COMPARISON OF PROTEIN STRUCTURES BY HIGH RESOLUTION SOLID STATE AND SOLUTION NMR

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1. Introduction

Comparisons of the crystal and solution structure of proteins are usually based on circumstantial evidence. Direct structure determination is possible only in the solid state, and to make the comparison, various spectroscopic findings (CD, ORD, NMR), or results of chemical modification experiments are tested for consistency with the known crystal structure [1]. To be rigorous a comparison should be based on data obtained by the same method on the same object under two different conditions. Here we present an experiment which makes such a direct comparison of the crystal and solution structure of proteins by high resolution ^{13}C NMR.

2. Experimental

Crystalline hen egg white lysosyme (Sigma Chem.) has been used in these experiments without further purification. Spectra were obtained on a Nicolet Model NT-150 NMR Spectrometer operating at 35.2 MHz for ^{13}C.

3. Results and discussion

Figure 1 shows a comparison of solid state and solution ^{13}C spectra of native lysozyme at 35.2 MHz. A similar comparison for lysozyme at 70°C with oxidized disulfide bridges is shown in fig.2. Oxidation of the disulfide bridges, in addition to heating, is necessary to produce complete unfolding of this molecule [4]. It is doubtful that complete unfolding has been achieved in these experiments, but several structural differences between the high and low temperature conditions are apparent.

It may be regarded as established that the complex high resolution NMR spectrum of a native protein reflects its secondary and tertiary structure [2]. The spectrum of the unfolded form is much simpler and can be approximated by a sum of the spectra of the constituent amino acids. Recent developments in NMR technology, using the well-known principle of magic angle spinning to average out dipole–dipole interactions which otherwise dominate the linewidths in solids [3], have made it possible to obtain high resolution spectra of moderately complex molecules in the solid state and to compare these to spectra obtained on solutions. If a solution NMR spectrum of a protein is identical to the solid state spectrum, it can be argued that the protein structures in the two phases are identical, since the specific pattern of shifts results primarily from secondary and tertiary structure. Conversely, differences in the spectra can be taken to reflect differences in ambient conditions (pH, ionic content), structure or dynamics. The probability that identical spectra in solution and in the solid could arise merely by accident is, roughly speaking, inversely proportional to the number of well-resolved lines in the spectra: The less resolved the spectrum, the more limited the accuracy of the conclusion.

Comparison of the high temperature solid- and broadened liquid- (fig.2C) state spectra reveals several groups of resonances shifted with respect to each other, suggesting that the degree of unfolding may indeed be different in the two phases. In contrast the difference between the room temperature solid- and liquid-state spectra is largely a difference in the intensity of groups of lines. This could result if

