Laser Raman studies of the 5 S rRNA–protein L5 complex of rat liver ribosomes

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The effects of ribosomal protein L5 on the conformation of 5 S rRNA in the 5 S rRNA–protein L5 complex extracted from rat liver ribosomes have been studied by laser Raman spectroscopy. A comparison of the spectra shows small protein-induced conformational changes in the 5 S rRNA, but most of the base-paired regions appear to be present in the complex with protein L5 as well as in the free 5 S rRNA. Furthermore specific interactions between 5 S rRNA and protein L5 are indicated. Cytosine (and/or uracil) residues in single-stranded regions and the N(7) of guanine are engaged in interactions with the protein as suggested by the Raman data.

5 S rRNA *Ribosomal protein L5* *RNA–protein complex* *Raman spectroscopy* *Rat liver*

1. INTRODUCTION

Ribonucleoprotein complexes containing 5 S rRNA have been released both from prokaryotic and eukaryotic ribosomes [1–7]. The 5 S rRNA has been an object of extensive biochemical and physical studies ([8–10] and references within). On the basis of these data and comparative sequence studies [10–16] it seems evident now that both for prokaryotic and eukaryotic 5 S rRNA a generalized secondary structure model with slight modifications for the different species can be constructed which indicates a high base-pairing content of about 65%. In contrast, few studies have been done on 5 S rRNA–protein complexes; until recently they were limited mainly to *Escherichia coli* 5 S rRNA–protein complexes [9,17–22] and yeast 5 S rRNA–protein complex [6,23]. Due to the relative simplicity of the complexes, consisting of a low-M_r RNA and a low-M_r protein component of 2 or 3 proteins in prokaryotes or one protein in eukaryotes [1–7], these complexes are especially suited to studying RNA–protein interactions. The 5 S rRNA–protein interactions may be expected to be representative for ribosomal RNA–protein interactions in general.

Treatment of the 60 S subunit of rat liver ribosomes with EDTA or formaldehyde leads to the release of a 7 S ribonucleoprotein complex [1,2,4,7] between 5 S rRNA and one ribosomal protein. This protein was identified [4] as protein L5 [24].

Raman spectroscopy has provided a useful means for obtaining structural information on nucleic acids (reviewed in [25,26]), including 5 S rRNAs [27–29], and proteins [25,26,30]. The intensities and frequencies of the lines in the Raman spectra of nucleoproteins can be related to the interactions and structures of the different subgroups of both the nucleic acid and protein component.

Here, Raman spectra have been obtained from the 5 S rRNA and the 5 S rRNA–protein L5 complex of rat liver ribosomes. Small protein-induced conformational changes in 5 S rRNA have been found but most of the base-paired regions of 5 S rRNA appear to be present in the complex with protein L5 as well as in the free 5 S rRNA. Fur-

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thermore, specific interactions between RNA and protein are indicated. Candidates for interactions with the protein appear to be cytosine (and/or uracil) residues in single-stranded regions and the N(7) of guanine as well in single-stranded as in double-stranded regions.

2. MATERIALS AND METHODS

The 5 S rRNA–protein L5 complex was extracted from 60 S ribosomal subunits with EDTA according to [1] as detailed in [7]. The complex was dialysed against 5 mM Tris–HCl (pH 7.8), 50 mM KCl, 1.5 mM $MgCl_2$, 5 mM 2-mercaptoethanol and sedimented in a SW 60 Ti rotor for 14 h at 50000 rev./min. The soft pellet was transferred in a Raman cell and centrifuged to remove air bubbles for 1 h at 5000 rev./min. The complex yields about 100 μg/μl using a specific absorption of 13.8 at 260 nm at 1.0 cm pathlength for a solution of 1 μg/μl. The 5 S rRNA was extracted from the complex by phenol/sodium dodecyl sulphate treatment and prepared for the Raman experiments as in [28]. The 5 S rRNA was dialysed against diluted buffers, lyophilized and dissolved in such volumes of twice distilled water that finally solutions of about 40 μg/μl 5 S rRNA in TKM buffer (5 mM Tris–HCl (pH 7.8), 50 mM KCl, 1.5 mM $MgCl_2$) were obtained using a specific absorption of 21.4 at 260 nm at 1.0 cm pathlength for a solution of 1 μg/μl. Raman spectra excited at 488 nm with an argon-ion laser (ILA-120, VEB Carl Zeiss, Jena) were recorded on a Raman spectrometer consisting of a double monochromator GDM 1000 (VEB Carl Zeiss, Jena), a photomultiplier with S20-photocathode and direct current amplification. Sample tubes (glass micro cells) were irradiated with 200–300 mW of laser power (6 cm^{-1} slitwidth) and kept at the indicated temperature by a circulating water bath. Scan speed was 0.2 $cm^{-1}.s^{-1}$ at a time constant of 3 s.

