SELECTIVE SPIN DIFFUSION. A NOVEL METHOD FOR STUDYING MOTIONAL PROPERTIES OF BIOPOLYMERS IN SOLUTION

K. AKASAKA*, M. KONRAD and R. S. GOODY

**Abteilung für Molekulare Physik* and *Abteilung für Biophysik, Max-Planck-Institut fur Medizinische Forschung, 6900 Heidelberg, FRG*

Received 2 October 1978

1. Introduction

The increasing use of high frequency spectrometers for the study of high molecular weight biopolymer systems is a recent aspect of NMR application in biology. Detection and characterization of internal mobility within a biopolymer is a major goal of such studies [1–3]. On the other hand, a question has been raised whether relaxation measurements in high polymer systems at high frequency yield useful information as to the differential mobility of the polymer because of the presence of spin diffusion in such systems [4,5].

Here we give a clear-cut example of the effect of spin diffusion among proton spin systems of a biopolymer in solution. At the same time, we would like to demonstrate that this very effect can be quite selective within a given biopolymer and may be utilized to differentiate the 'mobile' region of the polymer from the rest, or 'immobile' part, from the first principles of magnetic relaxation.

2. Materials and methods

In this paper, a particular example from myosin (from rabbit skeletal muscle, mol. wt 470 000) will be shown. The purified myosin [6] was dialyzed against D_2O containing 500 mM KCl, 5 mM $MgCl_2$ and 10 mM phosphate buffer (pH 7.0). Myosin was 31 mg/ml final conc. and 0.5 ml solution was used for measurement in a 5 mm o.d. tube.

The NMR spectra were recorded at 25°C on a Bruker HX-360 operating in the FT mode. The technique used to observe the spin diffusion was that of 'homo-nuclear gated decoupling', which allowed presaturation of the proton resonance at a certain frequency before the application of a non-selective sampling pulse.

Usually a presaturation pulse of one second duration was applied which was switched off immediately before the application of an ~45° sampling pulse. The cited power of a presaturation pulse was measured as a peak-to-peak voltage across a dummy load equivalent to the probe and given after converting to watts.

3. Results

Figure 1(a) shows the 360 MHz proton NMR spectrum of myosin without applying preirradiation (presaturation) pulse. It is noted that the major signal is extremely broad as may be expected from the very high molecular weight (470 000). On top of that, however, we also notice a number of unusually sharp lines. Figure 1(b) shows the spectrum of the same sample, but after application of a relatively strong (0.5 W) presaturation pulse at a position marked by f_2. The decrease of the resonance intensity of the broad component is quite dramatic in (b). On the other hand, the sharp component is practically unaffected. Thus the effect of preirradiation is to suppress the broad component selectively. Another

* On leave from: Department of Chemistry, Faculty of Science, Kyoto University, Kyoto 606, Japan

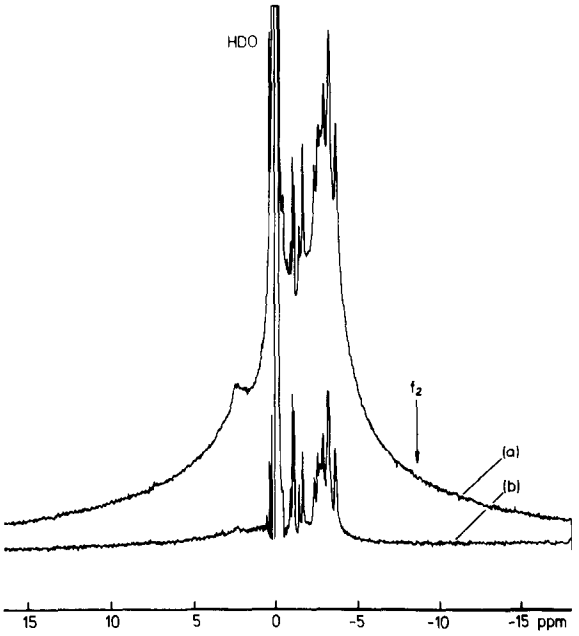

Fig.1. 360 MHz proton magnetic resonance spectra of myosin in a D_2O environment measured at 25°C. (a) Without presaturation pulse, (b) with presaturation pulse at the position shown. Two spectra are drawn with the same gain. See section 2 for solution composition and spectrometer condition.

important point noted in (c) is the fact that although the presaturation pulse is applied selectively at f_2, the whole of the broad component is almost equally suppressed.

