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NMR Relaxation and Water Self-Diffusion Studies in Whey Protein Solutions and Gels

ROXANE COLSENET, FRANÇOIS MARIETTE,* AND MIREILLE CAMBERT

Cemagref, Process Engineering Technology Research Unit, CS 64426, 17 Avenue de Cucillé, 35044 Rennes Cedex, France

The changes in water proton transverse relaxation behavior induced by aggregation of whey proteins are explained in terms of the simple molecular processes of diffusion and chemical exchange. The water self-diffusion coefficient was measured in whey protein solutions and gels by the pulsed field gradient NMR method. As expected, water self-diffusion was reduced with increased protein concentrations. Whatever the concentration, the water molecules were free to diffuse over distances varying from 15 to 47 μm. Water diffusion was constant over these distances, demonstrating that no restrictions were found to explain the water hindrance. The modification in protein structure by gelation induced a decrease in water diffusion. The effects of protein concentration on water diffusion are discussed and modeled. Two approaches were compared, the obstruction effect induced by a spherical particle and the cell model, which considered two water compartments with specific self-diffusion coefficients.

KEYWORDS: NMR; diffusion; transverse relaxation; whey; gel

INTRODUCTION

Bovine milk contains ~3.5% protein which falls into two main categories: caseins and whey proteins. Whey proteins are a mixture of β-lactoglobulin (β-lg), α-lactalbumin (α-la), bovine serum albumin (BSA), and immunoglobulins (Ig). They are widely used as food ingredients because they are highly nutritious and possess valuable functional properties (*1*). Whey proteins aggregate on heating, and a gel may be formed under suitable conditions. Three phenomena are involved in the aggregation of whey proteins, often simultaneously: i.e., conformational changes, chemical reactions, and physical interactions (*2*). The nature, extent, and rate of denaturation can be influenced by a number of factors (*3*) such as pH (*4*, *5*), ionic strength (*5*−*9*), protein concentration (*8*, *10*), and time and temperature of heating (*5*).

Translational diffusion is the most fundamental form of transport in chemical and biochemical systems. Pulsed field gradient nuclear magnetic resonance (PFG-NMR) provides a convenient means for measuring translational diffusion (*11*). The PFG-NMR technique was used because it permits nondestructive, fast, and precise water self-diffusion coefficient measurements. This technique has been used to study water diffusion in biopolymer systems such as polysaccharide gels (*12*), starch gels (*13*), cellulose (*14*) casein dispersion (*15*), and bovine serum albumin (*16*, *17*). The sensitivity of water diffusion to gel formation has led to contradictory results (*18*, *19*). For example, the water self-diffusion in a BSA system was independent of the association of globular BSA molecules after gel formation in the range of 5−20% (*17*). More recently, Mariette et al. (*15*) observed no modification of water diffusion in a casein system after renneting. In contrast, Hills et al. (*13*) reported self-diffusion measurements of water in a starch suspension and in starch gel in dry matter ranging from 15 to 35% (w/w). They observed wide variations in water diffusion behavior according to the granule structure. Anomalous water diffusion behavior was found in the case of native starch. When the internal granule structure was destroyed by gelatinization, the water diffusion then conformed to simple unrestricted diffusion in 3D space.

Various physical models of diffusion have been proposed (*20*, *21*) over the years. These models have generally been divided into three categories on the basis of the obstruction effect, the free volume effect, and the hydrodynamic interactions. In addition, new models of diffusion have been proposed such as the cell model diffusion based on Fick's first law (*22*), which takes obstruction as well as hydration into account.

Kimmich et al. (*16*) proposed three different regimes to explain water diffusion according to the gel concentration. In the first regime (up to 50% bw) the obstruction effect induced by the macromolecule dominates. The fraction of hydration water increases between 50 and 85% bw, and because the exchange between the two waters is fast, then the water diffusion can be written as the average weight of the diffusion coefficient in the free water and in the hydration water phase. This regime is called the "infinite-cluster limit". Above 85% bw the increase in protein concentration leads to unsaturated hydration shells. This is denoted "finite-cluster limit". For this concentration range, reduced water self-diffusion values are observed for the gelatin system compared to the BSA system. This phenomenon is related to the specific structure of the two systems, i.e., fibrillar

* Corresponding author. Telephone: 33 (0)223482121. Fax: 33(0)-223482115. E-mail: Francois.Mariette@cemagref.fr.

