Volume 8 Number 5 1980 Nucleic Acids Research

Nucleotide sequence of Lactobacillus viridescens 5S RNA

Leeson J.Alexander and Thomas S.Stewart

School of Biochemistry, University of New South Wales, Kensington, N.S.W. 2033, Australia

Received 2 January 1980

ABSTRACT

The nucleotide sequence of *Lactobacillus viridescens* ATCC 12706 5S RNA was determined to be pU-G-U-U-G-U-G-A-U-G-A-U-G-G-C-A-U-U-G-A-G-G-U-C-A-C-A-C C-U-G-U-U-C-C-C-A-U-A-C-C-G-A-A-C-A-C-A-G-A-A-G-U-U-A-A-G-C-U-C-A-A-U-A-G-C-G C-C-G-A-A-A-G-U-A-G-U-U-G-G-A-G-G-A-U-C-U-C-U-U-C-C-U-G-C-G-A-G-G-A-U-A-G-G-A C-G-U-C-G-C-A-A-U-G-C_{OH}. When compared with other published sequences of prokaryotic 5S RNA species, this sequence shows as much homology with that from *B. subtilis* (80% homology when all variations included) and *B. megaterium* (77% homology) as with the 5S RNA from another member of *Lactobacillaceae* family (*L. brevis*, 79% homology). The sequence contains the proposed tRNA binding site (CGAAC, positions 41-45) and can accommodate most, but not all, of the more recently proposed helical regions of secondary structure.

INTRODUCTION

The structure of 5S ribosomal RNA has attracted considerable attention in recent years (1). Comparative sequence analysis has been used to locate base pairing regions within the primary structure then these regions interpreted in possible functional secondary structures of 5S RNA (2,3,4).

During our studies of tRNAs from *L. viridescens*, the opportunity arose to sequence the 5S RNA from this organism. In this paper, the complete nucleotide sequence of *L. viridescens* 5S RNA is reported. One other 5S RNA sequence from a member of the *Lactobacillaceae* family has been published (5). The sequence provides another example for comparative analysis in defining functional secondary structures of 5S RNA. Variations in sequence do occur within the proposed helical regions of secondary structure. The effects on base-pairing interactions is discussed.

MATERIALS AND METHODS

General. Pancreatic RNase and bacterial alkaline phosphatase (BAPF) were obtained from Worthington Biochemical Corp. Spleen phosphodiesterase and T_4

© IRL Press Limited, 1 Falconberg Court, London W1V 5FG, U.K.

polynucleotide kinase were purchased from Boehringer Mannheim. T_1 and U_2 RNase (Sankyo) were from Calbiochem and T_2 RNase was obtained from Sigma. T_4 RNA ligase and Phy I RNase were purchased from P.L. Biochemicals. PEI (6) cellulose plates (Polygram Cel 300 PEI) were a product of Macherey-Nagel. Cellulose acetate strips were a product of Schleicher and Schnell. Whatman (DE 81) DEAE-cellulose paper and Whatman No. 1 paper were supplied by Balston Ltd. RX medical X-ray film was a product of Fuji Photo Co. with Dupont Cronex Lightening Plus and Quanta III intensifying screens being used when necessary. Sephacryl S-200 was a product of Pharmacia. [γ-^{32}P]ATP was prepared by the method of Schendel and Wells (7) using DEAE-cellulose chromatography to purify the ATP.

Isolation of 5S RNA. The 5S RNA for post-labelling experiments was first partially purified using a Sephacryl S200 column. Final purification of all 5S RNA was effected by two-dimensional polyacrylamide gel electrophoresis (8) using a 10% gel in the first dimension followed by a 20% gel in 6 M urea in the second dimension.

Post-labelling of RNA. The method of Silberklang *et al.* (9) was used for 5'-labelling whilst 3'-labelled RNA was prepared according to Peattie (10). Post-labelled 5S RNA was purified by polyacrylamide gel electrophoresis. The [5'-^{32}P]-pCp for 3'-labelling was prepared by the method of England and Uhlenbeck (11).

