Self-diffusion of Native Proteins and Dextran in Heat-set Globular Protein Gels

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Heat denatured β-lactoglobulin (β-lg) in aqueous solution aggregates and eventually gels. The fraction of residual nonaggregated proteins decreases with heating time but is still detectable at long heating times after the gels are formed. Aggregated β-lg does not have a significant NMR signal. Polysaccharide (dextran) with three different sizes was added to the system in small amounts so as not to perturb the gelation of the globular proteins. Pulsed field gradient NMR was used to determine the self-diffusion coefficient (D_s) of residual nonaggregated protein and dextran as a function of heating time. D_s of the protein increased with heating time, whereas that of the dextran decreased. The decrease stopped when the gel was formed, and D_s remains constant at longer heating times. The results indicate that the pore size of the protein gel is fixed close to the gel point, possibly involving a process of microphase separation. The pore size decreases with increasing protein concentration, and D_s is much larger at 0.5 M NaCl than at 0.1 M NaCl.

Introduction

Globular proteins in aqueous solution partially unfold when the temperature is raised. The unfolding often induces irreversible aggregation of the proteins, which may eventually lead to gelation.[1,2] This phenomenon has been investigated extensively, driven in part by the application of protein gels in the food industry. Heat-induced aggregation is observed for most globular proteins that have been studied, but the structure of the aggregates and the gels varies strongly depending on the type of protein and the external conditions (ionic strength, pH, etc).

One of the most studied globular proteins is β-lactoglobulin (β-lg), which is the major component of whey. It has a molar mass of 18 kg/mol and a radius of about 2 nm.[2,3] Heating aqueous solutions of β-lg above about 50 °C leads to the formation of aggregates and subsequently to more or less opaque gels, depending on the external conditions.[2] Here we report results of an investigation of heated β-lg solutions and gels at pH 7 using pulsed field gradient nuclear magnetic resonance (PFG-NMR). We have also studied the system in the presence of small amounts of dextran. We used the PFG-NMR signal of dextran to extract information about the structure of the heated β-lg solutions and gels. We have chosen this globular protein system because it has been investigated extensively in the past using other techniques and much is known about the kinetics of the aggregation[4] and the structure of the aggregates and the gel.[5−8] In addition, we have recently reported an investigation of the self-diffusion coefficient of native β-lg at pH 7 using PFG-NMR over a wide range of concentrations.[9]

It has been shown that at neutral pH the heat-induced aggregation of β-lg occurs in two steps.[7] In the first step, well-defined small clusters are formed with radii of about 15 nm containing about 100 proteins. In the second step, these small clusters aggregate into larger aggregates with a self-similar structure characterized by a fractal dimension $d_f = 2.0$. At concentrations above about $C = 10$ g/L the aggregates grow in size until a gel is formed, whereas at lower concentrations the growth stagnates when all of the native proteins have aggregated. In the absence of salt, the second step is inhibited by electrostatic repulsion and only small clusters are formed. For the present work it is important to realize that small oligomers of β-lg are not formed under the conditions we are considering here.

As the aggregation proceeds, the fraction of native proteins decreases even after the gel is formed. The fraction of native proteins at the gel point increases with increasing concentration. A combination of mechanical and permeability measurements shows that, although the elastic modulus continues to increase slowly well after the gel time, the pore size remains almost constant.[8] These results may be interpreted by assuming that the structure of the gel is fixed shortly after the gel time. The slow increase of the elastic modulus could be caused by further "decoration" by native proteins of this structure, which thereby reinforces it. Transmission electron microscopy shows that the pore size distribution is not very broad.[8,10] The formation of the gel structure possibly involves a microphase separation that determines the pore size. Increasing the ionic strength[8] or adding polysaccharides[10] leads to a larger average pore size of the gel, probably because the microphase separation is enhanced.

We will show that the NMR signal of even the smallest β-lg aggregates in the system is negligible, so that only the signals from the residual native proteins and added dextran can be observed. Therefore, we can measure the mobility of the native protein and dextran at different stages of the gel formation. We obtained in this way information about the structure of the heat-set β-lg gels. However, our main purpose here is not too investigate this particular system in great detail, but to demonstrate the utility of PFG-NMR for the investigation of globular protein gels in general.

Experimental Section

Materials. The β-lg used in this study was a gift from Besnier (batch no. 754). High-pressure liquid chromatography shows

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TABLE 1: Sample Characteristics

	M_w(Kg/mol)	R_{gz}(nm)	R_h[a](nm)	R_h[b](nm)
D1	1.7×10^3	44	40	24
D2	2.5×10^2	23	17	13
D3	62		7	6

[a] Obtained from DLS. [b] Obtained from PFG-NMR.

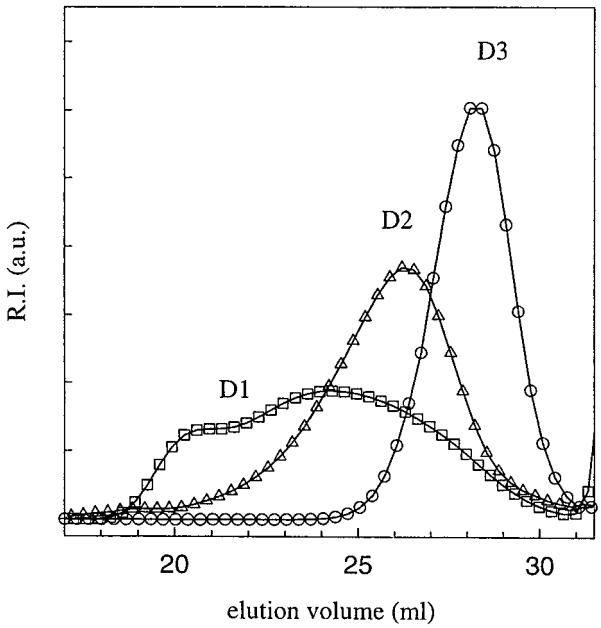

Figure 1. Chromatographs of the dextran samples.

