</td>
<td>2.6</td>
<td>0.63</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.63</td>
</tr>
<tr>
<td>Rat liver</td>
<td>1.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.01</td>
</tr>
<tr>
<td>Yeast</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Discussion

As noted by Benzer and Weisblum (2), the results presented here show that the incorporation of the amino acid in question into S-RNA depends not only on the source of enzyme and S-RNA but also on the particular amino acid. Although the species differences, wherever they appear to exist, are striking, a great number of homologies also are found. There are only a few cases in which an absolute specificity appears to be present, e.g., E. coli enzyme in the cases of aspartic acid, histidine, etc. Even in these cases, caution should be taken in the interpretation of the results, since it is quite possible that either the enzyme or S-RNA in question may have been damaged during the isolation procedures; this can be substantiated from the fact that Rendi and Ochoa (1) observed that rat liver or yeast enzymes failed to incorporate leucine into E. coli S-RNA. The results in Fig. 1 show that yeast enzyme incorporates leucine into E. coli S-RNA quite readily. Keller and Anthony (11) recently have shown similar incorporation of leucine into E. coli S-RNA by rat liver enzyme preparations.

The yeast aminoacyl RNA synthetases prepared by the methods described under "Experimental Procedure" were the least active of the three. The enzymes from the rat liver homogenates were more active whereas E. coli enzymes were the most active. The differences in various enzyme activities may be due to the presence of different amounts of unlabeled amino acids in the enzyme preparations. This may in part account for the lower amounts of amino acid incorporation with these enzyme preparations.

In many cases the amino acid incorporation into the S-RNA from the heterologous species is higher than into the corresponding S-RNA from the homologous species when assayed with homologous enzymes. From Fig. 1, it can be noted that out of 14 amino acids tested this appears to happen in only one case with E. coli enzymes (tryptophan incorporation into rat liver S-RNA), in no case with yeast enzymes, and in several cases with rat liver enzymes (alanine, arginine, isoleucine, and methionine incorporation into E. coli S-RNA; histidine, isoleucine, methionine, phenylalanine, tyrosine, and valine incorporation into yeast S-RNA). It is interesting to note that the frequency of such occurrence is so high with rat liver enzymes.

Several possible explanations may be advanced to explain these observations: (a) either the S-RNA or enzymes may have been damaged during the isolation; (b) the amount of a particular S-RNA may be different in different species; (c) there may be incorporation of a particular amino acid into a different heterologous amino acid acceptor RNA; (d) the heterologous aminoacyl RNA synthetases may recognize more than one component of a particular amino acid-specific RNA whereas this may not be possible with the S-RNA from the homologous species; or (e) the radioactive amino acid tested may have been contaminated by a different amino acid.

Isoleucine incorporation into rat liver, yeast, and E. coli S-RNA with rat liver enzyme was further investigated in order to explore the possibilities mentioned above. 14C-Isoleucine does not appear to have any contaminating materials when tested chromatographically. When isoleucine incorporation into the three S-RNAs was tested with rat liver enzyme in the presence of a mixture of the 19 other unlabeled amino acids, approximately 10 to 15% less incorporation was observed in all three cases. This rules out the possibility of isoleucine incorporation into any other S-RNA and leaves three other possibilities: (a) the amount of isoleucine S-RNA in different species may be different; (b) rat liver enzyme may recognize one or more components of isoleucine S-RNA in the heterologous system; or (c) rat liver isoleucine S-RNA may have been degraded during the isolation. These possibilities along with such possibilities in cases of other amino acid acceptor RNAs are under investigation.

The effect of small amounts of spermine on phenylalanine incorporation into yeast and rat liver S-RNA's with E. coli enzyme is quite striking. From the results in Table I, one can notice an almost 5- to 8-fold increase in the amount of phenylalanine incorporation into yeast or rat liver S-RNA. In similar cases with rat liver or yeast enzymes, spermine appears to have little effect on phenylalanine incorporation. Spermine is known to affect the thermal transition of DNA (12) and S-RNA (13). It is thus quite possible that the effect of spermine observed here may be due to its influence on the secondary structure of S-RNA. If this is the case, one is tempted to believe that species differences contained in phenylalanine S-RNA may reside in the secondary structure. This cannot be the general case, since no such effect of spermine is observed in parallel situations. It may be that the species differences contained in this S-RNA are in part due to the differences in secondary structure. A similar effect of spermine was observed, but to a lesser extent, in the cases of arginine incorporation into rat liver and E. coli S-RNAs with rat liver enzyme and of methionine incorporation into yeast and E. coli S-RNAs with E. coli enzyme.

In general, among the 14 amino acids tested under these conditions, it appears that E. coli enzymes are the most species-specific, the yeast enzymes the least, and rat liver enzymes in between. The data presented here show the extent of species specificity and the extent of incorporation of 14 amino acids into the S-RNAs by the respective enzymes isolated from three different sources. From the results presented here, it would be difficult to predict the number of S-RNAs which incorporate
individual amino acids in homologous or heterologous systems. One can obtain this information from experiments (which are in progress) in which the S-RNAs are separated and the amino acid incorporation into these separated S-RNAs is tested with the aminoacyl S-RNAs synthetases from various species.

Finally, the species specificity studies in this investigation shed light on that part of the RNA code which involves the recognition of amino acids and activating enzymes only, and do not furnish any information regarding the S-RNA code which is responsible for the transfer of amino acid to ribosomes and to protein. The existence of species specificity of these reactions has also been reported (1, 14).

**SUMMARY**

The specificity of incorporation of 14 amino acids into rat liver, yeast, and *Escherichia coli* soluble ribonucleic acids (S-RNAs) with the aminoacyl S-RNA synthetases from these three species have been studied. Various degrees of specificity appear to exist, but also many homologies. Caution should be taken in the interpretation of results on species specificity, since many variables in experimental conditions and reaction components appear to alter the extent of amino acid incorporation into various S-RNAs. The effect of spermine on phenylalanine incorporation into various S-RNAs is described.

**Acknowledgments**—The authors are indebted to Miss Carol M. Connelly of this laboratory for help and assistance, and to Dr. Marshall W. Nirenberg of the National Institutes of Health for help, suggestions, and discussions in carrying out this project and preparing this manuscript.

**REFERENCES**

Species Specificity of Amino Acid Acceptor Ribonucleic Acid and Aminoacyl Soluble Ribonucleic Acid Synthetases

B. P. Doctor and John A. Mudd