Water Binding of Polysaccharides—NMR and ESR Studies

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ABSTRACT: In this paper proton and deuteron NMR relaxation data of water in various physiologically important polysaccharides are presented as a function of the water content. The relaxation times are analyzed by assuming a fast exchange of water molecules between an unbound and a bound water fraction. From this model it can be estimated that less than two water molecules are bound per sugar ring. The water relaxation behavior varies considerably for the different polysaccharide solutions probably caused by the different polymer mobilities which are reduced by decreasing water contents. Microviscosities studied by ESR spectroscopy show that the bulk water mobility is only slightly affected by the water content. Mean rotational correlation times of the water molecules determined from deuteron relaxation increases from 3 ps in pure water to about 1 ns for 30 wt % water in dextran solution. The NMR relaxation times of the samples prepared by the osmotic stress technique are affected only slightly by varying Na^+ content. Hence, electrostatics plays only a minor role for the water binding properties of aqueous polysaccharide solutions.

I. Introduction

Water binding properties of water-soluble polymers and biological tissues are of great interest in modern physics, chemistry, biology, and food industries. Because of this importance, a large number of studies about water binding in biological structures have been performed (reviewed by Berendsen,[1] Comper and Laurent,[2] Wiggins,[3] and Pérez[4]). In two previous papers we could show that water binding properties in cartilage and aqueous polyethylene glycol (PEG) solutions can be well described by the proton and deuteron NMR relaxation of water.[5,6] The polymers in cartilage are strongly restricted in motion whereas the PEG chains are very flexible. In the present study we extend our investigations on water binding on polysaccharides (cartilage components and model systems) with intermediate mobilities.

Different methods have been used for studying water binding of polymers:[1,7] e.g., differential scanning calorimetry, X-ray scattering, neutron scattering, dielectric relaxation, viscosity measurements, and nuclear magnetic resonance (NMR). In the present study, NMR relaxation time measurements[8−16] are used because the relaxation times reflect directly the dynamical properties of water molecules. Different models have been developed to interpret the measured relaxation times.[17−27] They assume either an exchange between fractions of bound and unbound water molecules with distinct correlation times or a distribution of correlation times. In the present paper we used the simplest of those models: the fast exchange between a compartment of unbound water molecules and a compartment of water molecules bound to the macromolecular surfaces. This model can be used if the exchange is fast compared to the relaxation times. In this case the observed water relaxation is monoexponential, and the measured relaxation rates, which are defined as inverse relaxation times, are weighted averages of the intrinsic relaxation rates of the two compartments. The measured monoexponential relaxation rate is then a function of the number of bound water molecules and of the mobilities of the unbound and bound water molecules as well. Therefore, the water relaxation behavior is expected to change from polymer to polymer due to different polymer mobilities and water binding properties. Valuable information upon water binding of polymers can be obtained by studying the relaxation times as a function of the water content. Therefore, the water content of the polymer solutions was varied between 30 and 99 wt %. For the sample preparation the osmotic stress technique[5,28,29] was used which allows one to adjust water content, pH, and salt concentration in an easy way. This method allows to obtain a close relation between NMR relaxation and thermodynamic properties of water, for instance water activity. Some samples were prepared by adding definite amounts of water to the dry polymer.

From the large number of accessible polysaccharides five polymers were selected: dextran, dextran sulfate, chondroitin sulfate, heparin, and xanthan. They differ considerably in their backbone mobility and the number of negatively charged groups. A marked interaction of the negatively charged polysaccharides with cations is expected. Therefore, the Na^+ concentration was varied to elucidate the influence of negative charges on water binding of polyelectrolytes.

In addition to proton relaxation times, the deuteron relaxation times of water molecules were measured because deuteron relaxation is affected by processes which are mainly intramolecular in origin. Therefore, the H_2O in the samples was exchanged by D_2O.

The NMR relaxation times are very sensitive to changes in the rotational and diffusional mobilities of the water molecules. Therefore, in addition to the NMR studies, the microviscosities of the polymer solutions were measured as a function of the water content by ESR spectroscopy to obtain information about the influence of the polymer concentration on the bulk water mobility.