that the sample consists of equal fractions of genetic variants A and B. Three dextran samples were used in this study. Sample D3 was purchased from Sigma, and samples D1 and D2 were purchased from Pharmacia. The solutions were dialyzed against distilled water at pH 7, to which 200 ppm NaN_3 was added to avoid bacterial growth. The ionic strength (I_s) was set at 0.1 M with either CH_3COONH_4 or NaCl. The solutions were filtered through 0.2 μm or 0.45 μm pore size filters, depending on the concentration. The concentrations were determined after filtration by size exclusion chromatography (SEC) with refractive index detection using refractive index increment dn/dc = 0.189 g/mL for β-lg[11] and dn/dc = 0.15 g/mL for dextran.[12]

The characterization of native β-lg using static (SLS) and dynamic (DLS) light scattering and PFG-NMR was reported in ref 9. At pH 7 and I_s = 0.1 M, native β-lg is present mainly in the form of dimers. After filtration, the solutions still contain some large aggregates, but their weight fraction is very small and they can be detected only with light scattering.

The dextran samples were characterized in dilute solution using SEC and light scattering. The weight average molar mass (M_w) and z-averaged radius of gyration (R_{gz}) were determined from the angular dependence of the scattering intensity extrapolated to zero concentration in the standard manner.[13] The results are summarized in Table 1. The radius of gyration of D3 is smaller than 10 nm and cannot be obtained with light scattering. Chromatograms of the samples show that D3 has quite a narrow size distribution, whereas that of D1 and D2 is broad, see Figure 1. D1 contains material that is fully excluded, which explains the shoulder at elution volume V_e = 20 mL.

Methods. Proton PFG-NMR measurements were done on a Bruker AM 200 (4.7 T) wide bore NMR spectrometer controlled by an ASPECT 3000 computer system. The actively shielded gradient coil was designed and manufactured at Massey University, Palmerston North, New Zealand by Callaghan and co-workers. It was mounted in a 5 mm proton probe (Bruker). The carefully balanced gradient and shielding currents were generated by a Techron 7570 amplifier. The NMR signals were transferred to a PC system and analyzed with a data analysis program in a Matlab environment. In the case of a single diffusional process, the signal attenuation S is given by

$$\frac{S(U)}{S(0)} = \exp[-D_s(\Delta - \delta/3)\gamma_p{}^2G^2\delta^2] \qquad (1)$$

with Δ the diffusion delay time, γ_p the proton gyromagnetic ratio, G and δ the amplitude and the duration of the field gradient, respectively, and D_s the self-diffusion coefficient. For the experiments described here, the diffusion coefficient was determined on samples at T = 25 ± 0.2 °C using different diffusion delay times between 20 and 300 ms and a gradient

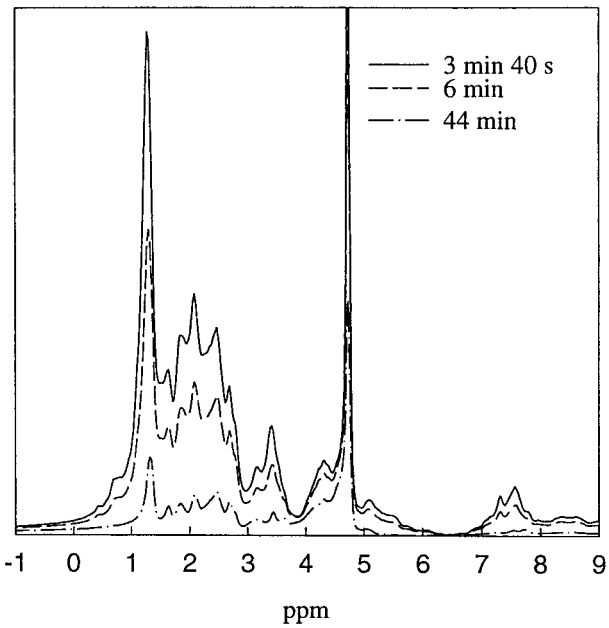

Figure 2. In-situ proton NMR spectra of a β-lg solution heated at 76 °C at three times indicated in the figure.

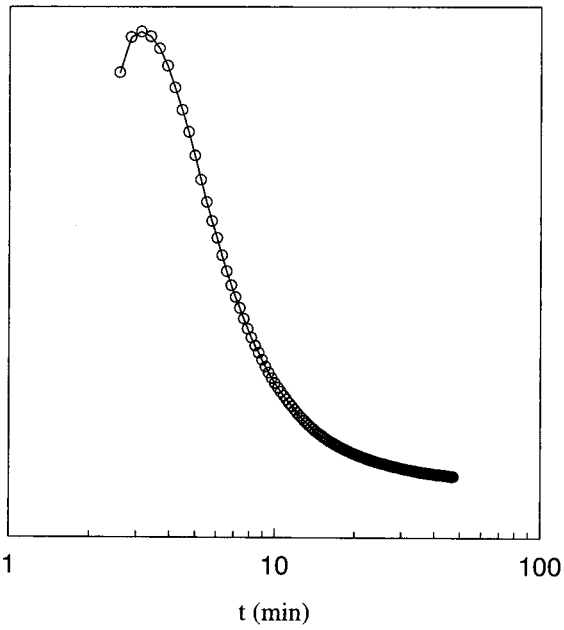

Figure 3. Heating time dependence on the intensity of the main protein peak at 1.2 ppm.