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Table 1. Composition of Whey Protein Powder

	whey protein powder
total solid ($g \cdot kg^{-1}$)	917.5
total nitrogen matter ($g \cdot kg^{-1}$)	886.1
noncasein nitrogen ($g \cdot kg^{-1}$)	809.4
nonprotein nitrogen ($g \cdot kg^{-1}$)	8.6
pure proteins ($g \cdot kg^{-1}$)	813.0

structure for gelatin and globular structure for BSA. The absence of network formation effect on water diffusion should therefore be interpreted with regard to the concentration regime proposed by Kimmich et al. (*16*). Another model has recently been proposed to explain water diffusion hindrance in casein systems (*23*). This model includes the effects of both obstruction and protein hydration on water self-diffusion. According to this model the water flow could be divided into two flows, one around the micelle and one through the micelle.

As casein micelles are large particles and highly porous to water, the aim of the present study was to extend the water self-diffusion results in a system such as globular whey proteins. In particular we focused on the effects of the whey protein concentration on water self-diffusion in order to analyze the findings with several diffusion models. Water self-diffusion in whey protein solution and gel systems was also compared and discussed.

MATERIALS AND METHODS

(1) Materials. Whey protein powder (INRA, Rennes, France) was used, and protein powder composition is summarized in Table 1. Sodium azide (Merck, Darmstadt, Germany) and NaN_3 were used without purification.

(2) Preparation of Solutions. Whey protein powder was conserved in darkness at room temperature before rehydration. Rehydration of the whey protein powder was performed at room temperature with a NaCl/water solution (0.1 M). Sodium azide was added (0.02% (w/w)) to each solution to prevent bacteria development. The solutions were studied without pH adjustment. For example, the pH of the whey protein solution was 6.58 for a concentration of 4.95 g/(100 g) and 6.54 at 37.40 g/(100 g).

(3) Preparation of Gels. Whey protein solutions were kept in a water bath at 70 °C for 30 min to induce gelation. Sample weight was measured before and after coagulation, and no significant dehydration was detected.

(4) Determination of Dry Matter. The dry matter of all samples was determined by measuring variations in weight after drying in an oven at 103 °C for 24 h. Protein concentrations were calculated from values of dry matter in each solution and pure protein percentage in powders (88.61% for whey proteins).

(5) NMR Measurements. All NMR measurements were performed on a 20 MHz Bruker spectrometer equipped with a pulsed field gradient probe. The Carr Purcell Meiboom Gill (CPMG) sequence (*24*, *25*) was used to measure spin−spin relaxation time T_2. NMR signals were analyzed by using a discrete fitting method such as the Marquardt method (least-squares nonlinear regression technique). The best mathematical model consisted of two exponential components (1 and 2):

$$I(t) = I_1 \exp\left[-\left(\frac{t}{T_2(1)}\right)\right] + I_2 \exp\left[-\left(\frac{t}{T_2(2)}\right)\right] \quad (1)$$

with $I(t)$ the intensity of the NMR signal given at t, I_1 and I_2 the intensities, and $T_2(1)$ and $T_2(2)$ the spin−spin relaxation time of each relaxation component, respectively.

Pulsed-gradient spin−echo (PGSE) and pulsed-gradient stimulated-echo (PGSTE) described by Tanner (*26*) sequences were used to measure the water self-diffusion coefficient. A PGSTE sequence is detailed in Figure 1. One important aspect of PGSTE experiments is that they can detect restriction to translational diffusion occurring during the diffusion time Δ and thus the restricted diffusion observed. The diffusion coefficient measured in a homogeneous system is independent of Δ.

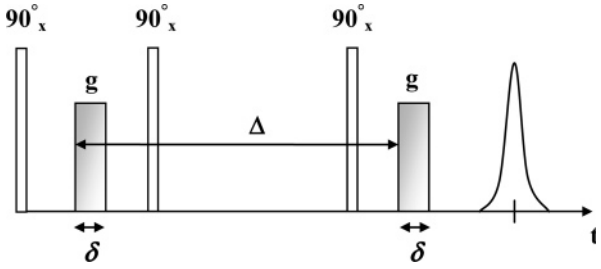

Figure 1. Stimulated echo pulse sequence used in NMR self-diffusion experiment.