The degree of saturation, however, depends on the frequency where the f_2 preirradiation pulse is applied (fig.2). The effect is almost absent when f_2 is applied very far from the central part of the resonance (a), but becomes progressively stronger as f_2 moves towards the central part of the spectrum (b,c). The degree of saturation is found roughly proportional to the intensity of resonance at the point where f_2 is applied.

Figure 3 shows the dependence of the degree of saturation on the power of f_2 applied at a constant frequency (in this particular case at the HDO peak). The result shows again that the saturation effect is shared all over the broad component even when a relatively lower power of f_2 is employed. Comparison of the resonance intensity in the convolution difference spectra shows that the intensities of the peaks in the sharp component are also affected by the application of the f_2 pulse, but the effect is far smaller compared to that on the broad component (in fig.3(d), 20–50% is suppressed).

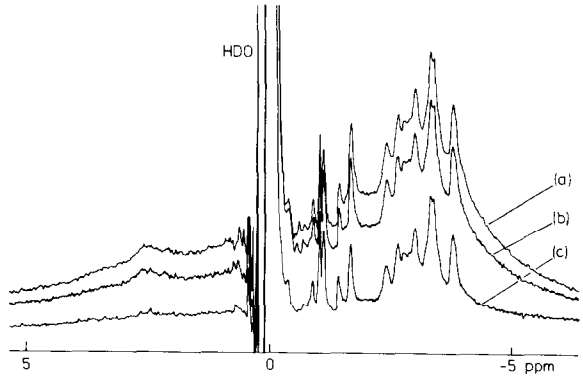

Fig.2. Dependence of the degree of saturation of the broad component of the myosin spectrum on the frequency of the presaturation pulse at constant power (0.5 W). f_2 was applied at (a) 120 ppm, (b) 37 ppm and (c) 8 ppm downfield from the HDO resonance. All the spectra are drawn with the same gain.

4. Discussion

The results shown above have several important implications.

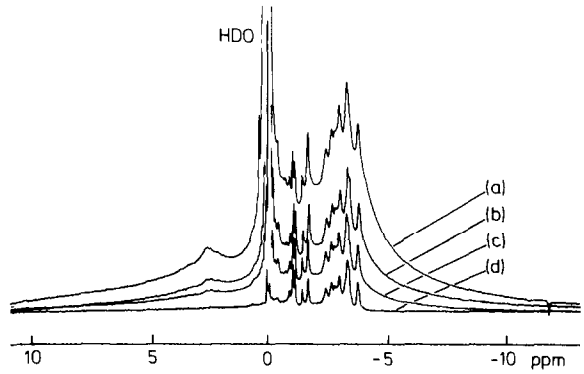

Fig.3. Dependence of the degree of saturation of the broad component of the myosin spectrum on the power of the presaturation pulse (f_2) at constant frequency (at the HDO position). The f_2 power applied was (a) 0 W (b) 0.05 W (c) 0.14 W and (d) 0.5 W. All the spectra are drawn with the same gain.

1. They give another clear-cut example of extensive cross-relaxation or spin diffusion among proton nuclei of a high molecular weight compound in solution studied at high magnetic field, as has been discussed [4,5,7]. In addition, however, they also show that this spin diffusion can be quite selective, confined to a certain region of the polymer.
2. They signal caution for intensity measurements in NMR of polymers even in a deuterated solvent when often the presaturation of the remaining undeuterated solvent signal is employed in the so-called gated decoupling mode. Although the saturation transfer effect from H_2O to the solute polymers was discussed recently for the H_2O environment [7], the same caution should be taken also in the D_2O environment.
3. Perhaps most importantly, this very effect, which selectively suppresses the broad component and leaves the sharp component to be seen clearly, may be utilized to differentiate the 'mobile' part of the polymer from the rest, the 'immobile' part. This effect should not be looked upon as equivalent to other line-narrowing techniques, such as convolution difference which manipulates the free induction signal after it is taken [8].