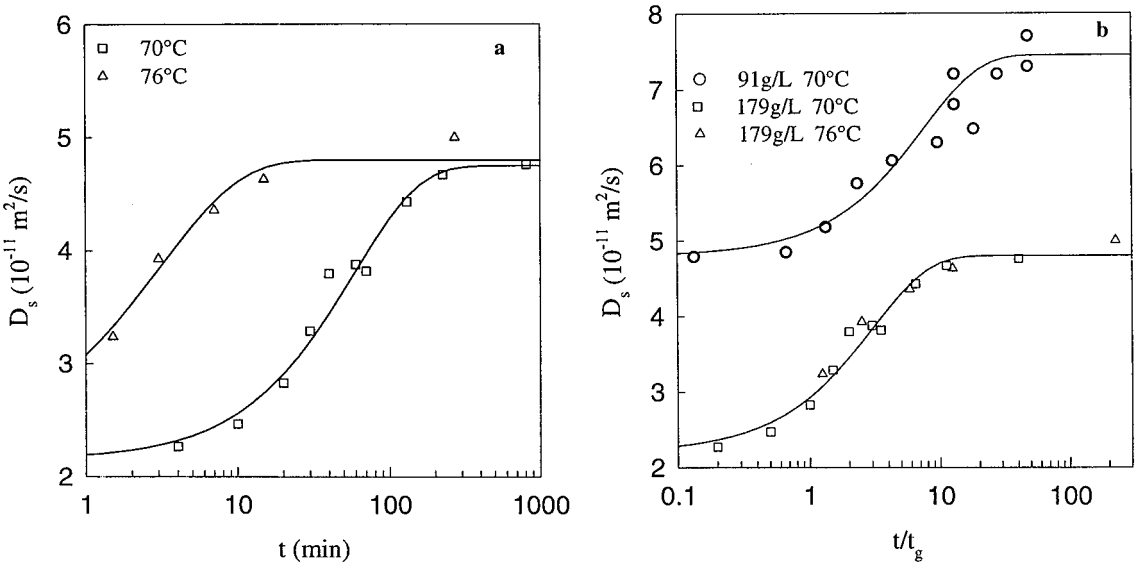

Figure 4. (a) Self-diffusion coefficient of native β-lg with $C = 179$ g/L as a function of heating time at two temperatures indicated in the figure. The solid lines are guides to the eye. (b) Self-diffusion coefficient of native β-lg as a function of heating time normalized by the gel time at two temperatures and two concentrations indicated in the figure. The solid lines are guides to the eye.

duration of 2 ms. The signal was recorded with a spectral width of 5 kHz. The gradient currents (0.5−12A) were chosen in such a manner that an exponential spacing in U was obtained. The magnetic field gradient varied between 0.018 and 0.43 T/m using this current range. A phase cycled stimulated echo sequence was optimal for the observed T_1 and T_2. Moreover, a longitudinal eddy current delay sequence with a 20 ms delay was added to reduce inductive artifacts.[14]

In-situ proton NMR measurements were made at 76 °C using a DPX300 spectrometer. The signals were recorded in a 5 mm high-resolution probe with presaturation of the H_2O signal. For the in-situ measurements, D_2O was added to the solutions (10%).

SLS and DLS measurements were made using an ALV-5000 multibit multi-tau correlator in combination with a Malvern goniometer and a Spectra Physics argon ion laser operating with vertically polarized light with wavelength $\lambda = 532$ nm. The range of scattering wave vectors covered was $3.0 \times 10^{-3} < q < 3.5 \times 10^{-2}$ nm^{-1}. The temperature was controlled by a thermostat bath set at 20 ± 0.1 °C. The electric field autocorrelation function ($g_1(t)$) is related to the normalized measured intensity autocorrelation function ($g_2(t)$) as $g_2(t) = 1 + g_1(t)^2$.[15] The $g_1(t)$ term was analyzed in terms of a distribution of relaxation times: $g_1 = \int A(\log \tau) \mathrm{d} \log \tau$. We have used the so-called generalized exponential to describe the distribution: $A(\log \tau) = k(\tau/\tau_g)^p \exp[-(\tau/\tau_g)^s]$, where k is a normalization constant and τ_g is the characteristic relaxation time. This versatile function has two parameters (p,s) to describe a wide variety of distributions such as the Schultz−Zimm and the Pearson distribution.[16]

Size exclusion chromatography experiments were carried out at room temperature with a TSK PW 5000 + PW 6000 column set (30 cm + 60 cm) in series and a differential refractive index detector SHODEX RI 71. The columns were eluted with a 0.1 M $NaNO_3$ solution at a flow rate of 1 mL/min. The injected volume was 300 μL, and the injected concentration was approximately 1 g/L.

Results and Discussion

Self-diffusion of Native β-lg. The proton NMR spectrum of aqueous β-lg shows a collection of peaks between 1 and 8 ppm due to the various protons on the proteins. Figure 2 shows in-situ measurements of the spectra of a solution of β-lg with C = 70 g/L at three times after setting the temperature to 76 °C. It appears that the signal of all peaks decreases, but their shape and position is not much modified. There is a small shift of the peak positions if we compare the spectra at 76 °C with the one before heating at 25 °C, but this shift is reversed when the samples are cooled back to 25 °C. Note that the *x*-axis of the proton NMR spectrum of β-lg at 25 °C shown in Figure 3a of ref 9 is incorrect.

