Proton NMR spin grouping and exchange in dentin

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ABSTRACT The nuclear magnetic resonance spin-grouping technique has been applied to dentin from human donors of different ages. The apparent T_2, T_1, and $T_{1\rho}$ have been determined for natural dentin, for dentin which has been dried in vacuum, and for dried dentin which has been rehydrated in an atmosphere with 75% relative humidity. All apparent spin relaxation has been analyzed for exchange between the spin groups in which the dentin protons exist; the analyses incorporate the results of selective inversion recovery T_1 measurements which better probe the effects of exchange. The exchange analyses of the high fields and rotating frame spin-lattice relaxation have also been correlated to determine uniquely the inherent relaxation parameters of the proton spin groups constituting the dentin magnetization. The natural dentin contains protons on water, protein, and hydroxy apatite; these spins contribute 50%, 45%, and 5% to the total dentin proton magnetization, respectively. The water exists in three distinct environments, the dynamics of each environment has been modeled. In the natural dentin 30% of the water undergoes uni-axial reorientation, 52% of the water has similar relaxation characteristics to bound water hydrating a large molecule, and the majority of the remaining water acts as bulk water undergoing isotropic reorientation. The results are independent of the age of the donor.

INTRODUCTION

Nuclear magnetic resonance (NMR) has been used extensively during the past two decades for investigation of biological systems. In particular, it has been used in studies of the nature of water in tooth enamel (1, 2). Recently, the use of magnetic resonance as a probe in dental research has increased (3). For example, the NMR spin-grouping (4) has made possible determination of the various types of water in human enamel (5) and a comparison of the sizes of the proton groups in human dentin from different donors (6). This initial work is now followed by a study of the water dynamics in dentin.

Dentin is the bone-like hard tissue immediately below the enamel; it constitutes the bulk of the tooth (7). The dentin consists of ~35% organic matter and water and ~65% inorganic material, mainly hydroxyapatite. Like bone, the structure of the dentin is provided by a solid network of collagen fibers embedded with hydroxyapatite crystals. Throughout this solid matrix extend channels (called dentinal tubules) which contain Tomes fibers, the cytoplasmic extensions of the specialized cells, the odontoblasts, which make dentin a living tissue. The main bodies of the dentin-forming odontoblasts are confined to the inner surface of the dentin at its interface with the dental pulp organs. Thus, dentin is much more complex tissue than the enamel.

It has been known since the discovery of NMR that various spins in a heterogeneous sample can be classified by their spin–spin relaxation times (T_2's). The sizes of the spin groups can be determined if the total free induction decay (FID) of the sample can be resolved into magnetization "components" decaying with the different T_2's. This separation is straight forward if the T_2's of the various spin groups differ by a factor greater than 4 or 5. The characterization of heterogeneous systems is enhanced by the spin-grouping NMR technique (4, 8) which enables an additional resolution based on the different spin-lattice relaxation times (T_1's or $T_{1\rho}$'s).

In this report the proton groups of dentin from human donors are classified using FID analysis and spin-grouping at both high fields (T_1) and in the rotating frame ($T_{1\rho}$) at three H_1's. The results for dentin from donors of different age groups are compared. A model is proposed for the proton relaxation in dentin in terms of the inherent relaxation of the separate spin groups and magnetization transfer between these groups. The NMR techniques give information on the dynamics of the dentinal water which cannot be determined by conventional x-ray or electron microscopic structural analysis of dentin. NMR spectroscopy also gives an indication of the criteria which must be met to develop a viable MRI for dentistry (3).

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THE SPIN-GROUPING TECHNIQUE

The proton relaxation times of the human dentin were measured with the spin-grouping technique (4, 8). This technique improves the NMR resolution of heterogeneous systems exhibiting pronounced nonexponential magnetization evolutions. With spin-grouping the total magnetization of the sample is resolved into a superposition of component magnetizations, each recovering with its own spin-lattice relaxation time. The relaxation measurement is performed as follows. The FID of the heterogeneous sample is divided into time intervals called "time windows," each window is usually a few microseconds in duration. The evolution of the magnetization upon a pulse excitation can then be monitored separately, yet simultaneously, at each of the windows along the FID. Therefore, at the end of a spin-grouping experiment the magnetization evolution has been recorded as the elements of a two time dimensional manifold which is dependent on the time between the pulses in the measuring sequence, as well as on the time window on the FID. Typically, the magnetization is recorded at 33 windows for 33 pulse spacings.

The nonmonoexponential spin-lattice evolution of the magnetization is decomposed using a graphically assisted least squares fit into resolvable magnetization components. The magnetization evolution is plotted semi-logarithmically and the user defines the time regimes of the evolution which are to be fitted with the least squares regression (9), beginning at the tail end of the evolution where only the slowest relaxation component is still present. Such a decomposition is not possible in all systems. However, the spin-lattice evolution can be unambiguously and uniquely broken down into a sum of exponentially relaxing magnetizations if the spin-lattice relaxation times of the different magnetization components of the composite evolution differ by a factor greater than five (8). At any particular window the slope of the fit of the recovery function for each magnetization component gives its relaxation time T_1 (or $T_{1\rho}$), while the intercept at zero pulse spacing gives its contribution to the total magnetization at the window. The fit is repeated at each time window along the FID.

The plot of the magnetization fractions vs. time window "position" then gives the decomposed FID's corresponding to the different spin-lattice relaxation times. The fraction of the total magnetization which corresponds to a particular spin-lattice relaxation time can be determined by extrapolating its FID to the zero time window. Such a fit also determines the spin–spin relaxation time, or times, of each FID component. The FID's are fit as the sum of exponentially decaying and Gaussian decaying components. The solid Gaussian FID's are described using the convention of Kubo and Tomita (10) where T_2 gives the time for the magnetization to decay to one half of the initial magnetization. The second moment for the Gaussian lineshape is then $M_2 = 1936/T_2^2$, where the units are Gauss^2 and microseconds for the second moment and T_2, respectively.