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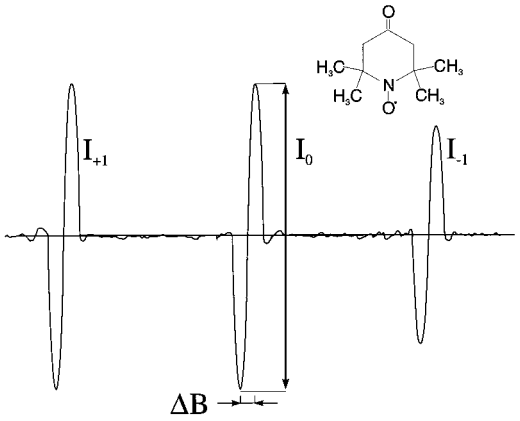

Figure 1. ESR spectrum of tempone in water.

II. Materials and Methods

Materials. The polymers dextran, dextran sulfate, heparin, xanthan, and chondroitin sulfate were purchased from Fluka. The samples with D_2O as solvent were prepared by adding definite amounts of water to the dry polymer. The H_2O samples, however, were produced by the osmotic stress technique[5,28,29] which uses the osmotic activity of poly(ethylene glycol) (PEG) with an average molecular weight of 20 000 (Fluka). Osmotic pressures up to 4.3 MPa were applied by aqueous PEG solutions of up to 40 wt % PEG.[5] By means of this technique it is possible to adjust pH and salt concentration in an easy way. For our experiments pH 7.0 and NaCl concentrations of up to 1 M in the PEG solutions were used.

To prevent mixing of PEG solution with the samples, the polymers were inserted into dialysis membranes (Spectrapor) with a molecular weight cutoff of 1 000. To guarantee thermodynamic equilibrium, the samples were incubated in the PEG solution for at least 48 h. Na-Azid at a concentration of 200 mg/L was added to prevent growing of microorganisms.

For the deuterium measurements D_2O with an isotopic purity of 99.9% (Wilmad) was used as solvent instead of H_2O.

For the NMR measurements the samples were removed from the PEG solution and placed into the NMR tubes. The water content and the number N_M of sugar rings per water molecule of the samples were determined by drying and weighing after the NMR experiments were carried out.

The ESR spin probe tempone (2,2,6,6-tetramethyl-4-oxo-1-piperidine-*N*-oxyl) was purchased from Sigma.

Viscosity Measurements. The macroviscosities of polymer solutions were measured using a Hoeppler viscosimeter (type BH2 from the Prüfgeräte-Werk, Medingen, Germany).

The microviscosities were determined by the lineshape analysis of ESR spectra (home-built ESR spectrometer of the Interdisciplinary Group "Time-Resolved Spectroscopy" at Leipzig University) of the spin probe tempone which was added to the polymer solutions at a concentration of 500 μM. The rotational correlation time τ_R can be calculated from the ESR spectrum of tempone (Figure 1) using

$$\tau_R = A\Delta B\left(\sqrt{\frac{I_0}{I_{-1}}} - 1\right) \tag{1}$$

The constant A equals 6.5×10^{-6} s/T, ΔB is the width of the central signal, and I_0 and I_{-1} are the intensities of the central signal and the high field signal, respectively.[30] The correlation time is connected with the microviscosity η_{mi} by the Debye equation:

$$\tau_R = \frac{4\pi\eta_{mi}a^3}{3kT} \tag{2}$$

a is the radius of the tempone molecule. Inasmuch as the macroviscosities and microviscosities are identical for glycerol/water solutions,[31] the tempone radius a can be determined by measuring the macroviscosities of glycerol/water mixtures by the Hoeppler viscosimeter. From these measurements it follows $a = 0.26$ nm. Hence for $T = 295$ K, eq 2 can be transformed into

$$\eta_{mi} = C\tau_R \tag{3}$$

The constant C equals 5.53×10^7 N/m^2. Equation 3 is useful for the calculation of the microviscosity from ESR measurements.

