Ellerton, N. F., and Isenberg, I. (1969), *Biopolymers 8*, 767.
Evett, J., and Isenberg, I. (1969), *Ann. N. Y. Acad. Sci. 158*, 210.
Gill, T. J., III, and Omenn, G. S. (1965), *J. Amer. Chem. Soc. 87*, 4188.
Gill, T. J., III, McLaughlin, E. M., and Omenn, G. S. (1967), *Biopolymers 5*, 297.
Gill, T. J., III, and Omenn, G. S. (1967), *in* Ordered Fluids and Liquid Crystals, Porter, R. S., and Johnson, J. F., Ed., Washington, D. C., American Chemical Society.
Gottlieb, Y. Y., and Wahl, P. (1963), *J. Chim. Phys. 60*, 849.
Huang, R. C. C., Bonner, J., and Murray, K. (1964), *J. Mol. Biol. 8*, 54.
Inoue, S., and Ando, T. (1968), *Biochem. Biophys. Res. Commun. 32*, 501.
Inoue, S., and Ando, T. (1970), *Biochemistry 9*, 395.
Jablonski, A. (1960), *Bull. Acad. Pol. Ser. Sci. Math. Astron. Phys., 8*, 259.
Katchalsky, E., and Sela, M. (1958), *Advan. Protein Chem. 13*, 394.
Knopp, J. A., and Weber, G. (1969), *J. Biol. Chem. 244*, 6309.
Leng, M., and Felsenfeld, G. (1966), *Proc. Nat. Acad. Sci. U. S. 56*, 1325.
Ohba, Y. (1966), *Biochim. Biophys. Acta 123*, 84.
Olins, D. E., Olins, A. L., and von Hippel, P. (1967), *J. Mol. Biol. 24*, 151.
Olins, D. E., Olins, A. L., and von Hippel P. (1968), *J. Mol. Biol. 33*, 265.
Omenn, G. S., and Gill, T. J., III. (1966), *J. Biol. Chem. 10*, 4899.
Raukus, E. (1965), *Biokhimiya 30*, 1121.
Rawitch, A. B., Hudson, E., and Weber, G. (1969) *J. Biol. Chem. 244*, 6543.
Shapiro, J. T., Leng, M., and Felsenfeld, G. (1969), *Biochemistry 8*, 3219.
Stewart, J. W., and Stahmann, M. A. (1962), *in* Polyamino Acids, Polypeptides and Proteins, Stahmann, M. A., Ed., Madison, Wis., Univ. of Wisconsin Press.
Stryer, L. (1968), *Science 162*, 526.
Tsuboi, M., Matsuo, K., and Ts'o, P. O. P. (1966), *J. Mol. Biol. 15*, 256.
Wahl, Ph., and Weber, G. (1967), *J. Mol. Biol. 30*, 371.
Wahl, Ph., Paoletti, J., and Le Pecq, J. B. (1970), *Proc. Nat. Acad. Sci. U. S. 65*, 417.
Weber, G. (1952a), *Biochem. J. 51*, 145.
Weber, G. (1952b), *Biochem. J. 51*, 155.
Weber, G. (1953), *Advan. Protein Chem. 8*, 415.

On the Basis of Specific Fragmentation of Ribonucleic Acid by Nucleases*

Jennifer C. Pinder and W. B. Gratzer†

ABSTRACT: The limited exposure of rabbit reticulocyte RNA to T_1 ribonuclease produces a series of stable intermediates of degradation, which can be characterized by electrophoresis in polyacrylamide gels. Explanations of this effect in terms of secondary or tertiary structures are ruled out by fragmentation patterns under conditions leading to the elimination of base-pairing. In particular, the RNA in the presence of 3% formaldehyde is much more rapidly degraded, but a sharp pattern of zones is formed which is very similar to that produced by the native material. No differences are observed when the digestion is performed at 63°, when single-strand stacking is to a great extent eliminated, or after chemical methylation of guanine residues, or after introduction of excess amounts of polycytidylic acid under conditions leading to binding. This last, as well as the absence of significant amounts of guanylic acid in the digests, renders any explanation of specific fragmentation in terms of long runs of guanine residues improbable.