Sequencing Techniques. Standard procedures (12) were used for enzyme degradation and fingerprinting of uniformly [^{32}P] labelled 5S RNA. The rapid sequencing methods involving partial enzymic degradations (13,14) were used for the 5'-labelled 5S RNA whereas the techniques of partial chemical degradations, developed by Peattie (10), were used for the 3'-labelled 5S RNA.

3'-Terminal Analysis. A sample of [3'-^{32}P] labelled RNA was digested with T_2 RNase and the products separated by electrophoresis at pH 3.5 on Whatman #1. The [^{32}P] labelled mononucleotide was located by autoradiography.

RESULTS

Pancreatic and T_1 RNase Digestion Products. Figures 1 and 2 show the autoradiogram resulting from fingerprint analysis of fragments from a complete pancreatic RNase or T_1 RNase digestion of uniformly [^{32}P] labelled 5S RNA. After the determination of molar yields, the digestion fragments were further analysed. For the pancreatic RNase end products, the sequence of fragments p1 to p12 could be elucidated by combined results of T_2 and T_1 RNase digestion.

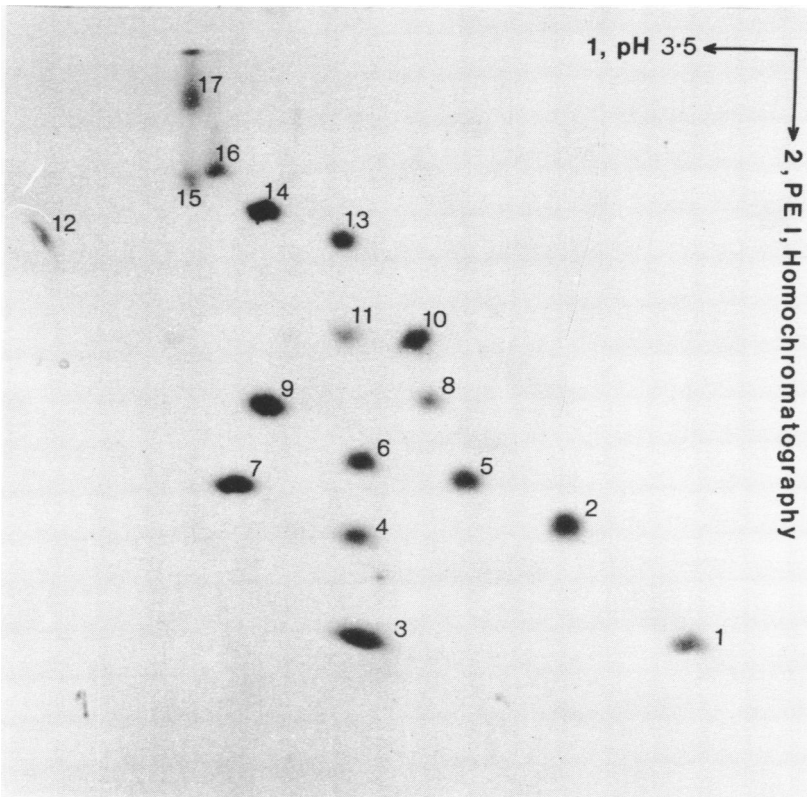


Figure 1.
Fingerprint of complete RNase A digest of *L. viridescens* 5S RNA.

