PROTON NUCLEAR MAGNETIC RESONANCE STUDY OF THE RIBOSOMAL PROTEIN L7/L12 IN SITU

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1. Introduction

The ribosome represents a multicomponent ribonucleoprotein complex; this greatly impedes the use of physical methods for the study of its components in situ. Therefore isolated ribosomal components are mainly studied. It is important to prove the identity of properties of the isolated components and those within intact particles.

One of the most interesting components of the prokaryotic ribosome is the protein complex consisting of 4 copies of protein L7/L12 and 1 copy of protein L10 [1]. Taking into account the remarkable position of protein L7/L12 on the ribosome [2] and facility of its removal without damaging the particles [3], we used proton NMR spectroscopy for comparative studies of *Escherichia coli* ribosomes containing the L7/L12 tetramer and those devoid of it.

Protein L7/L12 in situ makes a considerable contribution into the PMR spectrum of *E. coli* ribosomes. A comparative analysis of the PMR spectra allows us to assert that:

(1) Protein L7/L12 within the ribosome has the structure similar to that in solution;
(2) Just as in the isolated complex with protein L10 [4], protein L7/L12 is bound to the ribosome with its N-terminal part;
(3) Protein L7/L12, both in the isolated 50 S subunit and in the complete 70 S ribosome, has a considerable independent mobility.

2. Materials and methods

Ribosomes and their subunits were obtained as in [5,6]. Protein L7/L12 was removed from 50 S subunits by 50% ethanol with NH_4Cl [3].

70 S ribosomes and their subunits were controlled by velocity sedimentation on the UCA-10 model ultracentrifuge (USSR) with ultraviolet recording at 260 nm.

PMR spectra were recorded at 22°C in WH-360 spectrometer (Bruker, FRG) in the pulse mode. The sweepwidth was 12 000 Hz, the interval between pulses was 1.2 s. Spectra were recorded in standard 5 mm tubes at 6–30 mg/ml using 2,2-dimethyl-2-silapentane sodium sulphonate as an internal standard. To suppress the broad component, we used homonuclear-gated decoupling [7]. A pre-irradiation pulse of 1 s was applied at the frequency corresponding to the HDO signal.

Throughout the study we used the buffer solution consisting of 1 mM Na-phosphate (pH 7.4), 1–10 mM $MgCl_2$ and 175 mM KCl in D_2O.

3. Results and discussion

3.1. *PMR spectrum of the 30 S subunit*

The spectrum of the small subunit is represented in fig.1A. As it may be expected for systems with such a large M_r-value (of the order of 10^6), very broad signals >1 kHz wide are observed in the spectrum. In addition, there is a large number of rather narrow lines <60 Hz wide in the high-field region of the spectrum. In the low-field region there is only one narrow signal with the center at 7.25 ppm whose intensity is small. An earlier study on viruses and 70 S ribosomes has shown that narrow signals are due to protein, but not RNA, protons [8,9]. Accordingly, narrow signals in our spectrum can be attributed to protons of protein amino acid residues. The presence of narrow signals in the 30 S subunit spectrum sug-