It is suggested that the degree of lability of any bond is determined by subsites on the enzyme, which recognize a series of adjoining residues; such situations have been reported for other hydrolytic enzymes. This interpretation is supported by experiments with the closely related nuclease, N_1, which has the same primary specificity and other properties as T_1. Limited digestion with N_1 nuclease leads to a well-defined pattern of zones, which is however unrelated to that produced by T_1. Limited digestion of a specific tRNA by these two nucleases also reveals some differences. This indicates that, as expected, the systems of subsites on the two enzymes are not identical.

The ability of nucleases under the right circumstances to break down RNA into large fragments by cleavage at labile regions has been known for a considerable time (Huppert and Pelmont, 1962; McPhie *et al.*, 1966; Gould, 1966a,b; Delihas and Bertman, 1966, and much subsequent work). The use of polyacrylamide gel electrophoresis to provide high resolution according to molecular weight of RNA species over a wide size range revealed that the fragments were indeed highly stable intermediates, each essentially monodisperse (McPhie *et al.*, 1966; Gould, 1966a, 1967). This type of behavior has

* From the Medical Research Council Biophysics Unit, King's College, London, W.C.2. *Received February 25, 1970.*
† To whom correspondence should be addressed.

been noted in a variety of ribosomal RNA species (Gould *et al.*, 1966; Pinder *et al.*, 1969) and in viral RNA (Gould *et al.*, 1969; Thach and Boedtker, 1969; Nichols, 1970).

The formation of specific fragments, combined with their separation by gel electrophoresis has been of some interest in relation to sequence determination (Adams *et al.*, 1969; Nichols, 1970; Fellner *et al.*, 1970) and in comparative evolutionary studies of RNA (Gould *et al.*, 1966; Pinder *et al.*, 1969). The reasons for the altogether unexpected formation of intermediates of high relative stability in the digestion has remained obscure, however. The only working hypothesis that has been put forward is that the lability of certain parts of the RNA chain is a consequence of the manner of folding, or in other words that high molecular weight RNA species have a defined tertiary structure (McPhie *et al.*, 1966; Delihas and Bertman, 1966; Thach and Boedtker, 1969). In this communication we show that this is not in fact the basis of specific fragmentation, and we suggest an alternative explanation.

Experimental Section

Escherichia coli ribosomes were prepared from nuclease-deficient strain MRE 600, and the RNA was extracted by the detergent–phenol method (Nirenberg and Matthaei, 1961). Rabbit reticulocyte ribosomes were prepared as described by Arnstein *et al.* (1965) and in different preparations the RNA was extracted by phenol–detergent, or the guanidine hydrochloride method (Cox, 1966a). A sample of 95% pure valine tRNA of *E. coli* was generously provided by Dr. H. H. Paradies. Ribonucleases T_1 and N_1 were purchased from Sankyo Chemical Co. and Miles Laboratories Inc.

Digestions were performed for 10 min at 4°, at nuclease concentrations in the range 2.5–2500 units[1]/mg of RNA, using 0.4 mg of RNA for each digestion. Reaction with formaldehyde was achieved by heating RNA at 1 mg/ml in 3% formaldehyde–0.1 M phosphate buffer, pH 7.5, at 63° for 15 min and then quenching in ice water (Boedtker, 1968).

After formaldehyde treatment the nuclease concentration for digestion was decreased to 2.5–25 units/mg of RNA. Polyacrylamide gel electrophoresis was carried out in cylindrical gels, 7 mm in diameter. The acrylamide concentration was 5%, and the proportion of methylenebisacrylamide 5%. The gels were prepared with ammonium persulfate initiation and dimethylaminoethyl cyanide as catalyst. The buffer system was continuous 0.05 M diethyl barbiturate, pH 8.6; 400 μg of RNA was applied to each tube by layering the solution, containing 5% sucrose, on the surface of the gel. Bromophenol blue was incorporated as a marker for migration. The electrophoresis was allowed to proceed for 45 min at 5 mA per tube. The gels were then removed from the tubes, stained overnight in 1% pyronine Y, 15% acetic acid, and 2% lanthanum acetate, and destained electrophoretically. Formaldehyde-treated RNA was run in similar gels containing 3% formaldehyde and this concentration was also present in the buffer compartments. Methylation was performed by exposure to dimethyl sulfate exactly as described by Leng *et al.* (1969), modification of guanylic acid residues being detected by paper chromatography after acid hydrolysis. Analysis of digests for mononucleotides was performed by paper chromatography of the digest directly (Markham and Smith, 1951), and also by paper chromatographic estimation of free bases after treatment with alkaline phosphatase (Thach and Doty, 1965). Attempts to attach polycytidylic acid were made by the hybridization procedure of Moore and Asano (1966), involving incubation at 37° with 0.01 M magnesium.

