A Proton Water $T_1$-NMRD Study of Ganglioside Micelles

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Abstract Ganglioside GM1 ($G_{M1}$) micelles have been studied by means of water proton $T_1$ NMRD experiment. The field dependent spin-lattice relaxation rates were measured for Larmor frequencies ranging from 0.1 to 40 MHz and for two micelle concentrations at three temperatures (T=10,15,20°C). The proton $T_1$ NMRD-profiles are well described by assuming two proton pools for the dispersion curves. The proton pools are characterized by an effective correlation time and a proton fraction. The largest correlation time, $\tau_{c,1}$, is determined by the low field part of the NMRD profile. The second correlation time, $\tau_{c,2}$, is determined by the high field part of the NMRD profile. The radius of the ganglioside micelles has previously been determined as about 54 using fluorescence experiments and with Stoke-Einstein relation the reorientation correlation time becomes $\tau_R = 120-165$ ns depending on the temperature dependence of the water viscosity. It is thus plausible to identify one pool of water-protons, characterized by the largest effective correlation time, as corresponding to waters residing in the headgroup with an order parameter $S \neq 0$ and $\tau_{c,1} \approx \tau_R$ or corresponding to labile protons with a $\tau_{c,1}$ as the mean life time. The proton NMRD profile reveals a second Lorenzian which also can either be labile and exchanging Ganglioside protons or water molecules residing in the head group with a mean life time as approximately 12 ns. The proton NMRD experiment cannot discriminate between these two cases.

Keywords Proton $T_1$ NMRD Profiles, Proton Spin-lattice Relaxation, Ganglioside Micelles

1 Introduction

GM1 ganglioside are amphiphilic molecules with a hydrophobic part consisting of a sphingosine and a fatty acid. The bulky hydrophilic part is built up by several sugar units. This system is rather well studied and is comprised of large spherical micelles with a stable aggregation number of $\approx 168 \pm 4$ in the temperature interval $T_1 = 30^\circ C$ [1]. Consequently, we may study the information content of the water proton $T_1$-NMRD profiles obtained from fast field cycling (FFC)-NMR dispersion experiments. The proton spin-lattice relaxation rate profile is measured at 30 different proton Larmor frequencies ranging from $\omega_0 = 0.01$ to 40 MHz. The great advantage of this technique is that the relaxation rate is sensitive to molecular motions with correlation times in the range of a few ns to $\mu s$ [2],[3]. The aim of this work is to examine what molecular information can be extracted from a relatively simple relaxation model which describes the water proton spin-lattice NMRD profiles very well.

2 Materials and methods

The $G_{M1}$ lipids forms micelles over a broad range of concentrations ($\geq 2 \cdot 10^{-8}$M) mainly because of the bulky head group. The aggregation numbers (168 $\pm$ 4) of GM1 micelles have been determined by using fluorescence correlation spectroscopy (FCS) and electronic energy transfer experiments, as well as static/dynamic light scattering. The aggregation number is constant up to 30°C and it decreases almost linearly from 30 to 55 °C. The hydrodynamic radius was also determined to $R_H = 5.4nm$ [1].

2.1 Sample preparation

Two concentrations of GM1 ganglioside, 24 mM and 15 mM were used in preparing the micellar solution according to the procedure described in [1]. The main steps are dissolving, evaporation and hydration. GM1 was dissolved in a chloroformmethanol mixture (2:1, v/v). After evaporation of the organic solvents, sample dried under vacuum for 3 h. The lipid film was then hydrated to the desired concentration of accumulated transients was 4 for all samples. Sample temperatures were 10, 15 and 20 ±0.1°C, maintained using the

2.2 Proton spin relaxation measurements

Water $^1H$ longitudinal relaxation rates, $R_1^{\text{LPS}}(\omega)$ of figure 1, was measured on a 1T Stelar FFC2000 fast-field-cycling instrument with polarization at 25 MHz and detection at 16.29 MHz. Relaxation takes place at different proton Larmor frequencies ranging from 0.01 to 40 MHz. The switching time is 3 ms, with a 90° pulse length of 7.8 $\mu s$. The polarization and recovery times were set to 4 $T_1$ and the number of accumulated transients was 4 for all samples. Sample temperatures were 10, 15 and 20 ±0.1°C, maintained using the
temperature unit of the Stelar spectrometer. Set temperatures are given by the spectrometer with an accuracy of about ±1 degree. The temperature is maintained, however within ±0.1 degree. In Figure 1, the six experimental NMRD profiles are displayed.