3. RESULTS AND DISCUSSION

Raman spectra of rat liver 5 S rRNA and of the 5 S rRNA–protein L5 complex are shown in fig.1. The prominent Raman lines in the spectra are assigned to residues of RNA or protein, as indicated along the abscissa.

Raman spectra of the protein L5 alone could not be obtained due to its very low solubility. L5 aggregates in TKM-buffer already at much lower concentrations than necessary for Raman studies. The assignment of the Raman lines of the protein L5 arising from vibrations of the peptide backbone and specific amino acid residues, was performed on the basis of [30]. The position of amide I (1667 cm^{-1}) and the absence of a peak at 1235 cm^{-1} (fig.1b) suggest a mainly unordered structure of protein L5 in the complex. Infrared spectra and circular dichroic spectra indicate a mainly unordered structure and a portion of about 25% α-helical structure of protein L5 in the 5 S rRNA–protein L5 complex (unpublished).

A pair of Raman lines near 830 cm^{-1} and 850 cm^{-1} is the so-called 'tyrosine doublet'. Their ratio of intensities $R_{Tyr} = I_{850}/I_{830}$ has been correlated with the environment and state of hydrogen bonding of the OH group of Tyr [31]. The estimation of R_{Tyr} for the 5 S rRNA–protein L5 complex is difficult because the tyrosine lines near 830 cm^{-1} and 854 cm^{-1} are influenced by strong lines from the RNA. Nevertheless the Raman line at 854 cm^{-1} is pronounced and its intensity greatly exceeds the intensity of the lower frequency component near 830 cm^{-1} ($R_{Tyr} \sim 2.5$). According to [31], such a

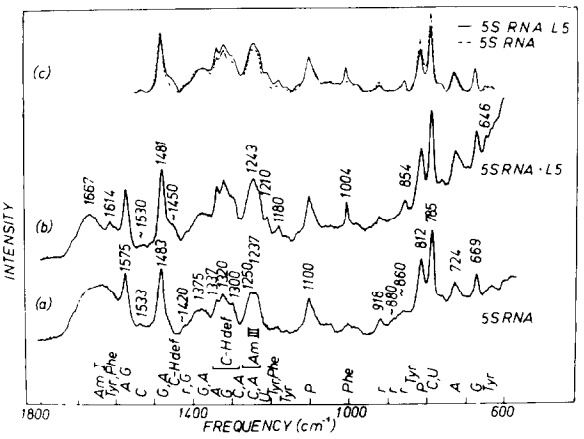

Fig.1. Raman spectra in the region of 600–1800 cm^{-1} of: (a) 5 S rRNA in TKM buffer at 20°C; and (b) 5 S rRNA–protein L5 complex pelleted from TKM buffer at 15°C. In (c) spectra (a) and (b) are retraced over a common base line with intensities normalized to the 1100 cm^{-1} line. *Abbreviations:* A, adenine; U, uracil; G, guanine; C, cytosine; P, phosphate group; r, ribose; Am I, amide I; Am III, amide III; C–H def, C–H deformation; Phe, phenylalanine; Tyr, tyrosine.

value for R_{Tyr} indicates that most tyrosines should be acceptors in strong hydrogen bonds with highly positive donor groups. Potential candidates for proton donating groups are $-NH_3^+$ (lysine) and $=NH_2^+$ (arginine) groups in protein L5 but an influence of RNA–protein interactions on R_{Tyr} can not be excluded.

Since the Raman scattering per nucleotide residue is substantially more intense than that per amino acid residue, the spectrum of the 5 S rRNA–protein L5 complex (fig.1b) is dominated for the most part by Raman scattering of the 5 S rRNA. Comparison with the protein-free 5 S rRNA (fig.1a) [28] shows noticeable differences especially concerning the intense Raman lines at 785 cm^{-1} (C,U), 812 cm^{-1} (C–O–P–O–C), and 1483 cm^{-1} (G,A) (fig.1c). While the intensity of these 3 lines significantly decreases, the intensity of the adenine line at 724 cm^{-1} increases slightly and coincides with the intensity for free 5 S rRNA at 35°C (not shown). The intensity of the guanine line at 669 cm^{-1} remains the same when 5 S rRNA is complexed with protein L5. The Raman line intensities of the 5 S rRNA–protein L5 complex significantly exceed those of protein-free 5 S rRNA in the region of 1004 cm^{-1}, 1200–1350 cm^{-1}, and 1450 cm^{-1}, but the differences can be attributed to the overlapping by Raman lines of protein L5.

