# Symmetrical structure of the L7 protein dimer

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500 MHz NMR studies of the L7 monomer (oxidized protein) and of the dimer (intact protein) show that its N-terminal sequence takes part in the dimer formation. The identical environment of equivalent amino acid residues in different polypeptide chains in the dimer is evidence of the symmetrical structure of the L7 dimer.

*NMR study* *L7 dimer structure* *L7 monomer structure*

## 1. INTRODUCTION

Protein L7 exists in solution in the form of a dimer. Its N-terminal sequence participates in formation of the dimer while the C-terminal moiety is globular (review, [1]).

Several different models of monomer arrangement in the L7 dimer have been proposed: (a) a symmetrical antiparallel model (head-to-tail arrangement of monomers), (b) a symmetrical parallel model, and (c) a staggered and parallel model (review, [1]). Despite numerous experimental data, the organization of the dimer structure is still uncertain.

If we assume that the dimer has a staggered parallel structure, the identical amino acids from different polypeptide chains participating in the dimer formation would have a non-equivalent environment due to their shift relative to each other.

To elucidate the amino acid environment in the L7 dimer and to choose the most realistic model we used the nuclear magnetic resonance (NMR) technique to study protein L7. The data obtained show that the identical amino acid residues from different polypeptide chains in the L7 dimer have an equivalent environment. Consequently, the dimer structure of protein L7 is a symmetrical one.

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## 2. MATERIALS AND METHODS

Isolation and oxidation of proteins L7 and L12 have been described in [2]. Proteins for NMR studies were lyophilized from a buffer solution (0.05 M sodium phosphate (pH 7.3), 0.3 M KCl) and dissolved in $^2H_2O$.

500-MHz $^1$H-NMR spectra were recorded at 27°C on a Bruker WM 500 spectrometer operating in the Fourier-transform mode. Chemical shifts are given relative to the internal standard 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The differential spin-echo method was used to find spin-coupling systems of amino acid residues and to define resonance multipliers in the protein spectra [3,4].

Spin-echo spectra were recorded using pulse sequence (90°-$\tau$-180°-$\tau$). The time $\tau$-0.06 s was chosen experimentally so that on the one hand, phase modulation was achieved and, on the other hand, the resonance intensity did not decrease significantly due to relaxation. In this case, singlet and triplet resonances with a spin-spin constant of ~7 Hz have a negative polarity while doublet resonances with the same constant have a positive one.

## 3. RESULTS AND DISCUSSION

Fig.1,2 represent the $^1$H-NMR spectra of the L7 protein dimer and monomer. The monomeric form

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of L7 was obtained by oxidation of methionine residues (in positions 14, 17 and 26 of the N-terminal sequence). Resonances of phenylalanine protons (Phe 30, Phe 54) and non-exchanged amide protons are observed in the low-field part of the spectra. NH-protons exchange completely in 1 h (after the protein has been dissolved in $^2H_2O$) for both the monomer and dimer forms of L7. Resonances from slowly exchangeable protons are practically identical in the NMR spectra (fig.2) for the monomer and the dimer. Therefore, as compared to the monomer, there is no additional shielding of amide protons from the solvent in the dimer. This also means that protons of the peptide groups which took part in the dimer formation exchanged rapidly.

Slowly exchangeable protons are characteristic of globular proteins and this means that either such amide groups are included in strong hydrogen bonds or that the NH-groups are shielded from the solvent [5].

The C-terminal part of the L7 protein molecule (residues 51–120) has a globular structure [6] and therefore resonances of slowly exchangeable amide protons can be assigned to the NH-groups of the L7 C-terminal sequence. The identity of these resonances in the spectra for the monomer and the dimer shows that the globular conformation remains unchanged in both samples. The assignment of resonances from phenylalanine residues has been done previously [7]. The Phe 54 resonances in the spectra for the L7 monomer and dimer have the same chemical shifts and line widths. Consequently, the local environment and mobility of Phe