Optical rotatory dispersion was measured with a Bellingham and Stanley "Polarmatic" instrument in cells of 1-cm path length. The cell housing was thermostatted at 24°. A Radiometer TTT1 pH-Stat was used to follow digestions. The RNA concentration was 3 mg/ml, and the nuclease 1000 units/ml. The titrant was 0.02 N sodium hydroxide.

Results and Discussion

A typical digestion pattern of rabbit reticulocyte ribosomal RNA by ribonuclease T1 is shown in Figure 1a,b. The molecular weight range based on previous calibrations (McPhie *et al.*, 1966) is as indicated. The most prominent components which define the pattern are seen to lie within the molecular weight range 50,000–300,000. This pattern is remarkably stable, and changes little over the range of enzyme concentrations 250–2500 units/mg of RNA for a 10-min digestion: it is readily identifiable, and may be regarded as a fingerprint for this combination of RNA and enzyme. At higher enzyme concentrations the pattern changes, and more extensive degradation occurs leading in due course to the disappearance of all zones (Figure 1c). It must be noted that the digestion is only arrested by the separation of enzyme and RNA in the electrophoresis. The position of the enzyme, which is a strongly acidic protein, is revealed by colorless zones in the background of pyronine stain, in the region of highly degraded low molecular weight RNA.

A pattern of a relatively small number of zones, such as that of Figure 1a,b reflects the presence of a few regions of high comparative lability in the RNA chain. That we are dealing here with regions rather than single labile phosphodiester bonds is clear from the stoichiometry of the degradation. In the first place, in the very early stages of the digestion (10 min, at 0.25 unit of enzyme per mg of RNA) the zones appearing in the acrylamide gels are overlaid by a smeared background of RNA. This subsequently gives place to the sharp patterns with no appreciable stain in the background, as shown in Figure 1a,b. This suggests that the fragments initially formed are of rather variable length, and are trimmed down to produce the stable components which then resist exposure to nuclease at much higher concentrations, or longer times. This is confirmed by pH-Stat experiments from which one may estimate the fraction of bonds broken (see, *e.g.*, Batt and Houck, 1964) under the conditions used to generate the fragments of Figure 1. For the RNA:nuclease ratio corresponding to Figure 1a,b, some 8% of phosphodiester bonds are broken. From the amount of nucleotide material not precipitated by ethanol from the digest (*ca.* 6%, assuming an average molar residue absorptivity of about 10^4) it appears that the fraction of RNA digested results largely in the formation of mononucleotides and small oligonucleotides. As in earlier work (Gould, 1967) the proportions of RNA in the major zones of the digest are compatible with 1 mole of fragments per mole of RNA. On this basis, within the generous cumulative error of molecular

[1] The unit of activity is defined in terms of the appearance of 1 absorbance unit of nonprecipitable oligonucleotide in 1 ml (Egami *et al.*, 1964). We find that 1 mg of enzyme is equivalent to some 1400 units.

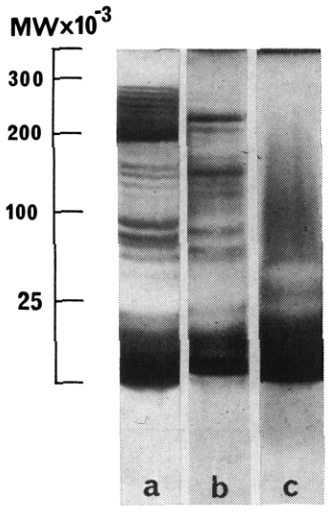

FIGURE 1: Polyacrylamide gel electrophoresis (5% gels) of ribonuclease T_1 digests of rabbit reticulocyte ribosomal RNA, showing molecular weight scale: (a,b) 100 units of enzyme added to 0.4 mg of RNA in 0.2 ml of 0.01 M Tris buffer, pH 7.6, and digested for 10 min at 0°; (c) 500 units of enzyme added under the same conditions. The zones near the top of (a) are unstable and difficult to reproduce; (a) and (b) were obtained in different experiments under nominally identical conditions.