The sequences of the two oligonucleotides in p14 were deduced from analysis of the T_1 RNase end products. For fragments p13 and p15 to p17, the "wandering spot" technique using partial spleen phosphodiesterase digestions followed by two-dimensional chromatographic analysis of the resulting fragments was used to sequence each fragment. The sequence of each of these fragments was later confirmed by the rapid gel techniques. The results are shown in Table I. For the T_1 RNase end products, the sequence of fragments t1 to t5, t7 to t11, t12a, t14 and t20 could be elucidated from the combined results of T_2 and T_1 RNase analysis. The sequence of fragment t15 was deduced from analysis of the pancreatic RNase end products. Except for fragments t18 and t19 the remaining T_1 RNase end products were sequenced by a combination of U_2 RNase

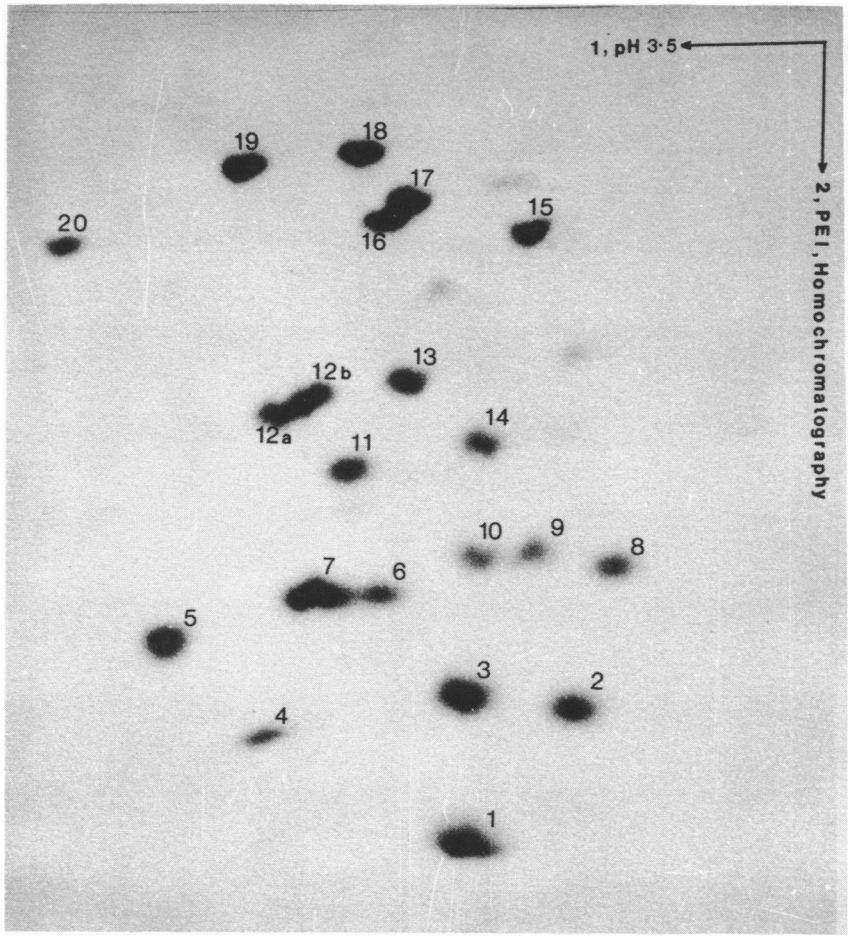


Figure 2.

Fingerprint of complete T_1 RNase digest of *L. viridescens* 5S RNA.

and spleen phosphodiesterase digestions.

The final sequences of fragments t18 and t19 were derived from the rapid gel sequencing results for the [3'-^{32}P]labelled RNA. The sequences of the T_1 RNase end products are given in Table II.

3'-Terminal Residue. A T_2 RNase digestion of 3'-labelled 5S RNA followed by analysis for the [^{32}P]labelled mononucleotide showed the 3-terminal residue to be $-C_{OH}$. The pancreatic RNase end product GpC_{OH} in the fingerprinting of uniformly labelled 5S RNA was not located.