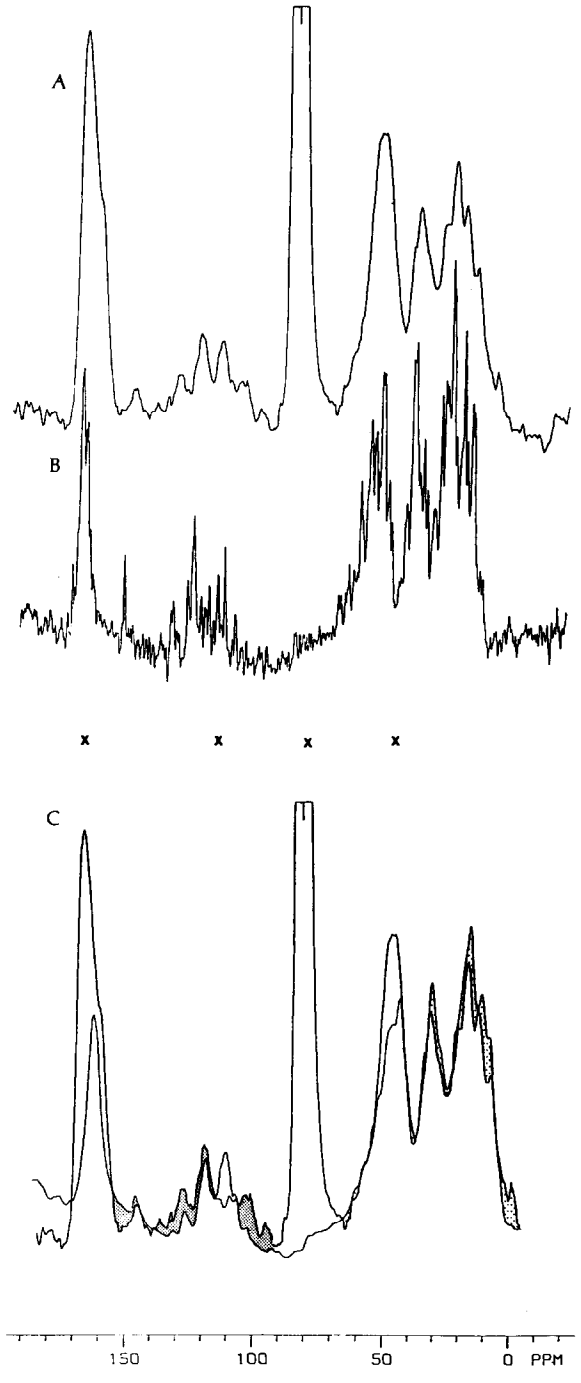

Fig.1. ^{13}C NMR spectra of lysozyme at 35.2°C and 25°C. (A) Crystal powder spectrum with magic angle spinning. (B) Aqueous solution (250 mg/ml, pH 7) spectrum. (C) Aqueous solution spectrum with 200 Hz line broadening superimposed on solid state spectrum. Position of peaks from rotor and spinning side bands are indicated by X.