By the spin-grouping procedure the sample is characterized in terms of the spin-lattice and spin–spin relaxation times of a number of spin groups along with the relative magnetizations (sizes) of these groups. The spin-grouping measurement of the NMR relaxation rates is obtained in the same total time required by standard detection; that is, the repetition time for the pulse sequence (e.g., $5 \times T_1$) times the number of pulse spacings times the number of averages taken for each spacing. All the advantages of the technique are obtained through the analysis of the 33×33 magnetization evolution manifold. Typically it takes 15 min to decompose the composite magnetization evolution with the existing software.

MATERIALS AND EXPERIMENT

Dentin fragments were obtained from healthy human incisors of 20, 35, and 50 yr old donors. The dentin was isolated using a water cooled diamond disk, each fragment was ~3 mm^3. The samples were stored in a 0.9% NaCl buffer. "Natural" dentin samples were prepared by blotting fragments of dentin from five donors of the same age group and sealing them into 8 mm OD NMR glass tubes with teflon. After the experiments were completed, these "natural" samples were dried at room temperature at atmospheric pressure for a period of 6 d and, subsequently, at a pressure of 10^{-3} Torr for 3 d. These samples are labeled "dry." After all "dry" experiments were completed, the dry samples were rehydrated in a constant atmosphere of 75% relative humidity (r.h.). This was achieved by placing the samples in a dessicator over a saturated aqueous sodium chlorate, $NaClO_3$, solution for 4 d (11). These samples are labeled "rehydrated."

The NMR measurements were performed at 20°C with an SXP Bruker pulse spectrometer (Bruker Canada Inc., Malton, Ontario) at 40 MHz. The signal averaging and the data analysis were done with a Hewlett-Packard model 9836 computer (Hewlett-Packard, Palo Alto, CA) interfaced with the spectrometer through a Biomation 805 transient waveform recorder (Biomation, Cupertino, CA). All NMR signals were recorded with an 8-μs delay after the r.f. pulse due to the deadtime of the receiver of the spectrometer. Typically 100 signals were averaged for a T_2 measurement, while 16 signals were accumulated for each pulse spacing in the spin-grouping T_1 experiments.

The T_2^* measurements were made by recording 110 points along the FID after a $\pi/2$ pulse. The T_2 for the natural dentin was determined using the $[\pi/2\text{-}\tau\text{-}(\pi\text{-}2\tau\text{-}\pi) \ldots]$ Carr-Purcell Meiboom-Gill (CPMG) spin-echo pulse sequence with the phase of the π pulses shifted by 90° (12). The high field T_1 spin-grouping was performed with the inversion recovery pulse sequence $[\pi\text{-}\tau\text{-}\pi/2]$. The T_1 experiments were performed under conditions of both nonselective (hard-hard [h-h]) and selective (soft-hard [s-h]) inversion with lengths of 2 and 200 μs for the hard (nonselective) and soft (selective) preparation π pulses, respectively. The relaxation times in the rotating frame, $T_{1\rho}$, were measured with the spin-locking pulse sequence (13); the field pulse was set at 5, 7, and 10 G.

RESULTS

The results of the T_2 measurements are presented in Table 1. The number of spin groups resolved by the FID decomposition alone is dependent on the hydration of the dentin (see Fig. 1). The dry samples are well characterized by two magnetization components having T_2's of ~65 and 12 μs. The rehydrated samples have an additional magnetization component with $T_2 \approx 200$ μs. The FID of the natural sample has yet another magnetization component with $T_2 \approx 1{,}000$ μs. It is interesting that the characterization is independent of the age of the donors from which the dentin was obtained. Within the accuracy of the experiment, the sizes of the magnetization components (each having a different T_2) are also independent on the age of the dentin. The true T_2 of the slowly relaxing magnetization of the natural sample, as determined separately by the CPMG measurements, is 38 ± 6 ms; therefore the T_2^* component of 1 ms is shortened by macroscopic (e.g., magnetic field) or microscopic inhomogeneities.

A T_2 FID experiment was performed also on a single fragment of natural dentin from one 35 yr old donor to establish whether the distribution of structural sites in the multidonor samples was an important factor in determining the line shape of the sample. No significant difference was seen in the shape of the FID. The decomposition of the FID of the single fragment into magnetization components also gave results identical to the decomposition of the FID of the multi-fragment samples.

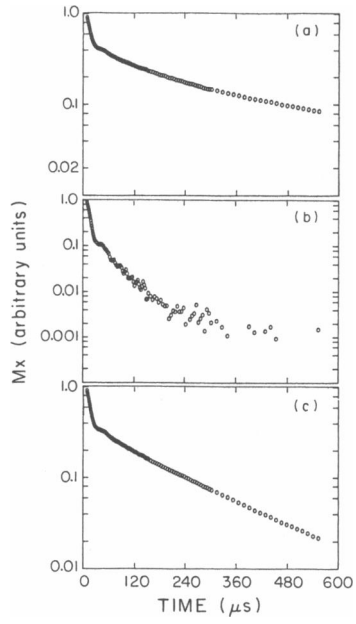

FIGURE 1 The proton Free Induction Decays (FID's) of the dentin from the 50 year old donors. (*a*) That of the natural blotted dentin characterized by four T_2's of about 1,000, 200, 60, and 12 μs; (*b*) that of the dry dentin characterized by two T_2's of ~60 and 12 μs; (*c*) that of the dentin rehydrated in the 75% r.h. atmosphere characterized by three T_2's of ~200, 60, and 12 μs. The FID's of the dentin from the 20 and 35 yr old dentins are identical to the 50 yr old dentin within the experimental accuracy. It should be noted for the dry dentin that there is a small fraction (1%) of the signal which is coherent noise. The noise is less significant in the other samples. It does not influence the results of the analysis.