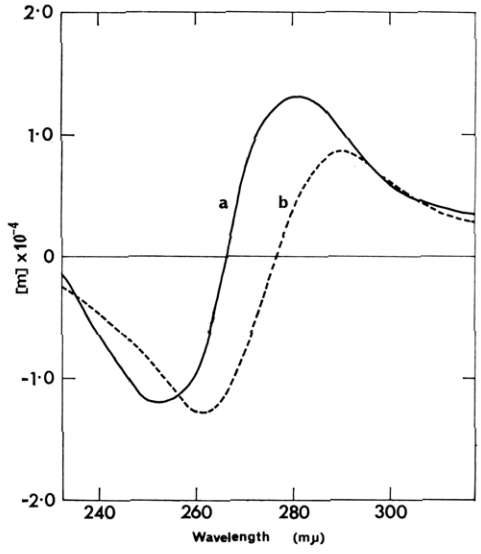

FIGURE 2: Optical rotatory dispersion of (a) native rabbit reticulocyte ribosomal RNA; (b) the same material after treatment with 3% formaldehyde at 63° for 10 min (both in 0.1 M phosphate, pH 7.7). The curves are similar in shape to those shown by Cox (1969) for similar samples, but our peak rotations are some 30% lower. We are unable to account for this discrepancy.

weight estimations for the fragments, little of the RNA could have disappeared as small fragments.

In seeking an explanation for the phenomenon one must consider first the absolute specificity of the nuclease for G residues and secondly the conformation of the RNA. Work involving several techniques on a variety of ribosomal RNA species (Cox, 1966b; Gould and Simpkins, 1969; Thomas, 1969; Cotter and Gratzer, 1969) places the fraction of paired bases in the range 50–70%, and it is clear from many lines of evidence (see, *e.g.*, Spencer and Poole, 1965; Cox and Kanagalingam, 1967) that the double-helical segments are short. For example, the estimate of Cox *et al.* (1968), that the lengths of the double helices lie in the range 6–16 base pairs, leads to 200–300 helices interspersed with loops. It is highly probable that a nuclease will cleave preferentially in unpaired regions. If however the loops as such represent labile points, the total number of fragments would run into 10^4–10^5, on the basis of the relation given above. It seems clear that no explanation for a small number of zones in terms of secondary structure alone can therefore be sought.

We have considered four possible mechanisms for specific cleavage: (1) a defined tertiary structure for the RNA such that certain parts of the chain are always on the surface and accessible, and the remainder masked; (2) unusual bases, in particular methylated G residues which might be better substrates; (3) long sequences of G residues to increase the probability of breaks within a narrow region; and (4) strong sequence specificity of the nuclease. It appears to be possible to eliminate the first three of these.

In order to study the effect of folding on the digestion pattern, digestion experiments were performed at an ionic strength sufficiently low (10^{-4} M EDTA in doubly distilled water) to cause the substantial loss of base pairing, as revealed by hypochromicity and optical rotatory dispersion measurements (Cantor *et al.*, 1966; Cotter *et al.*, 1967). Other experiments were done in the presence of ethylene glycol, which is an effective denaturant for RNA (*e.g.*, Fasman *et al.*, 1965) at concentrations up to 60% v/v, at which point the activity of the nuclease was lost, and also in various concentrations of dimethylformamide. In all cases, though the digestion proceeded much more rapidly, the patterns were indistinguishable from those of the RNA under standard conditions. The clearest demonstration however comes from digestion of formaldehyde-treated RNA. It is established (Penniston and Doty, 1963; Boedtker, 1967, 1968) that base pairing is fully eliminated by this reagent. After heating the ribosomal RNA to 63° for 15 min in 3% formaldehyde a large increase in absorbance is noted, and the optical rotatory dispersion corresponds, as Figure 2 shows, to a typical curve for a single-stranded, partially stacked, chain (*cf.* Cantor *et al.*, 1966). Ribonuclease T_1 contains only one lysine residue, but neither this nor the α-amino group are required for enzymic activity (Shiobara *et al.*, 1962; Kasai *et al.*, 1969). The enzyme is therefore fully active in the presence of formaldehyde. We find in the first place that for a given enzyme concentration the digestion proceeds very much faster, apparently by at least an order of magnitude. This is clear from a comparison of Figures 1a and 3a, which shows that under conditions leading to the standard zone pattern in native ribosomal RNA, the formaldehyde-treated material produces only low molecular weight fragments travelling near the marker. With a tenfold reduction in enzyme concentration, however, Figure 3 shows that a similar pattern of zones is generated. To demonstrate that the patterns in the presence and absence of formaldehyde can indeed be equated we have plotted the R_F values of the zones obtained in one system against those in the other. The reproducibility of the patterns is limited by the relative transience of some zones, so that the extents of digestion can never be precisely matched. It is however clear from Figure 4 that the two patterns are essentially congruent, since the

