Physical properties of ribosomal proteins isolated under different conditions from the *Escherichia coli* 50 S subunit

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Received 31 March 1983

Physical properties of ribosomal proteins obtained with or without denaturating agents were compared. CD measurements and NMR studies have shown that proteins L2, L19, L24 and L30 isolated under denaturing conditions have the same properties as those prepared avoiding denaturating agents. CD and NMR spectra of proteins L1, L6, L11, L23, L25 and L29 obtained by us under denaturating conditions practically coincide with the data for the same proteins reported under 'mild' conditions. These findings suggest that the differences of reported physical properties can be due to different procedures of protein renaturation rather than to the methods of their isolation.

Ribosomal protein *CD-, NMR-measurement* *Denaturation–renaturation, of protein*

1. INTRODUCTION

The use of ion-exchange chromatography, high salt concentrations, organic solvents and detergents for isolation of proteins often results in their partial or complete denaturation. In physico-chemical studies a question arises whether the data obtained reflect the properties of the unique protein structure in solution or are results of artefacts of protein isolation and sample preparation. It is usually presumed that the protein has been more or less completely renatured.

Renaturation of proteins with an enzymatic function can be tested by their biological activity. It is more difficult to check renaturation of proteins without any easily tested biological function. An example of such proteins are ribosomal proteins.

There are indications in the literature that differences in physico-chemical properties or ribosomal proteins may be due to different methods of their preparation [1,2].

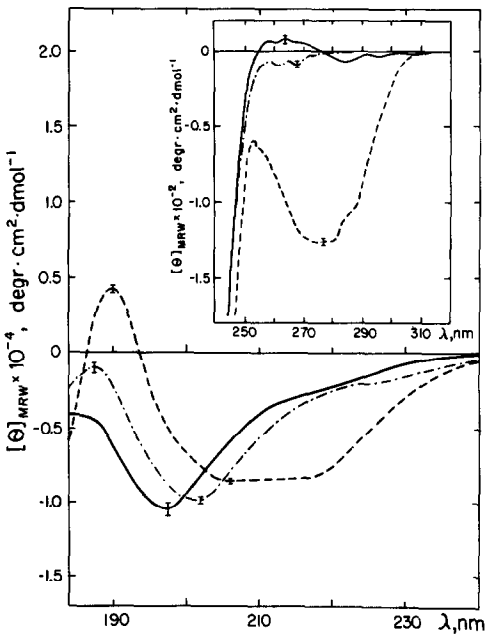

Fig.1. CD spectra of proteins (——) L2 in 0.1 M NaP, 0.001 M β-mercaptoethanol (pH 7.0); (– – –) L19 and (-·-·-) L24 in the far and near (insert) UV region.

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00145793/83/$3.00

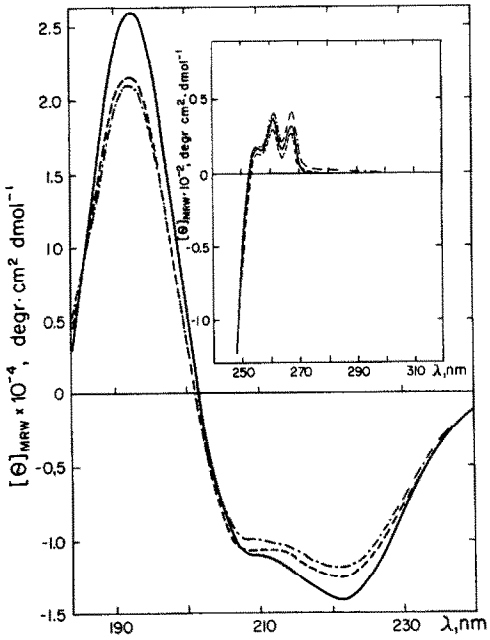

Fig.2. CD spectrum of protein L30 in the far and near (insert) UV regions (buffer as in fig.1): (A) (——) protein isolated without denaturating agents as in [6], stored at −70°C; (B) (– – –) protein isolated with the use of urea, lyophilized and dissolved with Gu·HCl; (C) (-----) the protein isolated with the use of urea, stored at −70°C.