We have monitored the decrease of the signal by integrating the main protein peak (1.2−1.4 ppm) at different times, see Figure 3. The initial rise is caused by the change in temperature of the sample. Subsequently, the signal decreases rapidly at first and then slowly decays to what appears to be a finite value at long times. This behavior mirrors the observed decrease of native proteins,[4] which implies that only the signal of nonaggregated β-lg can be observed. This is perhaps not so surprising because the smallest aggregates contain already about 100 proteins so that their mobility is very much reduced. At short times the decrease of the signal due to the decrease of native β-lg is confounded with the setting of the temperature. Therefore, it is difficult to determine the absolute amount of residual native β-lg as a function of time from this experiment. Nevertheless, it is clear that NMR is potentially a useful technique to determine the rate of the aggregation process.

Earlier,[9] we measured the self-diffusion coefficient (D_s) of native β-lg as a function of the concentration using PFG-NMR. D_s was found to decrease from 8.3×10^{-11} m^2/s at infinite dilution to 2.6×10^{-11} m^2/s at $C = 179$ g/L. Here we report measurements of D_s as a function of heating time (t) using the same method. These measurements were not done in-situ, because it takes a long time to obtain accurate results of the decay of the NMR signal. Instead, the samples were separately heated in the NMR tubes at a given temperature for varying duration. Subsequently, the aggregation was quenched by rapid cooling to room temperature. At room temperature the samples are stable and were studied with PFG-NMR at 25 °C within a few days of preparation. The decay of the protein NMR signal was close to a single exponential at all heating times.

Figure 4a shows the results of D_s obtained for β-lg with $C =$ 179 g/L heated at 70 °C and 76 °C. The D_s increases from the value obtained for the unheated sample β-lg (2.3×10^{-11} m^2/

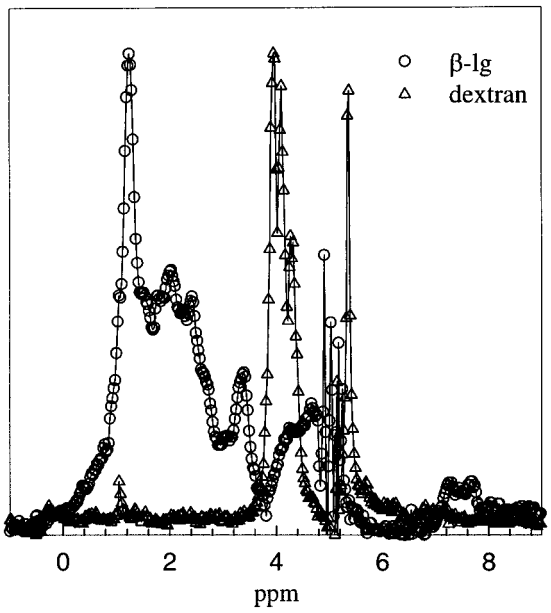

Figure 5. Comparison of the proton NMR spectra of β-lg and dextran.

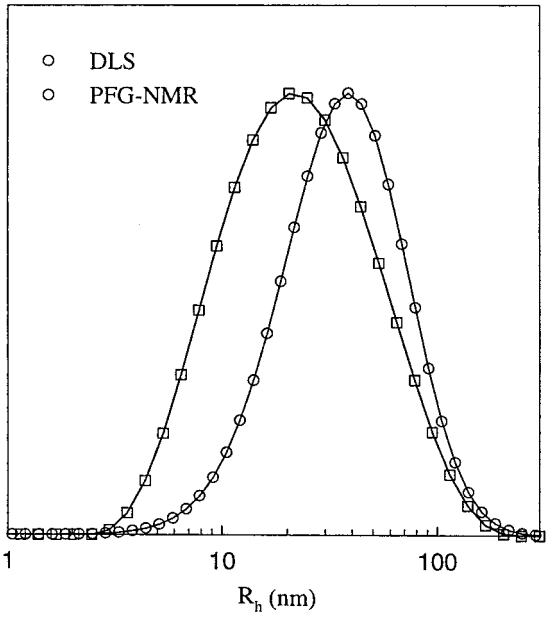

Figure 6. Comparison of the hydrodynamic radius distribution of dextran sample D1 obtained from DLS and PFG-NMR.

s) and stabilizes at a larger value after long heating times. It was shown in ref 4 that at a given concentration the fraction of residual native β-lg is the same if the heating time is normalized by the gel time (t_g). In addition, the structure and size of the aggregates at t/t_g are independent of the temperature. From these results it was concluded that increasing the temperature only increases the rate of aggregation, but does not modify the process of aggregation. Figure 4b shows that the evolution of D_s is also the same if it is plotted as a function of t/t_g.

At lower concentrations the same effect is observed. For comparison we show in Figure 4b the results obtained for $C =$ 91 g/L. The self-diffusion coefficient of unheated β-lg with C = 91 g/L is 4.9×10^{-11} m^2/s. At $C = 91$ g/L, D_s, after long heating times, is close to that of dilute native β-lg, which implies that the friction between the residual native proteins and the immobile skeleton of the gel is negligible. At even lower concentrations, the diffusion coefficient of the unheated samples is already closer to that of dilute β-lg so that the effect of heating