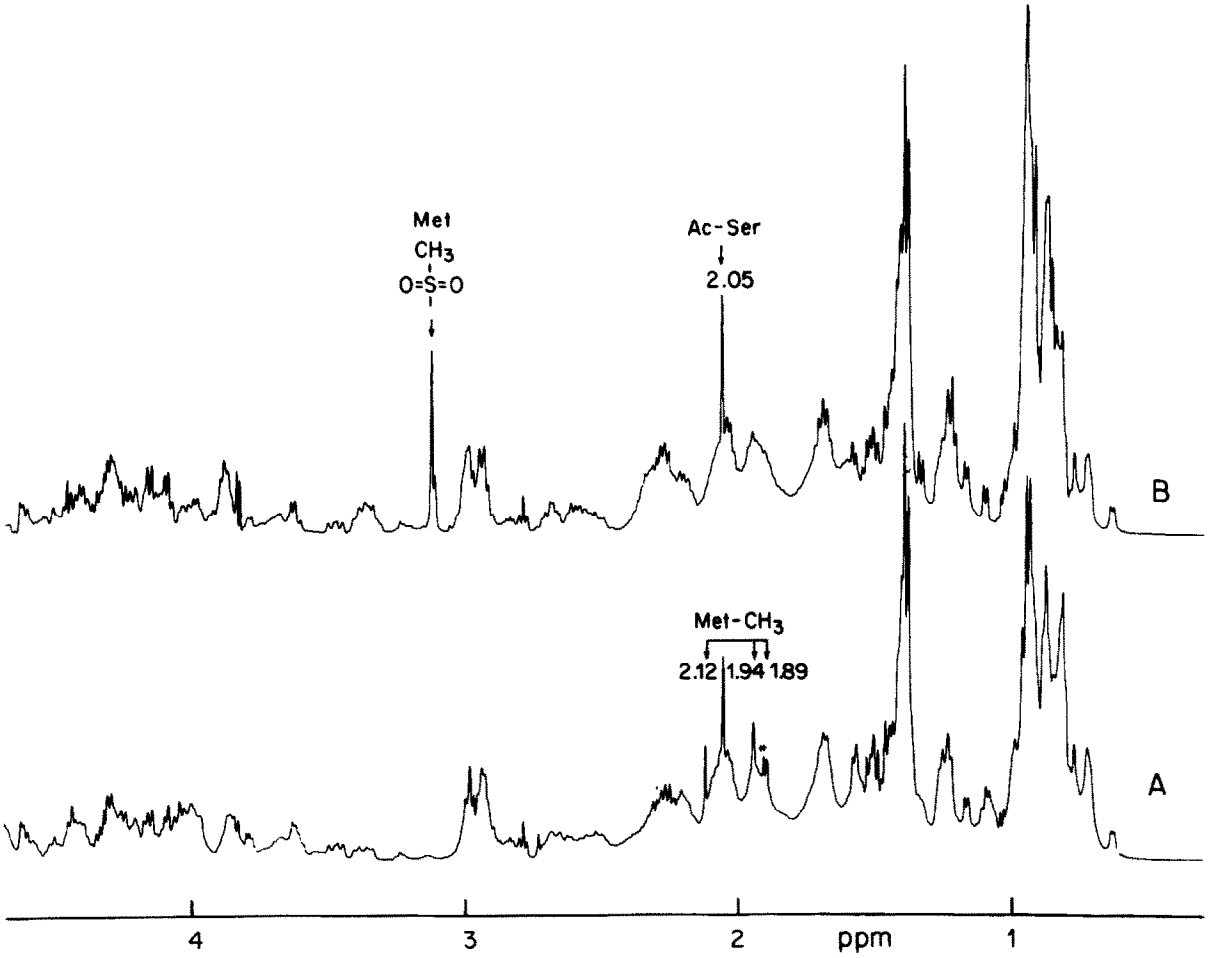


Fig.1. High-field region of $^1$H-NMR 500 MHz spectra of (A) the L7 dimer and (B) the monomer.