By analogy with model compounds [25] the constant intensity of the Raman lines at 669 cm^{-1} (ring stretching vibration of G) indicates a comparable stacking of G residues in the free 5 S rRNA and in 5 S rRNA complexed with protein L5 while the intensity at 724 cm^{-1} (ring stretching vibration of A) points to a protein-induced decreased stacking of A residues. According to studies on tRNAs [29] there is at least a qualitative correlation between the intensity of the Raman lines at 669 cm^{-1} and 724 cm^{-1} and the fraction of paired G and A residues, respectively, in the stem regions of RNAs. We suggest that the constant stacking of guanine (I_{669}) and decreased stacking of adenine (I_{724}) for 5 S rRNA in the complex in comparison to the protein-free 5 S rRNA correlates with the melting of weak secondary/tertiary structure elements containing mainly A residues. Because of base-pairing, the stacking of the C residues should correlate to some extent with that of G, and stacking of U with A, and on the basis of the intensities of the lines at 669 cm^{-1} and 724 cm^{-1} one would expect a higher intensity of the Raman line at 785 cm^{-1}. But the binding of protein L5 to 5 S rRNA produces a decrease of 20% in the intensity of the lines both at 785 cm^{-1} (ring stretching vibration of C and U) and 812 cm^{-1} (C–O–P–O–C stretching vibration of the phosphodiester group). These decreased intensities of both lines suggest a significantly increased stacking of C and/or U, on the one hand, but a pronounced rearrangement of the ribophosphate backbone, on the other. Since there is a small overlap between these two lines, a decrease of intensity by 20% at 785 cm^{-1} diminishes the intensity at 812 cm^{-1} to some extent too and vice versa, but never to the same degree. Alterations of the RNA backbone are further indicated by intensity changes in weak Raman lines near 880 cm^{-1} and 918 cm^{-1}, which have been assigned to ribose residues [32]. The Raman line at 880 cm^{-1} was found sensitive to protein extraction also in Raman studies of ribosomes [32] but investigations of model compounds are necessary as the basis for a more detailed interpretation.

While the intensities of the Raman lines at 669 cm^{-1} (G) and 1375 cm^{-1} (G,A) are the same in the protein-free and complexed 5 S rRNA, the intensity of the line at 1483 cm^{-1} is diminished and slightly, but significantly shifted to 1481 cm^{-1} (fig.1c). The 1483 cm^{-1} Raman line is assigned predominately to guanine with a slight contribution from adenine [25,33].

Summarizing, it can be stated that intensities of some Raman lines which are assigned to one nucleotide (e.g., guanine) are changed but others are not in the complex in comparison to the free 5 S rRNA. Furthermore, intensities of Raman lines characteristic for C and U indicate an increased stacking, but lines characteristic for A and G indicate a decreased and comparable stacking, respectively.

We never observed similar characteristic changes in the Raman spectra of rat liver 5 S rRNA solutions changing temperature and/or buffer conditions (unpublished). Such spectral effects should be interpreted in terms of specific interactions between distinct chemical groups of the protein and the nucleotides. First Raman evidence for such interactions has been found in DNA–protein complexes and was used to localize specific DNA–protein interactions [33,34].

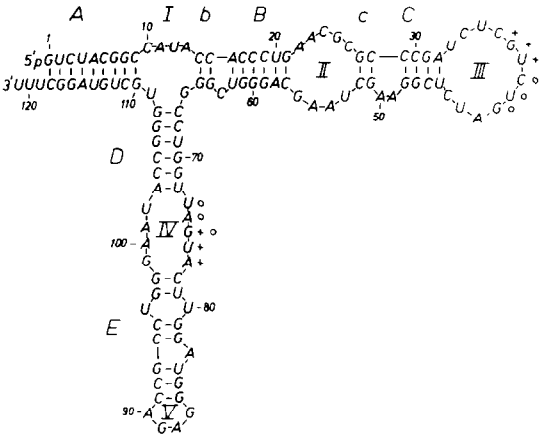

Fig.2. Proposed base-pairing scheme of rat liver 5 S rRNA. The model is adapted to the proposed generalized version of eukaryotic 5 S rRNAs [10] and illustrates (——) secondary structure base pairs and the proposed tertiary interactions: (+) parallel base pairing between C_{36}–U_{38} and G_{75}–A_{77}; (○) antiparallel base pairing between C_{39}–G_{41} and U_{73}–G_{75}; (···) between A_{11}–U_{109} and U_{12}–G_{77}; (A–E) helices A–E; (b,c) extended parts of helices B and C; (I–V) loop regions.