In order to reproduce the experimental NMRD profiles, with a reliable pure fit using Levenberg-Marquardt method, introduction of two types of β water pools was sufficient. However, this is of course a great simplification and one may expect a distribution of water residence times or mean life times of labile protons but it was not permissible to extract more proton pools because of the information content of the experimental NMRD profiles.

Intrinsic relaxation rates of the β waters are denoted $R_{1,\beta}$ and $R_{1,\beta}$ and the water fractions satisfy the relation: $f_{Bulk} = 1 - f - f_{\beta} - f_{\beta}$. Fast chemical exchange conditions mean that $R_{1,\beta} \cdot \tau_{\text{rel}} << 1$. In the high field limit of the NMRD profile relaxation rates display the characteristic temperature dependence as expected in the extreme narrowing regime. That is, the highest temperatures give shortest correlation times and the lowest relaxation rates.

The spectral density, describing water proton spin relaxation, has been separated into two parts assuming the presence of two distinctively different modulation time scales. One is a fast (local) anisotropic reorientational motion of water which thus introduce a partial average of the fluctuating dipole-dipole coupling. Consequently, an order parameter $S$, $(0<S<1)$ is introduced for the partial averaged interaction which is characterized by a correlation time in the ns time regime. The effective correlation time combines the micelle reorientation correlation time ($\tau_{R}$) and the water residence time($\tau_{w.i}$) i.e.,

$$\tau_{c,i} = \frac{\tau_{R} \cdot \tau_{w,i}}{\tau_{R} + \tau_{w,i}}. \tag{2}$$

This type of model was introduced in the 1980’s and sometimes named “the two step model”[8] or ”the model free approach”[9] because no detail diffusion or molecular dynamic description is introduced to describe the effect of the stochastic modulations[10]. The spectral densities for the β water pools (i=1,2) reads:

$$J_1(n\omega) = (1 - S_i^2) \tau_f + S_i^2 \tau_{w,i} \frac{1}{1 + (\tau_{w,i} n\omega)^2} \tag{3}$$

Field dependent relaxation is due to both the intra- and inter-molecular spin dipole-dipole relaxation mechanisms.

$$R_{1,\beta,i} = S_i^2 \tau_{c,i} \beta_{\text{Intra}} \left\{ \begin{array}{c} 0.2 \frac{1}{1 + (\omega_0 \tau_{c,i})^2} + \frac{0.8}{1 + 4(\omega_0 \tau_{c,i})^2} \\
+ 0.3 \frac{1}{1 + (\omega_0 \tau_{c,i})^2} + \frac{0.6}{1 + 4(\omega_0 \tau_{c,i})^2} \end{array} \right\} \tag{4}$$

The weight factor $W_D$ takes the value in the range 0.3 (protein)-0.6(bulk) thus relating the inter-molecular coupling constant to the intra-molecular dipole dipole coupling [3]. This assumption means that $\beta_{\text{Intra}} = \beta_{\text{Intra}} W_D$ and that both intra- and inter- molecular spin dipole-dipole couplings have the same type of spectral density. This simplification assumes that water reorientation is dominant. The intra-molecular spin dipole interaction constant is given by:

$$\beta_{\text{Intra}} = \frac{3 \mu_0}{4 \pi h} \gamma_H^2 H_H^2 = \beta_{\text{Intra}} W_D \gamma_H^2 H_H^2 \tag{5}$$

