LASER RAMAN STUDIES OF RAT LIVER RIBOSOMAL 5 S RNA

Heinz FABIAN, Siegfried BÖHM, Heinz WELFLE, Peter REICH* and Heinz BIELKA

Central Institute of Molecular Biology, GDR Academy of Sciences, DDR-1115 Berlin-Buch and **Central Institute of Physical Chemistry, GDR Academy of Sciences, DDR-1199 Berlin-Adlershof, GDR*

Received 10 November 1980

1. Introduction

The secondary and tertiary structure of ribosomal 5 S RNAs has been studied using a variety of physical and chemical techniques (review [1]). As part of our spectroscopic investigations on ribosomal RNAs we report here on Raman spectroscopic data of the native form of 5 S RNA of rat liver ribosomes. Laser Raman spectroscopy has proved to be a sensitive probe for the structure of RNAs, especially for the determination of the type and extent of base stacking, the amount of order in the ribophosphate backbone and the percentage of U residues in base-paired regions [2–5]. Raman data have been published for *E. coli* 5 S RNA [5] and yeast 5 S RNA [6]. Our Raman data for rat liver 5 S RNA indicate a very high regularity of the ribophosphate backbone in the native form of rat liver 5 S RNA, comparable to native tRNAs. The stacking efficiency of the guanine bases is much higher in 5 S RNA than in yeast $tRNA^{Phe}$, while that of the adenine bases is lower. The results support a base pairing pattern [7] which is very similar to that proposed in [8].

2. Materials and methods

Rat liver 5 S RNA was prepared by phenol/sodium dodecyl sulphate treatment of the ribosomal 7 S complex isolated as in [9] and subsequently separated by gel filtration on Sephadex G-100 in 1 M NaCl solution into 2 forms of 5 S RNA, characterized by different electrophoretic mobilities. The studies were performed with the 5 S RNA moiety of lower electrophoretic mobility, the so-called native 5 S RNA.

The 5 S RNA preparation was dialyzed against TKM buffer (5 mM Tris–HCl (pH 7.8), 50 mM KCl, 1.5 mM $MgCl_2$) and was either concentrated by ultrafiltration to ~4% (w/w) or lyophilized and subsequently dissolved in such volumes of twice-distilled water that finally a 4% (w/w) solution of 5 S RNA in TKM buffer was obtained. The purity of 5 S RNA was checked before and after the Raman experiments by polyacrylamide gel electrophoresis.

Yeast $tRNA^{Phe}$ was purchased from Boehringer (Mannheim) and was prepared for the Raman experiments as in [10].

Raman spectra were excited at 488 nm with an argon-ion laser (ILA-120, VEB Carl Zeiss, Jena). Spectra were recorded on a modified spectrometer as in [11] consisting of a double-monochromator with ruled diffraction gratings (blazed at 1.6 μm and used in the 4. order), a M 12 FC 52 photomultiplier with S20-photocathode and electronic photon counting. Sample tubes were glass microcells kept at 20 ± 1°C by a circulating water bath.

The intensity measurements for all resolved lines are peak heights above the background measured with respect to the intensity of the PO_2^- line at 1100 cm^{-1}. All peak heights were determined as in [5].

3. Results and discussion

Fig.1 gives the Raman spectra for rat liver 5 S RNA and $tRNA^{Phe}$ from yeast, respectively, and table 1 lists the normalized peak intensities and origins of the various resolved Raman lines.

The intense line at 813 cm^{-1} originates from the C–O–P–O–C stretching vibration of the phosphodiester group and has been shown to be very sensitive to the degree of order in the ribophosphate backbone in RNA [4]. The value of 1.68 for the intensity ratio I_{813}/I_{1100} points to a very high regularity in the back-