TABLE 1 The proton spin-spin relaxation times and magnetization fractions of human dentin from 20 and 50 yr old donors: results of the FID analysis

SAMPLE	20 yr old dentin		50 yr old dentin	
	T_2 (μs) or T_2^* (μs)	M_{oi} (%)	T_2 (μs) or T_2^* (μs)	M_o (%)
blotted§	~1000*‡	9 ± 2	~1000*‡	9 ± 2
natural	220 ± 20	28 ± 4	180 ± 20	28 ± 3
	56 ± 5	7 ± 2	57 ± 5	5 ± 2
	12 ± 1	56 ± 4	12 ± 1	58 ± 4
dry	68 ± 3	9 ± 2	65 ± 6	8 ± 2
	12 ± 1	91 ± 3	12 ± 1	92 ± 3
75% r.h.	215 ± 20‡	29 ± 2	205 ± 15‡	28 ± 3
rehydrated	69 ± 3	6 ± 2	70 ± 3	6 ± 2
	13 ± 1	65 ± 3	13 ± 1	66 ± 3

*Imposed from T_1 results and FID measured to 1.5 ms after the 90° pulse.

‡T_2^* due to macroscopic and/or microscopic magnetic field inhomogeneities.

§The results for the blotted dentin from one 30 yr old donor are essentially identical (T_2 = 900 ± 200 μs, 8 ± 2%, 186 ± 10 μs, 32 ± 3%; 55 ± 2 μs, 5 ± 1%; and 12 ± 2 μs, 55 ± 3%).

The results of the high field spin-grouping with the nonselective and selective inversion recovery pulse sequences are presented in Tables 2 and 3, respectively. Again the results for the dentins obtained from donors of different ages are identical. The magnetization recoveries of the dried and rehydrated dentin are well characterized by a single time constant (~300 and ~115 ms, respectively) when measured with the nonselective h-h pulse sequence. However, the s-h magnetization recoveries (by the selective inversion preparation) from the same samples are characterized by two T_1's. Examples are illustrated in Figs. 2–4. The slow T_1's are the same as those measured by the h-h experiments while the fast relaxing components of the magnetization have T_1's of ~5 ms. As discussed below, this is a demonstration of magnetization transfer between the spin groups within the dentin (14). The magnetization evolutions of the natural dentin from the h-h measurements are

TABLE 2 **Results of the nonselective (h-h) T_1 spin-grouping characterization of the dentin proton magnetization**

SAMPLE	20 yr old dentin			50 yr old dentin		
	T_1 (*ms*)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)	T_1 (*ms*)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)
blotted‡	480 ± 40	800 ± 200*	8 ± 2	460 ± 20	1,000 ± 200*	9 ± 2
natural	110 ± 5	250 ± 23*	31 ± 3	105 ± 7	200 ± 20*	30 ± 3
		55 ± 5	6 ± 1		62 ± 6	5 ± 2
		12 ± 2	55 ± 4		12 ± 2	56 ± 3
dry	300 ± 15	60 ± 5	8 ± 2	285 ± 20	59 ± 5	8 ± 2
		12 ± 1	92 ± 2		12 ± 2	92 ± 2
75% r.h. rehydrated	115 ± 10	185 ± 20*	30 ± 3	114 ± 9	205 ± 15*	28 ± 3
		60 ± 5	6 ± 2		67 ± 3	6 ± 2
		12 ± 1	64 ± 3		13 ± 1	66 ± 3

*T_2^* due to macroscopic and/or microscopic magnetic field inhomogeneities.
‡The results for one 30 yr old dentin sample are identical: T_1 = 458 ± 50, 96 ± 9 ms.

characterized by two spin-lattice relaxation times of ~470 and 107 ms. The magnetization recovering with the long T_1 = 470 ms is also characterized by a long T_2^* of ~1 ms. The component of the magnetization which recovers with $T_1 \approx 107$ ms has an FID identical to that of the 75% r.h. rehydrated sample. When measured with the selective inversion sequence the recovery of the magnetization of the natural blotted dentin is characterized by three T_1's of ~485, 106, and 4.5 ms. The decomposed FID corresponding to the magnetization recovering with the long T_1 is the same as that observed in the h-h experiment.

The results of the spin-grouping in the rotating frame are presented in Table 4; a typical spin-grouping is illustrated in Fig. 5. The magnetization fractions reported have been corrected for coupling of the Zeeman reservoir to the dipolar reservoir of the protons in the strong dipolar fields in the rigid lattice (15). Again the

TABLE 3 **Results of the selective (s-h) T_1 spin-grouping of the dentin proton magnetization**

SAMPLE	20 yr old dentin			50 yr old dentin		
	T_1 (*ms*)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)	T_1 (*ms*)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)
blotted natural	499 ± 80	500 ± 200*	9 ± 2	488 ± 51	630 ± 200*	12 ± 3
	109 ± 5	340 ± 18*	36 ± 3	106 ± 8	200 ± 50*	35 ± 3
		43 ± 5	6 ± 3		51 ± 2	5 ± 3
		12 ± 2	48 ± 4		12 ± 2	49 ± 5
	4.5 ± 0.4	235 ± 10	25 ± 2	4.4 ± 0.4	190 ± 5	26 ± 3
		13 ± 3	−24 ± 6		16 ± 3	−27 ± 5
dry				244 ± 32	63 ± 10	12 ± 3
					12 ± 2	68 ± 7
				3.2 ± 0.9	50 ± 10	15 ± 3
					11 ± 4	−12 ± 5
75% r.h. rehydrated	113 ± 5	197 ± 13*	30 ± 3	110 ± 3	179 ± 13*	33 ± 3
		59 ± 10	4 ± 2		41 ± 6	4 ± 2
		12 ± 1	59 ± 3		12 ± 2	56 ± 4
	5.2 ± 0.5	190 ± 20	28 ± 3	4.3 ± 0.4	171 ± 15	28 ± 3
		13 ± 3	−21 ± 5		13 ± 2	−21 ± 4

*T_2^* due to macroscopic and/or microscopic magnetic field inhomogeneities.

