PROTON MAGNETIC RESONANCE STUDIES TO COMPARE *ESCHERICHIA COLI* RIBOSOMAL PROTEINS PREPARED BY TWO DIFFERENT METHODS

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1. Introduction

The ribosomal proteins of *Escherichia coli* have been fractionated and purified by a variety of procedures (review [1,2]) all of which used denaturing conditions such as high concentrations of urea and low pH. Recently, a method has been developed for the purification of ribosomal proteins which avoids such denaturing conditions [3,4]. The proteins obtained in this manner behave differently from the previously prepared proteins in that they are more soluble at high ionic strength while their counterparts are more soluble at low ionic strength. This behaviour is interpreted as an indication of a more 'native' structure being preserved in the proteins prepared by this new method.

Methods such as hydrodynamic studies [5–11], neutron scattering [12,13] and low-angle X-ray diffraction [7,9,11,14–17] have been used to provide data on the shape of individual ribosomal proteins. High resolution proton magnetic resonance (PMR) supplies detailed information about specific interactions between amino acid side chains due to tertiary folding of the protein molecule. This method has been recently used to study protein S4 from the 30 S subunit [18] and many of the other ribosomal proteins [19] which were prepared by denaturing procedures [20,21]. With a few exceptions most of the proteins studied were found to have very little tertiary structure.

Proton magnetic resonance was therefore employed to analyze and compare proteins made by both the denaturing method (acid/urea proteins) and by the new procedure (salt extracted proteins). With the exception of proteins S20 and L27, which showed very few structural features, salt extracted proteins S8, S16, L1, L6, L11, L25 and L30 gave spectra, significantly more complex than those found for the corresponding acid/urea proteins. This suggests that they contain more specific tertiary structure than their acid/urea extracted counterparts. Salt-extracted S14, S17 and the complex of S13–S19 [22] were also studied and all showed complex spectra.

2. Materials and methods

Acid/urea proteins were extracted from the ribosomal subunits with 66% acetic acid in the presence of 0.37 M $MgCl_2$ [23]. They were fractionated by CM-cellulose chromatography in 6 M urea, followed by gel filtration on Sephadex G-100 in 15% acetic acid [20,21]. Protein L11 was provided by M. Dognin and the remainder of the acid/urea proteins by Dr H. G. Wittmann.

Salt-extracted proteins were obtained by a stepwise LiCl extraction of the subunits followed by chromatography on CM-Sephadex C-25 using LiCl gradients. Further purification was obtained by gel filtration on Sephadex G-100 at high ionic strength. In this method

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denaturing conditions such as the use of urea, low pH and lyophilization were avoided and the proteins were concentrated by gentle methods [3,4]. The identity and purity of the proteins were established by two-dimensional gel electrophoresis [24–26] and by one-dimensional slab gel electrophoresis in the presence of dodecylsulphate [27].

Acid/urea proteins were available in lyophilized form and were dissolved in ^2H_2O at concentrations of 2–5 mg/ml. Usually concentrated potassium phosphate buffer, pH 7.0, was added to a final concentration of 0.01 M. Any precipitate which formed after addition of the buffer was removed by centrifugation.

Salt extracted proteins were concentrated to 1–5 mg/ml by dialysis in Spectrapor 3 or 6 dialysis tubing (obtained from Spectrum Medical Industries Inc., Los Angeles; molecular weight cut-off of 3500 and 2000, respectively) against dry Sephadex G-150. They were then dialysed against 0.35 M KCl, 0.01 M potassium phosphate pH 7.0, 5×10^{-4} M dithioerythritol, 1×10^{-4} M benzamidine in ^2H_2O. In some cases the phosphate concentration was raised to 0.05 M which improved the solubility of some of the ribosomal proteins.

Protein solutions were centrifuged and transferred into standard 5 mm NMR tubes. Spectra were recorded at 270 MHz on a Bruker WH 270 magnetic resonance spectrometer operating in Fourier Transform mode, using a pulse-length of 12 μs and data collection over 0.5 s for each pulse. Spectra were obtained at 20°C over a period of 4–12 h which was equivalent to between 10 000 and 40 000 transients. The free induction decay pattern was multiplied by an exponential function equivalent to line broadening of approx. 2 Hz. Chemical shifts were measured relative to sodium 2.2, dimethyl-2-silapentane sulphonate (DSS). After the PMR spectrum had been recorded the proteins were checked for proteolytic degradation by slab gel electrophoresis in the presence of dodecylsulphate [27]. Protein concentrations were determined by a nitrogen assay [28].