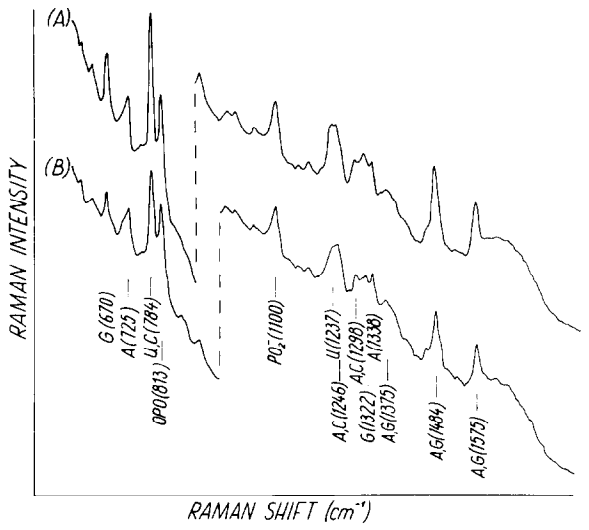

Fig.1. Raman spectra in the region of 600–1800 cm^{-1} of 4% aqueous RNA solutions kept at 20°C: (A) rat liver ribosomal 5 S RNA (5 mM Tris–HCl, pH 7.8, 50 mM KCl, 1.5 mM $MgCl_2$); (B) yeast $tRNA^{Phe}$ (2 mM $MgCl_2$). Instrumental conditions: laser power 200 mW (sample point); slit width 5 cm^{-1}; scan speed 0.2 $cm^{-1} . s^{-1}$; time constant 3 s; sensitivity 10^4 counts/s. Dotted vertical lines indicate change in the background.

Table 1
Frequencies and intensities of some Raman lines of rat liver ribosomal 5 S RNA and yeast $tRNA^{Phe}$

Frequencies (cm^{-1})	Origin of line	Relative intensity	
		$tRNA^{Phe}$	5 S RNA
670	G	0.50	0.75
725	A	0.64	0.62
784	C and U	2.16	2.50
813	–OPO–	1.65	1.68
1100	PO_2^-	1.00	1.00
1237	U	1.36	1.46
1246	C and A	1.59	1.53
1298	C and A	1.19	1.10
1322	G	1.39	1.41
1338	A	1.62	1.26
1375	G and A	1.12	0.92
1484	G and A	2.30	2.48

All values are averages of at least 3 spectra of 2 different batches of 5 S RNA and $tRNA^{Phe}$, respectively, and are within ±5% accuracy. The intensities of the Raman lines were corrected for the different sensitivity of the Raman spectrometer used for these studies and the Spex Raman spectrometer. The values for yeast $tRNA^{Phe}$ are within the accuracy limits of measurement of correction procedure in agreement with the results in [10]

bone of 5 S RNA comparable with yeast $tRNA^{Phe}$ and other tRNAs.

Several other lines of the Raman spectra provide information about the efficiency of base stacking in RNAs. Especially the separated lines at 670 cm^{-1} due to G, at 725 cm^{-1} due to A, and, to some extent, the line at 784 cm^{-1} due to C and U, can be used for this purpose [5]. The intensity of all these lines decreases with increased stacking at a given base concentration, except the line at 670 cm^{-1}, for which the opposite is valid [5,10].

After correction the difference in the G base content between 5 S RNA (32%) and $tRNA^{Phe}$ (29%) the intensity of the Raman line at 670 cm^{-1} for 5 S RNA is higher by 34%. Therefore the G bases are much higher effectively stacked on the average in 5 S RNA than in $tRNA^{Phe}$. The 725 cm^{-1} line in 5 S RNA is after correction for the different A base content more intense by 26%, which indicates a less stacking of A in 5 S RNA than in yeast $tRNA^{Phe}$.

There is at least a qualitative correlation between the stacking efficiency of G and A and the fraction of paired G and A residues, respectively, in the stem regions of RNAs [5]. Therefore, the very high stacking efficiency of G residues indicates that the fraction of paired G bases in the stems of 5 S RNA must be larger than in $tRNA^{Phe}$. Since ~59% of the G residues are paired in the stems in yeast $tRNA^{Phe}$ [5] our Raman data indicate that the fraction of paired G residues in rat liver 5 S RNA should be in the order of 75%. On the contrary, the fraction of paired A bases should be lower than in $tRNA^{Phe}$, that means, ~35% of the A residues should be paired in the stems of the 5 S RNA molecule.

