Two-dimensional Fourier transform ^1H NMR studies of ribosomal protein E-L30

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Two-dimensional Fourier transform ^1H NMR spectra of ribosomal protein L30 of *Escherichia coli* MRE 600 were recorded at 500 MHz both in H_2O and in ^2H_2O. From the available data we infer (i) the existence of one or more hydrophobic domains in the molecule, (ii) at least two helical regions and (iii) the presence of an antiparallel β-sheet involving two threonines.

Two-dimensional Fourier transform NMR *L30* *Ribosomal protein* *β-Sheet*
COSY *NOESY*

1. INTRODUCTION

The determination of the primary structures of all proteins of the *E. coli* ribosome has been completed [1] and one now faces the formidable task of elucidating their tertiary structures. The obvious method of choice is X-ray crystallography, but its application has been hampered by the difficulty in obtaining good crystals of *E. coli* ribosomal proteins. However, some proteins of *Bacillus stearothermophilus* have been crystallized successfully and the first protein whose low-resolution crystal structure has been described is protein L30 from this species [2].

Another method which may provide detailed information about protein structure is high-resolution 2D NMR [3]. Here we report results of the application of this method to L30 of *E. coli*. This is the first report of a 2D NMR study of a ribosomal protein.

Abbreviations: 2D FT NMR, two-dimensional Fourier transform nuclear magnetic resonance; NOESY, nuclear Overhauser enhancement spectroscopy; COSY, correlated spectroscopy

2. MATERIALS AND METHODS

Protein L30 was isolated from *E. coli* MRE600, and NMR samples prepared as in [4]. The samples consisted of about 10 mg lyophilized protein in 0.4 ml of buffer containing 20 mM potassium phosphate (pH 6.5), 100 mM KCl, 0.02% NaN_3 and 10% ^2H_2O or 99.9% ^2H_2O (Aldrich). COSY and NOESY spectra were recorded at 500 MHz on a Bruker WM500 spectrometer. The NOESY spectra were obtained using a mixing time of 150 ms for ^2H_2O and 100 ms for H_2O solutions. Experiments were performed as in [5] except that in ^2H_2O the COSY data were recorded during the mixing time of the NOESY experiment [6]. The solvent resonance was suppressed by continuous irradiation throughout the experiments except during the frequency-labelling and detection periods. Data manipulation consisted of sine-bell window multiplication and zero-filling prior to 2D Fourier transformation to obtain a final digital resolution of 4.88 Hz/pt. Chemical shifts are reported relative to 3-trimethylsilyl[2,2,3,3-^2H]propionate.

3. RESULTS AND DISCUSSION

Fig.1 shows a NOESY spectrum of L30 in ^2H_2O.