3. Results and discussion

All of the salt extracted protein preparations contained variable amounts of contaminants introduced mainly during the concentration step employing Sephadex G-150. These could not be removed by subsequent dialysis and gave strong PMR signals. Control experiments using a buffer solution concentrated in a similar manner showed that the signals from the contaminants were confined to the 3.5–4.5 ppm region For this reason only the low-field (aromatic amino acids) and the extreme high-field (apolar amino acids) regions of the spectra are presented here. These are the main areas of interest in this study and are not affected by the signals from the contaminants. The concentrations of the salt-extracted 30 S ribosomal proteins were, in general, somewhat lower than those of the 50 S proteins. This caused a lower signal/noise ratio in some of the 30 S protein spectra.

With the exception of protein S20, all of the salt extracted 30S proteins tested exhibited ring-current shifted resonances in the high-field apolar methyl region, accompanied by perturbations of the resonances in the low-field aromatic region (figs 1 and 2). These arise as the result of specific tertiary folding in the proteins whereby apolar methyl resonance peaks are shifted from their normal positions by ring-current fields induced in the π-electron systems of aromatic residues which, by virtue of the folding, are constrained to lie in close proximity to the methyl side chains. Of the corresponding acid/urea proteins which could be compared, only S16 exhibited any evidence for structure [19], and while the pattern of ring-current shifts is identical, salt extracted S16 showed a much enhanced peak intensity suggesting that the latter preparation contained a far higher proportion of 'native' structure (fig.1).

Figure 3 shows the 270 MHz PMR spectra of proteins L1, L6, L11, L25, L27 and L30 for both acid/urea and salt extracted material. With the exception of L27, which showed no indication of structure in either sample, the salt extracted proteins exhibited more complex PMR spectra than their acid/urea counterparts. This is particularly pronounced in the case of L11 and L30. L11 shows an extremely complex aromatic spectrum with numerous perturbed resonances and L30 displays several large ring-current shifted apolar methyl peaks. L25 is similar to S16 in that the salt extracted protein shows a ring-current shift pattern identical to that found for the acid/urea extracted sample but at much higher intensities.

The two groups of ribosomal proteins were investigated under different conditions, at low ionic strength