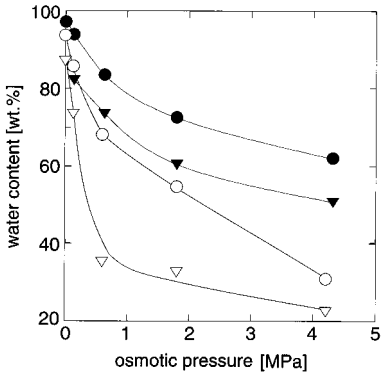

Figure 2. Water content of dextran (○, ▽) and heparin (●, ▼) solutions as a function of the osmotic pressure for varying Na^+ concentration: (○, ●) 50 mM Na^+; (▽, ▼) 1 M Na^+.

NMR Measurements. The proton and deuterium spin−lattice and spin−spin relaxation times, T_1 and T_2, respectively, were determined by using a Bruker AMX300 spectrometer (field strength 7 T). T_1 was obtained by the inversion recovery sequence (15 measured points, recycling time 10 s), whereas T_2 was determined by means of the Carr−Purcell−Meiboom−Gill sequence (echo time $\tau = 200$ μs; at least 256 echoes). The multiexponential analysis of relaxation data was performed by means of the least-squares curve-fitting software package Peakfit v.3 (Jandel Scientific). For all experiments presented in this paper, this analysis showed a monoexponential behavior; i.e., one single relaxation time could be observed for the water molecules in the polymer solutions under investigation.

All NMR measurements were conducted at a temperature of 300 K.

Water Binding Model. For the interpretation of the relaxation times the simplest model of a fast exchange of water molecules between a bound and an unbound state was assumed. This model is based on the equation

$$R_l = p_f R_{lf} + p_b R_{lb} \quad l = 1, 2 \tag{4}$$

R_l is the mean relaxation rate defined by $R_l = 1/T_l$ and R_{lf} and R_{lb} are the intrinsic relaxation times of unbound and bound water, respectively. p_f and p_b are the water fractions of the unbound and bound water compartment, respectively. For $R_{lf} \ll R_{lb}$, eq 4 can be transformed into[6]

$$R_l = N_b R_{lb} N_M + R_{lf} \tag{5}$$

The constant N_b is equal to the number of bound water molecules per sugar ring of the polymers under investigation, and N_M is the number of sugar rings per water molecule. Hence, R_l is a linear function of N_M with slope $N_b R_{lb}$ if a single bound fraction and one unbound water fraction are present.

III. Results

Water Contents. The water contents of all investigated polymers decrease considerably with increasing osmotic pressure. The water contents of dextran and heparin solutions as a function of the osmotic pressure are shown in Figure 2 as examples. In PEG-free incubation solution the polymers can swell almost without restriction resulting in very high water contents

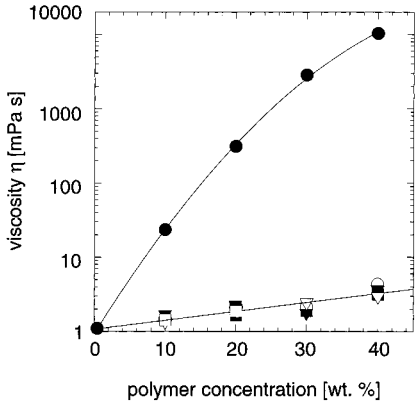

Figure 3. Microviscosities of dextran (○), dextran sulfate (▽), chondroitin sulfate (▼), heparin (□), and PEG (■) solutions as functions of the polymer concentration. The macroviscosities (●) of dextran solutions are also shown.

Table 1. Mass Densities ρ of Dextran, Dextran Sulfate, Chondroitin Sulfate, and Heparin

polymer	ρ (g/cm^3)
dextran	1.56[33]
dextran sulfate	1.96[33]
chondroitin sulfate	1.85−2[34,35]
heparin	2.17[32]

(up to 99 wt % water). Osmotic pressures of 4.3 MPa result in different water contents for the various polymers: 65 wt % water in heparin solution (Figure 2), 60 wt % water in chondroitin sulfate solution, 52 wt % water in xanthan solution, and 30 wt % water in dextran solutions (Figure 2). These large differences are caused by different water uptake properties of the several polymers or, in other words, by different osmotic pressures, e.g. due to the repulsion of charged groups on the polymers (cf. the paper of Peitzsch and Reed[32]).

Using the densities of the polymers taken from literature[32−35] (Table 1) we were able to calculate compressibilities for the different polymers. Their values are between 10^{-5} Pa^{-1} for small pressures and 2×10^{-7} Pa^{-1} for 4.3 MPa and are hence in good agreement with the data given by Peitzsch and Reed[32] for heparin.