Fig.2 shows a base-pairing scheme for rat liver 5 S rRNA based on infrared [35] and Raman data [28], biochemical studies [36,37] and a comparative sequence analysis of eukaryotic 5 S rRNAs [10,11]. The model modifies the first proposed generalized base-pairing scheme for eukaryotic 5 S rRNAs [38] in few positions only and is very similar to schemes in [9,12,13,15,16]. Tertiary base-pairing interactions between nucleotides located in the large loop III and loop IV (marked by rings and crosses in fig.2) and between nucleotides in loop I (marked by dots in fig.2) have been proposed [10,11,36]. Direct evidence for tertiary interactions in eukaryotic 5 S rRNAs has not been published but X-ray scattering data have shown that a Y-like structure is not an adequate model for rat liver 5 S rRNA in TKM buffer. The X-ray scattering data are more compatible with a compact spatial structural model which should include tertiary interactions [39]. The Raman data indicate, as discussed above, that most of the base-paired regions of the 5 S rRNA appear to be present in the complex with protein L5 as well as in the free 5 S rRNA. The most likely regions which could be responsible for the observed diminished stacking of A in the 5 S rRNA–protein L5 complex in comparison to the free 5 S rRNA are the melting of the proposed tertiary interactions and/or a stacking rearrangement of single-stranded A residues; e.g., A_{49}, A_{50} and the A residues in loop II. The Raman line near 1483 cm^{-1} results mainly from N(7) = C(8) stretching vibrations of guanine [33] and is greatly affected by molecular interactions with N(7) [33,34]. The diminished intensity of the Raman line near 1481 cm^{-1} in the spectrum of the 5 S rRNA–protein L5 complex in comparison to the free 5 S rRNA could be an indication of hydrogen bonding between suitable side-chain group(s) of protein L5 (e.g., arginine) and the N(7) of guanine. The guanine residues could be located both in double-stranded and single-stranded regions. In [19], the reagent dimethyl sulfate was used to probe interactions at N(7) of guanine in an *E. coli* 5 S rRNA–protein L18 complex. It was shown that the N(7) position of 8 guanines located both in double-stranded and single-stranded regions, which were accessible to methylation in *E. coli* free 5 S rRNA, were protected in the 5 S rRNA–protein L18 complex.

Raman studies on complexes of polyribocytidylic acid (poly(rC)) and polyriboadenylic acid (poly(rA)) with poly(L-lysine) (PLL) and poly(L-arginine) (PLA) as model compounds [32,40,41] offer a basis for the identification of further peptide–nucleotide interactions in RNA–protein complexes. However, Raman data for polyuridylic acid, poly(A) × poly(U) complexes or poly(G) × poly(C) complexes with polypeptides or poly(rC) with polypeptides other than PLL and PLA have not been published; therefore, the influence of such interactions on the intensity of the Raman lines at 785 cm^{-1} and 812 cm^{-1} is unknown. Among the complexes studied up to now the poly(rC) × PLA complex [41] and the 5 S rRNA–protein L5 complex (fig.1c) show similar spectra characteristics. The Raman spectra of the complexes exhibit a diminished intensity of the Raman lines at 785 cm^{-1} and 812 cm^{-1} in comparison to the spectra of free poly(rC) and free 5 S rRNA, respectively. This indicates that the kind of peptide–nucleotide interactions occurring in the poly(rC) × PLA complex could be at least one further possibility of interactions between 5 S rRNA

and protein L5. Support for the participation of C residues comes from changes of the weak Raman line near 1530 cm^{-1} (fig.1a,b) (assigned only to C), but an influence on the intensities of the Raman lines at 785 cm^{-1} and 812 cm^{-1} due to RNA–protein interactions including U residues can not be excluded. Looking at the generalized base-pairing schemes for 5 S rRNAs most of single-stranded cytosines (and uracils) are located in the large loop III [10]. The importance of nucleotides located in this large loop for complex formation [21] and at least a protection of some of these nucleotides against ribonuclease digestion by binding of ribosomal proteins were found in the *E. coli* 5 S rRNA–protein L18 complex [19,20,22] and the 5 S rRNA–protein (L5 + L18 + L25) complex [22]. Possibly any RNA–protein interactions with participation of cytosines (and uracils) located in the large loop around position 40 do exist in the 5 S rRNA–protein L5 complex of rat liver ribosomes as well as in 5 S rRNA–protein complexes from other species.

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