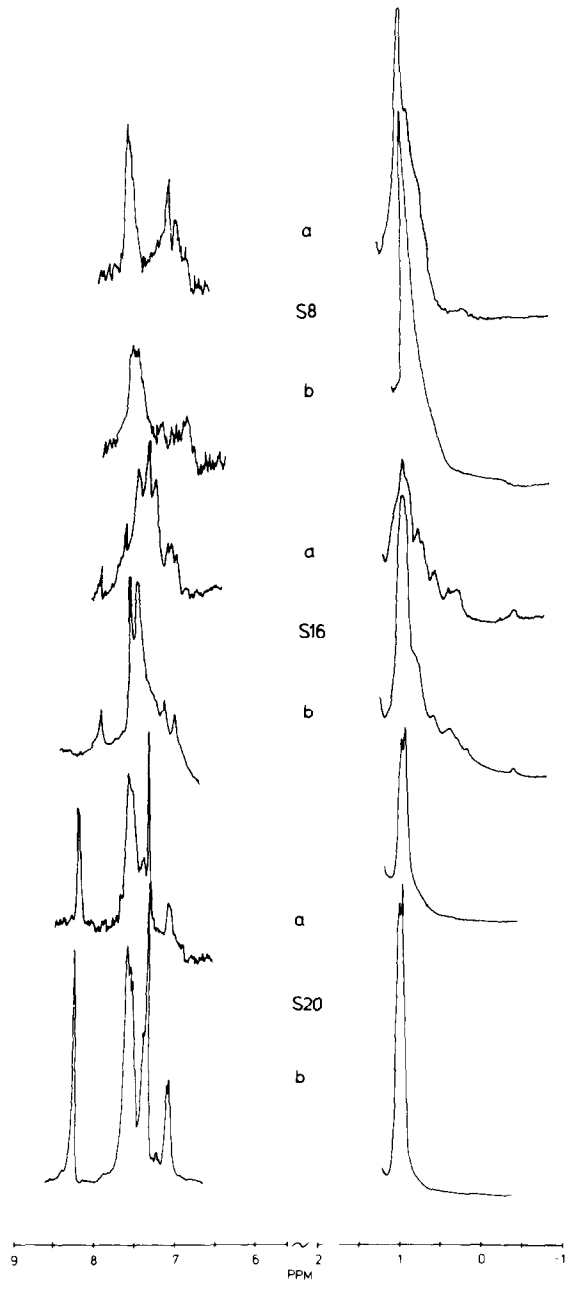

Fig.1. 270 MHz PMR spectra of ribosomal proteins from the 30 S subunit. Comparison of salt-extracted (a) and acid/urea-extracted (b) proteins S8, S16 and S20. Because of differences in protein concentrations the gain settings used for the spectra vary. The expansion of the aromatic region is 4-fold with respect to the high-field region.

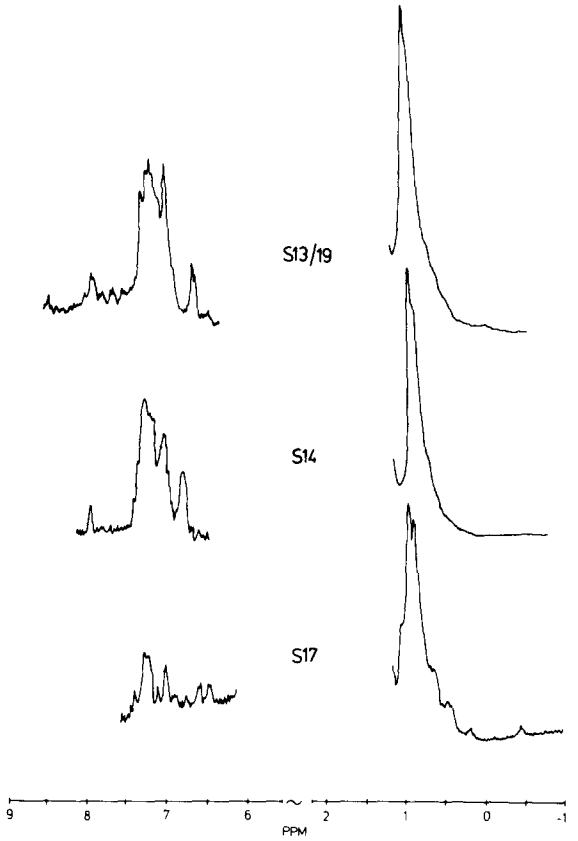

Fig.2. 270 MHz PMR spectra of ribosomal proteins from the 30 S subunit. Salt extracted proteins S14, S17 and the complex of S13/19. Other details are as described in fig.1.

for the acid/urea extracted proteins and at high ionic strength for the salt extracted proteins. Control experiments were therefore performed to eliminate the possibility that the observed structural differences were due to the change in ionic strength. It was necessary to conduct these experiments at low protein concentrations (approx. 1 mg/ml) to prevent aggregation and precipitation. PMR spectra were obtained from some acid/urea proteins at high ionic strength and from some salt extracted proteins at low ionic strength. The spectra obtained were the same as those presented above, indicating that the change in ionic strength had no effect on the protein structure. However, if acid/urea extracted S16 at higher protein concentration was dialysed from urea to high salt