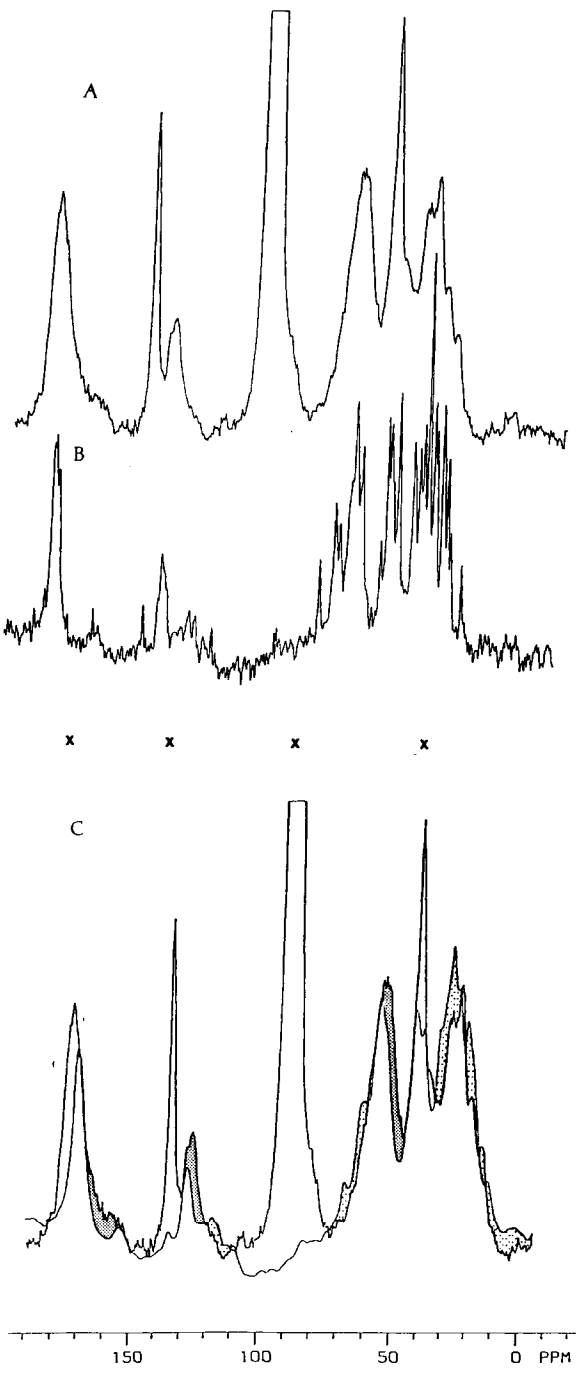

Fig.2. Same samples as fig.1, but at 70°C. (A) Crystal powder. (B) Aqueous solution. (C) Aqueous solution spectrum with 200 Hz line broadening superimposed on solid state spectrum. Dotted: difference spectrum. Dense dots: excess area in solid state spectrum. Sparse dots: excess area in solution spectrum. X: rotor peak (center) and sidebands.

the structures were identical, but the linewidths for different lines are different from each other and different in the two states of aggregation. A uniform broadening of 200 Hz was applied to generate the envelope of the solution spectrum. If the distribution of relaxation times throughout the spectrum were different in the solid and in solution such a procedure would distort the solution spectrum in the manner observed. The findings are therefore compatible with the notion that the ordered room temperature structures are very similar, although the possibility of several subtle but significant differences cannot be excluded at this level of resolution. The fact that the method is sensitive to major structural rearrangements is apparent from a comparison of the high and low temperature spectra for the solid (fig.1A, 2A) and the solution (fig.1B, 2B), respectively. It is not possible to generate the solid state spectrum of lysosyme at 25°C from the solution spectrum of lysosyme at 70°C or the solid state spectrum at 70°C from the solution spectrum 25°C simply by line broadening. Thus, the solid state spectrum in each case reflects the secondary and tertiary structure of the protein, despite the rather poor resolution. Since it can be closely approximated by simple line broadening performed on the corresponding solution spectrum, the structures in solution and in the solid state must be similar, at least in their principal features. Obviously, much more remains to be learned about protein structure from this type of experiment, and much more precise conclusions will become possible as the resolution of solid state spectra improves. For the present, we simply wish to draw attention to the fact that such experiments are possible and meaningful. Interpreted in the most conservative manner, they reveal at least no dramatic differences between the structures of lysozyme in the crystal and in solution. Preliminary experiments on ribonuclease A suggest a similar conclusion.

It bears emphasis that a comparison of this type provides a completely rigorous answer to the posed question, even though it involves no structure determination. It does not involve a comparison of structures (or structural features) deduced by different methods. Most existing evidence for the similarity of solution and solid state structures rests on such comparisons. While they are reasonable in most cases, they suffer from the drawback that different assumptions have to be made to derive structural information from different types of measurements. There is an everpresent danger of over-interpreting spectroscopic results in the light of X-ray findings and of becoming trapped in circular reasoning. In the experiment described here, the conclusion rests on a direct comparison of data obtained by the same method on the same subject in the two phases. Interpretation is required to explain differences, but not to establish identity of the structures if no spectral differences are found. The principal drawback of this procedure for the present is that the resolution of the solid state spectra obtainable on existing spectrometers does not compare favorably with that obtainable in solution spectra. To make a comparison at this time, it is therefore necessary to introduce artificial line broadening in the solution spectra and to compare the general features of the spectral envelope. This permits a conclusion concerning the gross features of the structure, but does not permit the observation or exclusion of more subtle differences.

Acknowledgements

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References

[1] Anfinsen, C. B. and Scheraga, H. A. (1965) Adv. Prot. Chem. 29, 205–300.
[2] Roberts, G. C. K. and Jardetzky, O. (1970) Adv. Prot. Chem. 24, 447–545.
[3] Andrew, E. R., Bradbury, A. and Eades, R. G. (1958) Arch. Sci. 11, 223–235.
[4] Cohen, J. S. and Jardetzky, O. (1968) Proc. Nat. Acad. Sci. USA 60, 92–96.