Table 2. Relaxation Times (T_2) According to Whey Protein Solution Concentrations at 20 °C

concn (g/(100 g of water))	$T_2(1)$ mean (ms)	amplitude(2) (%)	$T_2(2)$ mean (ms)
1.79		100.00	1774 ± 19
6.70		100.00	1044 ± 6
10.17		100.00	754 ± 9
17.86		100.00	418 ± 1
22.33	3.9 ± 0.2	91.42	309 ± 4
31.26	2.4 ± 0.1	89.14	171 ± 1
35.73	1.9 ± 0.1	88.41	127 ± 2
44.66	1.28 ± 0.01	87.60	80 ± 1

NMR tubes (5 mm) were used, and all the measurements were conducted at 20 ± 0.1 °C. Calibration of the pulse field strength gradients was performed with a sample of pure water of known self-diffusion coefficient ($D_{H2O} = 1.98 \times 10^{-9}\ m^2 s^{-1}$). The gradient strength, g, used in this study ranged between 0.1 and 2 T/m.

Diffusion coefficients were obtained using

$$I(\delta,\Delta,g) = I_0 \cdot \exp[\gamma^2 g^2 \delta^2 (\Delta - \delta/3) D] \quad (2)$$

where $I(\delta,\Delta,g)$ and I_0 were the echo intensities in the presence of gradient pulses of strength g and the absence of gradient pulses, respectively. The length of the pulse gradient was δ, Δ was the distance between the leading edges of the pulse gradients, γ was the gyromagnetic ratio (for protons, $\gamma = 26.7520 \times 10^7\ rad T^{-1} s^{-1}$), and D was the water self-diffusion coefficient.

With the PGSE sequence a total of four scans was collected with a recycle delay of 15 s and duration of gradient δ equal to 0.5 ms, and Δ was fixed at 7.5 ms. With the PGSTE sequence, eight scans were carried out with the same recycle delay of 15 s and phase cycling. The values of other parameters are recorded in parentheses, depending on the experiments: δ (0.05−0.16 ms) and Δ (40−410 ms). All of the other parameters were kept constant.

RESULTS AND DISCUSSION

(1) Relaxation. Spin−spin relaxation times were measured with the CPMG sequence for samples of whey protein concentration ranging between 1.79 and 44.66 g/(100 g of water) for solutions and between 10.17 and 35.73 g/(100 g of water) for gels. T_2 and relative intensities obtained are presented in Table 2 for solutions and Table 3 for solutions and gels. For the whey protein solutions, the relaxation presented a monoexponential behavior until 17.86 g/(100 g of water) (amplitude, 100%). Above this concentration a biexponential decay curve was observed, the first component representing 10% of the signal and the second 90%. For increasing whey concentrations, the relaxation time for the first component ($T_2(1)$) decreased from 3.9 to 1.28 ms and for the second ($T_2(2)$) from 1774 to 80 ms.

In the view of the relaxation time values and relative amplitudes, the first component could be attributed to nonexchangeable protein protons and the second to water protons

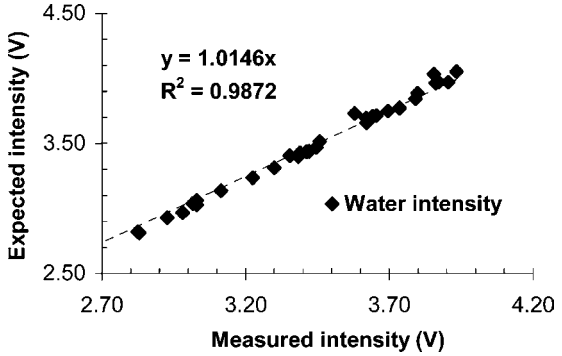

Figure 2. Expected intensity of water signal according tointensity of water signal measured for whey protein solutions. Dashed line is just for the eyes.

Table 3. Relaxation Time (T_2) of Whey Protein Gels and Solutions According to Concentrations at 20 °C

	concn (g/(100 g of water))	amplitude (%)	T_2 mean (ms)
solution	10.17	100	754 ± 19
gel		100	215 ± 18
solution	17.86	100	418 ± 9
gel		100	113 ± 1
solution	22.33	91.42	309 ± 4
gel		100	81 ± 1
solution	31.26	89.14	171 ± 1
gel		100	58 ± 1
solution	35.73	88.41	127 ± 2
gel		100	47 ± 1

including exchangeable protein protons. This assumption was validated by careful comparison between the experimental intensity and the expected intensity deduced from the water content. The NMR signal intensity (V) generated by 1 g of pure water was 7.75 ± 0.08 for the same acquisition conditions. The expected water signal intensity could then be estimated from the known water content in each sample and compared to the intensity measured (Figure 2). These intensities were equal after taking account of standard errors. This result confirmed that the second component described the water proton relaxation, whatever the protein concentration. The contribution to the NMR signal intensity from the exchangeable proton was too small to be detected at low field. Moreover, the signal from nonexchangeable protein protons could only be detected for a concentration above 17.86 g/(100 g of water).