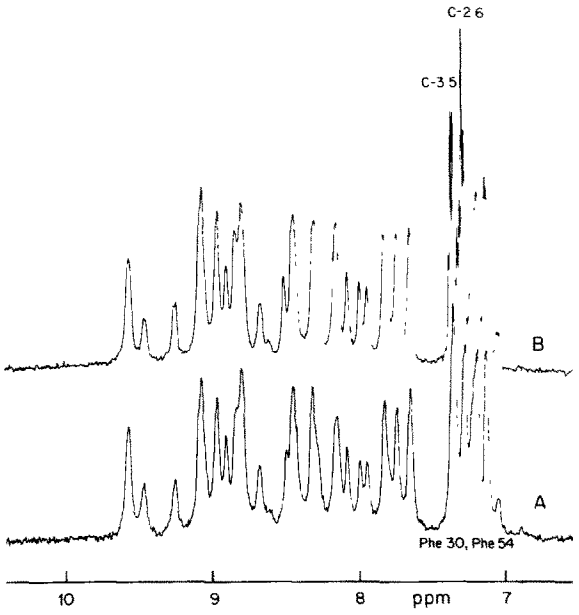


Fig.2. NH and aromatic protons region of the $^1$H-NMR 500 MHZ spectrum of (A) the L7 dimer and (B) the monomer. The resonance intensity is given at an 8-fold magnification as compared with that in the high-field region of the spectrum.

54 are the same in the monomer and dimer forms of L7. This is also a confirmation that the globular part of L7 remains practically unchanged upon dimerization.

Resonances of Phe 30 protons at C-2,6 in the dimer undergo an upfield shift while those at C-3,5 undergo a downfield one and have a broader linewidth than the shifts and breadth of Phe 30 resonances in the monomer form. These results confirm the data in [7], that the Phe 30 participates in intermolecular interaction in the L7 dimer.

There are 4 singlet resonances in the 2.2–1.8 ppm region (2.12; 2.00; 1.99; 1.89) of the L7 dimer spectrum. Three of these resonances disappear from this region after methionine oxidation and an additional single resonance with an intensity of 9 protons is observed at 3.12 ppm (fig.1B).

On the basis of these facts, we assigned 2.12, 1.94 and 1.89 ppm resonances to methyl protons of the methionine residues. The resonance at 3.12 ppm is assigned to $CH_3$-groups of oxidized methionine in monomeric L7. The resonance at 2.06 ppm is assigned to the methyl group of acetylated N-terminal serine of L7 because this resonance is not observed in the L12 protein spectrum and the acetylation of Ser 1 in L7 is the only difference between proteins L7 and L12 [9].

Methyl resonances of 3 methionines in the dimer spectrum differ in chemical shifts and linewidths (fig.1A). Resonance intensities of $CH_3$-groups at 1.94 and 1.89 ppm are weaker than that at 2.12 ppm for the dimer. In the monomer spectrum, the resonances of these 3 methionine $CH_3$-groups have practically the same shifts. Therefore, it is probable that the methionine residues play different roles in dimer formation.

Fig.3 represents the high-field region of the spin-echo spectra of the L7 dimer and monomer.

The resonance intensity is proportional, in the first approximation, to the mobility of the groups which give this resonance [8]. A resonance at 0.772 ppm with a negative polarity is observed in the high-field region of the spin-echo spectrum of the dimer. This resonance must have a triplet or a singlet structure as only triplet or singlet resonances have a negative polarity in a spin-echo spectrum. Proton resonances of isoleucine methyl