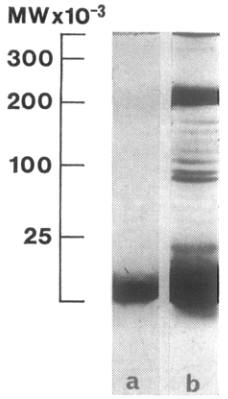

FIGURE 3: Polyacrylamide gel electrophoresis (5% gels) of T_1 digests of rabbit reticulocyte ribosomal RNA in 3% formaldehyde, after heating the RNA at 63° for 10 min in this reagent: (a) 100 units of enzyme with 0.4 mg of RNA, the conditions are identical with those of Figure 1a; (b) 10 units of enzyme under the same conditions of digestion.

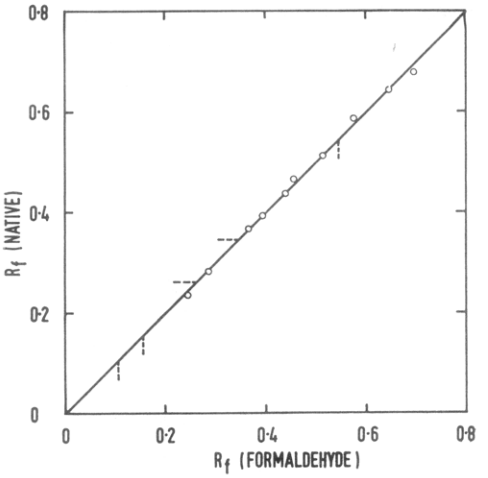

FIGURE 4: Plot of R_F values (relative to bromophenol blue marker) of electrophoretic zones in a T_1 digest of ribosomal RNA performed in the presence of 3% formaldehyde, after heating the RNA in this medium, against R_F values of zones in a T_1 digest prepared in the standard buffer system in the absence of formaldehyde. The line is of unit slope, passing through the origin, and therefore corresponds to congruent patterns. The broken lines indicate the positions of zones for which no clear counterpart could be found in the other digest.

points lie on a good straight line, of unit slope, passing through the origin. The extent of ambiguity in this procedure can be seen by the small number of zones in either pattern that cannot be matched; these are indicated on the plot. The pattern of Figure 3b is largely stable over a wide range of nuclease concentrations (though these are all relatively low). We conclude that the specificity of cleavage is unrelated to tertiary or, indeed, secondary structure of the RNA. It may be noted that the mobility in the gel is less for formaldehyde-treated RNA than native. This is a consequence of its larger Stokes radius (Boedtker, 1968). A linear law relating mobility with the logarithm of molecular weight still obtains (J. C. Pinder and W. B. Gratzer, unpublished observations), however. Explanations of specific cleavage in terms of tertiary structure had been previously invoked (McPhie *et al.*, 1966; Delihas and Bertman, 1966; Thach and Boedtker, 1969), and must now be discarded.

The possibility that preferential cleavage occurs at modified G residues, though remote, is hard to eliminate. At least one mammalian ribosomal RNA species has a low but measurable content of methylated bases (Brown and Attardi, 1965). In order to examine the effect of methylation on the digestion with T_1 ribonuclease, ribosomal RNA was treated with dimethyl sulfate under the conditions of Leng *et al.* (1969) for varying periods of time. The same paper chromatographic method was used to detect the presence of 7-methylguanylic acid, and though the resolution was insufficient to allow any precise estimates of its relative concentration, appreciable amounts were found to be present. After this treatment there was no detectable difference in susceptibility to T_1 ribonuclease, or in the electrophoretic pattern produced on digestion. The involvement of derivatives methylated at other positions, or of methylated sugar residues, cannot be wholly ruled out, but seems unlikely, more particularly in view of the absence of unusual bases at the terminal positions of the fragments isolated from mild T_1 digests of R17 virus RNA (Adams *et al.*, 1969; Nichols, 1970) and of *E. coli* ribosomal RNA (Fellner, 1969; Fellner *et al.*, 1970).