Here, $\beta_{\text{Intra}}=5.492036 \cdot 10^{10}$ where $\mu_0$, $h$, $\gamma_H$, and $\tau_{HH}(=1.58)$ refer to vacuum permeability, Planks
Consequently, the relaxation contribution \( \alpha \) can be summarized as a qualitative retardation correlation time \(<\tau_\alpha>\) due to surface water and local fast anisotropic motion summarized as: \( <\tau_\alpha> = f_{\text{bulk}} R_{1,\text{bulk}} + f_S R_{1,S} \). There is also a field-independent relaxation contribution from the \( \beta \) waters due to the fast anisotropic motion which is given by the expression: \( 1 - S_1^2 \tau_f \beta_{\text{Intra}} (1 + W_D) \). These contributions are added to form the \( \alpha \) relaxation term which is used as a fitting parameter and defined as:

\[
\alpha = <\tau_{1,\alpha}> + [f_{\beta_1} (1 - S_1^2) + f_{\beta_2} (1 - S_2^2)] \tau_f \beta_{\text{Intra}} (1 + W_D).
\]

Consequently, the relaxation contribution \( \alpha \) is expected to have a value larger than 0.5 in all fittings given in table 1. We also give the averaged water retardation reorientational correlation time which thus indicate the relaxation contribution due to surface water and local fast anisotropic motion summarized as a qualitative retardation correlation time \(<\tau_\alpha>\):

\[
<\tau_\alpha> = \alpha / \beta_{\text{Intra}}.
\]

The fraction of perturbed water, which hydrating the micelle head group is given by \( f_{\beta_0} = \frac{N_c}{N_\text{ag}} [\text{Gan}] \), where [Gan] is the molar concentration of ganglioside molecules, \( [\text{Micelle}] = \frac{[\text{Gan}]}{N_c} \) is the micelle concentration with an aggregation number \( N_\text{ag} = 168 \). In the analysis of the NMRD profiles the fitting parameters are the fraction of the \( \beta \) pools multiplied by a characteristic order parameter: \( S_1^2 \cdot f_{\beta_1} \) and \( S_2^2 \cdot f_{\beta_2} \) and the effective correlation times. In summary, the relaxation model has the following five fitting parameters: \( \tau_{\alpha}, \tau_{\beta_1}, \tau_{\beta_2}, (S_1^2 \cdot f_{\beta_1}), (S_2^2 \cdot f_{\beta_2}) \) and \( \alpha \). In figure 2 the different relaxation contributions are shown for a typical \( R_{1,\text{exp}}^\text{NMRD} \) profile (T=10°C, c=24mM).

### 4 Results and Discussion

One aim of the present study is to determine the information content in water spin-lattice NMRD profiles when applied to a rather well characterized micellar system. The aggregation number and the radius of the spherical ganglioside micelles are known to be temperature independent for \( T \leq 30 \) °C[1]. The hydration on the other hand is not well known and here the proton water \( R_{1,\text{NMRD}} \) experiment provide some new information about the hydration of the micellar head group.

The theoretical expression (Eq.(1)) for relaxation model is simplified as:

\[
R_{1}(\omega) = \alpha + f_{\beta_1} \cdot R_{1,\beta_1} + f_{\beta_2} \cdot R_{1,\beta_2}.
\]

![Figure 2](image_url)  

The measured proton \( R_{1,\text{exp}}^\text{NMRD} \) profile for \( T=10 \) °C and the concentration 24mM. The theoretical NMRD is composed by \( R_{1,\beta_1}(0) = S_1^2 f_{\beta_1} \beta_{\text{Intra}} \tau_{\beta_1} (1 + W_D) \) and \( R_{1,\beta_2}(0) = S_2^2 f_{\beta_2} \beta_{\text{Intra}} \tau_{\beta_2} (1 + W_D) \) and \( \alpha \). The theoretical predictions are shown in red points whereas the experimental values are in black.