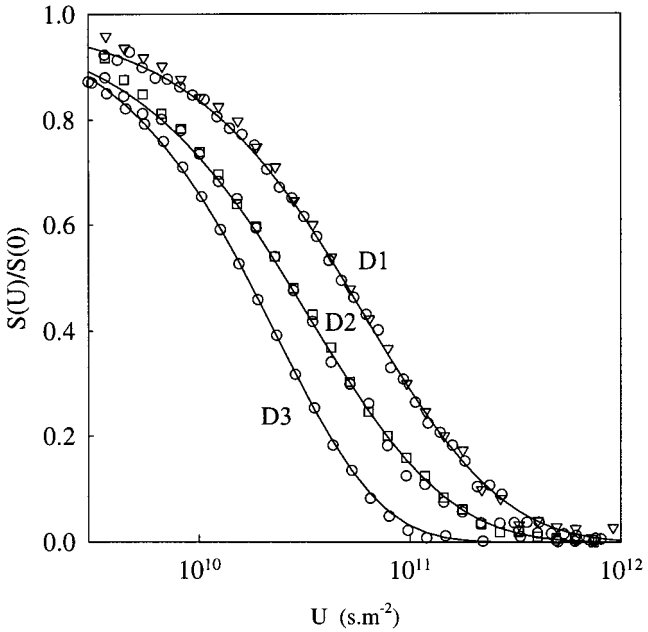

Figure 7. Decay of the NMR signal at 4 ppm for three dextran samples at 4.0 g/L. For comparison we also show the decay of sample D1 at 2.0 g/L (triangles) and sample D2 at 4.0 g/L plus 70 g/L β-lg (squares). The solid lines represent least-squares fits to the generalized exponential distribution, see text.

is less important. The observation that D_s of dilute β-lg does not decrease after heat treatment is another proof that with NMR we do not observe aggregated β-lg, which has, of course, a smaller self-diffusion coefficient.

To explain the variation of D_s of the native proteins we have to consider the friction of the native proteins with other native proteins and with the larger aggregates and the gel. The friction of the native proteins with the solvent is not influenced by the heat treatment because they have the same hydrodynamic radius before and after heating. On one hand, the friction with other native proteins decreases in proportion to the fraction of residual native protein.[9] On the other hand, the friction with large aggregates and the gel is smaller than with native proteins for two reasons: (1) the native proteins easily penetrate the gel and the large aggregates because the pore size is much larger than the proteins, and (2) the friction with mobile particles is larger than the friction with the same volume fraction of immobile particles. As a consequence, the increase of D_s mirrors the decrease of the fraction of native proteins reported in ref 4. The time after which half of the proteins have aggregated (t_h) for a solution containing 176 g/L β-lg at 0.1 M CH_3COONH_4 is 3 min at 76 °C and 35 min at 70 °C. The values of t_h correspond approximately to the midpoint of the increase of D_s. The increase of D_s stabilizes at long times because the fraction native protein stabilizes, or at least the decrease becomes very slow.

The results shown in Figure 4 were obtained using a delay time $\Delta = 60$ ms, which corresponds to a diffusion length of the proteins in the gel of about 5 μm. For one sample we varied the delay time between 20 ms and 300 ms, which corresponds to a variation of the diffusion length by a factor 4. We obtained the same results for all delay times, which is probably because the diffusion length is much larger than the average pore size of the gels, so that the proteins probe the average structure of the gel.

Self-diffusion of Dextran. The proton NMR spectrum of dextran is compared with that of β-lg in Figure 5. The spectra were obtained at a value of $U = (\Delta - \delta/3)\gamma_P^2 G^2 \delta^2$, where the

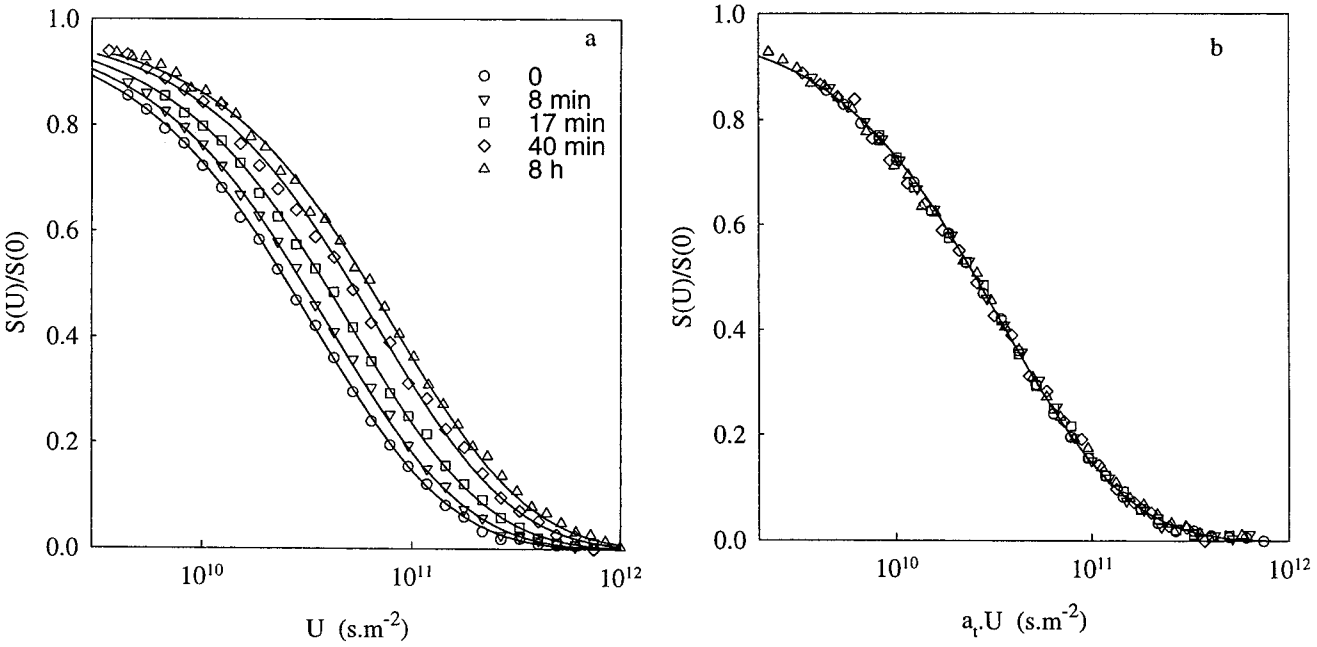

Figure 8. (a) Decay of the NMR signal at 4 ppm for sample D2 in 70 g/L β-lg at 0.1 M NaCl after heating at 70 °C during different times indicated in the figure. The solid lines represent least-squares fits to the generalized exponential distribution, see text. (b) Same data as in Figure 8a after horizontal shifts.

