PROTON NMR OBSERVATION OF THE *ESCHERICHIA COLI* RIBOSOME

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1. Introduction

Proton magnetic resonance has proven to be a very fruitful tool for the study of both the structure and dynamics of small to moderately sized biological macromolecules. Some ^{1}H NMR spectra of the *Escherichia coli* ribosomal proteins, most of which are <20 000 M_r, have appeared [1,2]. The preliminary analysis of these spectra reveals that the ribosomal proteins in solution are mostly unfolded, only 6 of the 24 studied exhibiting any well-defined tertiary structure. Detailed investigation by NMR methods may be expected to yield important information on the protein chemistry of the ribosomal components but may not be of direct consequence to an understanding of the intact organelle for two reasons:

(i) The structure of the proteins in solution and in place in the ribosome may be entirely different;
(ii) The individual ribosomal proteins are almost certainly not the units of function in the organelle and have no biological activity when not part of an intact ribosome.

One might not expect the ribosome to reveal a resolved proton NMR spectrum due both to the many thousands of potential resonances and to the slow rotational correlation time of the structure. However, here *E. coli* ribosomes are shown to display a partially resolved ^{1}H spectrum suggestive of internal mobility within the organelle.

2. Materials and methods

Escherichia coli ribosomes and subunits were purified and prepared for NMR spectroscopy as in [3]. The buffer used in all experiments was 1.0 mM potassium phosphate (pH 7.4), 10 mM $MgCl_2$ and 175 mM KCl in D_2O. Spectra were acquired on a Bruker HX-270 instrument with quadrature detection.

3. Results and discussion

In a study of the interaction of chloramphenicol with *E. coli* ribosomes [3], we were observing the proton resonances of the drug present in large molar excess over the ribosomal receptor. In investigating the concentration dependence of the drug spectrum, we noticed the existence of resonances not accountable for by chloramphenicol, when attempting to acquire spectra at low drug/ribosome ratios. We reasoned that these resonances could be from the ribosome itself and consequently obtained spectra like that shown in fig.1A when observing the 70 S ribosome alone in solution at 20°C. This spectrum reveals a number of resolved, albeit broad, resonances on top of an envelope in the 0–5 ppm range (aliphatic). No aromatic resonances are observed at 20°C, although this region does show resolved resonances at 70°C where the ribosome is unfolded (fig.1B).

These observations are interesting for several reasons. First, the ribosome is so large (2.5×10^6 M_r) that, were it rigid, no useful spectral data could be obtained. Thus the data already suggest some degree of internal flexibility in the ribosome; that is, there are groups within the ribosome with a correlation time faster than the tumbling of the overall organelle in solution. The relationship between the linewidth, relaxation time and correlation time of the system can be expressed as:

$$\frac{1}{T_2} = \pi W_{1/2} = K \tau_r$$

where T_2 is the spin–spin relaxation time, $W_{1/2}$ the

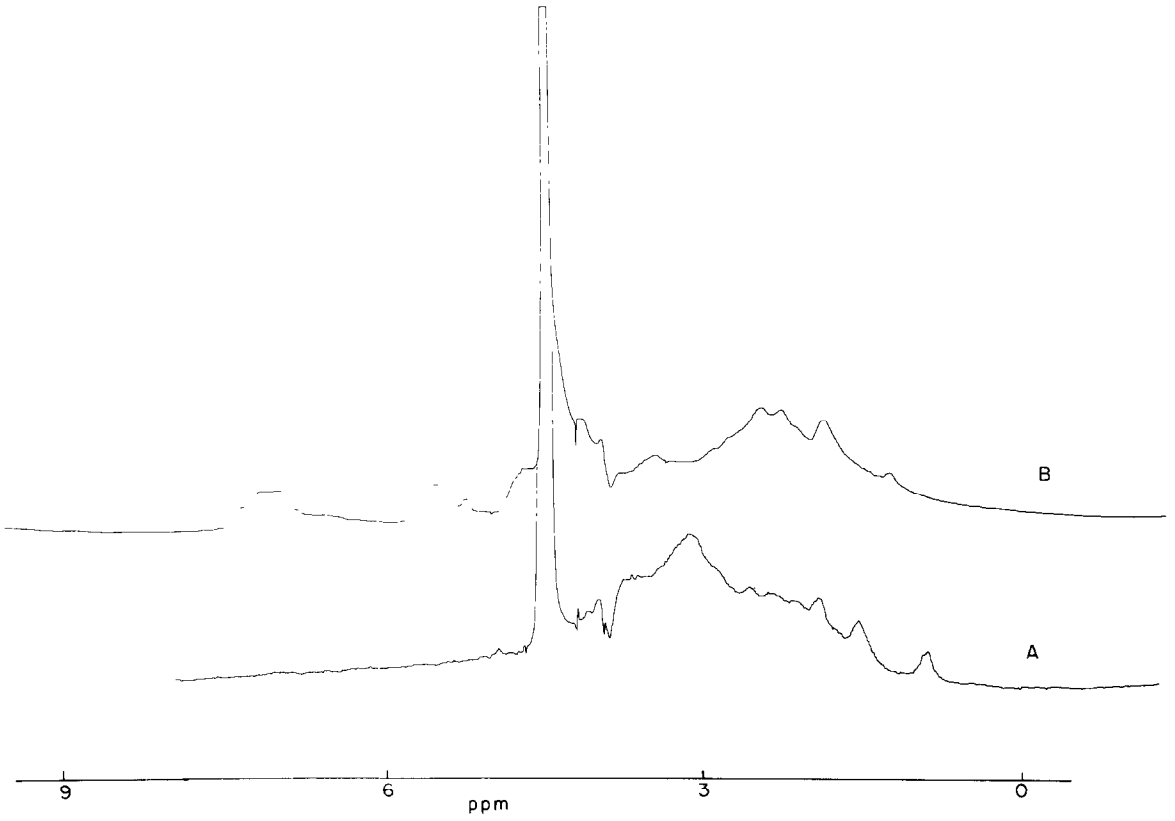

Fig.1. Proton NMR spectra of *E. coli* 70 S ribosomes at 20°C (A) and 70°C (B). The large resonance is residual water in the D_2O sample. The ribosome concentration is 1.25×10^{-5} M. A sweepwidth of 3000 Hz was sampled with 1000 free induction decays.

linewidth at half-height, K a collection of constants and τ_r the rotational correlation time. Taking the measured value of τ_r for the 70 S ribosome [5] as 2.5 μs and assuming reasonable values for K [4], we calculate that the linewidth for proton resonances to be several kHz. Since the actual maximum linewidth of the narrow resonances in the ribosome spectra are on the order of 100 Hz, the correlation time for motions within the ribosome must be 1–2 orders of magnitude faster than the overall rotational correlation time. A similar conclusion concerning tobacco mosaic virus has been reached [4] from ^{13}C and ^{1}H NMR studies. Thus one is tempted to generalize that assemblies of biological macromolecules are not necessarily rigid structures and that individual domains may exist with independent mobilities.

Another notable point about these spectra concerns the lack of resolution in the aromatic region at room temperature. This suggests to us that some of the more hydrophobic regions within the ribosome have a longer correlation time and are thus relatively more ordered. In turn, this implies that there are different rates of relaxation among the different spin systems of the ribosomal structure. It had been pointed out [6] that spin diffusion in polymeric systems may limit the ability to gain information on differential mobility within the system. This example shows that it is possible to utilize NMR on macromolecular assemblies and that spin diffusion effects, if present, do not necessarily dominate the relaxation mechanism.

The major problem with these proton spectra of ribosomes lies in the assignment of resonances. Clearly the resolved resonances in the spectra in fig.1 do not represent single protons, but rather large groups of protons in magnetically similar environments. It is possible to further improve the resolution in these spectra by computer manipulation of the signals and thereby make some assignments to the ribosomal subunits. The spectra in fig.2 were obtained by trapezoidal multiplication of the original free induction