Very high Na^+ concentration influences all the interactions which contribute to water binding of polymers resulting in a very strong decrease in water contents even for solutions of the uncharged dextran when using 1 M NaCl (Figure 2).

Viscosity. Figure 3 shows the macro- and microviscosities of dextran solutions for polymer concentrations up to 40 wt %. As expected the macroviscosity strongly increases with rising polymer concentration up to 9 Pa s at 40 wt % dextran. This increase is a result of reduced polymer mobilities. On the other hand the microviscosities represent the mobilities of small solutes in the bulk water. It is a well-known phenomenon that polymer solutions are characterized by different macro- and microviscosities since the mobilities of small solutes and long polymer chains differ considerably. Hence the microviscosities in Figure 3 are much smaller than the corresponding macroviscosities. However, a remarkable slope can be observed also for the microviscosities determined by ESR spectroscopy of tempone. This indicates reduced bulk water mobilities with increasing dextran concentration.

The comparison of microviscosities of dextran solutions with the other polymer solutions (PEG, dextran sulfate, chondroitin sulfate, and heparin) is also shown in Figure 3. It can be seen that all measured polymer solutions have about the same microviscosity at the same polymer concentration. This indicates that only the amount of macromolecular structures is important for the microviscosities. The type of the polymer is of less importance. It has been found[36,37] that self-diffusion coefficients which reflect mostly the bulk water properties depend only on the polymer content regardless of the polymer type.

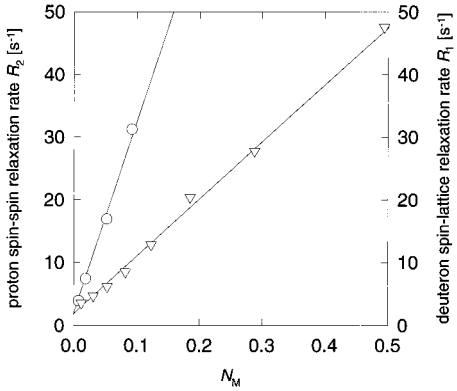

Figure 4. Proton spin−spin relaxation rates R_2 (○) and deuteron spin−lattice relaxation rates R_1 (▽) of aqueous dextran solutions as a function of the number N_M of sugar rings per water molecule. The H_2O samples were prepared by the osmotic stress technique (50 mM Na^+) whereas the D_2O samples were prepared by direct addition of D_2O to the dry polymer. The linear curves have slopes of 333 s^{-1} and 90 s^{-1} for proton R_2 and deuteron R_1, respectively.

NMR Relaxation. Dextran. Figure 4 shows proton R_2 and deuteron R_1 of water in dextran solutions. For the proton relaxation time measurements the samples were prepared by means of the osmotic stress technique whereas in order to measure the deuteron relaxation times definite amounts of D_2O were added to the dry polymer. No homogeneous samples could be produced for water contents lower than 30 wt %. For small numbers of sugar rings per water molecule ($N_M < 0.1$) a linear behavior for both relaxation rates in Figure 4 as well as for deuteron R_2 (data not shown) as a function of N_M can be observed whereas the transverse relaxation rates for higher N_M are considerably larger than the initial linear behavior suggests (data not shown in Figure 4). This is probably a result of strong restrictions in polymer mobility as indicated by the macroviscosities. Such slow processes affect the longitudinal relaxation only slightly so that the linearity in deuteron R_1 continues also for $N_M > 0.1$. The slopes of the linear curves are 333 s^{-1} for proton R_2 and 90 s^{-1} for deuteron R_1 and R_2. The linearity of deuteron R_1 can be observed for the whole range of water contents studied, i.e. for R_1 in the interval of at least 0 to 50 s^{-1} (Figure 4). Therefore, fast exchange can be assumed for $R_1 \leq 50$ s^{-1}. Probably this linearity could be observed for even higher R_1, but as mentioned above no homogeneous samples could be prepared for lower water contents. Hence, no statement can be made for $R_1 > 50$ s^{-1}. Nevertheless, it can be said that R_{1b} should be greater than 50 s^{-1} because R_1 should reach a maximum value and the linearity should discontinue at $R_1 = R_{1b}$. Hence using this value of $R_{1b} > 50$ s^{-1} and the slope of 90 s^{-1}, the number N_b of bound water molecules per dextran sugar ring is smaller than 2. Using this estimated maximum N_b, we get a proton R_{2b} of greater than 166 s^{-1}, which is in the expected order of magnitude.