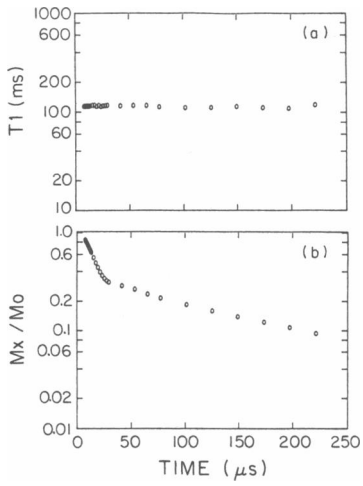

FIGURE 2 The results of the h-h T_1 spin-grouping of the rehydrated 50 yr old dentin at 75% r.h.: (*a*) the variation of T_1 with window position, only one T_1 (114 ± 9 ms) is needed to characterize the decay; (*b*) the corresponding FID. The results for the 20 and 35 yr old dentin are identical to the 50 yr old dentin within the experimental accuracy.

$T_{1\rho}$'s and the FID decomposition are independent of the age of the dentins. As is usually the case for tissues and dense solutions, the values of the spin-lattice relaxation times in the rotating frame are considerably shorter than those measured at high fields. The $T_{1\rho}$'s of the dry sample are "1/*e*" values obtained by determining M_x/e of the

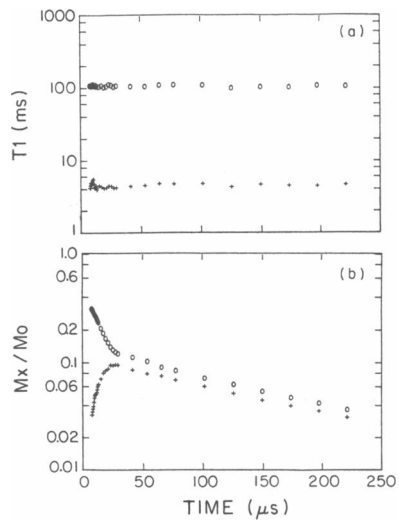

FIGURE 3 The results of the s-h T_1 spin-grouping of the rehydrated 50 yr old dentin: (*a*) the variation of T_1's with window position, two time constants (T_1 = 4.3 ± 0.4 and T_1 = 110 ± 3 ms) characterize the recovery at all windows; (*b*) the corresponding FID's. The growth of the magnetization component relaxing with the fast spin-lattice relaxation time is manifestation of magnetization transfer within the dentin.

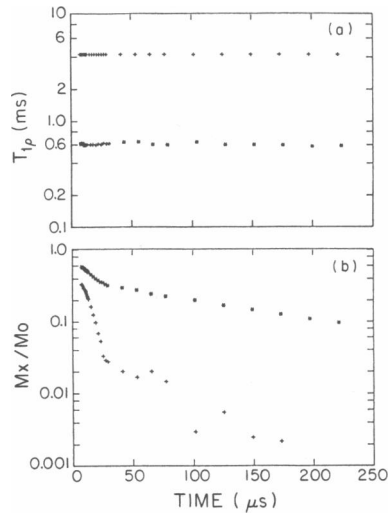

FIGURE 4 The results of the rotating frame spin-grouping of the rehydrated 50 yr old dentin: (*a*) the variation of $T_{1\rho}$'s with window position; two time constants (4.2 ± 0.2 and 0.6 ± 0.1 ms) characterize the decay at all windows; (*b*) the FID's corresponding to the different relaxation components. The results for the 20 and 35 yr old dentin are identical to these results within the experimental accuracy.

magnetization decay in the rotating frame. The spin-grouping could not reliably resolve the decay into components since the fast and slow $T_{1\rho}$'s differed by only a factor of about three (8). In addition, the magnetization fraction corresponding to the slowly relaxing component was <20%. Even though the tail of the magnetization evolution is not fitted, the 1/*e* fit characterizes the decay of each dry sample quite well (i.e., to below 10% of the initial magnetization). The 1/*e* $T_{1\rho}$ values for the dry dentin are ~4.8, 3.8, and 2.9 ms at H_1 = 10, 7, and 5 G, respectively. Upon rehydrating the sample in the 75% r.h. atmosphere, an additional distinct magnetization component with $T_{1\rho}$ of ~0.7 ms appears at both H_1 = 7 and 10 G. The remainder of the magnetization continues to relax with $T_{1\rho} \approx 4.4$ ms. There are two further components of the magnetization of the natural blotted dentin which relax with $T_{1\rho} \approx 30$ ms and $T_{1\rho} \approx 5$ ms. These components of the magnetization are characterized by a long $T_2^* \geq 500$ μs.

DISCUSSION

Apparent NMR relaxation of heterogeneous systems undergoing exchange

It is well known that the results of NMR relaxation measurements in heterogeneous systems are difficult to interpret not only because of the distribution of protons