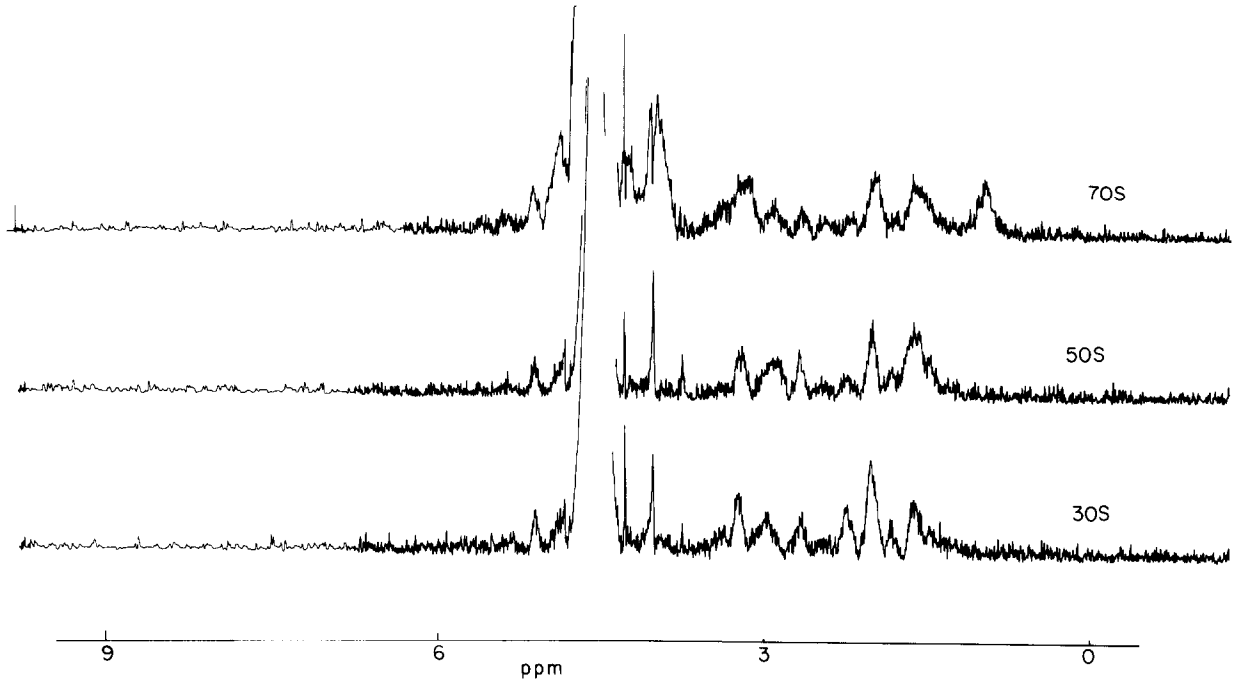

Fig.2. 30 S, 50 S and 70 S ribosome spectra plotted after trapezoidal multiplication of the accumulated free induction decays. This manipulation was accomplished using a Bruker BNC-12 computer equipped with Nicolet software. The TM parameters were T_1 = 50 and T_2 = 10.

decay so as to remove the broad components of the spectrum and selectively emphasize the narrower ones. This is essentially the same as convolution difference spectroscopy [7]. It is apparent from these spectra that some of the resonances are due to either the 30 S or 50 S subunit or are common to both, while others arise from the interaction of the two subunits to form the 70 S ribosome.

Another aid in assignment of the resonances is to acquire spectra of the isolated ribosomal RNA separate from the total ribosomal proteins. In doing this experiment (not shown) we found that the RNA shows no resolvable spectrum at 20°C while the mixture of ~50 ribosomal proteins portrays a spectrum with numerous resonances, particularly in the aliphatic region where the intact ribosome has a spectrum as well. This result suggests that the internal motion which leads to the 70 S spectrum is present largely in the protein neighborhoods of the ribosome, although we recognize that separation of the RNA and protein moieties may destroy the structure responsible for the resonances seen in intact ribosomes.

Another approach to the problem of assignment has been to hydrolyze the 70 S ribosome into nucleotides and amino acids, using a mixture of nucleases and proteases. All the higher order structural constraints are released and each proton should be in a relatively mobile environment. ^{1}H NMR spectra of such mixtures reveal the expected complexity from a variety of monomeric or oligomeric units in solution. By comparing the integrated total intensities of digested versus intact ribosomes we can tentatively conclude that ~75% of the protons seen in the monomers are so immobilized that they do not contribute to the spectrum of the functional organelle. Thus at this level we can divide the ribosome into at least two kinds of neighborhoods with differing degrees of internal motion:

(1) One component (~25%) has high to moderate flexibility and leads to the broad envelope and resolved resonances seen in fig.1;
(2) The second component (~75%) is relatively rigid and proton resonances from these regions are so broad that they do not contribute to the ^{1}H NMR spectrum.

High resolution NMR of the ribosome cannot be

expected at this stage to yield the detailed kinds of information obtained with small proteins. Rather, we are looking to identify and characterize internal mobility within this assembly of macromolecules. This communication in conjunction with ^{31}P [8], ^{13}C [9] NMR and spin labeling ESR [10] studies demonstrate that the ribosome possesses a variety of kinds and time scales of motion and raise the intriguing possibility that such motion may be essential to the correct functioning of the ribosome in protein synthesis.

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