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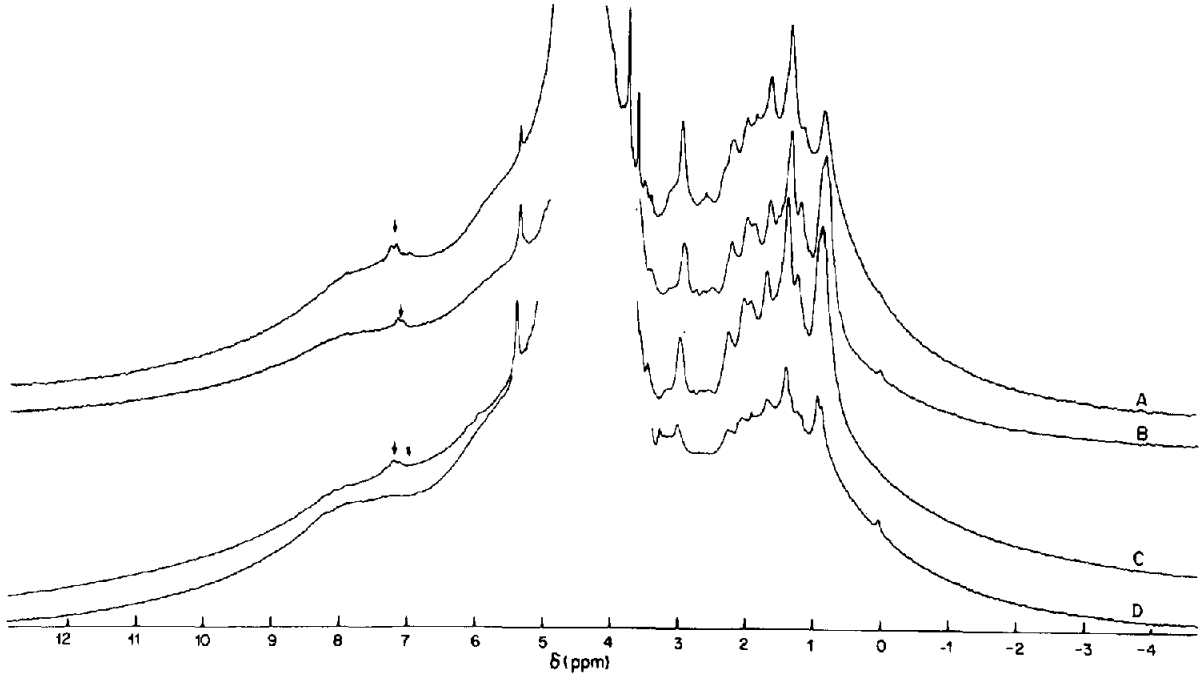

Fig.1. 360 MHz proton NMR spectra of ribosomal subparticles at 22°C: (A) 30 S subparticles, 7 mg/ml, 5700 transients; (B) 50 S subparticles, 10 mg/ml, 3100 transients; (C) 70 S ribosomes, 10 mg/ml, 3000 transients; (D) 50 S subparticles without protein L7, 5.3 mg/ml, 11 000 transients.

gests a relatively high internal mobility of a part of amino acid residues of some proteins within 30 S subunits. We cannot yet say whether these signals can be attributed to an individual protein or a group of proteins of the 30 S subunit. In the aliphatic region, the intensity of the narrow component is <15%. This result seems to reflect the rigid packing of most proteins within the 30 S subunit.

30 S subunit spectra in 1 mM and 10 mM Mg^{2+} does not display considerable differences over 6–30 mg/ml.

3.2. *Spectra of the 50 S subunit and 70 S ribosome*

PMR spectra of 50 S and 70 S particles, just as those of 30 S subunits, contain broad and narrow lines (fig.1B,C). However, narrow lines from protons of 50 S and 70 S particles are considerably more intensive than in the spectrum of 30 S subunits and make up >20% of the overall intensity of protein proton signals. A comparison of the spectra of 30 S, 50 S and 70 S particles (fig.1A–C) shows that the narrow lines in the spectrum of 70 S particles are very similar to those in the spectrum of 50 S subunits (see also the spectra of 50 S and 70 S particles in fig.2C,D). Thus, it can be concluded that the main contribution into the narrow signals of the spectrum of 70 S particles is made by proteins which are also contained in the 50 S subunits.

In contrast [10], the spectrum of 70 S particles over 6–30 mg/ml does not contain overt signals which would not be present in the spectra of 30 S and 50 S subunits, i.e., in the first approximation, the spectrum of 70 S ribosome is a sum of spectra of its 30 S and 50 S subunits. Therefore, the conclusion made in [10] on the increase of mobility of protons of some proteins upon association of 30 S and 50 S subunits is not corroborated.

3.3. *Spectra of the $(L7)_4$–L10 complex and the 50 S subunits deprived of protein L7*

The removal of proteins L7/L12 results in a sharp decrease of intensity of narrow lines in the PMR spectrum of 50 S subunits (cf. fig.1B,D). This strongly suggests that the main contribution in the narrow component of the 50 S subunit spectrum is made by protein L7/L12. Since the PMR spectrum of 70 S par-

ticles is very similar to that of the intact 50 S subunits it can be assumed that the most mobile component of 70 S particles and 50 S subunits is the protein L7/L12 complex.