After coagulation, monoexponential behavior was observed for the spin−spin relaxation decay curve whatever the protein concentration. This single component described the water proton relaxation, since the relaxation times from the nonexchangeable protein protons were reduced by the coagulation and were not detected in the CPMG decay curve. The water relaxation time values were also smaller after heat treatment. This effect increased with the protein concentration.

The variation in water relaxation rate according to the protein concentration was also modified by the protein state. In solution a linear dependency was observed below 0.17 g/g. Nonlinear increase was observed above this protein concentration. Water relaxation in the gel increased linearly from 0.1 to 0.36 g/g (Figure 3).

Proton relaxation times have been used extensively to study the state of water in food. Different models are reported in the literature for the interpretation of water relaxation rate data in proteins. Differences in interpretation revolve around three major issues: (1) the nature of the protein-associated water which contributes to the relaxation dispersion (its location, orientational order, and residence time), (2) the relative importance of direct contributions to the ^{1}H relaxation rate observed from labile protein protons exchanging with water (*27*−*29*), and (3) the effects of cross-relaxation between longitudinal magnetizations associated with water protons and protein protons (*30*). Venu et al. (*31*) showed that cross-relaxation is generally negligible for the ^{1}H relaxation for solutions of freely tumbling proteins. The NMRD profiles of all three nuclei (^{1}H, ^{2}H, ^{17}O) were compared and they showed that the difference between ^{1}H and ^{2}H or ^{17}O relaxation could be quantitatively accounted for in terms of exchanging internal water molecules and labile protons, with no need to invoke cross-relaxation.

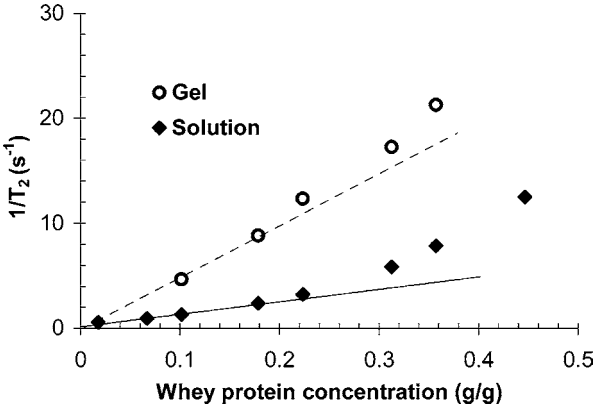

Figure 3. Transverse water proton rate $1/T_2$ (s^{-1}) according to whey protein concentration (g/(g of water)) for solutions and gels at 20 °C.

Hills et al. (*27*) suggested that spin−spin water proton relaxation in native bovine serum albumin dispersions could be quantitatively interpreted in terms of chemical exchange between protein and water protons. Assuming a simple two-site exchange process, the relaxation rate at low field is given by the corrected Carver−Richards expression:

$$\frac{1}{T_2} = \frac{P_a}{T_{2f}} + \frac{P_b}{T_{2b} + \frac{1}{k_b}} \quad (3)$$

where T_2 is the proton relaxation time observed, T_{2f} and T_{2b} are the transverse relaxation times of the bulk water protons and the exchangeable protein protons, respectively, P_a and P_b are water protons and the exchangeable protein protons, respectively, and k_b is the chemical exchange rate. This model has been used to explain the water relaxation in numerous protein and carbohydrate systems (*27*, *28*, *32*−*36*).

This two-site exchange model was in agreement with the effect of the protein concentration on the water relaxation. In solution, and below 0.17 g/g, the two-site exchange model assumed a linear variation since the exchangeable protein proton relaxation rate and the chemical exchange rate were assumed to be independent of the protein concentration in dilute systems. The latter assumption was only valid when the protein−protein interactions were negligible. Above a critical protein concentration, when this assumption is no longer valid, a nonlinear behavior should be observed, explained by dependency of the protein relaxation on the protein concentration. According to our findings, this critical protein concentration was 0.17 g/g. This value was in agreement with the results of Le Bon et al. (*37*). Using diffusion coefficient measurements of β-lactoglobulin in solution, they estimated a critical concentration limit of 0.16 g/g, above which the protein−protein friction coefficient could no longer be ignored compared to the protein−solvent fraction coefficient.

