NUCLEAR MAGNETIC RELAXATION STUDY OF TOBACCO MOSAIC VIRUS SOLUTIONS

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ABSTRACT The molecular order of water in liquid-crystalline 5–28% tobacco mosaic virus (TMV) solutions was studied by proton spin-spin, spin-lattice, and translational self-diffusion coefficient measurements at various concentrations as well as by deuteron D_2O nuclear magnetic resonance (NMR) studies. The results show that the average H_2O molecule spends less than 1% of its time in an ordered state bound to the TMV backbone. The protons on the TMV molecules themselves, on the other hand have a very short spin-spin relaxation time T_2 of about 20 μs, demonstrating the existence of a high degree of liquid-crystalline order.

The state of water in biological systems has been the subject of many investigations. TMV solutions, in particular, which form a lyotropic, liquid-crystalline "middle" mesophase, were studied in great detail, but rather conflicting results were obtained (1, 2). X-ray studies (3) showed that the TMV molecules form a two-dimensional hexagonal lattice of parallel cylinders with intervening water channels and freedom of movement in the third dimension, i.e., along the axis of the cylinders. Whereas the long-range order of the TMV backbone is fairly well understood, little is known about the molecular order of water in these systems. Jardetzky and Jardetzky (1) concluded on the basis of high resolution proton magnetic resonance studies that as much as 20% of water molecules in a 1.9% TMV solution exists in an ordered, "icelike" structure. Douglass et al. (2), on the other hand, concluded from self-diffusion measurements that at most 3% of water in a 4% TMV solution is immobilized in a bound, icelike state.

In this paper, the aim of which is to resolve the above discrepancy, we report the results of water proton spin-spin (T_2) and spin-lattice (T_1) relaxation measurements as a function of TMV concentration, water self-diffusion measurements, and D_2O deuteron NMR measurements as well as of T_2 measurements on the TMV protons themselves. T_2 was measured by the Carr-Purcell and free induction decay method, whereas a 90°–90° sequence was used for T_1 studies. The self-diffusion coefficients were obtained by the variable field gradient proton spin-echo method. All these

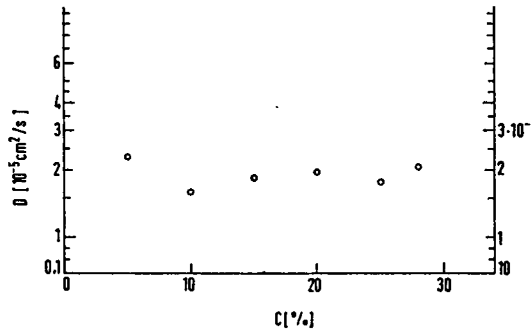

FIGURE 1

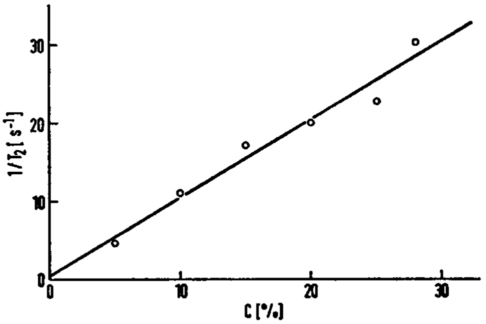

FIGURE 2

FIGURE 1 Concentration dependence of the self-diffusion constant of H_2O molecules in liquid-crystalline TMV solutions at room temperature.

FIGURE 2 Concentration dependence of the H_2O proton spin-spin relaxation rate (T_2^{-1}).

data were taken at 37 MHz, whereas the attempt to observe the quadrupole splitting of the D_2O NMR spectrum was made at 9.5 MHz. The main results are as follows.

(*a*) *Deuteron D_2O Spectrum.* No quadrupole splitting of the deuteron magnetic resonance spectra of the D_2O molecules in a 28% TMV solution in heavy water was observed. This is in sharp contrast to the observed quadrupole splitting in the sodium palmitate-heavy water system (4) where the middle mesophase has a structure similar to that in the present case.

Assuming fast chemical exchange between free D_2O molecules and those rigidly bound to the TMV molecules, we can write the effective quadrupole coupling constant e^2qQ/h of D_2O molecules in the TMV solutions as:

$$(e^2qQ/h) = p\cdot(e^2qQ/h)_{\text{bound}}, \tag{1}$$

where p represents the fraction of time of observation an average D_2O molecule is associated with the virus backbone, and the quadrupole coupling constant of free, isotropically moving D_2O molecules is zero. As $(e^2qQ/h)_{\text{bound}}$ should be of the order of 200 kHz as in ice and the upper limit of the observed value of e^2qQ/h is of the order of the line width, i.e. 0.1–0.2 kHz, we conclude that p, which is also the average fraction of bound water molecules, is less than 1%.

The deuteron NMR spectra of 4% TMV with 25% D_2O gave a line width of 89.5 Hz whereas the 20% TMV with 10% D_2O gave a line width of 145 Hz. The normal D_2O gave a line width of about 72.5 Hz. The small increase in line width and the absence of quadrupole splitting is indicative of the weak interaction of the D_2O molecules with the TMV backbone.

(*b*) *Self-Diffusion Measurements.* The above conclusion agrees with the results of H_2O self-diffusion measurements. As it can be seen from Fig. 1, the observed values of D are within the limits of experimental error independent of the concentration c of the TMV solutions. The magnitudes of D ($\approx 2 \cdot 10^{-5}$ cm^2/s) are practically equal to those in pure water and much larger than the ones of the TMV molecules themselves ($\approx 3 \cdot 10^{-8}$ cm^2/s) as observed by light scattering (5). The presence of TMV has thus minimal effect on the free water structure.