TABLE 4 **The results of the spin-grouping in the rotating frame**

Sample		20 yr old dentin			50 yr old dentin		
	H_1 (G)	$T_{1\rho}$ (ms)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)*	$T_{1\rho}$ (ms)	T_2 (μs) or T_2^* (μs)	M_{oi} (%)*
blotted natural	10	30 ± 3	600 ± 200	5 ± 2	29 ± 3	700 ± 200	5 ± 2
		4.4 ± 0.2	1,000 ± 200	5 ± 3	4.8 ± 0.3	900 ± 200	5 ± 3
			12 ± 2	42 ± 4		12 ± 2	4 ± 4
		0.8 ± 0.1	190 ± 15	28 ± 3	0.7 ± 0.1	200 ± 15	39 ± 3
			41 ± 7	4 ± 2		37 ± 10	3 ± 2
			12 ± 2	16 ± 2		12 ± 2	14 ± 2
	5	36 ± 3	700 ± 300	4 ± 2	30 ± 5	650 ± 300	3 ± 2
		4.6 ± 0.5	500 ± 100	6 ± 3	4.5 ± 0.7	1,000 ± 400	5 ± 2
			12 ± 2	37 ± 4		13 ± 2	36 ± 5
		0.5 ± 0.1	230 ± 35	30 ± 3	0.5 ± 0.1	208 ± 7	32 ± 3
			30 ± 10	6 ± 2		43 ± 15	4 ± 2
			14 ± 2	17 ± 4		11 ± 3	20 ± 4
dry	10	4.8 ± 0.4‡	75 ± 5	6 ± 3	4.4 ± 0.4‡	74 ± 16	7 ± 3
			12 ± 2	94 ± 3		12 ± 1	93 ± 2
	7	3.8 ± 0.4‡	70 ± 14	7 ± 3	3.8 ± 0.3‡	65 ± 8	6 ± 3
			12 ± 1	93 ± 3		12 ± 2	94 ± 2
	5	2.9 ± 0.3‡	60 ± 10	9 ± 3	2.8 ± 0.2‡	60 ± 5	6 ± 3
			12 ± 2	91 ± 2		12 ± 1	94 ± 3
75% r.h. rehydrated	10	4.6 ± 0.2	75 ± 25	2 ± 2	4.5 ± 0.3	70 ± 30	2 ± 2
			13 ± 2	36 ± 3		12 ± 2	38 ± 1
		0.7 ± 0.1	195 ± 15	32 ± 4	0.8 ± 0.1	180 ± 15	30 ± 3
			50 ± 10	5 ± 2		47 ± 20	4 ± 2
			13 ± 2	26 ± 2		12 ± 2	26 ± 3
	7	4.3 ± 0.1	76 ± 20	2 ± 2	4.2 ± 0.2	76 ± 15	2 ± 2
			12 ± 2	37 ± 3		12 ± 2	39 ± 4
		0.6 ± 0.1	180 ± 10	33 ± 3	0.6 ± 0.1	180 ± 12	32 ± 3
			60 ± 15	3 ± 2		45 ± 15	3 ± 2
			13 ± 2	25 ± 2		13 ± 2	24 ± 3

*The magnetization fractions have been corrected for coupling to the dipolar reservoir.
‡"$1/e$" values, see text.

in several environments but also because of the exchange of magnetization between these environments (14, 16–19). This exchange modifies the observed (or "apparent") relaxation so that it is dependent not only upon the true (or "inherent") relaxation of each spin group in its specific environment, but also upon the rate of transfer of the magnetization between the spin groups.

For example, if the magnetization transfer within a system with two spin groups is sufficiently fast only one relaxation time will be apparent for the system. This is the so-called Zimmerman-Brittin fast exchange limit (17) with:

$$1/T_1 = M_a/T_{1a} + M_b/T_{1b}, \quad (1)$$

where M_a and M_b are the inherent spin magnetizations of the two spin groups, T_{1a} and T_{1b} are their respective inherent relaxation times, and T_1 is the apparent spin-lattice relaxation time. In the fast exchange limit Eq. 1 is valid also for $T_{1\rho}$ and T_2. Empirically, the exchange rate appears fast if it satisfies (16):

$$k_a^2/[M_b(T_{1a}^{-1} - T_{1b}^{-1})^2] \geq 20, \quad (2)$$

with k_a the exchange rate from spin group a to b, M_b the true fraction of spins in the group b (normalized so that: $M_a + M_b = 1$), and the T_1's the inherent relaxation times of Eq. 1.

The equations for the apparent relaxation times and magnetization fractions in the limits of slow and interme-

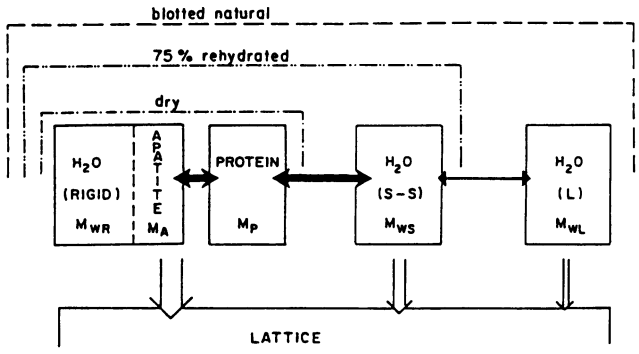

FIGURE 5 The inherent spin magnetizations, relaxation rates, and exchange parameters in the proposed relaxation model for dentin. The dotted lines define the spin groups present in the different samples. The magnetization of the spin groups were determined by the exchange analysis of the spin-grouping experiments (see Table 5).

diate exchange are also well known (16–19). These limits are not presented here. Although their forms are more complicated than that of Eq. 1, they are tractable and it is possible to solve for the inherent relaxation parameters if the apparent relaxation and the apparent magnetization are determined. For two spin groups the inherent relaxation times are given in terms of the apparent parameters by:

$$1/T_{1a} = [(\lambda^+ - \lambda^-)C^- + M_b\lambda^- - M_a\lambda^+ - k_a]/(M_b - M_a) \quad (3)$$

and

$$1/T_{1b} = (\lambda^+ + \lambda^-) - T_{1a}^{-1} - k_a - k_b; \quad (4)$$

together with the condition of dynamic equilibrium:

$$k_aM_a = k_bM_b. \quad (5)$$

The inherent relaxation times and magnetization fractions of the *a* and *b* spin groups (T_{1a}, T_{1b}, M_a, and M_b) are defined as in Eq. 1 and 2. These equations are again valid also for T_2 and $T_{1\rho}$. The inherent times provide information on the molecular dynamics (in the case of spin-lattice relaxation times T_1 or $T_{1\rho}$) or the structure (for T_2) within each spin group. The k_a and k_b are the exchange rates from the *a* group to the *b* group and vice versa. λ^+ and λ^- are the relaxation rates corresponding to the short and long apparent relaxation times, respectively, while C^- is the apparent magnetization fraction of the magnetization relaxing with the long time constant $(\lambda^-)^{-1}$. If the inherent fractions are known a priori, the inherent relaxation parameters can be obtained directly from the results of the spin-grouping as all apparent quantities are well determined. This step is termed the exchange analysis of the apparent relaxation. However, if M_a and M_b are not known, the solutions can be parametrized for example in terms of M_a. It is then possible to search for a unique solution by correlating the exchange analyses of different spin-grouping experiments on the same sample. Since the relaxation times measured in the rotating frame are typically two orders of magnitude shorter than those at high fields, this analysis is particularly reliable because the exchange is probed independently at two time scales. It should be noted that it is also necessary to determine the spin-lattice relaxation at high fields and in the rotating frame to better model the molecular dynamics of the system.