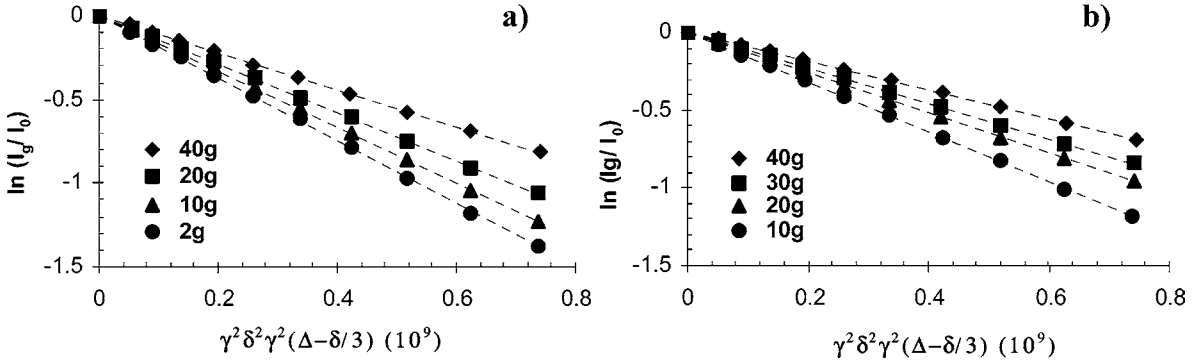

Figure 4. Echo attenuation of water for different concentrations of whey protein solutions (a) and gels (b) (g of whey protein/(100 g of water)) according to $\gamma^2\delta^2g^2(\Delta - \delta/3)$ at 20 °C.

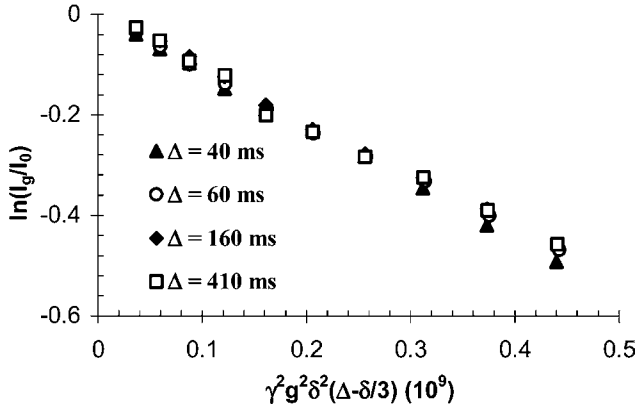

Figure 5. Echo attenuation of water in 40 g of whey protein gel/(100 g of water) according to $\gamma^2\delta^2g^2(\Delta - \delta/3)$ for different diffusion time Δ at 20 °C in gel.

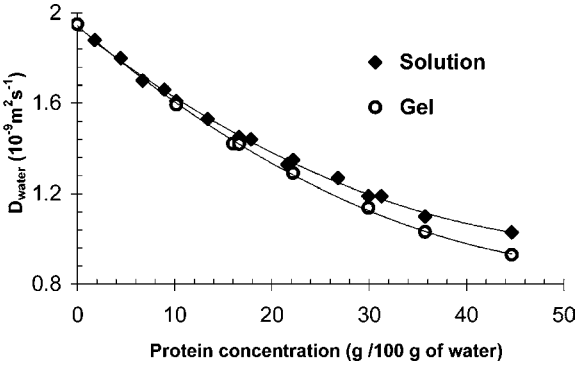

Figure 6. Whey protein concentration (g of protein/(100 g of water)) according to the self-diffusion coefficient in whey protein solutions (♦) and gels (○) at 20 °C measured by PGSE.

The increased water proton relaxation rate induced by aggregation could be explained by the simple molecular processes of diffusion and chemical exchange (Hills et al. (*27*, *28*) and Lambelet et al. (*38*)). The main effect of aggregation is to reduce the protein proton transverse relaxation time because the dipole−dipole interactions between the protein protons are no longer as efficiently averaged by rotational motion. This in turn causes a reduction in water proton transverse relaxation time since water protons are in fast chemical exchange with the protein protons.

Moreover, these results demonstrated that the water self-diffusion could be estimated with a low-field NMR spectrometer only if the echo time was sufficiently high to reduce the protein signal intensity into the echo intensity. Since the protein relaxation in solution did not exceed 4 ms, an echo time of TE = 15 ms was chosen.