and the quality of the fitting is expressed as

\[
\chi^2 = \frac{1}{N} \sum_j \frac{[R_{1,\text{exp}}(\omega_j) - R_{1}^j(\omega_j)]^2}{\sigma_j^2}
\]

where \( j \) runs over ca 30 Larmor frequencies from 0.01 MHz to 40 MHz. From a single \( T_1 \) NMRD experiment that deviation \( \sigma_j^2 \) of a relaxation rate \( R_{1,\text{exp}}^j(\omega_j) \) for each field is given as a result of a fitting procedure using about 1000 FID amplitudes. However, this deviation was much to small compared to the relaxation deviation obtained from three independent \( T_1 \) NMRD experiments. Actually, the \( \sigma \)-vector of a single NMRD experiment has to be multiplied with 10 before included in Eq.(9). Then the \( \chi^2 \) value is about 1.0(0.99-0.95) in all performed fittings. Consequently, one cannot motivate to include a third Lorentzian representing an additional pool of protons. The results of the NMRD profiling fit using a Levenberg-Marquardt(L-M) method where obtained using four parameters. The maximum relaxation rate at low field was determined experimentally which equals the sum of \( \alpha \) and two \( \beta \)-relaxation contributions \((S_1^2 f_{\beta_1}, S_2^2 f_{\beta_2})\) thus making the \( \beta \) fractions dependent. The fitting were performed for three values of the factor \( W_D \) (cf Eq.(4)) determine the amount of inter-molecular proton dipole dipole relaxation present. Clearly the WD factors introduce very small changes in the extracted fractions and correlation times. The dispersion at low field determine \( \tau_{\alpha} \) and the end of the dispersion at high fields determine the second correlation time \( \tau_{\beta_2} \). All parameters with their variations obtained from the L-M fitting procedure are summarized in Table 1.

### 4.1 Reorientation correlation times of the micelles

The reorientation of the spherical ganglioside micelles is expected to be well described using the Stoke-Einstein-Debye(SED) theory, which predicts that the reorientation correlation time, \( \tau_{\text{SED}} \), is proportional to the water viscosity, \( \eta_{\text{H}_{2}\text{O}} \) and the volume of the micelle:

\[
\tau_{\text{SED}} = \frac{4 \pi \eta_{\text{H}_{2}\text{O}} R^3_{\text{SED}}}{kT}
\]
Table 1. The model parameters obtained from a non-linear least-square fit by Levenberg-Marquardt method using 4 fitting parameters with $W_D$ constant at 0.1, 0.3 and 0.4 and three temperatures and two micelle concentrations.

| Sample $W_D$,0.1 | $\tau_{c,1}$ (ns) | $\tau_{c,2}$ (ns) | $\alpha$ | $S_f^2$ | $S_f^2(x10^4)$ |
|------------------|------------------|------------------|---------|---------|----------------|
| T=10±1°C, c=24mM | 176.4±4.0        | 13.5±1.4         | 0.79±0.02(14.4) | 0.303±0.005 | 1.01±0.04 |
| T=15±1°C, c=24mM | 151.9±3.7        | 10.3±1.2         | 0.73±0.02(13.3) | 0.287±0.005 | 1.02±0.04 |
| T=20±1°C, c=24mM | 141.9±3.9        | 12.7±1.7         | 0.68±0.02(12.4) | 0.240±0.005 | 0.79±0.06 |
| T=10±1°C, c=15mM | 151.9±4.4        | 10.5±1.7         | 0.67±0.02(12.2) | 0.170±0.005 | 0.63±0.05 |
| T=15±1°C, c=15mM | 151.9±4.0        | 9.4±1.6          | 0.61±0.02(11.2) | 0.170±0.005 | 0.58±0.04 |
| T=20±1°C, c=15mM | 122.6±3.8        | 10.4±1.7         | 0.56±0.02(10.2) | 0.147±0.005 | 0.50±0.04 |

For ganglioside micelles with a radius of 54, the reorientation correlation time, $\tau_{w,E}$, approximates 125 – 165 ns depending on the temperature dependence of the water viscosity $\eta$ = $10^{-3}$(kg/sm), $T$=10°C-1.02 - $10^{-3}$, $T$=20°C. The effective correlation time $\tau_c,1$ may thus be identified as the reorientation correlation time of the micelle which also is consistent with the reorientation correlation time determined by other methods[1].