An answer to the question on renaturation can be provided to some extent by physical methods (NMR, CD etc.). This approach has been applied to ribosomal proteins L11 and L25 [3,4].

Here, we present the results of physical studies of some ribosomal proteins obtained under 'mild' and denaturating conditions. This comparative study suggests that the protein properties coincide, irrespective of the way the proteins have been obtained. This indicates that in both cases the proteins after renaturation have the same conformation in solution.

2. MATERIALS AND METHODS

The proteins in denaturating conditions were obtained as in [5]. To obtain proteins L12, L19, L24, L30 under conditions avoiding strong denaturating agents we used the procedure proposed in [6]. The identity and purity of the individual proteins were established by two-dimensional electrophoresis [7]

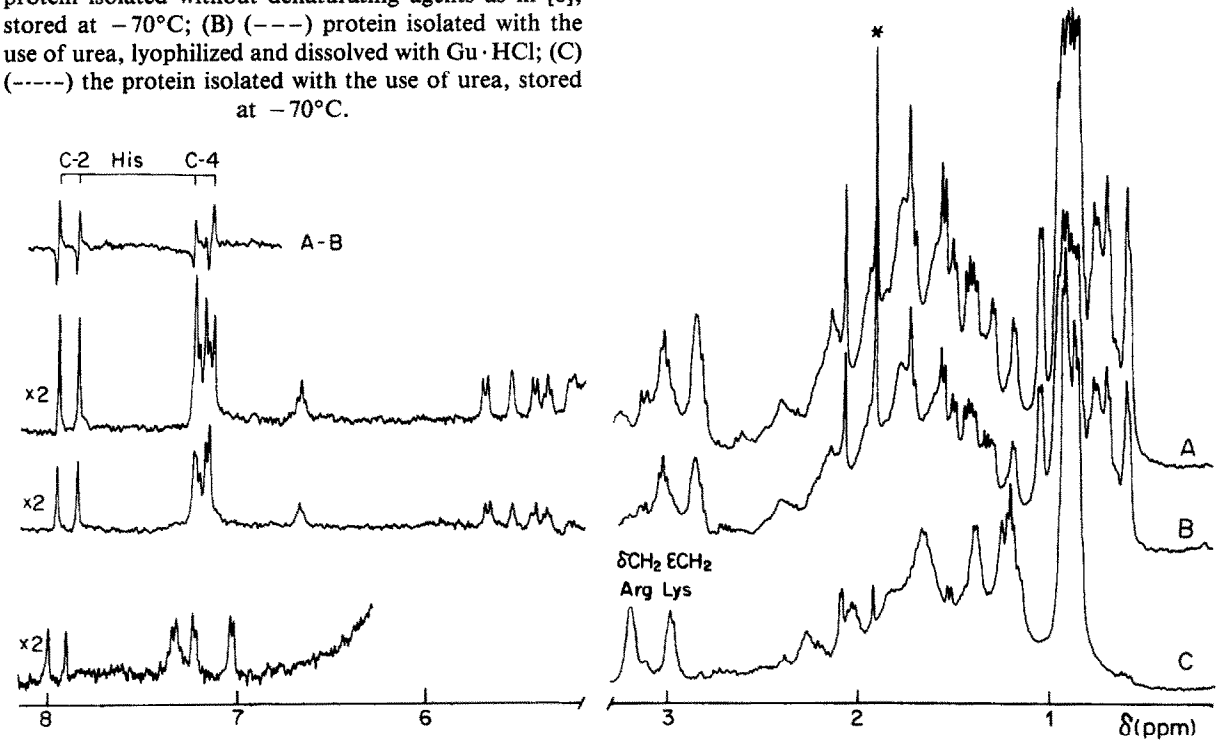

Fig.3. ^{1}H-NMR 360 MHz spectra of protein L30 at $T = 300$ K: (A) protein L30 isolated without denaturating agents; (B) protein L30 isolated with the use of urea; (C) protein L30 in the presence of 6 M urea; a difference spectrum (A − B) is given in the upper part of the aromatic region. The expansion of the aromatic region is 2-fold with respect to the aliphatic region (0.05 M sodium phosphate, 0.3 M KCl, pH 6.7–6.8 in ^2H_2O).