An explanation of the specific fragments in terms of long runs of G residues is in principle possible. Because of the stability of many of the intermediates over nearly two orders of magnitude of nuclease concentration (Figure 1) and the absence of a staining background of heterogeneously degraded material it appears however that such a mechanism would require of the order of 10–100 clustered G residues. Evidence for any such sequences is absent, although fragments containing as much as 40% G have been isolated from digests of ribosomal RNA from other mammalian sources (Delihas, 1967; Wikman *et al.*, 1969). If there were indeed long essentially uninterrupted runs of G residues, one would expect that the digestion patterns would not persist after introduction of polycytidylic acid under conditions of binding to ribosomal RNA in the presence of 10^{-2} M magnesium (Moore and Asano, 1966). Again, however, apart from the overlay of polydisperse homopolymer material there is no noticeable deterioration in the digestion patterns. Furthermore, analysis of the digests by two methods revealed no significant concentration of guanylic acid monomers.

In order to rule out the participation of single-stranded stacking in determining the enzyme specificity, digestions were carried out at a temperature of 63°, at which the enzyme still functions, but the majority of the stacking hypochromicity is lost. There is no change or deterioration in the digestion pattern.

We conclude from these results that there is a high likelihood that the specificity of digestion results from sequence dependence of the T_1 function. The rates of digestion of dinucleoside phosphates by T_1 has been studied (Whitfield and Witzel, 1963). The greatest difference, which is less than fourfold, is between GpU and GpC. This is altogether insufficient to account for our results, and we infer that some longer range sequence dependence must be involved. The existence of subsites with high affinity for particular residues in a substrate chain has been established in a number of situations. Thus papain has seven recognizable subsites (Schechter

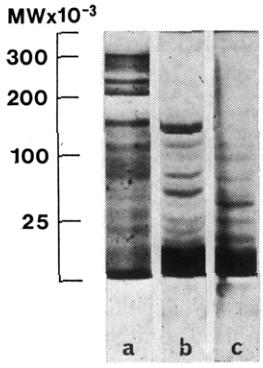

FIGURE 5: Polyacrylamide gel electrophoresis (5% gels) of ribonuclease N_1 digests of rabbit reticulocyte ribosomal RNA: (a) 0.01 units; (b) 0.1 units; (c) 1 unit enzyme, added in each case to 0.4 mg of RNA in 0.2 ml and digested 10 min at 37°.

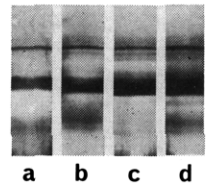

FIGURE 6: Polyacrylamide gel electrophoresis (15% gels) of T_1 and N_1 digests of *E. coli* valine tRNA: (a) T_1 2.5 units/mg of RNA; (b) T_1, 25 units/mg of RNA; (c) N_1, 0.025 units/mg of RNA; (d) N_1 0.25 units/mg of RNA. The split zone in the undigested tRNA reveals the presence of an equilibrium proportion of the denatured form (Adams *et al.*, 1967). The prominent minor component nearer the origin is 5S RNA, and its satellite is presumed to be the denatured form, which separates from it under these conditions (Aubert *et al.*, 1968).

and Berger, 1967), carboxypeptidase A five (Abramowitz *et al.*, 1967), subtilisin six (Morihara *et al.*, 1969), *Bacillus subtilis* amylase nine (Thoma *et al.*, 1970), and a number have also been reported in lysozyme (Dahlquist and Raftery, 1969). With five or more subsites of even quite small binding constants, very large increases in Michaelis constant will ensue. As the work of Thoma *et al.* (1970) on *B. subtilis* amylase shows, subsites can function in a favorable or unfavorable sense. An unfavored residue in the substrate chain at a point several positions removed from the active center can cause the enzyme to reject the region of the substrate. Thus the existence of subsites on the enzyme can lead to very high resistance of parts of the substrate, as well as unusual lability, and the specific RNA fragments can be viewed in terms of either. This therefore seems a probable explanation for the specificity of cleavage. The much more rapid velocity of fragmentation in the presence of formaldehyde presumably represents loss of the energy of activation for unfolding of the native RNA to make the whole of the chain accessible.

To support the hypothesis of subsites, experiments were also performed with a different nuclease, which however is related to T_1 and has the same specificity for guanine residues. This is ribonuclease N_1 (Takai *et al.*, 1966), which derives from *Neurospora crassa*, whereas T_1 is from *Aspergillus oryzae*. A well-developed pattern of zones is produced (Figure 5), which is different from that generated by T_1. Attempts to construct a plot such as that in Figure 4 relating the pattern given by N_1 nuclease and that from T_1 gave a maximum of four points on a line of unit slope through the origin. Thus, despite the identical primary specificity of the two enzymes the cleavage patterns are unrelated and this is as expected if multiple subsites are the agents controlling specificity of fragmentation.