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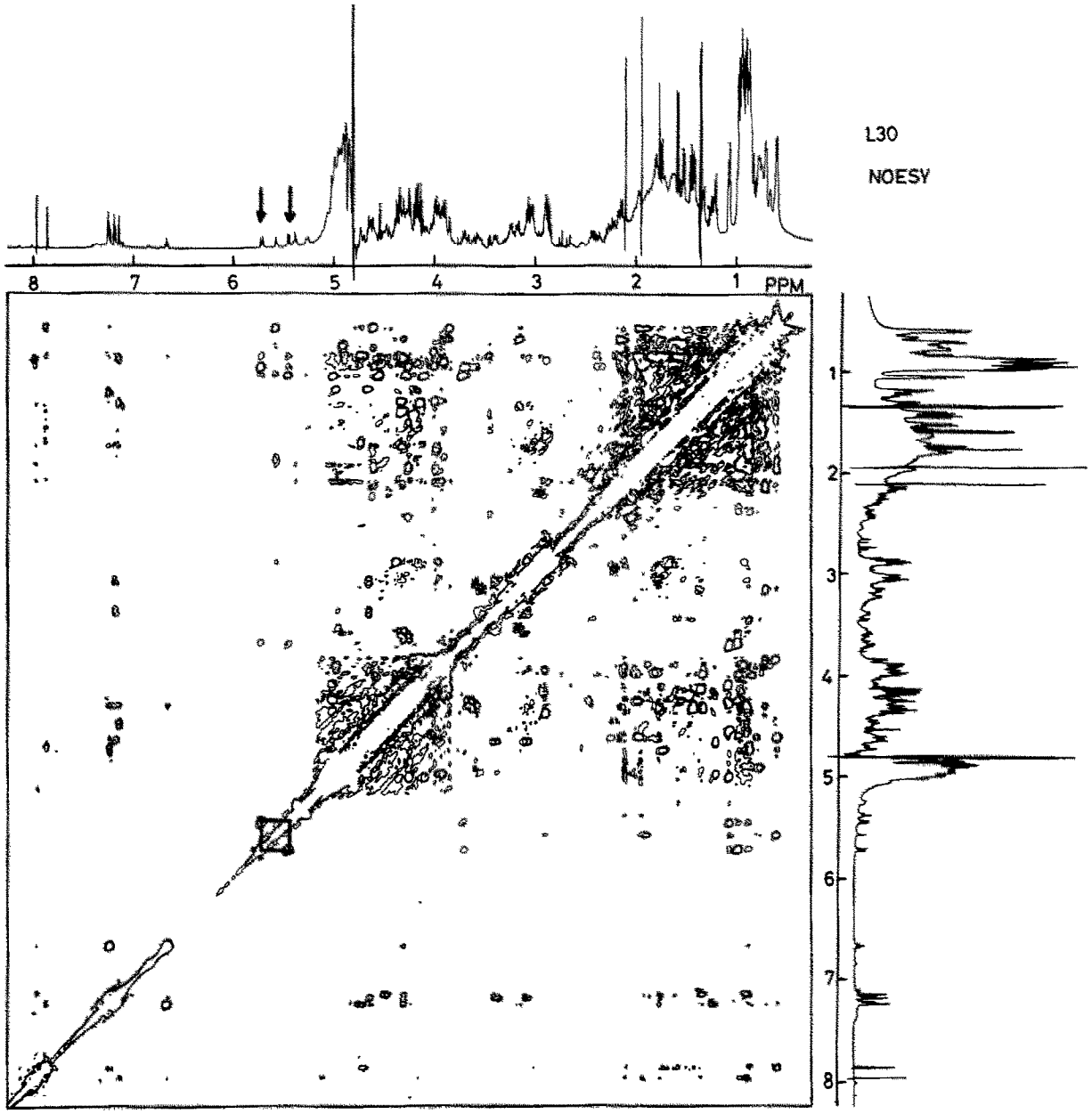

Fig.1. 2D NOESY of protein L30 in ^2H_2O. A mixing time of 150 ms was used. The recording of the spectrum took 30 h. An NOE connectivity (drawn line) is indicated between the doublets at 5.44 and 5.70 ppm (arrows).

In this spectrum the non-diagonal cross-peaks indicate connectivities of resonances of protons at distances of about 3.5 Å or less. The 1D spectrum has been plotted along both axes to facilitate interpretation. A complete description of this figure is beyond the scope of this paper and we will limit the discussion to two outstanding features.

Firstly, a number of cross-peaks are observed connecting the regions 6.5–8.2 ppm and 0.5–1.0 ppm. The first region is often referred to as the 'aromatic part' of the spectrum and contains the resonances of the aromatic ring protons; for L30 these are His[19], His[33] and Phe[52] [7]. The spectral region upfield from 1.0 ppm contains almost