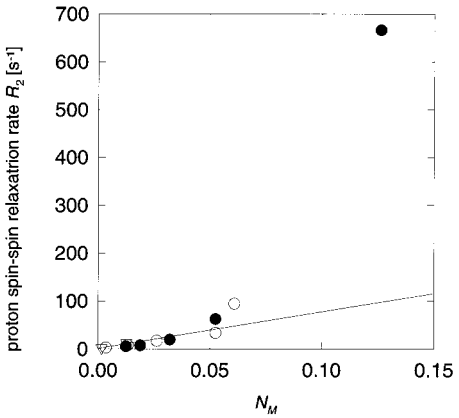

Figure 5. Proton spin−spin relaxation rates R_2 of dextran sulfate solutions as a function of the number N_M of sugar rings per water molecule. The samples were prepared by the osmotic stress technique (▽, without Na^+; ○, 50 mM Na^+; ●, 1 M Na^+). The linear curve has a slope of 770 s^{-1}.

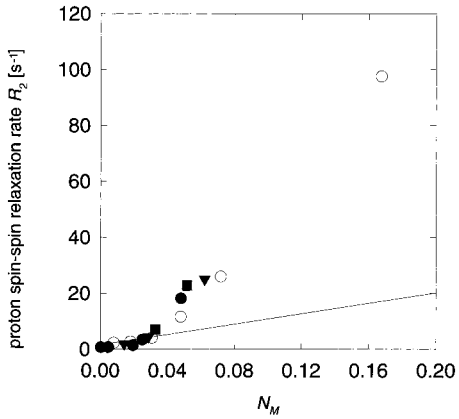

Figure 6. Proton spin−spin relaxation rates R_2 of chondroitin sulfate solutions as a function of the number N_M of sugar rings per water molecule. The samples were prepared by the osmotic stress technique (●, without Na^+; ▼, 50 mM Na^+; ■, 1 M Na^+) and by direct addition of H_2O to the dry polymer (○), respectively. The linear curve has a slope of 95 s^{-1}.

Table 2. Water Contents, Number of Sugar Rings N_M per Water Molecule, Deuteron Relaxation Times T_1 and T_2, and Rotational Correlation Times τ_q of Water in Dextran Solutions

water content (wt %)	N_M	T_1 (ms)	T_2 (ms)	τ_q (ps)
90	0.012	281	244	5.6
80	0.031	213	194	7.2
70	0.053	163	144	9.7
60	0.082	118	100	13.9
50	0.123	78	8.8	158
40	0.185	49	2.6	549
30	0.288	36	1.4	1120

Independent investigation of the sorption behavior of dextran indicated that one water molecule is bound per sugar ring,[33,38] supporting the results of the present paper.

From the deuteron R_1 and R_2 we could determine mean rotational correlation times using the same procedure as described in a recent paper[6] (Table 2). These correlation times increase from about the typical value of pure water (3 ps) to about 1 ns for water contents as low as 30 wt %.

Dextran Sulfate. The relaxation behavior of dextran sulfate solutions is very similar to that of dextran solutions. For $N_M < 0.1$ all measured relaxation rates are linear functions of N_M with slopes of 640 s^{-1} for proton R_2 (Figure 5) and 70 s^{-1} for deuteron R_1 and R_2 (data not shown). For larger N_M a strong deviation from this linearity can be observed. We obtained for dextran a maximum number of two bound water molecules per sugar ring. Using this value, we get the following results for dextran sulfate: proton $R_{2b} > 320$ s^{-1}, deuteron $R_{1b} > 35$ s^{-1}, and deuteron $R_{2b} > 35$ s^{-1}.

Varying Na^+ concentration does not affect the water relaxation times in dextran sulfate solutions (Figure 5), indicating that water binding of dextran sulfate is not changed by different salt concentration.

