Antibodies to Yeast Phenylalanine Transfer Ribonucleic Acid Are Specific for the Odd Nucleoside Y in the Anticodon Loop

(Y base/tRNA fractionation)

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ABSTRACT Antibodies with specificity to a single species of tRNA were elicited in a goat by immunization with a glutaraldehyde conjugate of yeast phenylalanine transfer RNA with bovine gamma globulin. The specificity of the antibodies was studied by a radioimmunoassay measuring the direct binding of [3H]tRNA\(^{\text{Ph}}\) or the inhibition of the binding. The antibodies formed are predominantly directed towards the characteristic highly modified nucleoside Y, which is located right next to the anticodon. The antibodies bind specifically to tRNA\(^{\text{Ph}}\), to oligonucleotides derived by enzymatic digestion from the anticodon loop of tRNA\(^{\text{Ph}}\), and to the Y nucleoside itself. tRNA species which do not contain Y in their sequences, or tRNA\(^{\text{Ph}}\) from which the Y base has been excised, do not bind to the antibodies. Yeast tRNA\(^{\text{Ph}}\) can be separated from other tRNA species with an immuno-adsorbent of antibodies to tRNA\(^{\text{Ph}}\).

Antibodies capable of reacting immunospecifically with single-stranded nucleic acids and with double and triple-stranded synthetic polynucleotides have been prepared experimentally. Such antibodies were elicited upon immunizing either with electrostatic complexes of the nucleic acid with methylated bovine serum albumin (MBSA) or with conjugates in which nucleosides, mono-, di-, and trinucleotides have been bound covalently to proteins or to synthetic polypeptides (1–3). No experimental antibodies have yet been reported against double-stranded DNA, even though they may be produced spontaneously in patients with systemic lupus erythematosus and with other autoimmune diseases (3).

Within the family of nucleic acids tRNA seems to be a good candidate for further immunological studies. Specific tRNAs may now be isolated in substantial amounts (4, 5). They are well-defined molecules of molecular weight 25,000, containing single-stranded as well as double-stranded regions (4–6). These basic structural elements of all nucleic acids are folded into a well-defined three-dimensional structure in any tRNA (7, 8). In addition, tRNA contains rare bases (9), some of which are buried inside the structure; others are outside and may thus be immunopotent determinants.

We have previously reported on the production of antibodies to tRNA such antibodies were obtained by immunization with covalent tRNA–protein conjugates in which Escherichia coli tRNA was linked to a protein carrier either via a water-soluble carbodiimide (10) or via glutaraldehyde (11). By a different approach it was demonstrated that antibodies prepared against rare nucleosides present in tRNA may react with the specific tRNA that contains these nucleosides. Thus, antibodies against inosine (12) and isopentenyladenosine (13), prepared by immunization with conjugates of these nucleosides with carrier proteins, react specifically with E. coli tRNA\(^{\text{ATG}}\) (14) and with yeast tRNA\(^{\text{TYT}}\) (13), respectively. We are now reporting on the production of antibodies against a specific tRNA, namely, yeast phenylalanine transfer RNA (tRNA\(^{\text{Ph}}\)) by immunization with the intact molecule of the tRNA, and conclude that the specificity of the antibodies formed is predominantly directed towards the characteristic Y nucleoside, which is located right next to the anticodon.

MATERIALS AND METHODS

Yeast tRNA\(^{\text{Ph}}\) was purified as described elsewhere (5). Yeast [3H]tRNA\(^{\text{Ph}}\) was prepared by incorporating [3H]ATP into a tRNA\(^{\text{Ph}}\) lacking its 3’ end (15). Yeast tRNA was purchased from Boehringer Mannheim GmbH (Mannheim, Germany), and nucleosides from Sigma (St. Louis, Mo), Glutaraldehyde (practical) was obtained from Fluka (Switzerland) as a 25% solution in water, bovine gamma globulin (BGG) from Schwarz/Mann (Orangeburg, N.Y.) and methylated bovine serum albumin from Nutritional Biochemical Corp. (Cleveland, Ohio).

Preparation of tRNA\(^{\text{Ph}}\)-bovine gammaglobulin conjugate was achieved by reacting 30 mg of yeast tRNA\(^{\text{Ph}}\) and 3 mg of BGG in 1 ml of 0.2 M sodium acetate buffer, pH 5.5, with 0.2 ml of glutaraldehyde solution (2.5%), by a procedure similar to that described elsewhere (11).

Immunoglobulin-Sepharose immunoadsorbsents were prepared by reacting 250 mg of the immunoglobulin fraction with 10 g of CNBr-activated Sepharose (14, 16) in 0.1 M NaHCO\(_3\).

Immunization Procedure. A goat was injected intradermally at multiple sites with tRNA\(^{\text{Ph}}\)-BGG conjugate. The antigen (0.3 mg) was complexed with equal weight of methylated bovine serum albumin and given as an emulsion with complete Freund’s adjuvant. Three immunizations, 3 weeks apart, were given.