(*c*) *Spin-Spin and Spin-Lattice Relaxation.* The effect of bound water molecules is expected to be most pronounced on the proton spin-spin (T_2^{-1}) and spin-lattice (T_1^{-1}) relaxation rates of TMV solutions (Figs. 2 and 3). Due to fast chemical exchange the observed relaxation rates will also represent an average of the relaxation rates of free and bound H_2O molecules:

$$\frac{1}{T_i} = \frac{p}{T_{i,\text{bound}}} + \frac{1-p}{T_{i,\text{free}}}, \qquad i = 1, 2. \tag{2}$$

As the correlation times of free water are of the order of 10^{-12} s, whereas the correlation times of the bound water molecules are much larger and should be of the order of the inverse Larmor frequencies, we expect that $(T_i)^{-1}_{\text{bound}} \gg (T_i)^{-1}_{\text{free}}$ and that moreover $(T_2)_{\text{bound}} \ll (T_1)_{\text{bound}}$ whereas $(T_1)_{\text{free}} \approx (T_2)_{\text{free}}$. As it can be seen from Figs. 2 and 3 the ratio T_1/T_2 is indeed much larger than 1 and varies between 10 and 20, indicating a large contribution of the bound water molecules to the average relaxation rate.

The experimental data can be represented by the expression:

$$1/T_i = (1/T_{i,\text{free}}) + k_i \cdot c, \qquad i = 1, 2, \tag{3}$$

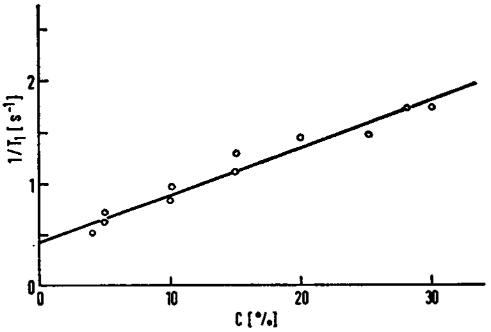

FIGURE 3

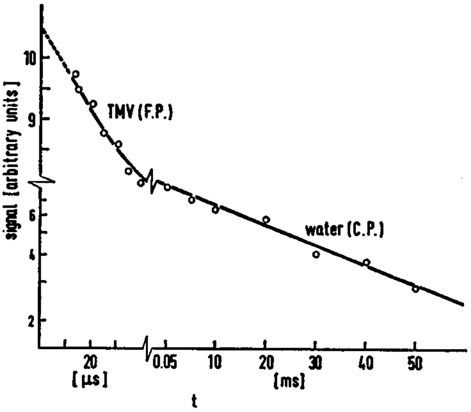

FIGURE 4

FIGURE 3 Concentration dependence of H_2O proton spin-lattice (T_1^{-1}) relaxation rates in liquid-crystalline TMV solutions at room temperature.

FIGURE 4 Proton-free induction decay signal and Carr-Purcell spin-echo envelope as a function of time in a 20% TMV solution.

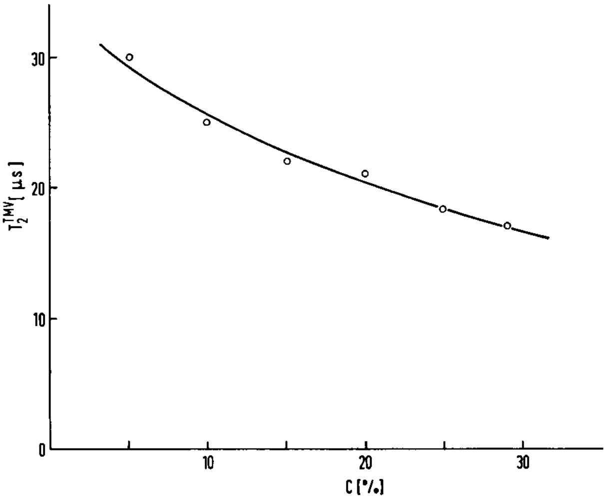

FIGURE 5 T_2 of TMV protons as a function of TMV concentration in liquid-crystalline TMV solutions.

where $T_{1,\text{free}} = T_{2,\text{free}} \approx 3$ s, c is the concentration of the TMV solution (expressed in weight fractions), and the values of k_i, which are proportional to $T_{i,\text{bound}}^{-1}$, are: $k_1 \approx 4.2\ \text{s}^{-1}$ and $k_2 \approx 100\ \text{s}^{-1}$. Expression 3 is, of course, equivalent to expression 2 as long as $p = (n/c_p) \cdot c \ll 1$. Here n is the number of protons rigidly bound to a TMV molecule, c is the concentration of TMV particles and c_p the concentration of water protons in the solution.

In summary, the above results show that the fraction p of bound, ordered water in TMV solutions is much smaller than 1% and that the liquid crystalline long range order of TMV solutions does not seem to arise from interactions of hydration shells of the virus particles (5, 6).

(*d*) *TMV Proton Spin-Spin Relaxation.* Superimposed on the long decay of the H_2O molecules is a very short decay ($T_2 \approx 20$ μs) due to the TMV protons themselves (Fig. 4). As it can be seen from Fig. 5, the spin-spin relaxation time of the TMV protons is rather short and varies between 17 and 30 μs. This demonstrates a high degree of liquid-crystalline order of the TMV backbone.

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