and SDS electrophoresis in 15% polyacrylamide gel [8]. The concentration of individual proteins was determined from the nitrogen content according to [9].

The proteins stored in the lyophilized form were thoroughly mixed with dry guanidine hydrochloride (to prevent gel formation) and dissolved in the buffers used for their study. Dithiothreitol up to 10 mM was added to the protein solution and incubated at room temperature for about 1 h. Then the protein solutions were centrifuged at 16000 × g at +4°C for 20 min and dialyzed into the required buffers at +4°C.

The proteins frozen in solution with urea and LiCl were thawed, centrifuged as indicated above, incubated with dithiothreitol and dialyzed into required buffers at +4°C. CD- and NMR-measurements were done as in [10].

3. RESULTS AND DISCUSSION

3.1. *CD measurements*

Fig.1 presents CD spectra of proteins L2, L19 and L24. These curves are averaged for the preparations obtained under denaturing conditions. Vertical bars in this figure show the maximum differences of ellipticity values for the proteins obtained in 'mild' conditions as in [6]. It is seen that these deviations for all the samples are <5% of the mean ellipticity value.

Fig.2 represents CD spectra of three different preparations of protein L30. In all the spectra the positions of the extrema (max at 192.5 nm, shoulder at about 208 nm, min at 222 nm and 3 max at 256 nm, 261 nm and 267.5 nm) and the crossovers (at about 201.5 nm and 253 nm) coincide. The biggest deviations for different samples