Immunoglobulin fractions were obtained from the sera by ammonium sulfate precipitations at 40% saturation.

Antigen Binding Assay. Antigen binding assay was performed by coprecipitation of labeled tRNA with immuno-

Abbreviations: BGG, bovine gamma globulin; Y, a highly modified nucleoside characteristic of yeast tRNA\(^{\text{Ph}}\).
globulins by 50% saturated ammonium sulfate (11). Reaction mixtures of 0.2 ml containing the immunoglobulins in phosphate-buffered saline (PBS; 0.14 M NaCl, 0.01 M sodium phosphate buffer, pH 7.2), and 0.025 ml of labeled tRNA (0.04 μg of yeast [1H]tRNA^Phe with 10,000 cpm) were incubated for 1 hr at 4°. Saturated ammonium sulfate was added to final 50% saturation, the precipitates were collected on glass-fiber discs (GF/c, Whatman) and washed with 50% saturated ammonium sulfate before measurement of radioactivity in toluene scintillation solution.

For inhibition of antigen binding, the mixture of the immunoglobulins and the inhibitor was preincubated 15 min at 4°. Labeled antigen was added and the assay was followed as for the direct binding.

RESULTS

The antiserum from the goat immunized with tRNA-BGG conjugate contains antibodies specific to yeast tRNA^Phe. Both the antiserum and the immunoglobulin fraction prepared from it were found to bind yeast [1H]tRNA^Phe (Table 1). In most experiments the immunoglobulin fraction was used, as this probably contains less nucleolytic activity than the unfractionated serum. In addition, in order to minimize nuclease activity the incubation for the binding assay was performed at 4°.

The binding of radioactive yeast tRNA^Phe by the goat antibodies was inhibited by preincubation with unlabeled yeast tRNA^Phe (Fig. 1). Unfractionated yeast tRNA also inhibited the binding; however, about 30-fold higher concentrations than that of yeast tRNA^Phe were required to achieve a 50% inhibition (Fig. 1). These results suggest that the antibodies are specific to yeast tRNA^Phe and probably do not cross-react with other tRNA species. As can be seen in Fig. 1, E. coli tRNA or E. coli ribosomal RNA did not cause any significant inhibition. The RNA from these two sources does not contain Y. On the other hand, wheat germ tRNA, which contains a modified form of Y (17), gave a certain amount of inhibition at high concentrations. The common ribonucleosides, adenosine, guanosine, cytidine and uridine, did not give any inhibition at a concentration as high as 100 μg per assay mixture. Of special significance is the lack of crossreaction with guanosine, which is considered to be a precursor of Y (18, 19). It thus appears that the specificity of the antibodies is towards the unique tricyclic imidazapurine nucleus of the Y base or towards its side chain.

In order to elucidate the role of Y in the antigenic specificity of the anti-yeast tRNA^Phe antibodies, the following experiments were carried out: yeast tRNA was treated with hydrochloric acid (pH 2.9, 37°, 4 hr) (20) to excise the Y base. This treatment abolished the binding of the tRNA to the antibodies. On the other hand, the material extracted into the chloroform phase, which contains the excised Y base, retained the binding to the antibodies. In a control experiment when tRNA was treated under conditions where no excision of the Y base occurs (pH 7.0, 37°, 4 hr and then chloroform extraction), the activity of the tRNA was not altered and no binding was detected in the chloroform extract.

The specificity of the antibodies was further tested by measuring the ability of several oligonucleotides derived from tRNA^Phe to inhibit the binding of yeast [1H]tRNA^Phe to the antibodies. These oligonucleotides were the dodecanucleotide A-Cm-U-Gm-A-A-Y-A-Y-A-Ψ-m5C-U-Gp, obtained by T1 RNase digestion of yeast tRNA^Phe and the hexanucleotide Gm-A-A-Y-A-Ψp obtained by pancreatic RNase digestion of tRNA^Phe (21). The oligonucleotide Gm-A-A-Rib-A-Ψp was prepared from the hexanucleotide after removal of Y as described (20, 21). Further digestion of the hexanucleotide with T2 RNase yielded Y-Ap and Y>Ψ (F. v.d. Haar, to be published).

As can be seen in Table 2, all the Y-containing oligonucleotides

TABLE 1. Binding of yeast [1H]tRNA^Phe to anti-tRNA^Phe-BGG immunoglobulin

<table>
<thead>
<tr>
<th>Immunoglobulin from</th>
<th>Immunoglobulin added (mg)</th>
<th>[1H]tRNA^Phe binding (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-tRNA^Phe-BGG</td>
<td>0.01</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>45</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>100</td>
</tr>
<tr>
<td>Normal goat serum</td>
<td>0.1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

inhibition of the binding of [1H]tRNA^Phe to anti-tRNA^Phe-BGG immunoglobulin by yeast tRNA^Phe (O), unfractionated yeast tRNA (●), unfractionated E. coli tRNA (○), E. coli ribosomal RNA (■), and wheat germ tRNA (▲). The inhibitor was incubated with 0.1 mg of the immunoglobulin for 15 min at 4°. [1H]tRNA^Phe (0.04 μg with 10,000 cpm) was then added and the reaction mixtures were incubated for an additional hour at 4°. The bound antigen was precipitated and estimated as described in Materials and Methods.