The variation of the molecular weight of dextran sulfate from 8000 to 500 000 had no influence on water relaxation (data not shown).

The mean rotational correlation times increase up to 234 ps for 20 wt % D_2O due to the increasing influence of bound water and reduced polymer mobility (Table 3).

Chondroitin Sulfate. The relaxation data of chondroitin sulfate solutions are shown in Figure 6. (The filled symbols represent the samples prepared by the osmotic stress technique. The other samples were produced by adding definite amounts of water.) No homogenous samples could be prepared below 30 wt % water. We could not observe any evidence for the constancy of proton spin-spin relaxation times in the concentration range 0.3−10 mg/mL chondroitin sulfate as reported by Cole *et al.*[39]

Table 3. Water Contents, Number of Sugar Rings N_M per Water Molecule, Deuteron Relaxation Times T_1 and T_2, and Rotational Correlation Times τ_q of Water in Dextran Sulfate Solutions

water content (wt %)	N_M	T_1 (ms)	T_2 (ms)	τ_q (ps)
90	0.006	413	386	3.6
80	0.014	369	331	4.2
70	0.024	316	275	5.1
60	0.038	244	214	6.5
50	0.084	84	83	16.7
40	0.131	34	25	56
30	0.225	12	6	234

Similar to dextran and dextran sulfate solutions a linear increase of proton R_2 as a function of N_M (slope 95 s^{-1}) can be observed. Caused by reduced polymer mobility the relaxation rates are far above the linear curve for low water contents. For the coarse estimation of $R_{2b} = 50$ s^{-1}, two water molecules were bound per sugar ring.

Very high Na^+ content (1 M) results in a considerable decrease of water contents as well as relaxation times (Figure 7). However, this variation with Na^+ concentration cannot be seen if the relaxation rates are represented as a function of N_M (Figure 6). A possible explanation for this behavior is that the ions can contribute to the osmotic pressure of the polymer solution but do not influence water binding as indicated by the identical slopes in Figure 6. Hence identical numbers of bound water molecules per sugar ring independent of the Na^+ concentration are observed.

Heparin. Figure 8 shows proton spin−spin relaxation times of heparin solutions prepared by the osmotic stress technique. The data are shown as a function of the osmotic pressure for varying Na^+ content. In analogy to water content (Figure 2) a decrease in proton T_2 can be observed for increasing ion concentration caused by a reduced contribution of the ions to the osmotic pressure. Higher osmotic pressures result in a

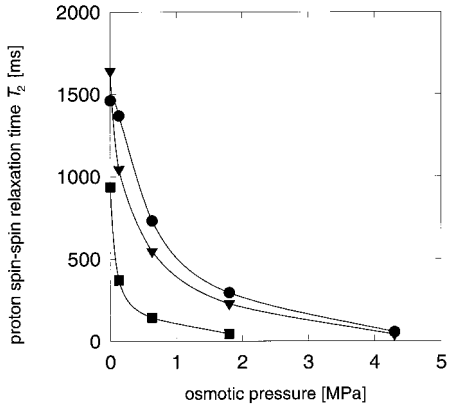

Figure 7. Proton spin−spin relaxation times T_2 of chondroitin sulfate solutions as a function of the osmotic pressure. The samples were prepared by the osmotic stress technique (●, without Na^+; ▼, 50 mM Na^+; ■, 1 M Na^+).

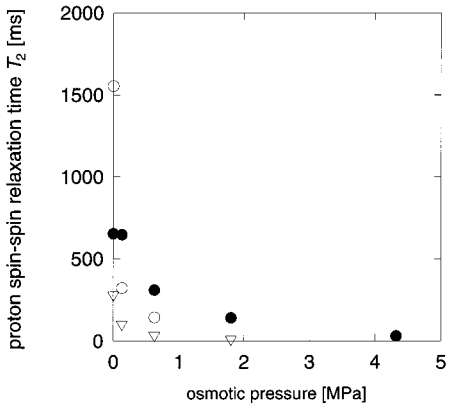

Figure 8. Proton spin−spin relaxation times T_2 of heparin solutions as a function of the osmotic pressure. The samples were prepared by the osmotic stress technique (○, without Na^+; ●, 50 mM Na^+; ▽, 1 M Na^+).