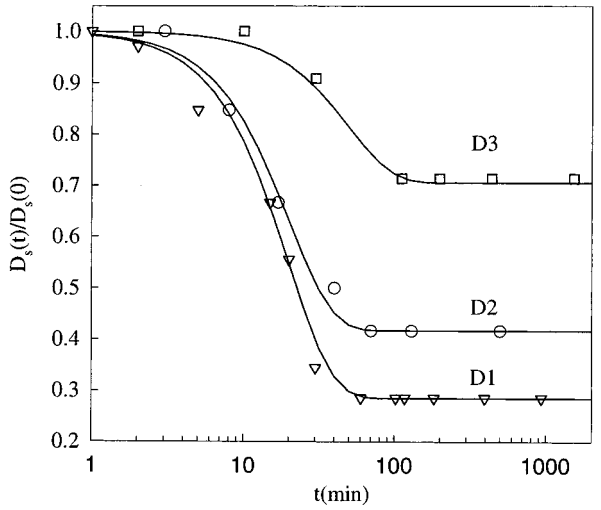

Figure 9. Self-diffusion coefficient of three dextran samples in 70 g/L β-lg at 0.1 M NaCl as a function of heating time at 70 °C.

large water signal at about 5 ppm has already mostly relaxed but the signal from dextran and β-lg not yet. The dextran spectrum is characterized by a distinct triplet with a maximum at 4 ppm where the signal from β-lg is weak. This fortunate coincidence allows for a simultaneous measurement of the self-diffusion coefficient of both dextran and β-lg in mixtures of the two components, by measuring the decay of the peaks at 4 ppm and at 1.2 ppm. Of course, in practice the feasibility of determining D_s of both components in the same experiment depends on the ratio of the signal amplitudes and the values of D_s. If the signal of one of the components dominates it is difficult to detect the other, and if the values of D_s are very different one cannot determine both accurately with the same gradient.

Here we wish to study the self-diffusion of dextran in heat-set β-lg gels. We envisage using dextran as a probe to investigate the structure of the gels, so we need to use low dextran concentrations to ensure that interaction between dextran chains can be neglected. On the other hand we need to use dextran concentrations that are sufficiently high so that we obtain a reasonable signal-to-noise ratio within a reasonable time. The concentration dependence of D_s has been studied in the past by several groups,[17,18] and it was shown that D_s depends weakly on the concentration below the so-called overlap concentration, C^*, and decreases rapidly at higher concentrations. The concentration where the chains begin to overlap can be estimated as $C^* = 3M/(N_a 4\pi R_g^3)$, with N_a Avogadro's number. C^* decreases with increasing molar mass, which means that sample D1 will show the strongest concentration effect. Using M_w and R_{gz} we find $C^* = 7.7$ g/L, which indicates approximately the concentration above which D_s is expected to decrease strongly. In the following we have used a dextran concentration of 4.0 g/L. At this concentration the effect of interactions between the dextran chains on D_s is still very small, see Figure 7. However, it has been shown that even at low concentrations polysaccharides may accelerate the gelation process[19] and also modify the structure of the protein gels.[10] We have found that dextran does indeed influence the gelation of β-lg, but only at concentrations higher than used here.

We have investigated the diffusion of dextran in the absence of proteins using PFG-NMR and DLS. With DLS, one probes the decay of concentration fluctuations, which is due to cooperative diffusion if $qR_g < 1$.[15] For monodisperse dilute solutions, the electric field autocorrelation function ($g_1(t)$) is characterized by a single-exponential decay, with relaxation time $\tau = 1/(D_0.q^2)$. D_0 is related to the hydrodynamic radius (R_h) as $D_0 = k_B T/(6\pi\eta_s R_h)$, where η_s is the solvent viscosity and k_B Boltzmann's constant. If the sample is polydisperse, $g_1(t)$ is characterized by a relaxation time distribution corresponding to the distribution of sizes.

We have analyzed the autocorrelation functions in terms of a generalized exponential relaxation time distribution, see Experimental Section. If interactions and internal dynamics are negligible, the relaxation time distribution may be transformed in the corresponding hydrodynamic radius distribution. In Figure 6 we show the hydrodynamic radius distribution for D1 obtained from a DLS measurement at 4.0 g/L and at $q = 0.01$ nm^{-1}. As expected in view of the SEC results, we obtain a broad