An essential tool in the analysis of exchange within a heterogeneous biological system is the use of the selective inversion technique (14) in which the proton relaxation of the system is studied with initial conditions of the magnetization different than those used in conventional relaxation spectroscopy. The modification of the initial conditions is brought about usually by the use of the so-called selective (or "soft") π pulses of duration δ which inverts the magnetization having $T_2 > \delta$ but leaves the solid protons with $M_2^{-1/2} \approx T_2 \ll \delta$ essentially undisturbed (at equilibrium with the lattice). The evolution of the magnetization is then monitored with a short (or "hard") $\pi/2$ pulse so that all of the magnetization is measured. The selective inversion (or "s-h") pulse sequence enables the direct observation of magnetization transfer between the protons in the solid-like environments and those in the liquid-like spin groups. In the case where all the spin groups are in fast exchange (so that the conventional nonselective "h-h" measures a single apparent relaxation time), the s-h measurement will show an additional short component with T_{1s} which is directly related to the exchange rates of the magnetization transfer (16):

$$1/T_{1s} = k_a + k_b = k_a/M_b. \quad (6)$$

With the selective inversion techniques and the correlation of the exchange analysis of all spin-grouping and T_2 experiments the inherent relaxation parameters of the various protons in dentin can be evaluated.

NMR characterization of dry dentin

The FID of the dry dentin is characterized by two T_2's of ~60 and 12 μs; these values are true T_2's. The exchange analysis when correlated with the T_1 analysis indicates that the spin groups in the dried dentin are in the slow exchange limit, on the time scale of T_2, so that the apparent and inherent spin–spin relaxation parameters are identical. This decomposition of the FID is similar to that of human enamel which has been dried at a temperature of 200° and contains some rigid water, apatite, and small traces of the organic matrix. The magnetization fractions of each T_2 component in dentin

are different, however, from that in enamel. This is to be expected because the protein content of dentin (in weight percent) is ~100 times that of enamel (20). Therefore, the majority of the dry dentin proton magnetization results from the protons on the protein. The FID of the rigid structural protein is characterized mainly by a T_2 of ~10 μs; however, a part of it has a longer T_2. The protons on the protein contribute to both magnetization components of the dentin (with T_2 of 60 and 12 μs). The remainder of the 60 μs component of the dentin is due to the biological apatite (5). The dried dentin may also contain some structural (rigid) water which is closely associated with the solid apatite and not with the protein matrix. This rigid water is characterized in enamel by a T_2 of ~12 μs (5). Because the structural water and the majority of the protein magnetization have about the same T_2's, no direct resolution of the water component is possible from the FID analysis.

The dried dentin is also characterized by a single T_1 of 290 ± 20 ms. Therefore, the magnetization transfer amongst the apatite, rigid water, and protein spin groups is in the fast limit on the time scale of its T_1. The T_1 of biological apatite has been determined in our laboratory (for the 925° sample in reference 5) to be 580 ± 75 ms. The T_1 of the dentin protein, which is predominantly the fibrous structural collagen (7), is also expected to be on the order of 500 ms or longer (16, 21, 22). Therefore, the apparent spin-lattice relaxation of the dry dentin cannot be explained in terms of exchange between the apatite protons and protein protons groups alone. That is, one spin-group in the system must serve as the relaxation sink to bring the apparent T_1 down, recall Eq. 1. Thus the T_1 measurement on the dry dentin confirms the presence of an additional spin-group in the sample; from the previous study of enamel (5) this group is identified with water. Because the dried dentin was prepared by exposing the dentin fragments to a 10^{-3} Torr vacuum for 3 d, it is assumed that the water remaining in the sample is associated with the apatite matrix. The proton magnetization of this water group can be estimated from the spin-lattice relaxation expected for the water protons. The minimum intramolecular T_1 for water undergoing reorientation is $T_{1\min} = 2\pi\nu/kA$, where ν is the frequency of the spectrometer, A is the dipolar constant ($1.097 \times 10^{+10}$ s^{-2}) of water, and k is a constant derived by minimizing the spectral density function of the particular dynamical model for the molecular reorientation. In our case at ν = 40 MHz, water undergoing isotropic reorientation has k = 1.426 and $T_{1\min}$ = 16 ms (23). The water associated with the apatite matrix is not expected to reorient isotropically; with anisotropic reorientation k decreases slightly (16) and $T_{1\min}$ increases to ~25 ms. Therefore, to explain to observed T_1 of the dry dentin, at least ~5% of the total proton mass must be protons on water. The NMR characterization of the dry dentin water spin group can be further determined by assuming that the stoichiometry of the apatite/water matrix for enamel (5) is maintained, i.e., that the dry dentin differs from the enamel only by the 100-fold increase in the protein content. Under these assumptions, the water proton content of the dried dentin would be 22 ± 2% of the total spin mass and, by Eq. 1, the T_1 of the water 110 ± 25 ms.

The rate for the magnetization transfer within the dry dentin is measured by the s-h experiment. The magnetization transfer between the protons on the water and those on the apatite is considered to be fast enough so that these protons constitute one spin group. The rate of the magnetization transfer between this group and the protein protons is then related to the relaxation rate of the short component in the s-h magnetization recover (see Eq. 6). The exchange rate from the water/apatite spin group to the protein spin group is 270 ± 60 s^{-1}.