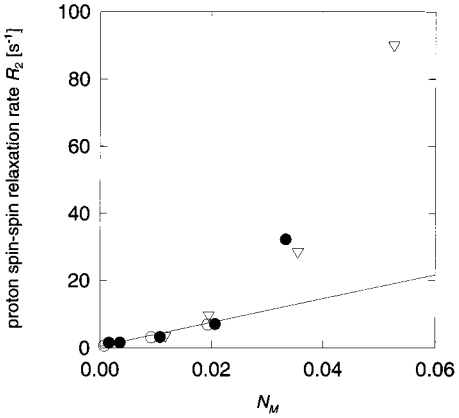

Figure 9. Proton spin−spin relaxation rates R_2 of heparin solutions as a function of the number N_M of sugar rings per water molecule. The samples were prepared by the osmotic stress technique (○, without Na^+; ●, 50 mM Na^+; ▽, 1 M Na^+). The linear curve has a slope of 375 s^{-1}.

decrease of unbound water in the polymer solution and hence in a reduced proton T_2 in heparin solutions like in all the other studied polymer solutions. The Na^+ influence vanishes if proton R_2 is shown as a function of N_M (Figure 9) because water binding properties are not affected by the Na^+ concentration. Hence, heparin, like the other studied polymers, binds the same amount of water for all used Na^+ contents. Proton R_2 shows a linear increase for low N_M (high water contents) in Figure 9. For higher N_M large deviations from this linearity can be observed, probably caused by a changed polymer mobility. The initial slope is 375 s^{-1}. If R_{2b} would be 200 s^{-1}, about two water molecules would be bound per sugar ring. This value, however, can only be understood as an estimation of the order of bound water molecules, because R_{2b} is unknown.

Xanthan. Proton spin−spin relaxation rates of xanthan solutions prepared by the osmotic stress technique represent a linear function of N_M with a slope of 1380 s^{-1} (data not shown). With R_{2b}= 600 s^{-1}, a xanthan sugar ring would bind 2.5 water molecules. The deviation from the linear behavior for higher N_M can also be observed for xanthan solutions. The proton spin−lattice relaxation rates are a linear function of N_M with a slope of 135 s^{-1} (data not shown). For 2.5 bound water molecules per sugar ring, R_{1b} would be in the expected order of about 60 s^{-1}.

IV. Discussion

Water binding in cartilage and in aqueous PEG solutions could be successfully described by assuming a fast exchange between a bound and an unbound water fraction.[5,6] Therefore, we use this model to explain the water relaxation in polysaccharide solutions which have an intermediate polymer mobility in comparison with the motionally restricted polymers in cartilage and the very flexible PEG chains in solution. Similar to cartilage and PEG solutions the fast exchange model can be used for high water contents indicated by the linear dependence of the proton spin−spin relaxation rates on the ratio of water molecules to sugar rings. If the used exchange model is valid, the slopes of these curves are equal to the product of the intrinsic relaxation rates of bound water (R_{2b}, e.g.) and the number N_b of bound water molecules per sugar ring. Unfortunately, R_{2b} is unknown. Hence, the number of bound water molecules cannot be calculated but can only be coarsely estimated by using reasonable R_{2b}'s.

Qualitatively, the proton spin−spin relaxation rates behave very similar for all studied polymers. They all show an initial linear dependence with more or less deviation from that linearity for lower water contents. The slopes in the linear range, however, differ considerably. The slope for xanthan is larger than that of dextran and chondroitin sulfate by about 2 orders of magnitude. The number of bound water molecules per sugar ring may vary from polymer to polymer by a few water molecules but not by 2 orders of magnitude. The ESR experiments showed that tempone has very similar reorientational rates in all polymers at the same water content indicating similar water mobilities in the bulk. This is supported by the diffusion coefficients of water measured by pulsed-field-gradient NMR, which show the same behavior for all studied polymers.[36,37] Hence, the intrinsic NMR relaxation time of bulk water (R_{2f}) should be the same for all the polymers. Therefore, in order to explain the strong differences in the NMR relaxation behavior of the various polymers, the intrinsic relaxation rates of the bound water fraction, R_{2b}, should differ strongly from polymer to polymer.