Rather, the effect arises directly from the differential relaxation behaviour among the spin systems of the polymer; while the spin temperature of a part of the system becomes extremely high by the application of the presaturation pulse, the rest of the spin system stays cool. Therefore the effect is more essentially related to the differential mobility within the polymer system than a simple line-narrowing technique.

The basic principle giving this selectiveness of saturation can be qualitatively discussed in the following manner:

The time dependence of longitudinal magnetization of a proton in a multi-proton system follows the equation:

$$dI_i(t)/dt = \sum_{j \neq i} \rho_{ij} (I_o - I_i(t)) + \sum_{j \neq i} \sigma_{ij} (I_o - I_j(t)) \quad (1)$$

where I_o is the magnetization of a proton in thermal equilibrium with the lattice (the bulk solution) [9]. The sign and the relative magnitude of σ_{ij} with ρ_{ij} determines the relative importance of cross-relaxation. Particularly, when σ_{ij} takes a negative value, the negative nuclear Overhauser effect arises, and this can lead to saturation transfer or spin diffusion in a multi-proton system. From the functional dependence of σ_{ij} on the dipolar correlation time (τ_c) and the measuring frequency (ω), i.e.:

$$\sigma_{ij} \propto \{-1 + 6/(1 + 4\omega^2 \tau_c^2)\} \quad (2)$$

a negative value of σ_{ij} is seen to occur when $\omega\tau_c > 1.12$.

The τ_c giving rise to the broad component in the spectrum is likely to be governed by the rotational correlation time of the whole protein molecule and, for the present example of myosin, an estimate of τ_c is given to be 4.5×10^{-7} s [10]. Clearly, $\omega\tau \gg 1$ ($\omega = 2.26 \times 10^9$ s^{-1} for 360 MHz) is realized, and the extensive spin diffusion is likely to take place. On the other hand, the lack of the transfer of saturation onto the sharp component indicates that such a condition is apparently not satisfied with this component, i.e., the effective correlation time for the dipolar interaction giving rise to the sharp component must be of the order of ω^{-1} ($\sim 10^{-9}$ s) or less.

The selective saturation of the broader component as shown above was also clearly observed in lower molecular size proteins, such as subtilisin BPN' (mol. wt 27 500) and its complex with *Streptomyces* subtilisin inhibitor (mol. wt 78 000 as E_2I_2). Thus the methodology shown here appears to be widely applicable to distinguish between the rigid core and the flexible region of a protein.

In the case of myosin, the results reported here confirm the earlier conclusion, based on line width considerations, that a part of the myosin molecule (specifically part of the myosin head) must show an unexpected degree of internal freedom [11].

Acknowledgements

K. A. is a recipient of a fellowship from the Alexander-von-Humboldt Foundation.

References

[1] De Wit, J. L., Hemminga, M. A. and Schaafsma, T. J. (1978) J. Magn. Res. 31, 97–107.
[2] Jardetzky, O., Akasaka, K., Vogel, D., Morris, S. and Holmes, K. C. (1978) Nature 273, 564–566.

[3] King, R. and Jardetzky, O. (1978) Chem. Phys. Lett. 55, 15–18.
[4] Kalk, A. and Berendsen, H. J. C. (1976) J. Magn. Res. 24, 343–366.
[5] Sykes, B. D., Hull, W. E. and Snyder, G. H. (1978) Biophys. J. 21, 137–146.
[6] Holtzer, A. and Lowey, S. (1959) J. Am. Chem. Soc. 81, 1370–1377.
[7] Stoesz, J. D., Redfield, A. G. and Malinowski, D. (1978) FEBS Lett. 91, 320–324.
[8] Campbell, I. D., Dobson, C. M., Williams, R. J. P. and Xavier, A. V. (1973) J. Magn. Res. 11, 172–181.
[9] Akasaka, K., Imoto, T., Shibata, S. and Hatano, H. (1975) J. Magn. Res. 18, 328–343.
[10] Mendelson, R. A., Morales, M. F. and Botts, J. B. (1973) Biochemistry 12, 2250–2255.
[11] Akasaka, K., Konrad, M., Goody, R. S., Holmes, K. C. and Jardetzky, O. (1978) abstr. 6th Int. Biophys. Congr. Kyoto.