(2) Water Self-Diffusion Coefficient in Whey Protein Solutions and Gels. Examples of semilogarithmic plots of echo intensities according to ($\gamma^2\delta^2g^2(\Delta - \delta/3)$) are given in Figure 4for solutions (a) and gels (b). A linear variation was observed for all samples of whey protein concentrations in solutions and gels. A water self-diffusion coefficient was measured in whey protein gel (40 g/(100 g)) with the PGSTE sequence for values of Δ = 40, 60, 160, and 410 ms. The logarithm of the echo attenuation according to k is given in Figure 5. A straight line was observed for all values of Δ, and then water self-diffusion coefficients were equal.

To probe the structure of porous media accurately in diffusion experiments, the diffusion distance must be greater than the characteristic size of the pore structure. The characteristic distance of water molecules measured by NMR is $(6D\Delta)^{1/2}$ (*39*). According to the value of Δ, the diffusion distance varied from 15 to 47 μm for the most concentrated sample. This value was much larger than the sub-micrometer protein size. In native state the radius of β-lactoglobulin is 2 nm (*40*), and for α-lactalbumin it is 1.7 nm (*41*). After heat treatment a gel network is formed which is usually composed of aggregates, the diameters of which are in the order of 40−60 nm (*42*, *43*). Moreover, since a straight variation in echo attenuation was observed and the self-diffusion was independent of the diffusion time Δ, this demonstrated that most water molecules were not confined in compartments or affected by the presence of barriers and could diffuse freely over 47 μm. The formation of the gel induced no restricted diffusion of the water molecules in the gel.

The normalized water self-diffusion coefficients in whey protein solutions and gels according to protein concentrations are shown in Figure 6. Normalization was performed with the experimental value of water self-diffusion of pure water added to NaCl (0.1 M), $D_0 = 1.95 \times 10^{-9}$ m^{2}s^{-1} at 20 °C. As expected, the water self-diffusion coefficient in both solutions and gels decreased with the increase in whey protein concentration, and as expected at very low whey protein content (1 g/(100 g)), the self-diffusion coefficient in whey protein solutions and gels approached the pure water self-diffusion coefficient. For protein concentrations below 20 g/(100 g of water), the water self-diffusion coefficients were insensitive to the protein state, and no difference was observed between the solution and gel state. Above this concentration the water self-diffusion was reduced after gel formation. This effect was clearly dependent on the protein concentration, and the reduction in water self-diffusion increased with the protein concentration. This indicates that the 3D network of gels formed by gelation changes the physical structure of the local environment through which the water molecules diffuse.

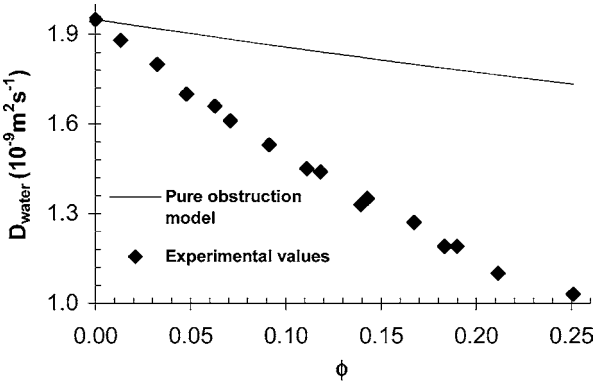

Figure 7. Water self-diffusion coefficients in whey protein solutions according to whey protein volume fraction: The full line is the fit to eq 4 (pure obstruction model) for whey proteins.

(3) Description of Water Mobility in Whey Protein Solutions with Diffusion Models. A number of physical models of diffusion have been proposed to describe molecular diffusion in polymers, each having its particular advantages and limitations. They are based on different concepts such as the obstruction effects, hydrodynamic interactions, and free volume concept. In 1999, Masaro and Zhu (*20*) reviewed some of these models and indicated their usefulness and applications.

Obstruction Effects. The obstruction model considered that water transport is only affected by the impenetrable slow-moving protein molecules. The diffusion of water in the system is hindered due to the obstruction by proteins, and no interactions between water molecules and proteins needed to be taken into account. If we assume that a protein could be described as a spherical object, then the pure obstruction model is expressed by

$$\frac{D}{D_0} = \frac{1}{1 + \frac{\Phi}{2}} \tag{4}$$

The volume fraction ϕ occupied by whey proteins can be written as follows:

$$\phi = \frac{m^{\text{prot}} v^{\text{prot}}}{m^{\text{prot}} v^{\text{prot}} + m^{\text{water}} v^{\text{water}}} \tag{5}$$

where m^{prot} and v^{prot} are the protein mass (g) and the specific volume of whey proteins, 0.75 cm^3g^{-1} (*37*, *44*), respectively, and v^{water} is the specific volume of water (1 cm^3g^{-1}).