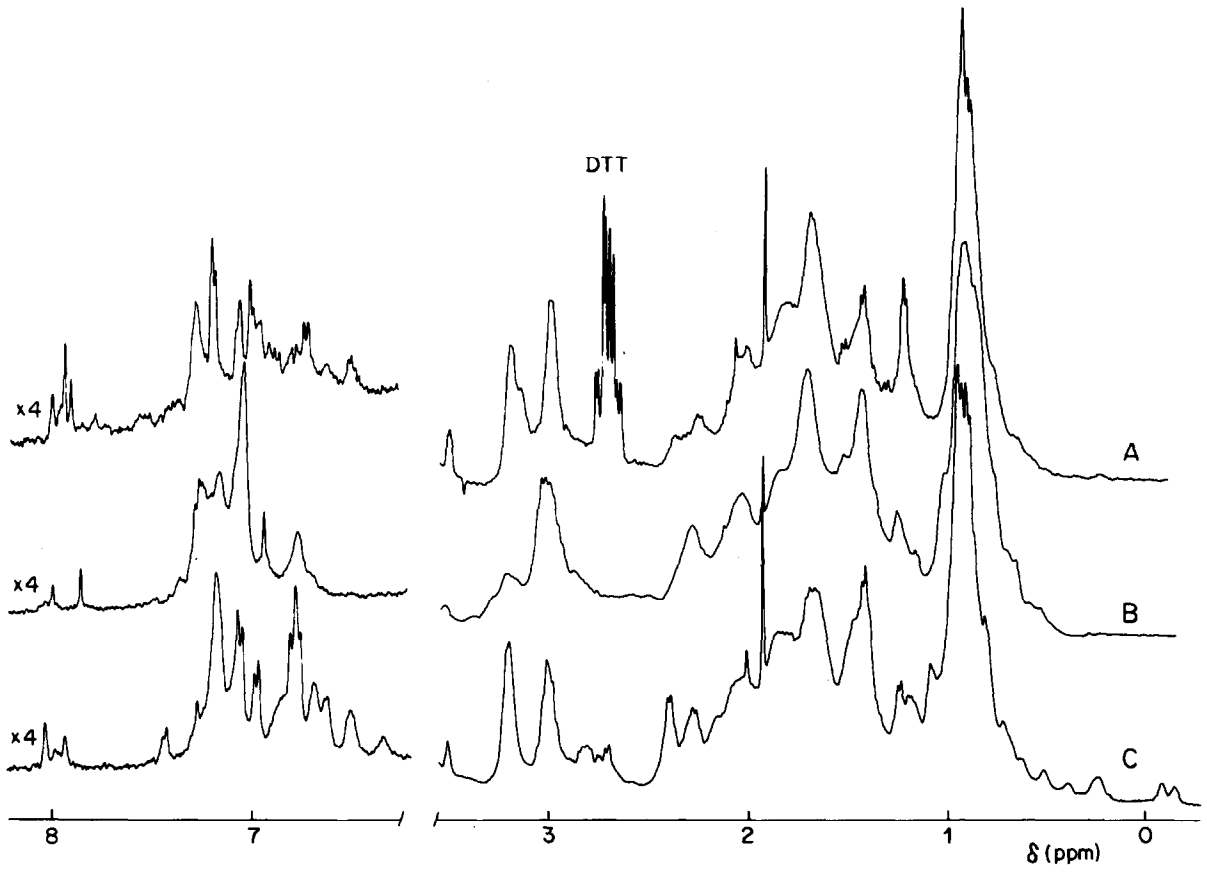

Fig.4. ^{1}H-NMR 360 MHz spectra of proteins L2 (A), L24 (B), L19 (C). All the spectra were recorded in the buffer as given in fig.3. Proteins were isolated under denaturating conditions.

of L30 with a [θ] value are at 192.5 nm (15–18%). But if the ratio $\theta_{max}/\theta_{min}$ is taken into consideration (parameter not depending on the concentrations) the difference is only 5–7%. Examination of the positions of extrema, crossovers and $\theta_{max}/\theta_{min}$ ratios for proteins L2, L19, L24 and L30 suggests that the CD properties of these proteins do not depend on the manner of their isolation.

The CD spectra of lyophilized proteins L11, L23 and L29 [10,11] also coincide with those obtained in 'mild' conditions and given in the review [12].

3.2. *NMR studies*

Fig.3A and 3B present spectra of protein L30. A comparison of these spectra indicates their practically complete coincidence.

A slight difference between them in the 8.0 and 7.3 ppm region is due to different pH-values of the protein solutions. The difference spectrum (A–B) allowed us to isolate proton signals from the C-4 carbon atom of histidine residues which overlap with the signals from the Phe-52 residue. In the difference spectrum there are no signals from Phe-52 protons; this is an indication of identity of the Phe-52 residue environment in both preparations. Besides protein L30, we have also carried out comparative studies of proteins L2, L19 and L24 isolated by different methods. The spectra of these proteins obtained under denaturating conditions are given in fig.4. These spectra practically coincide with those of proteins isolated under 'mild' conditions (see fig.5). The features of the NMR spectra obtained by us for 4 proteins L2, L19, L24 and L30 (signals in the high field, wide range of chemical shifts from aromatic amino acid and histidine residues) indicate the presence of a tertiary structure in the proteins studied. The identity of the NMR spectra for proteins obtained by