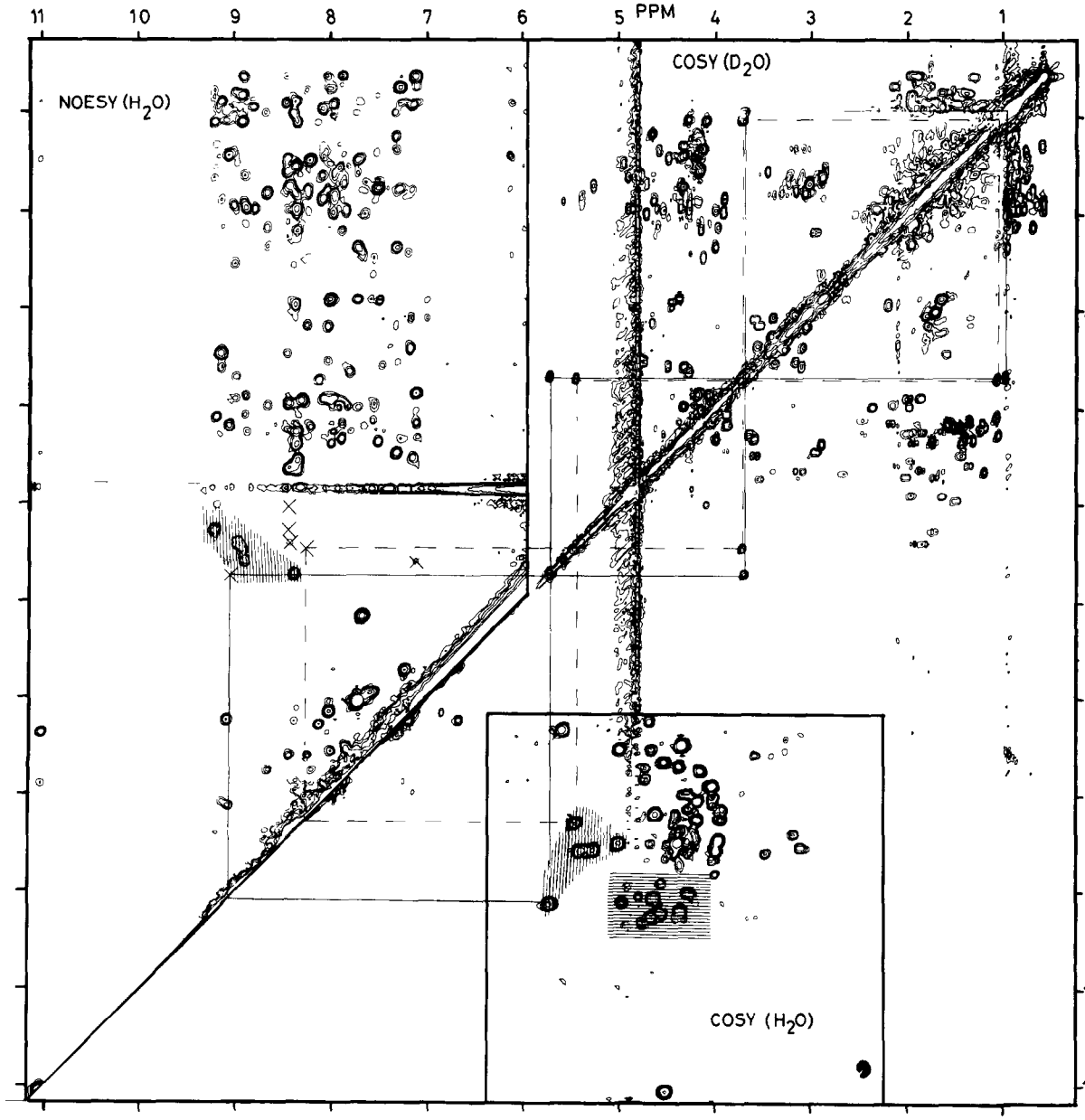

Fig.2. Combination of 3 different 2D NMR spectra as described in the text. COSY(H_2O) and NOESY(H_2O) were recorded in 24 and 48 h, respectively. The mixing time of the NOESY experiment was 100 ms. COSY(D_2O) was recorded simultaneously with the NOESY spectrum of fig.1. The $N_\alpha H-C_\alpha H-C_\beta H-C_\gamma H_3$ patterns of two threonines are indicated by a drawn and a dashed line. The small crosses and the shaded areas refer to typical features of antiparallel β-strands discussed in the text.

exclusively the resonances of the methyl protons of valines, leucines and isoleucines. Hence, these NOESY cross-peaks give direct evidence for the interactions of aromatic with aliphatic residues and thus suggest the existence of one or more hydrophobic domains in the molecule.

Secondly, a cross-peak is observed connecting the doublets at 5.40 and 5.77 ppm, both

originating from $C_\alpha H$ protons, showing a downfield shift of about 1 ppm with respect to their random coil positions. The implications of this finding will be discussed below.

Fig.2 is composed of 3 different sets of data: (i) downfield from 6 ppm and above the diagonal a NOESY spectrum is presented recorded in 90% H_2O, (ii) the inset below the diagonal represents part of a COSY spectrum also recorded in 90% H_2O and (iii) the remaining part of the figure shows a COSY spectrum recorded in ^2H_2O. The first feature of this figure that we wish to discuss is the presence of about 20 cross-peaks lying close to the diagonal in the 7–11 ppm region of the NOESY (H_2O) spectrum. These indicate the short $N_{\alpha i}H-N_{\alpha i\pm 1}H$ distances characteristic for α-helices [8]. A detailed analysis of this part of the NOESY(H_2O) spectrum strongly suggests the existence of two α-helices, each 5 or more residues long, which is in good agreement with the crystal data of L30 from *B. stearothermophilus* [2].