The results in Figure 7were fitted using the pure obstruction model. Fitting of eq 4 is outside of experimental values. This pure obstruction model cannot be used to explain water diffusion in whey protein solutions. This model has been successfully applied to describing water diffusion in oil in water (o/w) emulsions (*45*) and in PMMA latex particles (*22*). For such a system the hydration effect that corresponds to the lower mobility of the water molecule in the vicinity of the particle can be ignored. Nevertheless, with such a system, if the concentration of the obstructing particle increases above 20%, the diffusion coefficients measured are not correctly predicted by the obstruction model. For such concentrations the water hydration effect should be included in the model. In the case of protein solutions or gels this concentration limit appears to be very low. Moreover the hard sphere approximation is only valid for very dilute systems.

Cell Model of Jönsson. The cell-diffusion model of Jönsson et al. (*22*) takes obstruction as well as hydration into account.

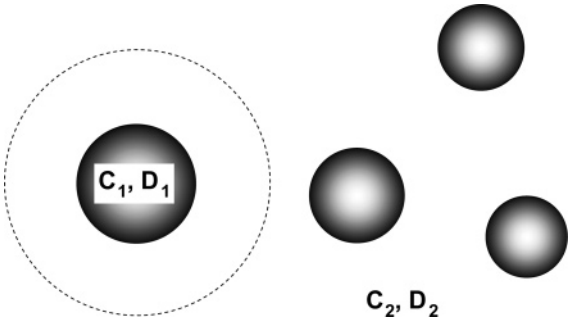

Figure 8. Illustration of the two water regions according to the cell model. The cell is divided into two regions, 1 and 2. Region 1 corresponds to water molecules inside the micelle and region 2 to water molecules outside the micelle (pure water). The regions together represent the macroscopic behavior of water diffusion.

This model has previously been used to evaluate water diffusion data in casein dispersions (*23*) and has been successfully applied to surfactant−water systems (*46*). According to this model, we considered a spherical shape for the whey protein particles, and two water compartments, i.e., bulk water and hydration water, characterized by different but constant water concentrations and self-diffusion coefficients (Figure 8). Using this model, obstruction effects and water−protein interactions were taken into account in the values of self-diffusion coefficients in the two different regions. When the self-diffusion coefficient of the particle is small compared to the water diffusion coefficient, the self-diffusion coefficient D_{eff} observed is written

$$D_{\text{eff}} = D_2 \frac{1}{1 - \left(1 - \frac{C_1}{C_2}\right)\phi} \frac{1 - \beta\phi}{1 + \frac{\beta\phi}{2}} \tag{6}$$

with

$$\beta = \frac{D_2 C_2 - D_1 C_1}{D_2 C_2 + 0.5 D_1 C_1} \tag{7}$$

where C_1 and D_1 are the water hydration concentration and self-diffusion coefficient, respectively, and C_2 and D_2 the same properties for the bulk water surrounding the hydrated particle. We emphasize that water hydration diffusion is not necessarily "homogeneous" because it is described by the same local diffusion coefficient in all parts. A situation with different sites in which water diffusion has different values is perfectly possible, if the exchange between the water and the protein are fast and the distance diffused by the water is greater than the region, so that any inhomogeneities are averaged out.

If ϕ occupied by the aggregates is written as in eq 5, the other parameters in eqs 8 and 9 can be written as follows:

$$C_1 = \frac{m^{\text{prot}} H^{\text{prot}}}{m^{\text{prot}} v^{\text{prot}} + m^{\text{prot}} H^{\text{prot}} v^{\text{eau}}} \tag{8}$$

It is assumed that there is pure water surrounding the whey protein particle, $D_2 = 1.95 \times 10^{-9}$ m^2s^{-1} and $C_2 = 1$ g/cm^3. If these values plus the expressions in eqs 7 and 8 are used in eq