The same conclusion can be made from the analysis of PMR spectra of the isolated protein L7, the pentamer complex $(L7)_4$–L10, and the 50 S and 70 S particles (fig.2). For reasons of clarity, only narrow lines have been isolated (by homonuclear-gated decoupling [7]) in the spectra of 50 S and 70 S particles. The spectrum of the $(L7)_4$–L10 complex does not contain signals which were absent from the spectrum of protein L7 (fig.2A,B). There is a slight change of the signal structure at 0.91 ppm and that of the ratio of signal intensities at 0.91 and 1.41 ppm.

As shown in [4], changes in the aromatic region of the PMR spectrum of protein L7 are caused by the interaction of the N-terminal part of the L7 molecule with protein L10. In the pentamer complex $(L7)_4$–L10 the signal from residue Phe 30 of protein L7 is shifted to the higher field as a result of interaction of this residue with L10. A somewhat larger width of all signals in the spectrum of the complex $(L7)_4$–L10 can be due to a higher M_r-value of the complex as

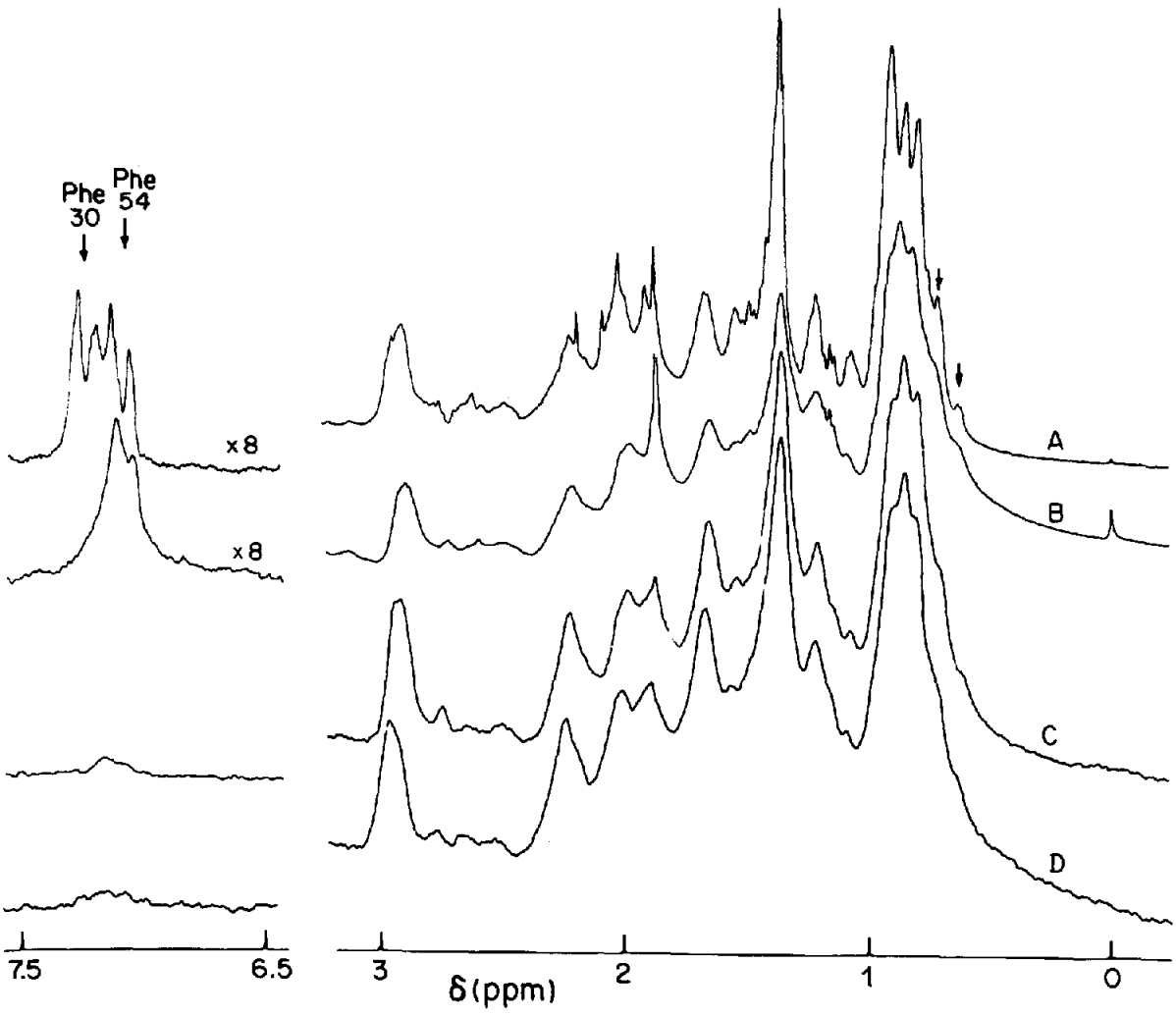

Fig.2. 360 MHz proton NMR spectra at 22°C: (A) protein L7 in D_2O buffer containing 300 mM KCl, 20 mM Na(P), pH 6.6, 3 mg/ml; (B) $(L7)_4$–L10 complex, 2 mg/ml in the same buffer as in (A); (C) 50 S subparticles, 10 mg/ml; (D) 70 S ribosomes, 10 mg/ml. 50 S and 70 S spectra were obtained in a gated decoupling mode of the Bruker WH-360. The saturating pulse was applied for 1 s at a frequency f_2 corresponding to the HDO signal.

compared with that of protein L7. Signals from residues Phe and Tyr in protein L10 are not observed in the spectrum of the complex. Thus, the main contribution into the spectrum of the complex is made by 4 copies of protein L7. This fact can be due to a lower molar amount of protein L10 in the complex and, probably, to a larger linewidth of protein L10 resonances in the spectrum of the complex than in the spectrum of L10 in the free state.