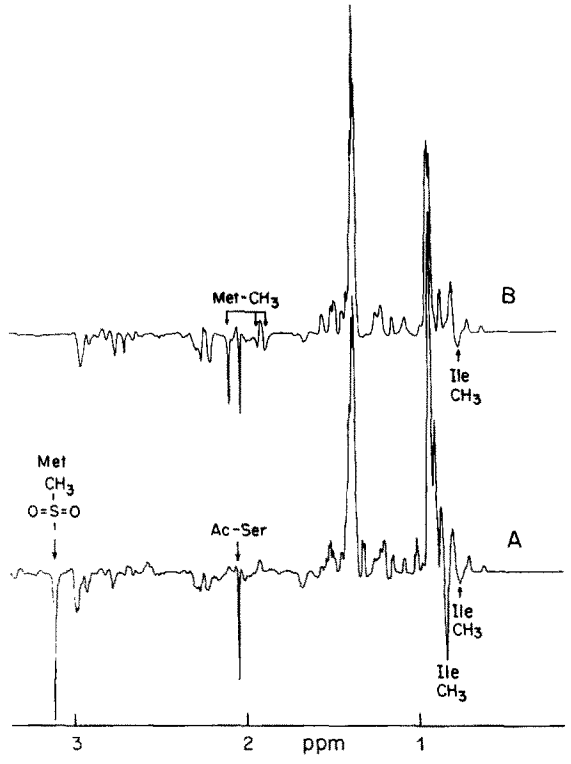


Fig.3. $^1$H-NMR spin-echo spectra of (A) the L7 monomer and (B) the dimer. Time $\tau$ in the pulse sequence (90°-$\tau$-180°-$\tau$) is 0.06 s.

Table 1
Chemical shifts (ppm) and relative intensity of $CH_3$-protons in spin-echo spectra for the L7 monomer and dimer

| Type of residue | Monomer | | | | Dimer | | | |
|---|---|---|---|---|---|---|---|---|
| | $\gamma$-$CH_3$ | $\beta$-CH | $\alpha$-CH | Relative intensity | $\gamma$-$CH_3$ | $\beta$-CH | $\alpha$-CH | Relative intensity |
| Thr I | 1.244 | 4.285 | 4.617 | 30 | 1.244 | 4.285 | 4.617 | 30 |
| Thr II | 1.161 | 4.398 | 4.500 | 40 | 1.161 | 4.398 | 4.500 | 40 |
| Thr III | 1.208 | 4.236 | 4.376 | 180 | 1.258 | 4.667 | 4.520 | 20 |

groups have a triplet structure while singlet resonances cannot be observed in this region of the spectrum. Consequently, the 0.772 ppm resonance can be assigned to the isoleucine $CH_3$-group. There are 6 isoleucine residues at positions 2, 7, 8, 23, 57 and 62 of the L7 sequence [9]. In the spectrum of the L7 monomer (fig.3A), besides a 0.772 ppm signal, there is also a narrow triplet resonance at 0.871 ppm which is the sum of at least two methyl resonances. On the grounds that the C-terminal sequence has the same structure in both the monomer and dimer form of L7, the resonance at 0.772 ppm can be assigned to Ile 57 or Ile 69.

The appearance of an isoleucine narrow resonance at 0.871 ppm in the monomer spectrum shows that at least 2 of the 4 isoleucine residues (nos 2,7,8 and 23) change their mobility and environment after conversion of the dimer into a monomer. Hence, the sequences containing these residues take part in dimerization.

Resonances of all 3 threonine residues (nos 3,52 and 76) were found in the spin-echo spectra of the L7 dimer and monomer. The chemical shifts and resonance intensity of threonyl methyl groups are given in table 1. It can be seen that resonances for Thr I and Thr II have the same chemical shifts and relative intensity in both the dimer and the monomer. As the C-terminal sequence of L7 has the same conformation in both the dimer and the monomer, Thr I and Thr II are threonine residues in the C-terminal part (Thr 52 and 76). Thr III resonances should be assigned to threonine in the third position (Thr 3) of the L7 polypeptide chain as it has different characteristics in the dimer and monomer. In the monomer spectrum, signals from Thr 3 have features characteristic of threonine residues in denatured proteins [8].

From the whole set of these data, it follows that the N-terminal sequence of L7 (at least sequences 3–8, 14–30) takes part in its dimerization. Amino acid residues in these sequences have a lower mobility in the dimer than in the monomer form.

As every L7 molecule contains 3 methionine residues and one acetyl-serine, one should observe more than 4 singlet signals in the dimer spectrum of L7 in the case of an asymmetrical (staggered) structure of the dimer. But the number of resonances in the dimer spectrum does not increase either for the methionine or the other assigned resonances (Phe 30, Thr 3, 52, 76, Ac-Ser), hence, the L7 dimer structure must be symmetrical. It is impossible from these data to choose between the parallel (head-to-head orientation of the monomers) and the antiparallel (head-to-tail) models because both are symmetrical models.

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