The relaxation rate of the bound state is determined by the mobility of the bound water molecules and by possible further exchange phenomena. The mobility of the bound water molecules is affected by reorientational processes of these water molecules with respect to the binding site and by the mobility of the polymer back-

bone. That means that solutions with a stiff polymer backbone show much larger R_{2b}'s than those of flexible polymers if the rotational motion of the bound water molecules is not very fast with respect to the polymer backbone motions. Xanthan is a very stiff polymer with a known macromolecular structure in aqueous solution, whereas other polymers like dextran and chondroitin sulfate should be much more flexible. This is a possible explanation of the varying slopes for the different polymers.

Because of the unknown relaxation rate of the bound state, the number of bound water molecules can be estimated only very coarsely. From the water relaxation in dextran solutions it can be concluded that less than two water molecules are bound per sugar ring. This value is lower by a factor of about 2 than the number of bound water molecules per sugar ring in cartilage that we estimated in an earlier paper.[5] This might be due to the fact that other macromolecular structures in cartilage—like collagen—can also bind water molecules. The number of bound water molecules given in the present paper is in very good agreement with sorption isotherm studies.[38] In addition molecular dynamics studies[40] showed that bound water molecules in dilute solution are also very mobile. The number of hydrogen-bonding sites on these polysaccharides is much higher. However, for the calculation of the average number of bound water molecules it has to be taken into account that the lifetime of a hydrogen bond is very short, i.e., on the order of some picoseconds.[40]

By assuming about two bound water molecules per sugar ring in all the studied polymer solutions, one can determine the relaxation rates of water in the bound state which increase in the following order: dextran—chondroitin sulfate—heparin—dextran sulfate—xanthan. This indicates that the water binding site and hence the polymer backbone are rather mobile for dextran and very stiff for xanthan whereas chondroitin sulfate, heparin, and dextran sulfate have intermediate mobilities. The mobility of dextran should be considered as mobile only in comparison with the other polysaccharides under investigation. For instance, the bound water in PEG, a linear, very mobile water-soluble polymer, has a proton R_{2b} of 5.6 s^{-1} which is lower by one order of magnitude than R_{2b} of dextran solutions.[6] In addition high-resolution ^{1}H NMR spectra of polysaccharides show very broad signals of the sugar regions[41−46] whereas the CH_2 signals of the PEG chains are very well resolved[47] and have relatively long relaxation times.[6] Therefore, the reorientational mobility of polysaccharides is considerably lower than that of PEG while there are also large differences in the mobilities of the various polysaccharides.

In addition to bound water on the polar sites of the polysaccharides, unbound water is taken up from the environment due to the osmotic pressure of the polymer solution. This water uptake is driven by contributions of the mixing entropy of polymer and water due to the large number of possible configurations.[48] In addition, the polyanionic systems possess an electrostatic contribution which arises from the osmotic pressures due to the Donnan equilibrium of the mobile cations between polysaccharide and incubation solution.[32] The small influence of Na^+ on the water uptake and hence the water relaxation times vanishes completely if the relaxation data are considered as a function of the water content. This fact leads to the conclusion that water binding properties of the polymers are not influenced by varying Na^+ contents.

The microviscosity of bulk water is strongly influenced by the collisions of water molecules and neighbouring polymer chains. Hence, the microviscosities might influence the water relaxation in polymer solutions. However, reduced water contents result in only a slight increase of the microviscosities for all polymers under investigation. Therefore, the strong deviations of water relaxation rates from the exchange model for low water contents reported here and previously[5,6] cannot result from the bulk water alone. On the other hand, the polymer mobilities are strongly reduced with decreasing water contents indicated by rising macroviscosities. Hence, the water binding sites reorient more slowly, and a stronger influence of the bound water molecules on the water relaxation is expected. This might be the reason for the strong deviation of the relaxation times from the exchange model in concentrated solution.

The mean rotational correlation times of the water molecules determined from deuteron spin—spin relaxation of water in dextran and dextran sulfate increase considerably with rising polymer concentration. They reach values of up to 1 ns for water contents of 20 wt % in dextran solutions. Hence, the water mobility is reduced to values which are also observed for protein side chain carbons of dilute aqueous protein solutions.[49]

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