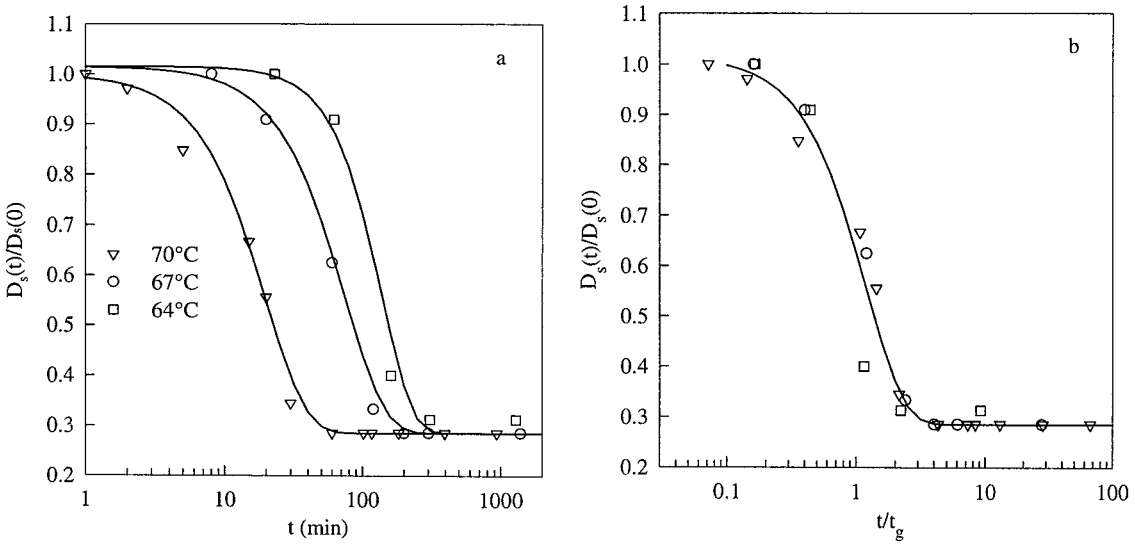

Figure 10. (a) Self-diffusion coefficient of dextran D1 as a function of heating time at three temperatures indicated in the figure. The solid lines are guides to the eye. (b) Same data as in Figure 10a as a function of heating time normalized by the gel time. The solid line is a guide to the eye.

distribution of sizes centered at 40 nm. Because the scattered light intensity is proportional to the concentration times the molar mass (M·C), one determines with DLS the *z*-averaged hydrodynamic radius. Values of the *z*-averaged hydrodynamic radii for the three samples are given in Table 1.

Figure 7 shows the NMR decay of the dextran signal at C = 4.0 g/L for the three samples. For sample D1 we also show the decay at $C = 2.0$ g/L in order to demonstrate that the concentration dependence is small at the low concentration used here. The decay is much broader than a single exponential and was again analyzed using a generalized exponential distribution. The corresponding hydrodynamic radius distribution for sample D1 is compared with that obtained from DLS in Figure 6. The difference between the distributions is caused by the different sensitivity of the two techniques. The DLS signal is proportional to the scattered light intensity, i.e., to M·C, whereas the NMR signal (and also the refractive index signal used in SEC) is proportional to C. This means that large particles are more prominent in the size distribution obtained from DLS. With PFG-NMR one obtains a weight-averaged hydrodynamic radius, and the values obtained for the three dextran samples are given in Table 1.

We studied the self-diffusion coefficient of dextran in mixtures containing 4.0 g/L dextran and 70 g/L β-lg. Before heating, the self-diffusion coefficient of dextran is not significantly influenced by the presence of β-lg. This is shown in Figure 7 where we compare the decay of sample D2 with and without 70 g/L β-lg. We have checked with DLS in dilute solution that there is no specific interaction or complex formation between dextran and β-lg. The samples were heated during set periods and then cooled rapidly to room temperature. PFG-NMR measurements were done at 25 °C shortly after the heat treatment. The heated samples very soon became opalescent and we were not equipped to do useful light scattering measurements on samples that showed multiple scattering.

Figure 8a shows the decay of the NMR signal sample D2 at different heating times at 70 °C. Initially, the decay shifts to larger values of *U*, which means that the self-diffusion slows down, but it stabilizes at longer times. Figure 8b shows that the data can be superimposed by simple horizontal shifts. The shift factor represents the relative variation of the self-diffusion coefficient caused by the heat treatment. The measurements were done with delay time $\Delta = 140$ ms, which corresponds to a diffusion length of 3 μm for D1 in the gels and somewhat larger distances for the smaller dextrans. In principle, variation of the diffusion length using different delay times is a method to study the heterogeneity of the gels. Unfortunately, with shorter delay times we could only detect the initial decay of the signal. For larger delay times up to 300 ms we did not observe a significant effect on D_s, but it should be realized that the variation of the diffusion length is small.

In Figure 9, we have plotted the relative variation of the D_s of the three dextran samples as a function of heating time at 70 °C. D_s decreases with increases heat treatment, and the effect is stronger the larger the dextran. The effect of heating temperature is shown in Figure 10a. As for the self-diffusion of β-lg, the temperature influences only the kinetics and the data obtained at different temperatures superimpose if the heating time is normalized by the gel time, see Figure 10b. Figure 10b shows that the self-diffusion of dextran decreases rapidly around the gel time and then remains constant. Note that whereas D_s is constant for $t > 3t_g$, the shear modulus continues to increase.

Native β-lg is small and can easily penetrate the dextran chains, so the friction between dextran and native β-lg is small, as shown in Figure 7. Therefore reduction of the concentration of native proteins does not have a significant influence on the self-diffusion coefficient of dextran. However, with increasing heating time, more and larger β-lg aggregates are formed, which increases the effective volume fraction of β-lg and thus the friction with the dextran chains. The decrease of D_s for dextran is caused by the friction between dextran and aggregated β-lg. Naturally, the effect is more important for larger dextran chains.