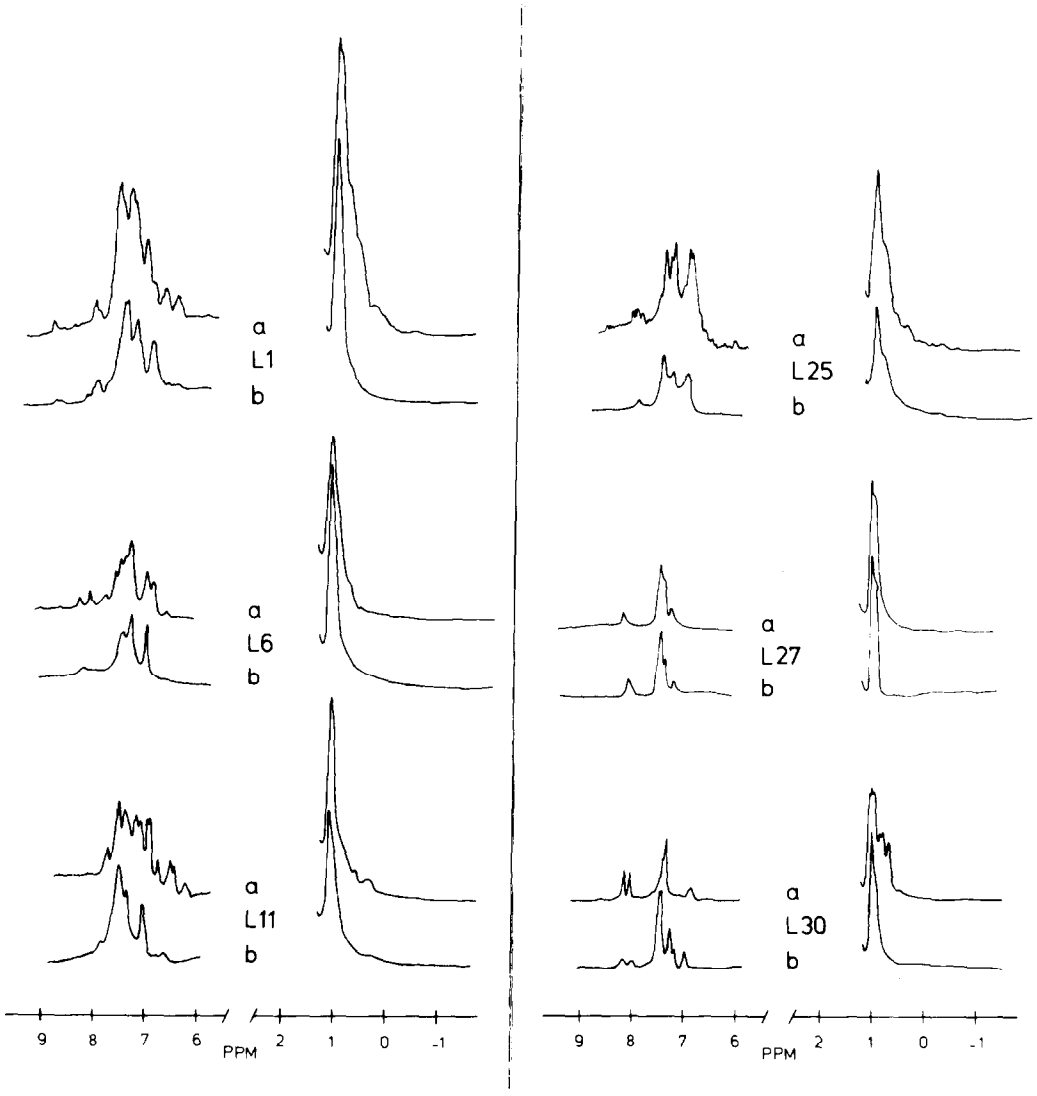

Fig.3. 270 MHz PMR spectra of ribosomal proteins from the 50 S subunit. (a) salt-extracted proteins, (b) acid/urea-extracted proteins. Varying gain settings were used to obtain the spectra. The aromatic regions are shown at a 8-fold expansion with respect to the high-field region, except for protein L25 where a 4-fold expansion is used.

buffer a substantial amount of protein precipitated and the PMR spectrum of the supernatant was identical to that obtained for salt extracted S16. Presumably, selective precipitation of denatured material occurred and the supernatant was enriched for 'native' protein.

Assignment of the perturbed resonances in the spectra to specific amino acid residues is not possible at this stage. Larger amounts of material are essential for such studies which could be pursued using proteolytic fragments of the various proteins, selective deuteration of residues, spin-decoupling and other special NMR techniques. Simple assignments are, however, possible. For example, in the case of L30 the splitting in the His C_2H resonance may be attributed to the occurrence of His-19 in a predominantly apolar and His-33 in a polar environment [29].

In conclusion, all salt extracted ribosomal proteins investigated, with the exception of S20 and L27, contain more structured molecules than their acid/urea extracted counterparts. In contrast to the histones [30] no structural changes were induced by variations in the ionic strength for the proteins tested. The presence of tertiary structure cannot be ruled out for proteins S20 and L27 since this method only detects interactions of aromatic residues and apolar side chains.

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