4.2 The $\alpha$ relaxation contribution

The field independent relaxation contribution denoted $\alpha$ and defined in Eq.(6) represent bulk water, weakly perturbed water at the micelle water interface as well as $\beta$-waters. Qualitatively, the retardation correlation times range between 10-13 ps. This relaxation contribution can be analysed at higher fields where it may display frequency dependence. Such studies may thus result in detail information about weakly perturbed interfacial water.

4.3 The hydration of the Ganglioside micelle or proton exchange with labile non-water protons

The bulk water proton spin-lattice relaxation observed as the NMRD profile is due to the influence of the micelle. One mechanism is bulk(B) water exchanging with long-lived(II) water residing in the micellar head group: $H_2^0 O)B \rightarrow H_2^0 O(II)$ where the II-waters are characterized by an order parameter S and a distribution of different residence times ($\tau_{w,i}$) in the ns time regime. It is expected, from a NMRD/MD study of water residing in a protein, where no exchange between labile non-water protons is present, that the NMRD profile becomes rather stretched (rather broad distribution of residence times) with three or more Lorentzian needed in order to describe the NMRD profile[11].

The second mechanism is bulk water exchanging with intermediate II-waters which are in material exchange with labile non-water protons: $H_2^0 O)B \rightarrow H_2^0 O(II) \leftrightarrow H - X$.

Now $\tau_{w,i}$ is referring to the life-time of the non-water proton in $H - X$. The correlation time characterize the modulation of the dipole-dipole interaction according to the EMOR model[4]. Thus for $\tau_{w,i} \leq \tau_R$ in eq.(2) is determined by $\tau_{w,i}$ which is characterizing mean life time of labile protons. However, distribution of different life times of labile $H - X$ is expected to be more narrow thus resulting in a less stretched NMRD profile. The fraction of protons for the Lorenzian describing the NMRD profile refer to the number of II-water in material exchange with labile protons (one-to-one relation).

The fraction of water molecules is given by $S_f^2 f_R = N_{w,i} [\text{Micelle}] / 55.56 (i = 1, 2)$ per micelle This number is estimated for the two concentrations and a consistent number $N_{w,i}$ is obtained as 110-120 water molecules at $T=10$ C and about 90-100 at $T=20$ C. The effective correlation time $\tau_c = \tau_R$ The number thus corresponds to slightly less than one water for each ganglioside lipid. For the second pool of water, $N_{w,2}$ with $\tau_c (12 ns) \leq \tau_R$, is about 400 at 10 C and 300 at 20 C.

These estimated values of $N_{w}$ represent a lower limit. The unknowing values of the order parameters may be estimated using NMR relaxation measurement of heavy water in ordered ganglioside lamellar phase samples or very large vesicles, where the reorientation is in the static regime. One may observe a quadrupole splitting of a powder spectra and the order parameter can be estimated from line shape analysis[12]-[15].

5 Conclusions

Using a relatively simple relaxation model our analysis of water proton $T_1$ - NMRD profiles of the ganglioside micelles give the micelle reorientation correlation time $\tau_R$. However the two fractions of protons may be interpreted in terms of two cases: as exchangeable labile protons or "buried" waters. In the case of waters the fraction is given times the order parameter may thus be estimated without knowing the water order parameter. Temperature dependence of the fractions or $N_{w}$
may indicate as observable difference between the two cases where the number of labile protons is expected to be constant in the temperature interval studied. However, data is not sufficient to give the relative importance of the two cases. However, if we consider the simplicity of the proton $T_1$ NMRD dispersion profile, it indicates that it is due to labile protons with effective correlation time determined by the mean life time of the labile protons and fractions giving the number of intermediate waters exchanging with $H - X$ protons (600 (T=20C)-800(T=10C)).

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