The majority of the magnetization of the dry dentin decays in the rotating frame with a single $T_{1\rho}$. Therefore, it is assumed that the magnetization transfer amongst the various spin groups of the dry dentin is close to the fast exchange limit even on the time scale of $T_{1\rho}$ (~4 ms). The $T_{1\rho}$ of the fibrous dentin protein is taken to be the same as that of oriented DNA (16, 19); $T_{1\rho}$ = 7, 6, and 4 ms at H_1 = 10, 7, and 5 G, respectively. The $T_{1\rho}$ of apatite is assumed to be >10 ms. Eq. 1 then yields that the rigid water has $T_{1\rho}$ ~ 2.0, 1.7, and 1.4 ms at H_1 = 10, 7, and 5 G, respectively. The inherent relaxation times and magnetization fractions for the dry dentin are summarized in Table 5 along with the inherent parameters of the other samples.

NMR characterization of rehydrated dentin

The analysis of the results for the 75% rehydrated dentin proceeds in the same fashion. In this case the T_2 data show an additional component of the magnetization compared with the dry dentin FID; this component accounts for ~30% of the total dry dentin proton signal. The new magnetization is identified with the water absorbed in the dentin during the sample rehydration. This water is termed structural water, it is characterized by a quasi-liquid T_2 of ~250 μs.

The apparent high field spin-lattice relaxation is well described by a single relaxation time of ~115 ms; therefore the spin-groups of the rehydrated dentin are undergoing fast exchange on this time-scale. The T_1 of the structural water can be calculated using Eq. 1 for fast exchange together with the magnetization fraction de-

TABLE 5 **The inherent magnetization fractions and relaxation times of the dentin spin groups**

Sample	Proton spin group	Magnetization %	T_2 (μs) or T_2^* (μs)	T_1 (ms)	$T_{1\rho}$ (ms)
dry	Protein	$M_p = 71 \pm 3$	~12	>500*	~6*
	Rigid water	$M_{WR} = 22 \pm 2$	~ {60 / 12 ± 2}	110 ± 25	2 ± 1
	Apatite	$M_A = 7 \pm 2$	~60	580 ± 80	≥10*
75% rehydrated	Protein	$M_p = 49 \pm 3$	~12	>500*	~6*
	Rigid water	$M_{WR} = 16 \pm 2$	~ {60 / 12 ± 2}	110 ± 25	2 ± 1
	Apatite	$M_A = 5 \pm 2$	~60	580 ± 80	≥10*
	Structural water	$M_{WS} = 30 \pm 3$	215 ± 20	46 ± 4	{0.7 ± 0.1‡ / 0.6 ± 0.1}
blotted natural	Protein	$M_p = 45 \pm 3$	~12	>500*	~6*
	Rigid water	$M_{WR} = 15 \pm 2$	~ {60 / 12 ± 2}	110 ± 25	2 ± 1
	Apatite	$M_A = 5 \pm 2$	~60	580 ± 80	≥10*
	Structural water	$M_{WS} = 26 \pm 2$	215 ± 20	46 ± 4	{0.7 ± 0.1 / 0.6 ± 0.1}
	Dentinal tubule water	$M_{LW} =$ {8 ± 2 / 1 ± 1}	1,000 ± 200§ / 200 ± 50	1,000 ± 300 / 50 ± 10	≥30 ± 3 / ≥5 ± 1

*Values from the literature (see text).
‡0.7 ± 0.1 at 10 G, 0.6 ± 0.1 at 7 G.
§This is T_2^*, the value obtained with spin-echo(CPMG sequence) is $T_2 \approx 38$ ms.

rived from the FID analysis. The T_1 of the structural water is 46 ± 4 ms.

The spin-lattice relaxation in the rotation frame, however, is not in the fast exchange limit and the magnetization decay in the rotating frame is characterized by two $T_{1\rho}$'s of ~0.7 and 4.5 ms. This is a typical observation in biological systems and dense aqueous solutions in which the $T_{1\rho}$'s are one or two orders of magnitude less than the T_1's (8, 16, 19). The inherent spin-lattice relaxation times in the rotating frame are found by solving the general Eqs. 4–5. The solution is made unique by correlating the exchange rates required for the rotating frame solution with those determined in the s-h selective inversion T_1 measurements with Eq. 6 (16, 19). The structural water has inherent $T_{1\rho}$'s of 0.7 ± 0.1 and 0.6 ± 0.1 ms at 10 and 7 G, respectively. It should be noted that the observed magnetization fraction of the short apparent $T_{1\rho}$ component corresponding to the structural water of the rehydrated dentin is larger than the magnetization expected from the stoichiometry as determined from the FID analysis. Therefore, the protons on the structural water are very strongly coupled to some of the protons of the remaining rehydrated dentin spin groups even on the time scale of 5 ms (16, 19). However, the correlation of the exchange analysis for the rotating frame and the s-h T_1 data indicates that the average exchange rate for the magnetization transfer from the water spin group to the rest of the protons in the rehydrated dentin is only 300 ± 50 s^{-1}. This inherent relaxation of the rehydrated dentin is reviewed in Table 5.

NMR characterization of the natural blotted sample

The natural blotted sample has an additional component of the magnetization not observed in the dry or rehydrated samples; it constitutes ~9% of the total proton signal and is characterized by a T_2^* of 1,000 μs. Therefore, this magnetization is from protons which must have been easily removed from the natural dentin in the preparation of the dry and rehydrated samples. These protons are identified with water in the dentinal tubules which traverse the dentin. The magnetization of the tubular water relaxes with a single apparent T_1 (~480 ms) and with two apparent $T_{1\rho}$'s (~5 and ~30 ms). The additional water spin group of the natural dentin does not alter the high field spin-lattice relaxation of the remainder of the dentin protons (as observed in the rehydrated sample). Also, the apparent spin-lattice times of this magnetization component measured with the h-h and the s-h inversion recovery experiments are identical. Therefore, the exchange processes between the tubule water protons and the rest of

the dentin spin mass are slower than the 500 ms. Because the magnetization of the tubular water protons relaxes with two apparent $T_{1\rho}$'s, these protons are considered to be in at least two distinct subgroups. The proton masses and the inherent relaxation times for these two subgroups are determined by correlating the fast exchange analysis (with Eq. 1) for the apparent T_1 and T_2 measurements, with the general exchange analysis for the $T_{1\rho}$'s (with Eqs. 4–5). The results of the correlated exchange analysis for the tubular water are summarized in Table 5. The inherent relaxation characterization is consistent with the dentinal tubular water existing in bulk and bound fractions (defined here as water able or not able to undergo isotropic reorientation, respectively). However, the analysis is tentative since the protons on the tubule water contribute only a minor fraction of the total natural dentin magnetization.