FIG. 1. Inhibition of the binding of [1H]tRNA^Phe to anti-tRNA^Phe-BGG immunoglobulin by yeast tRNA^Phe (O), unfractionated yeast tRNA (●), unfractionated E. coli tRNA (○), E. coli ribosomal RNA (■), and wheat germ tRNA (▲). The inhibitor was incubated with 0.1 mg of the immunoglobulin for 15 min at 4°. [1H]tRNA^Phe (0.04 μg with 10,000 cpm) was then added and the reaction mixtures were incubated for an additional hour at 4°. The bound antigen was precipitated and estimated as described in Materials and Methods.

TABLE 2. Inhibition of the binding of [1H]tRNA^Phe to anti-tRNA^Phe antibodies by oligonucleotides

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Amount of inhibitor added (A₄₅₅ units)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 × 10⁻¹  2 × 10⁻²  2 × 10⁻³  2 × 10⁻⁴</td>
</tr>
<tr>
<td>Gm-A-A-Rib-A-Ψp</td>
<td>0 0 0 0</td>
</tr>
<tr>
<td>Y-Ap</td>
<td>97 93 88 67</td>
</tr>
<tr>
<td>Y&gt;Ψ</td>
<td>90 85 55 11</td>
</tr>
</tbody>
</table>

* The numbers in the table represent the percent of inhibition obtained for each inhibitor concentration. Inhibition experiments were carried out as described in legend to Fig. 1.

were potent inhibitors, whereas Gm-A-A-Rib-A-Ψp, which has lost the Y base by chemical treatment, did not inhibit at all.

An immunoadsorbent prepared by the conjugation of the immunoglobin fraction from anti-tRNA^Phe-BGG serum to CNBr-activated Sepharose was shown to retain the antibody activity, as it bound radioactive yeast tRNA^Phe. A similar adsorbent prepared with normal goat immunoglobin did not bind the radioactive tRNA. The anti-tRNA^Phe immunoadsorbent was used for specific fractionation of yeast tRNA^Phe. Yeast tRNA was applied to the immunoadsorbent, which was packed in a column. The majority of the material applied came through the column without being adsorbed to it. However, the ability to inhibit the binding of ^[3H]tRNA^Phe to the specific antibodies, as well as the capacity to accept phenylalanine, was lost (Table 3), demonstrating that tRNA^Phe was bound to the column. On the other hand, the capacity to accept other amino acids was retained by the nonadsorbed material.

In a control experiment where yeast tRNA was chromatographed on a column of normal immunoglobulins bound to Sepharose, there was no loss in inhibitory capacity or in amino acid acceptance activity (Table 3).

**DISCUSSION**

The experiments described in the present study show for the first time the production of antibodies against a specific tRNA, yeast tRNA^Phe, following an immunization with the intact tRNA molecule. The antibodies obtained are directed towards the highly modified Y nucleoside present in the anticodon loop of yeast tRNA^Phe, right next to the anticodon (22). It thus appears that the anticodon is conformationally accessible, in agreement with the recent reports on the x-ray diffraction pattern (8). It seems that the anticodon loop may be an immunopotent determinant in tRNA, especially if it contains some unusual nucleoside.

Antibodies to rare nucleosides may be useful reagents for studying nucleic acids, as they play an important role in defining their antigenic specificity. An advantage of our method is that the intact tRNA molecule may be used effectively for immunization and it is not necessary to fractionate the Y nucleoside, or the base derived from it, for preparation of immunogens leading to Y-specific antibodies. A similar approach may be used for the preparation of specific antibodies towards other rare nucleosides.

The anti-tRNA^Phe immunoadsorbent proved useful for specific binding and fractionation of yeast tRNA^Phe (Fig. 1, Table 3). In addition to tRNA^Phe other nucleic acids and oligonucleotides containing the Y nucleoside can also be fractionated utilizing this anti-tRNA^Phe column. Phenylalanine tRNAs isolated from other eukaryotic cells like wheat germ, beef-, rat-, and chicken-livers were shown to contain the Y nucleoside or a modified form of it (18, 24). Indeed, we have shown that the antibodies against yeast tRNA^Phe cross-react to a certain extent with wheat germ tRNA (Fig. 1). Thus, antibodies to yeast tRNA^Phe may have a wide use in the research of tRNA, as they can be applied for fractionation of tRNA molecules on the basis of their Y content, and for studying the biosynthesis of Y and its biological function.

**DISCUSSION**

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