The very high regularity in the backbone of 5 S RNA indicates a high number of base pairs. This fact is supported by the G residues which are relatively uniformly distributed over the molecule and of which a high fraction should be paired according to our Raman data. Two of the most discussed secondary structure models with a large number of base pairs are the cloverleaf model proposed in [6] (LM) and the model in [8] (NT).

The LM model contains 22 GC base pairs (56%) and 10 AU base pairs (45%), respectively. According to our Raman data the cloverleaf model is probably not correct due to the too low amount of GC base pairs and the too high amount of AU base pairs.

The Raman data are more compatible with the base pairing pattern of 5 S RNA of rat liver shown in

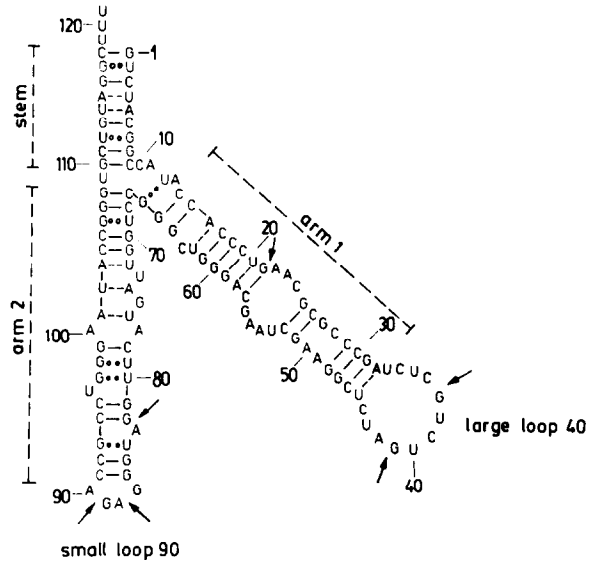

Fig.2. Base pairing pattern of the native form of rat liver ribosomal 5 S RNA. Arrows indicate RNase cleavage points according to [12].

fig.2, which is very similar to the NT model [8] for eukaryotic ribosomal 5 S RNA. It contains 40 base pairs, of which only 8 are AU base pairs (36%) but 25 are GC base pairs (64%). The number of paired A residues is quite consistent with the Raman data. The very high stacking efficiency of G seems to indicate a higher fraction of paired G residues (close to 75%). We assume, however, that a lot of segments of two or more consecutive GC base pairs existing in the stems of the proposed model can produce this large stacking effect.

Beside the Raman data, further support for the proposed model arises from infrared data, model building studies and phylogenetic data [7].

Acknowledgements

We wish to thank Dr W. Pilz for his advice in obtaining Raman spectra, and Miss B. Rösner for skillful technical assistance.

References

[1] Erdmann, V. A. (1976) Prog. Nucleic Acid Res. Mol. Biol. 18, 45–90.
[2] Thomas, G. J. jr and Kyogoku, Y. (1977) in: Vibrational Spectroscopy, Handbook of Practical Spectroscopy (Brame, E. G. jr and Graselli, J. G. eds) vol. 1C, pp. 717–872, Dekker, New York.
[3] Chen, M. C. and Thomas, G. J. jr (1974) Biopolymers 13, 615–626.
[4] Thomas, G. J. jr and Hartman, K. A. (1973) Biochim. Biophys. Acta 312, 311–322.
[5] Chen, M. C., Giege, R., Lord, R. C. and Rich, A. (1978) Biochemistry 17, 3134–3138.
[6] Luoma, G. A. and Marshall, A. G. (1978) J. Mol. Biol. 125, 95–105.
[7] Böhm, S., Fabian, H., Welfle, H. and Bielka, H. (1980) Acta Biol. Med. Germ. 39, K1–K6.
[8] Nishikawa, K. and Takemura, S. (1974) J. Biochem. 76, 935–947.
[9] Behlke, J., Welfle, H., Wendel, I. and Bielka, H. (1980) Acta Biol. Med. Germ. 39, 33–40.
[10] Chen, M. C., Giege, R., Lord, R. C. and Rich, A. (1975) Biochemistry 14, 4385–4391.
[11] Drommert, H., Kleimon, E. and Pilz, W. (1969) Exp. Tech. Phys. 17, 493–498.
[12] Vigne, R. and Jordan, B. R. (1977) J. Mol. Evol. 10, 77–86.