In a COSY spectrum a cross-peak is observed between the resonances of protons when the number of chemical bonds between them is 3 or less. Thus COSY allows identification of the contributions of individual amino acid residues to the whole protein spectrum. Since the peptide-bond $N_\alpha H$ protons are replaced by deuterons in ^2H_2O, $C_\alpha H-N_\alpha H$ connectivities cannot be observed in this solvent but they do become visible in the COSY spectrum recorded in 90% H_2O. The 'spin-systems' to which the two $C_\alpha H$ doublets at 5.44 and 5.70 ppm, mentioned above, belong are indicated in fig.2. Only threonines, valines or isoleucines may give rise to such patterns. To the best of our knowledge no $C_\beta H$ resonances of valines or isoleucines have been reported downfield from 3.5 ppm so we assign the doublets (in ^2H_2O) at 5.44 and 5.70 ppm to $C_\alpha H$ protons of two threonines. The data presented in fig.1,2 demonstrate that the L30 molecule contains antiparallel β-strands, arranged in such a way that these two threonines are on opposite positions with their $C_\alpha H$ protons in the inter-strand interface. To illustrate how antiparallel β-strands are manifested in 2D NMR spectra, fig.3 shows a schematic representation of their peptide backbones. This conformation gives rise to a number of spectral features which will be discussed in arbitrary order. First, the peptide-bond carbonyl groups facing the

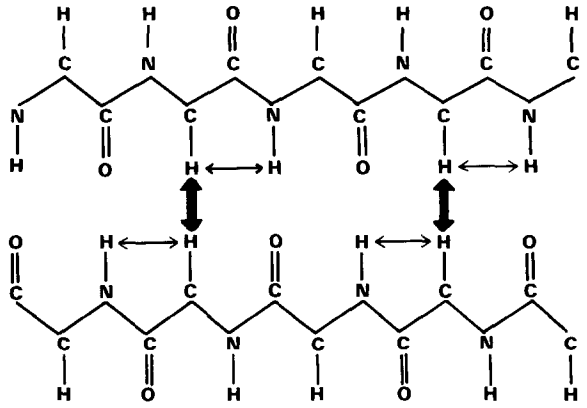

Fig.3. Schematic representation of the conformation of the backbones of two antiparallel β-strands. The arrows indicate proton pairs showing typical inter-residual NOEs.

partner strand often induce downfield shifts of resonances of $N_\alpha H$ and $C_\alpha H$ groups of the partner strand [9]. Especially for $C_\alpha H$ resonances downfield shifts may be considered characteristic for β-sheets [9]. In COSY, downfield-shifted resonances of $C_\alpha H$ protons show cross-peaks (vertically shaded, COSY-H_2O, fig.2) to resonances of $N_\alpha H$ protons which are located at the outside of the β-sheet (and most of which are likely to resonate at more or less normal positions). By the same token, downfield-shifted resonances of $N_\alpha H$ protons show cross-peaks (horizontally shaded) to $C_\alpha H$ resonances of normal positions. Secondly, the intra-residual NOEs between $C_\alpha H$ and $N_\alpha H$ are expected to be very weak or absent in the antiparallel β-sheet because these protons are in *trans* positions (see fig.3). In fig.2 the locations of these $N_{\alpha i}H-C_{\alpha i}H$ cross-peaks (predicted from cross-peaks in COSY(H_2O) for the downfield-shifted $C_\alpha H$ resonances) are indicated in NOESY(H_2O) by crosses. Consistently, only two of the 6 downfield-shifted $C_\alpha H$ resonances show a (very weak) intra-residual NOE cross-peak at these positions. Thirdly, one expects strong inter-residual NOEs between $C_{\alpha i}H$ and $N_{\alpha i+1}H$ because these protons are in *cis* positions [8]. Especially the sequential NOEs connecting downfield-shifted $C_\alpha H$ with downfield-shifted $N_{\alpha i+1}H$ resonances ($\leftrightarrow$ in fig.3) are easily discernible; these are indicated by shading in NOESY(H_2O) in fig.2.

Finally, one expects to find strong NOEs between downfield-shifted resonances of $C_\alpha H$ pro-

tons of opposite chains (↕ in fig.3). Such a NOESY-cross-peak is the one found between the $C_\alpha H$ resonances of two threonines (vide supra, fig.1).

It may be noted that our 2D NMR data present the first evidence for antiparallel β-strands in a prokaryotic ribosomal L30 protein. Further data concerning the structure of L30 in solution are currently being acquired using sequential resonance assignment procedures [5].

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