^{1}H NMRD Relaxation Dispersion Profiles of Solid BSA

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Introduction

Bovine Serum Albumin is a representative of nearly globular proteins of intermediate molecular weight. During the last two decades, a considerable amount of research [1-5] has been dedicated to collecting and interpreting its NMR relaxation data, in particular the longitudinal-relaxation dispersion profiles of both solid BSA and of its solutions. Although there is so far no convincing consensus on the detailed nature of relaxation processes in BSA (and in proteins in general), one feels that an in-depth understanding might be in reach.

Here we present high quality proton NMRD profiles of solid BSA, measured over more than five decades of relaxation fields (**Larmor frequencies ranging from 5 kHz to 600 MHz**) which, hopefully, might contribute to reaching such an understanding.

Experimental methodology

Lyophilized, ultra-pure (>99%) and globulin-free BSA purchased from Sigma (product number A7638) was kept for 3 hours in a 10 mm NMR sample tube under high vacuum in order to remove all free/adsorbed oxygen and any traces of humidity before sealing. FID's, NMRD profiles, CPMG decays and other NMR signals of the sample were measured using the following instruments:

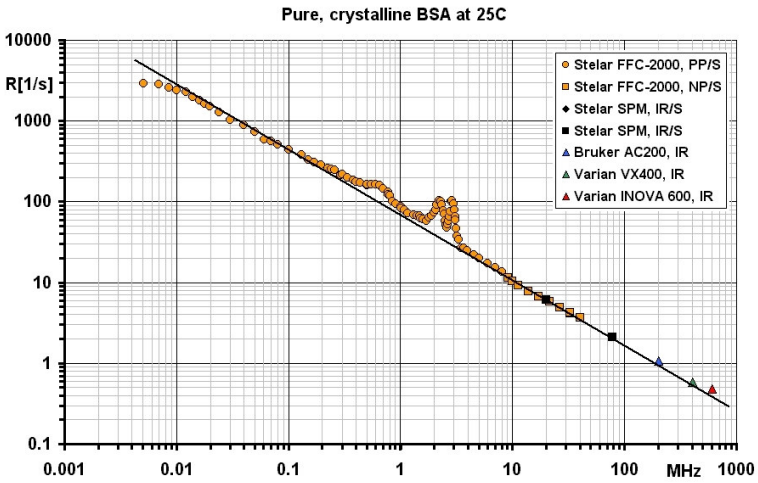
(a) Frequency range of 5 kHz - 30 MHz: fast-field-cycling relaxometer (1Tesla SpinMaster FFC-2000 produced by Stelar Srl, Mede, Italy).

(b) Frequency range 15 - 50 MHz: Stelar SpinMaster relaxometer of traditional type used with a recycled Jeol electromagnet.

(c) Fixed frequencies of 200, 400 and 600 MHz: respectively, Bruker AC200, Varian Mercury VX 400 and Varian INOVA 600.

Sample temperature has been carefully calibrated and rigorously controlled during all measurements. Since the FID's of the dry, solid BSA are very short and decay below the noise level in about 25 µs, we have used at frequencies below 100 MHz low Q-factor probes (~10) with short dead-times (~7 µs). For experiments at higher resonance frequency fields, standard HR probes were used.

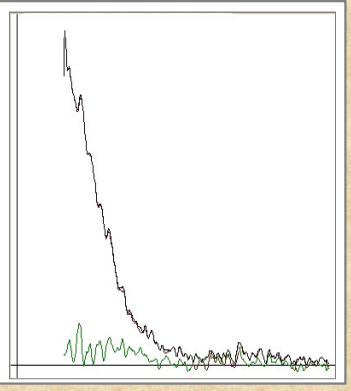
Fig.1. Experimental 1H NMRD relaxation profile

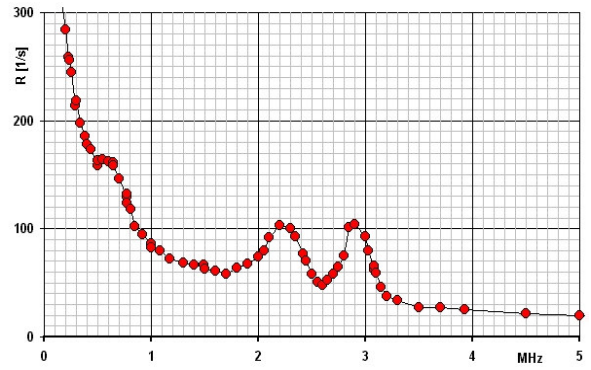


FID

The FID shown here starts at 9.0 µs after the pulse and terminates at 59.9 µs. It consists of 512 points taken with DW = 100 ns (SW of 10 MHz). The shape of the FID was found to be virtually independent of the observe frequency.

The brief duration of the FID indicates a rigid structure (crystalline-like solid) and a shape totally determined by strong internal dipole-dipole interactions. The decay is apparently close to a Gaussian but the reproducible beat in its trailing part (not to be mistaken for offset mis-setting or an instrumental artifact) indicates the presence of a more complex solid-state spectrum.