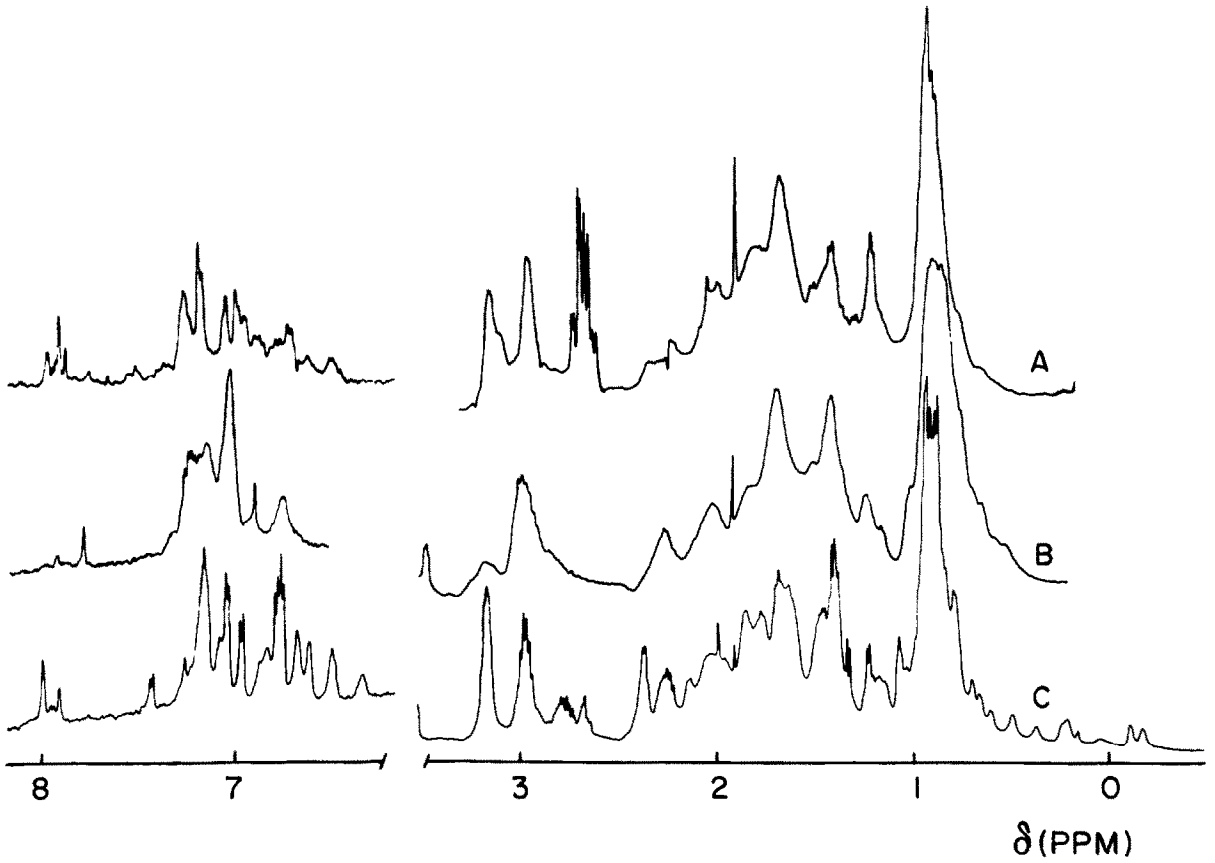

Fig.5. ^{1}H-NMR 360 MHz spectra of proteins L2 (A), L24 (B), 500 MHz spectrum of protein L19 (C) in buffer as given in legend to fig.3. The proteins were isolated under 'mild' conditions.