TABLE I

PANCREATIC RNase END PRODUCTS

Fragment Number	T_1 RNase Products	Sequence	Molar Yields	
			Measured+	From Final Sequence
p1	C-	C-	10.2	13
p2	A-C-	A-C-	3.7	4
p3	U-	U-	11.8	11
p4	A-U-	A-U-	2.6	2
p5	G-(0.9), C-(1)	G-C-	3.3	3
p6	A-A-U-	A-A-U-	2.2	2
p7	G-(1.0), U-(1)	G-U-	4.4	4
p8	A-G-(1.0), C-(1)	A-G-C-	1.0	1
p9	A-G-(0.6), A-U-(1.5), G-(1.3), U-(1)	A-G-U- G-A-U-, 2	3.0	3
p10	A-A-G-(0.9), A-A-C-(1), G-(1.0), C(1.1)	G-A-A-C-, A-A-G-C-	1.6	2
p11	G-(1.8), C-(1)	G-G-C-	0.9	1
p12	pUp-	pUp	0.8	1
p13	A-G-(1.0), A-C-(1), G-(1.0)	A-G-G-A-C-†	1.1	1
p14	A-A-A-G-(1), A-A-G-(1.0), A-G-(1.0), U-(2.5), G-(1.3)	G-A-A-A-G-U* A-G-A-A-G-U*-	2	2
p15	A-G-(1), G-(2.1), U-(0.9)	G-A-G-G-U-†	0.9	1
p16	A-G-(1), A-U-(1.0), G-(2.1)	G-A-G-G-A-U-†	1.0	1
p17	A-G-(1), A-U-(1.0), G-(2.9)	G-G-A-G-G-A-U-†	0.9	1
		Total Number of Bases		115

* Deduced from T_1 RNase fragments

† Final sequence from partial spleen phosphodiesterase digestions

\+ Average from several fingerprints

Final Sequence. In order to place the oligonucleotides of the pancreatic and T_1 RNase fingerprints into the final sequence, the rapid gel sequencing methods (10,13,14) on intact 5'- or 3'- end labelled 5S RNA were applied (15).

The total nucleotide sequence of *L. viridescens* 5S RNA is shown in Figure 3a with the complementary base pairing scheme for 5S RNA suggested by Fox and Woese (2) shown in Figure 3b.

DISCUSSION

The 5S RNA from *L. viridescens* contains 117 nucleotides, the same number as reported for *Lactobacillus brevis* 5S RNA (5). It is interesting to

TABLE II

T_1 RNase END PRODUCTS

Fragment Number	Pancreatic RNase Products	Sequence	Molar Yields	
			Measured+	From Final Sequence
t1	G-	G-	7.2	6
t2	C-(1.2), G-(1)	C-G-	2.5	2
t3	A-G-	A-G-	3.8	3
t4	U-(0.8), G(1)	U-G-	1.7	1
t5	U-(1.9), G(1)	U-U-G-	1.8	2
t6	U-(1.1), C-(0.9), G-(1)	U-C-G-*	1.0	1
t7	A-U-(1.9), A-G-(1.2), U-(0.9), G-(2)	2A-U-G, U-A-G-	3.0	3
t8	C-(2.5), G(1)	C-C-G-	0.9	1
t9	A-C-(1.6), G(1)	A-C-G-	1.0	1
t10	A-A-G-	A-A-G-	1.1	1
t11	A-U-(0.9), A-G-(1)	A-U-A-G	1	1
t12a	A-U-(1.0), U(1.5), C-(0.9), G-(1)	C-A-U-U-G*	1.2	1
t12b	A-A-G-(1), U-(1.9)	U-U-A-A-G-	0.9	1
t13	A-A-U-(1.1), C-(1.2), G(1)	C-A-A-U-G*	0.9	1
t14	A-A-A-G-	A-A-A-G-	1.0	1
t15	A-A-C-(1.0), A-C-(1.0), A-G-(1)	A-A-C-A-C-A-G-†	0.9	1
t16	A-A-U-(0.8), C-(1.7), U-(1.2), A-G(1)	C-U-C-A-A-U-A-G*	0.9	1
t17	A-C-(2.2), C-(2.0), U-(1.8), G(1)	U-C-A-C-A-C-C-U-G*	0.9	1
t18	A-U-(1.0), A-C-(1.1), C-(4.2), U-(2.0), G(1)	U-U-C-C-C-A-U-A-C-C-G*	0.9	1
t19	A-U-(1.0), C-(4.2), U-(3.5), G-(1)	A-U-C-U-C-U-U-C-C-U-G*	1.2	1
t20	pUp(1.0), G-(1)	pUpG-	0.9	1
		Total Number of Bases		116

* Sequence determined using U_2 RNase and partial spleen phosphodiesterase digestions and/or from sequencing gels.