Figure 6 shows digestion patterns of *E. coli* valine tRNA produced by T_1 and N_1 nucleases. Four fragments only can be recognized in the digests. It is reproducibly found however that whereas with N_1 all four zones appear together, T_1 produces in the first place only the two fastest zones, the two slower zones appearing only at a later stage of digestion. Thus, the susceptibilities of at least two points in the chain toward the two nucleases differ quantitatively. In formaldehyde after heating the rate of degradation of valine tRNA by T_1 is greatly accelerated, but the same pattern of zones is generated.

One may hope that the different digestion pattern in ribonuclease N_1 digests might be of value for the preparation of fragments overlapping with those from T_1 digestion for purposes of sequence determination. It is implicit in our results that the degree of susceptibility of a given bond to hydrolysis by T_1 or N_1 ribonuclease is not exclusively influenced by base pairing. It is of course possible that the inherently labile sequences are also those which are unprotected by base pairing in the native RNA.

Acknowledgment

We are grateful to Dr. H. H. Paradies for a gift of purified valine tRNA, and to Professor Sir John Randall for support.

References

Abramowitz, N., Schechter, I., and Berger, A. (1967), *Biochem. Biophys. Res. Commun. 29*, 862.

Adams, A., Lindahl, T., and Fresco, J. R. (1967), *Proc. Nat. Acad. Sci. U. S. 57*, 1684.

Adams, J. M., Jeppesen, P. G. N., Sanger, F., and Barrell, B. G. (1969), *Nature (London) 223*, 1009.

Arnstein, H. R. V., Cox, R. A., Gould, H. J., and Potter, H. (1965), *Biochem. J. 96*, 500.

Aubert, M., Monier, R., Reynier, M., and Scott, J. F. (1968), *in* Structure and Function of Transfer RNA and 5S RNA, Fröholm, L. O., and Laland, S. G., Ed., Oslo, Universitetsforlager, p 150.

Batt, C. W., and Houck, J. C. (1964), *Biochim. Biophys. Acta 89*, 90.

Boedtker, H. (1967), *Biochemistry 6*, 2718.

Boedtker, H. (1968), *J. Mol. Biol. 35*, 61.

Brown, G. M., and Attardi, G. (1965), *Biochem. Biophys. Res. Commun. 20*, 298.

Cantor, C. R., Jaskunas, S. R., and Tinoco, I. (1966), *J. Mol. Biol. 20*, 39.

Cotter, R. I., and Gratzer, W. B. (1969), *Nature (London) 221*, 154.

Cotter, R. I., McPhie, P., and Gratzer, W. B. (1967), *Nature (London) 216*, 864.

Cox, R. A. (1966a), *Biochem. Prepn. 11*, 104.