A model for water dynamics in dentin

The results of the proton NMR (consisting of the FID, spin-grouping, and exchange analysis [16]) of dentin samples are summarized in Table 5. This proposed model is also illustrated in Fig. 5. Within the accuracy of the experiments, the results are independent of the age of the donor. The proton magnetization of dentin can be resolved into magnetizations of protons on apatite, protein, and water. In addition, the water exists in three environments, depending on the sample hydration. All samples have a small rigid water component which has a magnetization evolution characterized by $T_2 \sim 12$ μs, $T_1 = 110 \pm 25$ ms, and $T_{1\rho} = 2 \pm 1$ ms. The two samples with the most water, the 75% rehydrated and the natural dentins, also contain water which has $T_2 = 215 \pm 20$ μs, $T_1 = 46 \pm$ ms, and $T_{1\rho} = 0.6 \pm 0.1$ ms. We have termed this water structural water. Finally, the natural sample also contains water in the dentinal tubules which has a distribution of water existing in the bulk and on the surface of the tubules.

The behavior of water in the three different environments can be modeled from the inherent relaxation parameters evaluated above. The spin-relaxation is assumed to be due to either the isotropic or anisotropic reorientation of the water molecules, a common approach in the modeling of bulk and so-called bound water, respectively (16, 23–25). The isotropic motion is characterized by a single correlation time, τ_c. The anisotropic motion is characterized by two correlation times: τ_r, which describes the fast reorientation about an axis which tumbles slowly, and τ_t, which characterizes the tumbling. The inherent spin-relaxation times of the rigid water correlate well with the model when $\tau_r \sim 10^{-8}$ to 10^{-7} s with τ_t at least seven orders of magnitude slower. That is, the relaxation of the rigid water suggests that the water molecules are restricted essentially to reorientation about a fixed axis. The inherent spin-lattice relaxation determined for the structural water is similar to that of water adsorbed in oriented DNA salts at low hydrations (16, 19). The inherent relaxation is that predicted by the model of anisotropic reorientation with $\tau_t = (5 \pm 0.5) \times 10^{-7}$ s and with the fast reorientation ~ 300 times faster. This motion is typical of the bound water. It should be noted that the ranges of the correlation times quoted reflect two limitations of the current study. Firstly, it is expected that the dynamics of the water should be better represented by a distribution of correlation times, although the distributions may be quite narrow (26). Secondly, the relaxation should also be measured as a function of either temperature or frequency to better determine the molecular dynamics. However, the correlation times quoted above do describe the dynamics rather well. In particular, the differences in the dynamics of the rigid water and the structural water are clearly seen. One support of the determination of the dynamics presented above is that the model of anisotropic motion predicts $T_{1\rho}$ to be nondispersive over the range of correlation times given for the rigid water and the structural water. This is observed; the slight apparent dispersion is a result of the coupling of the Zeeman and the dipolar energies.

The dentinal tubule water is considered to exist in two environments. The majority of the tubule water is in the bulk, and has a proton magnetization characterized by long values of T_1, $T_{1\rho}$, and T_2. The remainder of the tubule water is moderated by contact with the walls of the Tomes fibers in the tubules. The magnetization of this water evolves much as the structural water. The correlation of the exchange analysis of the different spin relaxation measurements gives the bulk water fraction to be the majority ($\sim 95 \pm 3\%$) of the tubule water.

CONCLUSIONS

The NMR correlation consists of three main steps. Firstly, the apparent spin-spin and spin-lattice relaxation of the system is determined. The spin-lattice relaxation at high fields and in the rotating frame are measured with the spin-grouping technique. Additional selective inversion (s-h) experiments are performed to probe the effects of exchange on the apparent T_1. Next, the inherent spin relaxation times and proton magnetizations of the different spin groups in the system are obtained by the exchange analysis of all the apparent data. The exchange analyses for the different apparent measurements (e.g., T_2, T_1, and $T_{1\rho}$) are correlated. Because these measurements probe the system at different time scales (e.g., the $T_{1\rho}$'s are one or two orders of

magnitude less than the T_1's) the correlation of the exchange analysis determines the inherent relaxation of the different spin groups well. Finally, the dynamics of the protons in the different spin groups can be modeled from their inherent relaxation characterization.

In this study the inherent NMR behavior of dentin, the bulk material of the human tooth, has been reported. The natural blotted dentin contains protons on water, protein, and apatite; these spin groups contribute 50%, 45%, and 5% to the total dentin proton magnetization, respectively. 30% of the water is not removed from the dentin even when evacuated. This water undergoes uni-axial reorientation and is strongly bound to the apatite matrix. 52% of the water in the dentin has similar spin-lattice relaxation characteristics to bound water hydrating a large molecule. This water is associated with the surfaces of hydroxyapatite and with the protein matrix of the dentin. The remaining 18% of the water is trapped in the dentinal tubules. The results are identical for dentin from 20, 30, and 50 yr old donors.

Finally, the apparent NMR characterization of the dentin, which has the largest proton magnetization in the human tooth, suggests that the development of magnetic resonance imaging systems for dentistry will require considerable improvements over existing systems. The main limitation is the short T_2 with which the majority of the proton magnetization decays; only $\sim 10\%$ of the magnetization has $T_2 > 250$ μs.

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