† Sequence deduced from Pancreatic RNase fragments.

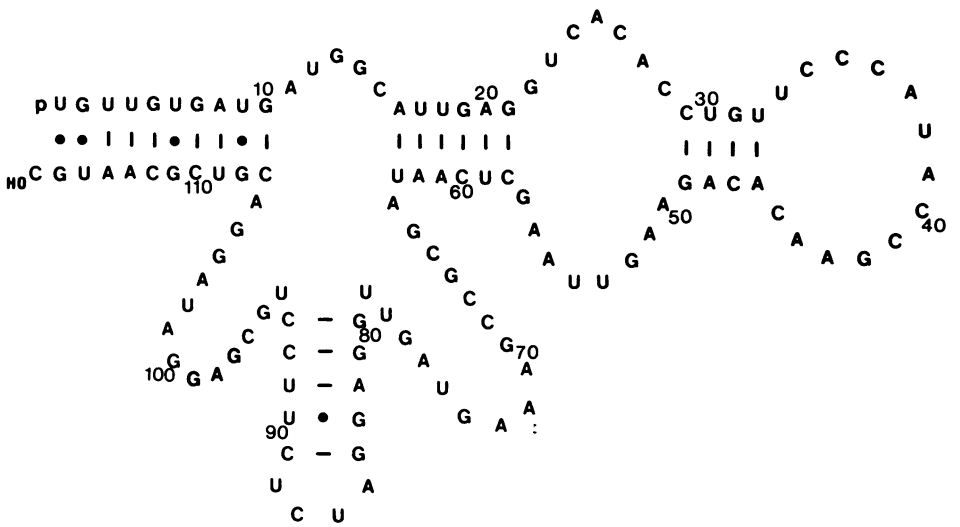
\+ Average from several fingerprints

note that there is as much homology with several *Bacillus* 5S RNA species as with *L. brevis* 5S RNA (79% homology). For example, there is 80% homology with *B. subtilis* 5S RNA (16) if one includes sequence variations occurring in minor species (17) and 77% homology with *B megaterium* 5S RNA (18).

As shown in Figure 3b, the sequence can be arranged in the secondary

pUGUUGUGAUGAUGGCAUUGAGGUCACACCUGUUCCCAUAC
CGAACACAGAAGUUAAGCUCAAUAGCGCCGAAAGUAGUUG
GAGGAUCUCUUCCUGCGAGGAUAGGACGUCGCAAUGC$_{OH}$

(a)



(b)

Figure 3.

(a) Complete nucleotide sequence of *L. viridescens* 5S RNA.
(b) *L. viridescens* 5S RNA drawn in the Fox and Woese model.

structure proposed by Fox and Woese (2) which is based on four conserved helices. The ten base pairs of the 'molecular stalk' (1-10/116-107) are conserved even though there are four changes in the sequences between positions 1 and 10 as compared to several *Bacillus* 5S RNAs.

The additional A residue at position 82 is unique for a prokaryotic 5S RNA. Usually three or four adjacent G residues occur in this region with a corresponding run of C residues closely following which form the stable 'prokaryotic loop'. *L. viridescens* 5S RNA can form a similar helical region of five base pairs (80-84/93-89) which includes the unique A residue at position 82.