At the gel point an immobile porous structure is formed. The fact that D_s remains constant for $t > 3t_g$ implies that the average pore size of the protein gel varies relatively little with further heating. Verheul et al.[8] came to the same conclusion based on a comparison of permeability measurements. Nevertheless, the shear modulus continues to increase slowly, which they attribute to the reinforcement of the gel structure by the residual proteins that continue to "decorate" the gel structure. The smallest dextran diffuses almost freely through the gel, which implies that the pore size is much larger than the diameter of D3, which is about 15 nm. It also proves that dextran does not form a complex with the proteins. On the other hand, the diffusion coefficient of D2 with a diameter of about 45 nm is already

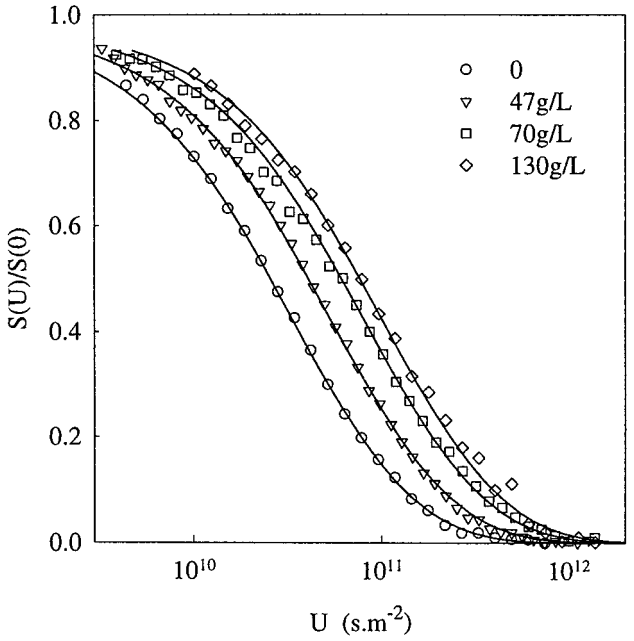

Figure 11. Decay of the NMR signal at 4 ppm for sample D2 in various concentrations of β-lg at 0.1 M NaCl after heating for several hours at 70 °C. The solid lines represent least-squares fits to the generalized exponential distribution, see text.

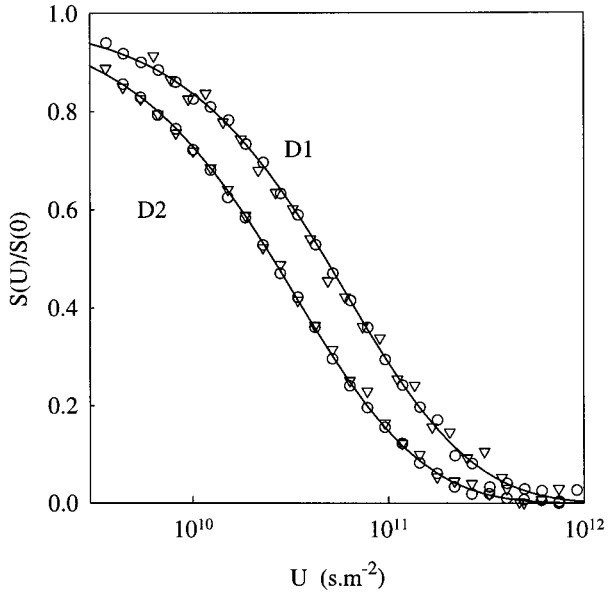

Figure 12. Decay of the NMR signal at 4 ppm for samples D2 and D1 in 70 g/L β-lg before (circles) and after (triangles) heating for several hours at 70 °C. The solid lines represent least-squares fits to the generalized exponential distribution, see text.

reduced by a factor of 2.4, and that of D1 with a diameter of roughly 80 nm is reduced by a factor 3.5. We may conclude that the pore size of the gels formed under the present conditions is of the order 50−100 nm. If the pore size were much larger, then we would expect no influence on D_s, whereas if it were much smaller we would expect an effect on the self-diffusion of D3.

The self-diffusion of dextran in heat-set protein gels is determined by the pore size. The pore size can be varied by varying the protein concentration or the ionic strength. Figure 11 shows the decay of sample D2 in β-lg gels formed by heating different β-lg concentrations for several hours at 70 °C. The decay is slower with increasing protein concentration, as might be expected for denser gels. Figure 12 shows that the diffusion of dextran is the same before and after heat treatment if the protein gels are formed in 0.5 M NaCl. This result implies that the pore size of β-lg gels formed in 0.5 M NaCl is much larger than 80 nm. Verheul et al.[8] also concluded that the pore size is much larger at 0.5 M NaCl than at 0.1 M NaCl, based on microscopy and permeability measurements.

Conclusions

The main conclusion of this work is that it is possible to determine with PFG-NMR the self-diffusion coefficient of both native protein and dextran in heat-set globular protein gels. We have shown that PFG-NMR is a powerful technique to study the structure of protein gels.

Aggregated β-lg does not show a significant NMR signal, so that only residual native protein is observed. The pore size of the gel is much larger than the diameter of the native proteins. As a consequence, D_s of native β-lg increases after heat treatment because the friction caused by the immobile structure of the gel is much smaller than that caused by the mobile native proteins.

The influence of native β-lg on the self-diffusion coefficient of dextran is small, at least for protein concentrations below 100 g/L. D_s of trace dextran in β-lg solutions decreases after heat treatment due to the friction with aggregated β-lg. Once the protein gel is formed, D_s remains approximately constant, indicating that the pore size of the gel is fixed close to the gel point and does not change much upon further heating. The pore size of the heat-set β-lg gels decreases with increasing protein concentration and is much larger at 0.5 M NaCl than at 0.1 M NaCl.

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