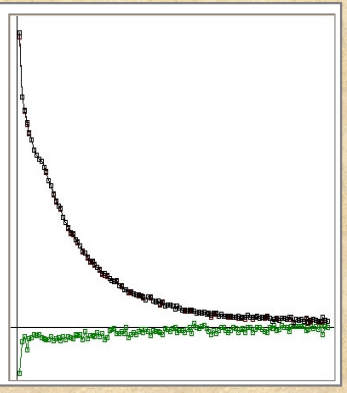


The log-lin graph on the left is an expansion of the region of ^{14}N glitches where proton Larmor frequency comes to match the ^{14}N quadrupole-dominated transitions and thus opens a new proton relaxation path through the ^{14}N.

There are four such glitches: two resolved ones between 2 and 3 MHz and two overlapped ones between 500 and 700 kHz. Analysis of their shape and intensity provides additional data for any theoretical model of relaxation processes in BSA.

CPMG

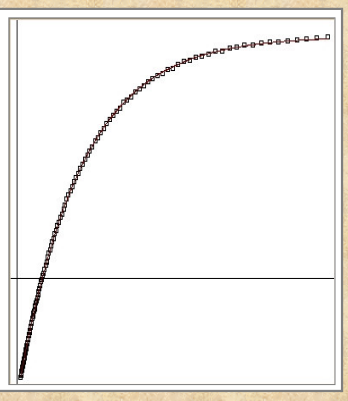
Example of a classical LR-CPMG decay of the same sample at 16.8 MHz. The Figure shows 128 echos taken at 20 µs intervals for a total X.axis span of about 2.58 ms. *The CPMG pulse train slows the decay of the signal more than 50 times* which can explained only by the presence of important internal motions with frequencies around and above 50 kHz. When the echo separation is increased to 60 µs, the decay becomes again very short and only very few echoes have an appreciable intensity, indicating that the increased interval is long enough for most of the internal motions to average out. *Note:* the intriguing shape of the starting portion of the CPMG decay may be an artifact due to the imperfect onset of spin-locking in a CPMG carried out in such extreme conditions.



IR

The Inversion Recovery curve shown here was taken at 20 MHz using 128 blocks with τ-values distributed logarithmically from 10 ms to 200 ms (in other cases we went logarithmically from 0.1 ms to 1s). It is perfectly mono-exponential. *No statistically significant deviation from mono-exponentiality could be detected, in fact, at any relaxation field.* Attempts to reveal a dignificant spread of relaxation rates by means of the UPEN decomposition program [6] also failed.

This illustrates the efficiency of spin-diffusion over the typical intra-molecular distances in BSA (≤ 6 nm). The spin-diffusion process, propelled by the rich pool of dipole-dipole interactions, is responsible for averaging all longitudinal-relaxation processes to a single relaxation rate value.



References

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Discussion of the results

The collected data represent perhaps the first NMRD profile measured for relaxation field values ranging from 5kHz to 600 MHz. Considering that the sample behaves as a rigid solid with a usable FID duration of just about 20 µs, the fact that it could be measured successfully over such a range of fields and instruments - and that the data are mutually very coherent - is by itself quite unique.

The NMRD profile has a steep dispersion extending from less than 10 kHz up to almost 400 MHz. The log-log plot of the relaxation rate dispersion profile reveals a nearly linear region (the classical power-law dependence) which extends from 10 kHz to 400 MHz. Superposed over the power-law dispersion are the four ^{14}N glitches which can be approximately modelled as four partially overlapping Lorentzians.

The equation of the straight line shown in the log-log profile graph is

$$\mathrm{Log}_{10}(R) = 6.6988 - 0.8110\ \mathrm{Log}_{10}(\nu)$$

with ν denoting the ^{1}H Larmor frequency in the relaxation field, expressed in Hz.

Notice that the log-log slope is much smaller than the value of -2 which characterizes the down-sloping portion of the BPP formula. Slopes of this value are typical, for example, of most bulk polymers. In particular, there is an extreme, almost-quantitative similarity between the profiles of solid BSA profile and those of bulk Nylon which merits further investigation.

Below 10 kHz the profile flattens towards a zero-field limit of $R \approx 3000\ s^{-1}$ (T_1 of about 333 µs). Reliable measurements of such high relaxation rates are possible only with the newest Fast Field Cycling relaxometers and they still require extreme care, especially when the sample is a solid with very short FID.

At the highest field of 600 MHz, there is a hint that the profile might be bottoming-out to a high-field plateau of R close to perhaps $0.2\ s^{-1}$ (T_1 of 5 s). The fact that at 600 MHz we have actually measured the value of R = 0.48 (T_1 of 2.07 s) without hitting a real plateau is a reward for the extreme care taken to vacuum-dry the sample. A trace of water and/or oxygen would have almost certainly caused the high-field plateau to move to higher R values.

Preliminary data not reported here indicate that the temperature dependence of the BSA NMRD profile is surprisingly small. At 40°C the central portion of the profile (around 1 MHz) does not seem to change at all (this is by itself amazing, considering the large variations one encounters in many elastomers). The 'wings' of the profile (at 10kHz on one side and >30 MHz on the other) both decrease a few percent, breaking the straight-line approximation into two nearly linear portions with slightly different slopes. Looking closely, even the data reported here have in the region of 10-300 kHz an approximately linear segment with a slope which is slightly smaller than that of the overall linear fit.

Note: In the above, magnetic field induction values are systematically expressed in terms of ^{1}H Larmor frequency, 42.578 MHz corresponding to 1 Tesla.