The 'tuned helix' of the Fox and Woese model can be accommodated in the sequence (16-21/63-58). The U residues (positions 17 and 18) replace A residues in the corresponding positions in the sequenced *Bacillus* 5S RNAs with the reverse occurring at positions 61 and 62 (As replace Us). This provides strong support for the 'tuned helix' being part of 5S RNA secondary structure.

The remaining helical region, forming the 'common arm base' of the Fox and Woese model is very similar in *L. viridescens* 5S RNA to that in *E. coli* 5S RNA (U=A replaces A=U between positions 32 and 46).

Weidner *et al.* (3) propose two interchangeable secondary structures for 5S RNA. The alternative structure (B form) consists of a 'central helix' of seven base pairs in *E. coli* 5S RNA (33-39/88-82). *L. viridescens* 5S RNA cannot form a helical region of such stability, a maximum of three consecutive base pairs are possible (35-37/81-79). The sequence of *L. viridescens* 5S RNA thus supports the Fox and Woese model for secondary structure but cannot accommodate the alternative 'B form' suggested by Weidner *et al.* (3) to allow rearrangement of 5S RNA secondary structure during protein synthesis.

ACKNOWLEDGEMENTS

This work was supported by a grant from the Australian Research Grants Committee. The authors wish to express their appreciation to Cheryl Hutchins for the preparation of [γ-^{32}P]ATP and 3'-labelling experiments.

REFERENCES

1. Erdmann, V.A. (1976) *Progress in Nucleic Acid Research and Molecular Biology* (Cohn, W.E., Ed.) *Vol. 18:* pp 45-90. Academic Press, New York.
2. Fox, G.E. and Woese, C.R. (1975) *Nature, 256:* 505-507.
3. Weidner, H., Yuan, R. and Crothers, D.M. (1977) *Nature, 266:* 193-194.
4. Hori, H. and Osawa, S. (1979) *Proc. Nat. Acad. Sci. U.S.A. 76:* 381-385.
5. Woese, C.R., Luehrsen, K.R., Pribula, C.D. and Fox, G.E. (1976) *J. Mol. Evol. 8:* 143-153.
6. Abbreviations: PEI, polyethyleneimine. All nucleotide abbreviations are according to the recommendations of the IUPAC-IUB Commission on biochemical nomenclature [*Biochemistry 9:* 4022 (1970)].
7. Schendel, P.F. and Wells, R.D. (1973) *J. Biol. Chem. 248:* 8319-8321.
8. Ikemura, T. and Dahlberg, J.E. (1973) *J. Biol. Chem. 248:* 5024-5032.
9. Silberklang, M., Prochiantz, A., Haenni, A.L. and RajBhandary, U.L. (1977) *Eur. J. Biochem. 72:* 465-478.
10. Peattie, D.A. (1979) *Proc. Nat. Acad. Sci. U.S.A. 76:* 1760-1764.
11. England, T.E. and Uhlenbeck, O.C. (1978) *Biochemistry, 17:* 2069-2076.
12. Barrell, B.G. (1971) *Procedures in Nucleic Acid Research* (Cantoni, G.L. and Davies, D.R., eds) *Vol. 2*, pp 751-779, Harper and Row, New York.
13. Donis-Keller, H., Maxam, A.M. and Gilbert, W. (1977) *Nucleic Acids Res. 4:* 2527-2538.
14. Simoncsits, A., Brownlee, G.G., Brown, R.S., Rubin, J.R. and Guilley, H. (1977) *Nature, 269:* 833-836.

15. Sequencing gels are not included but were provided for the referees.
16. Marotta, C.A., Varricchio, F., Smith, I., Weissman, S.M., Sogin, M.L. and Pace, N.R. (1976) *J. Biol. Chem. 251*: 3122-3127.
17. Raué, H.A., Rosner, A. and Planta, R.J., (1977) *Molec. Gen. Genet. 156*: 185-193.
18. Pribula, C.D., Fox, G.E., Woese, C.R., Sogin, M. and Pace, N. (1974) *FEBS Lett. 44*: 322-323.