In the spectrum of complex $(L7)_4$–L10 (fig.2B) there are a number of signals in the extreme high field; their chemical shifts are the same as in the spectrum of protein L7 (0.64 and 0.74 ppm). Therefore it can be assumed that protein L7 within the complex, as well as in the free state, has a globular structure including residues from 50–120 [11]. This has been confirmed by X-ray analysis data [12].

A comparison of spectra of the $(L7)_4$–L10 complex (fig.2B) over 0–4 ppm with the spectra of 50 S and 70 S particles (fig.2C,D) shows their astonishing coincidence to minute detail. In the spectrum of 50 S and 70 S particles there are the same high-field (0.64 and 0.73 ppm) resonances as in the spectra of protein L7 and complex $(L7)_4$–L10. In the aromatic region of the spectrum of 50 S subparticles there is a signal at 7.15 ppm which is absent from the spectra of the particles devoid of protein L7 (fig.1D). It is natural to presume that the signal at 7.15 ppm in the spectrum of 50 S subparticles as well as in the spectrum of the complex $(L7)_4$–L10 [4] corresponds to residues Phe 30 and Phe 54 of protein L7. Hence we can assert that the interaction of protein L7 with protein L10 in the complex and in 50 S particles involves the N-terminal part of L7 whereas the C-terminal part is rather independent.

Since the spectra of the complex $(L7)_4$–L10, the 50 S and 70 S particles are similar, it can be concluded that protein L7 does not participate directly in the association of 30 S and 50 S subunits (at least in the absence of a template, substrates and other factors of translation).

4. Conclusion

Thus, the above data lead to the following conclusions:

(1) Most proteins of the ribosome are rigidly fixed within the particles;
(2) At the same time, protein L7/L12 in situ has a rather great mobility which produces the corresponding narrow lines in the PMR spectra of 50 S and 70 S particles;
(3) Protein L7/L12, both in the $(L7/L12)_4$–L10 complex and within the ribosome, has the same structure as in the free state;
(4) Protein L7/L12 does not seem to take part in the association of ribosomal subunits and does not change its structure and mobility upon association, at least under non-translating conditions.

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