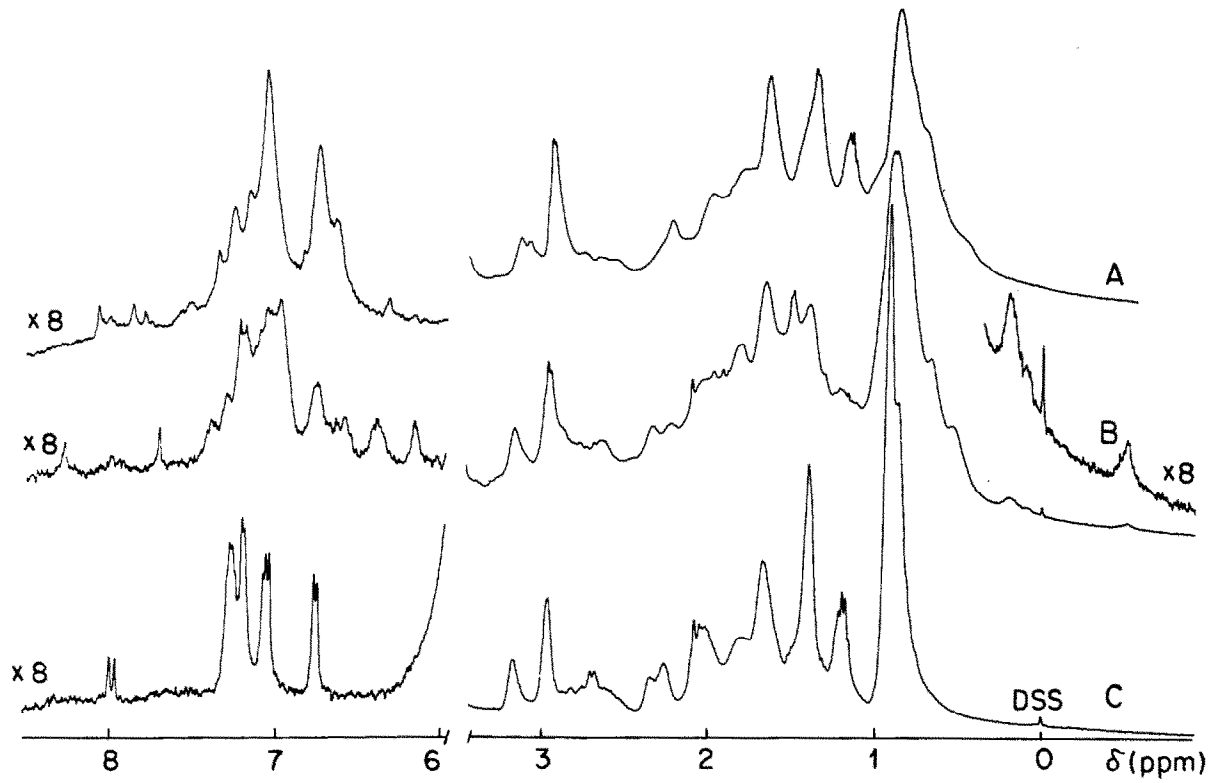

Fig.6. ^{1}H-NMR 360 MHz spectra of proteins L6 (A), L1 (B) at T = 294 K; (C) spectrum of protein L1 with 6 M at T = 294 K; buffer was the same as in fig.3.

different methods suggests a coincidence of their tertiary structures.

We have also compared the physical properties (CD and NMR) of proteins L1, L6 (fig.6), L11 [3,11,13], L23, L25 and L29 [10] obtained under denaturing conditions with the data reported in the literature for the same proteins obtained under 'mild' conditions [1,12]. The coincidence of these data also indicates, in our opinion, that the protein properties are independent of the method of isolation and storage. Even lyophilized proteins display the same properties though they require a more thorough preparation.

These results contradict the reports [1,2,12] on the considerable structural differences between proteins obtained under 'mild' and denaturating conditions. The data obtained for 10 proteins suggest that the differences are not caused by the isolation procedures, but by the preparation of proteins for the studies; i.e., the renaturation of proteins. This conclusion also agrees with the NMR data for proteins L11 and L25. Proteins L11 and L25 obtained under denaturating conditions do not differ by their properties after renaturation from those obtained under 'mild' conditions (cf. [1,3,4,10,13]).

ACKNOWLEDGEMENTS

The authors express their gratitude to Professor A.S. Spirin for constant interest and to Dr L.A. Sibeldina for providing the opportunity to record NMR spectra.

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