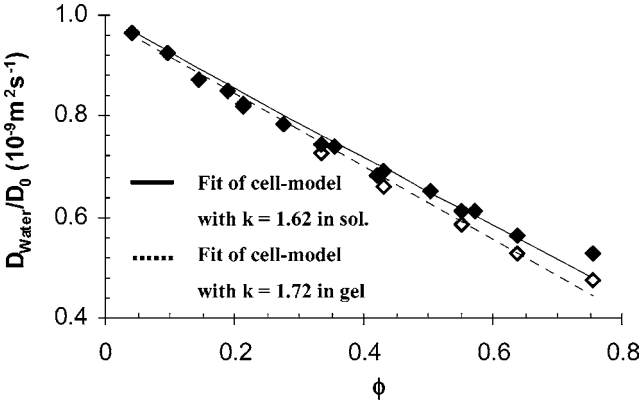

Figure 9. Normalized self-diffusion coefficient of water in whey protein solutions (♦) and gels (◇) according to the volume fraction of whey proteins. The full line is the fit to eq 9 for whey protein solution with $k = 1.62$, and the dashed line is the fit to eq 9 for whey protein gel with $k = 1.72$.

9, the concentration dependence of the effective water self-diffusion coefficient becomes

$$D_{\text{eff}} = 1.95 \times 10^{-9}\left(1 + 0.75\frac{m^{\text{prot}}}{m^{\text{water}}}\right)\frac{\left(1 + 0.75\frac{m^{\text{prot}}}{m^{\text{water}}} - k\frac{m^{\text{prot}}}{m^{\text{water}}}\right)}{\left(1 + 0.75\frac{m^{\text{prot}}}{m^{\text{water}}} + 0.5k\frac{m^{\text{prot}}}{m^{\text{water}}}\right)} \quad (9)$$

where

$$k = (\nu^{\text{prot}}/\nu^{\text{water}} + H^{\text{prot}})\beta \quad (10)$$

k is the only unknown parameter in eq 9, and its value can be obtained by fitting the experimental data with this equation. The result of the fitting process is presented in Figure 9, with the k value for the best fit equal to 1.62. This value was lower than the value of k determined in a micellar casein dispersion, for which the value of k was 1.76 at 20 °C (*47*) and no difference was observed between the water diffusion in solutions and gels.

However, it is not possible to determine both the concentration and the self-diffusion coefficient of hydration water from this value. One of the parameters needs to be specified. If we assume a reduction factor $D_{\text{bulk}}/D_{\text{hyd.}}$ in the range of 3−6, then the water hydration diffusion was between 0.32×10^{-9} and 0.65×10^{-9} m^2s^{-1}, which corresponds to hydration water between 1.14 and 1.58 g of water/(g of protein). This value was in agreement with the whey protein voluminosity, i.e., 1.51 for β-lactoglobulin and 1.135 for α-lactalbumin (*44*). This value of water hydration estimated from water diffusion could be considered as an upper limit since a spherical shape was assumed for the whey particle. Indeed, the obstruction effect is sensitive to particle shape, and this effect is greater for prolate and oblate particles (*22*). If other shapes were considered, the contribution to the diffusion of the hydration interaction would be reduced.

When we applied the cell model equation to the water self-diffusion coefficients in gels, the value of k obtained was 1.72 (Figure 9). Consequently, the results show that the self-diffusion coefficient in protein systems is mainly affected by protein concentration, whereas protein structure has minor effect. For casein micelles, which are large and highly porous, water accessibility to the protein surface is high. The structural changes induced by coagulation with rennet are therefore too small to induce a significant decrease in water self-diffusion. In other words, the water molecule accessibility to the protein surface is the same as that of the casein micelle structure.

For granular proteins, gelation by heating increased the probability of water protein proton bonding and induced a reduction in water self-diffusion.

Moreover, it should be mentioned that the water self-diffusion coefficient for hydration water ($(0.32-0.65) \times 10^{-9}$ m^2s^{-1} for a protein concentration of 66 g/(100 g)) was in agreement with the water self-diffusion coefficient measured in BSA (*16*).

We conclude that the water diffusion in protein systems follows a general trend whatever the protein system studied, and some differences in water diffusion could reflect the change in the water accessibility to the amino acid backbone of protein.

(4) Conclusions. We have shown here that the structural changes between a whey protein solution and a whey protein gel can be analyzed by transverse relaxation and diffusion. The main effect of this heat-induced aggregation and gelation process is to reduce the protein proton transverse relaxation time dramatically by hindering the rotational averaging of the protein dipolar interactions.

The water self-diffusion coefficient has been described using the obstruction model and the cell model. The cell model provided the best description of the concentration effects and was consistent over the whole concentration range. Water diffusion can be described by two self-diffusion flows, one around the proteins and one close to the proteins. No specific water−protein "binding" is needed to describe the reduction in water mobility, and these results are in accordance with the transverse relaxation measurements.

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