Cox, R. A. (1966b), *Biochem. J. 98*, 841.
Cox, R. A. (1969), *Biochem. J. 114*, 743.
Cox, R. A., Gould, H. J., and Kanagalingam, K. (1968), *Biochem. J. 106*, 733.
Cox, R. A., and Kanagalingam, K. (1967), *Biochem. J. 103*, 431.
Dahlquist, F. W., and Raftery, M. A. (1969), *Biochemistry 8*, 713.
Delihas, N. (1967), *Biochemistry 6*, 3356.
Delihas, N., and Bertman, J. (1966), *J. Mol. Biol. 21*, 393.
Egami, F., Takahashi, K., and Uchida, T. (1964), *Prog. Nucleic Acid Res. 3*, 59.
Fasman, G. D., Lindblow, C., and Seaman, E. (1965), *J. Mol. Biol. 12*, 630.
Fellner, P. (1969), *Eur. J. Biochem. 11*, 12.
Fellner, P., Ehresmann, C., and Ebel, J. P. (1970), *Nature (London) 225*, 26.
Gould, H. J. (1966a), *Biochemistry 5*, 1103.
Gould, H. J. (1966b), *Biochim. Biophys. Acta 123*, 441.
Gould, H. J. (1967), *J. Mol. Biol. 29*, 307.
Gould, H. J., Bonanou, S., and Kanagalingam, K. (1966), *J. Mol. Biol. 22*, 397.
Gould, H. J., Pinder, J. C., Matthews, H. R., and Gordon, A. H. (1969), *Anal. Biochem. 29*, 1.
Gould, H. J., and Simpkins, H. (1969), *Biopolymers 7*, 223.
Huppert, J., and Pelmont, J. (1962), *Arch. Biochem. 98*, 214.
Kasai, H., Takahashi, K., and Ando, T. (1969), *J. Biochem. (Tokyo) 66*, 591.
Leng, M., Rosilio, C., and Boudet, J. (1969), *Biochim. Biophys. Acta 174*, 574.
Markham, R., and Smith, J. D. (1951), *Biochem. J. 49*, 401.
McPhie, P., Hounsell, J., and Gratzer, W. B. (1966), *Biochemistry 5*, 988.
Moore, P. B., and Asano, K. (1966), *J. Mol. Biol. 18*, 21.
Morihara, K., Oka, T., and Tsuzuki, H. (1969), *Arch. Biochem. 132*, 498.
Nichols, J. L. (1970), *Nature (London) 225*, 147.
Nirenberg, M. W., and Matthaei, H. (1961), *Proc. Nat. Acad. Sci. U. S. 47*, 1588.
Penniston, J. P., and Doty, P. (1963), *Biopolymers 1*, 145.
Pinder, J. C., Gould, H. J., and Smith, I. (1969), *J. Mol. Biol. 40*, 289.
Schechter, I., and Berger, A. (1967), *Biochem. Biophys. Res. Commun. 27*, 157.
Shiobara, Y., Takahashi, K., and Egami, F. (1962), *J. Biochem. (Tokyo) 52*, 267.
Spencer, M., and Poole, F. (1965), *J. Mol. Biol. 11*, 314.
Takai, N., Uchida, T., and Egami, F. (1966), *Biochim. Biophys. Acta 128*, 218.
Thach, R. E., and Doty, P. (1965), *Science 148*, 632.
Thach, S., and Boedtker, H. (1969), *J. Mol. Biol. 45*, 451.
Thoma, J. A., Brothers, C., and Spradlin, J. (1970), *Biochemistry 9*, 1768.
Thomas, G. J. (1969), *Biopolymers 7*, 325.
Whitfield, P. R., and Witzel, H. (1963), *Biochim. Biophys. Acta 72*, 338.
Wikman, J., Howard, E., and Busch, H. (1969), *J. Biol. Chem. 244*, 5471.

2′-*O*-Methyloligoadenylates as Templates for the Binding of Lysyl Transfer Ribonucleic Acid to Ribosomes*

Alan R. Price† and Fritz Rottman‡

ABSTRACT: Certain oligonucleotides containing only 2′-*O*-methyladenosine have been found to stimulate the binding of lysyl-tRNA to ribosomes. The stimulatory activity of these oligonucleotides has been compared to oligonucleotides containing only adenosine and 2′-deoxyadenosine. All adenosine oligomers were highly active. All 2′-deoxyadenosine oligomers were inactive.

The 2′-*O*-methyladenosine triplet without terminal phosphate, AmpAmpAm, was inactive. However, pAmpAmpAm and AmpAmpAmpAm were approximately 30–40% as active as the corresponding adenosine oligomers. Neomycin and streptomycin enhanced the template activity of 2′-*O*-methyloligoadenylates. Thus, free 2′-hydroxyl groups are not essential for codon recognition on the ribosome, and the presence of a 2′-*O*-methyl residue may not block ribonucleic acid codon translation.

The template activity of oligonucleotides in directing the binding of aminoacyl-tRNA to ribosomes is sensitive to modifications in the codon structure. For example, the template activity of ApApA for the ribosomal binding of lysyl-tRNA is enhanced by adding a 5′-phosphate and is diminished by adding a 2′- or a 3′-phosphate. A trinucleotide

* From the Department of Biochemistry, Michigan State University, East Lansing, Michigan 48823. *Received June 22, 1970.* This work was supported by Grant GB-7543 from the National Science Foundation, and by a National Institutes of Health postdoctoral fellowship (5-FO2-GM40, 157-02) from the National Institutes of General Medical Sciences to A. R. P. Michigan Agricultural Experiment Station Journal No. 5123.

† Present address: Department of Biological Chemistry, University of Michigan, Ann Arbor, Mich. 48104.
‡ To whom to address correspondence.