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Registry No. Linolenic acid, 463-40-1; linoleic acid, 60-33-3; stearic acid, 57-11-4; dodecanol, 112-53-8; tetradecanol, 112-72-1; pentadecanol, 629-76-5; heptadecane, 629-78-7; octadecane, 593-45-3; tetracosane, 646-31-1.

Approaches to Protein Hydration and Water Activity in Food Proteins by Nuclear Magnetic Relaxation and Vapor Pressure Equilibration Techniques

Patricia A. Myers-Betts and Ion C. Baianu*

Department of Food Science, AFC NMR Facility, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801

^1H and ^{17}O NMR relaxation rates for two proteins, lysozyme and corn zein, were treated by employing nonlinear regression analysis and a protein activity model. The dependence of the NMR transverse relaxation rates on protein concentration was markedly nonlinear due to interactions between protein molecules. Using a virial expansion, protein activities were calculated for the entire concentration range. From the protein activities, theoretical water activity (a_w) curves were derived with a simple hydration model. Comparison of the a_w values as a function of protein concentration to the published sorption isotherms showed significant differences between the expected RVP and sorption isotherms, especially at protein concentrations >0.2 g/mL. This difference is likely to be caused by the fact that sorption "equilibration" depends on the (relatively slow) translational diffusion of water molecules whereas NMR relaxation monitors primarily the fast motions of water.

1. INTRODUCTION

Recently, there has been an increasing interest in the study of protein hydration (Kumosinski and Pessen, 1982; Kumosinski et al., 1987, 1988; Lioutas et al., 1986, 1987; Myers-Betts and Baianu, 1990; Kakalis and Baianu, 1988). The importance of investigating the hydration properties of proteins in food systems has its basis in practical applications. For example, the functionality of many food proteins depends, in part, on their hydration characteristics. In muscle products, the interactions between myofibrillar proteins, solute, and water will determine the functional behavior of the food, such as heat gelling, hardening, texture changes, and binding of water (Woeff, 1982). In addition to these functional implications, deteriorative reactions in foods depend greatly on the characteristics of the water in the food system. Microbial growth and undesirable chemical reactions (e.g., Maillard browning, lipid oxidation) can have an adverse effect on the stability and shelf life of the food product in addition to affecting the organoleptic properties. Therefore, it is important to investigate the dynamics of protein hydration and to determine the range of water perturbations induced by proteins.

Traditionally, sorption isotherm "equilibrium" methods have been used to investigate the retention of water by proteins (Bull, 1944), and several different models have been proposed to interpret the data in terms of the "water activity", often identified with the relative vapor pressure (RVP) (Brunauer et al., 1938; D'Arcy and Watt, 1970). (Quotes are employed throughout the text to indicate a generic use of the terms in quotes for foods that differs significantly from the original, precise definitions that are strictly valid only for simpler systems such as electrolytes solutions. The terms in quotes are, therefore, often misused in the context of food systems.) At thermodynamic equilibrium, the "water activity", a_w, is related to the equilibrium relative humidity (ERH) by

$$a_w = (p/p^0) = \text{ERH}\,(\%)/100 \quad (1)$$

where p and p^0 are the water vapor pressures in the food

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and of the pure water, respectively. However, the concept of nonequilibrium behavior due to restricted translational diffusion mechanisms of water molecules in concentrated carbohydrate systems has apparently led some researchers to question the usefulness of "water activity" measurements as RVP (Franks, 1982; Slade and Levine, 1987). Also, information concerning the mechanisms and molecular interactions involved in protein hydration cannot be directly or readily obtained by such hydrodynamic "equilibrium" techniques. Of particular interest are the protein–water and protein–protein interactions in relation to the protein activity.

Because proteins are polyampholytes, they can be expected to exhibit attractive and repulsive interactions in solution (Kirkwood and Shumaker, 1952), which can perturb local water motions and affect water binding (Kumosinski and Pessen, 1982). Hence, the protein hydration and water activity should both be affected. Therefore, it is important to be able to quantitate these charge, dipolar, polar, and nonpolar interactions and to determine separately how they influence the water activity and the relative vapor pressure.

Nuclear magnetic resonance (NMR) relaxation techniques provide useful, nondestructive methods for studying protein hydration, protein–protein interactions, and the molecular dynamics of water in complex food systems. NMR has been used to study several aqueous protein systems including β-lactoglobulin A (Kumosinski and Pessen, 1982), bovine caseins (Kumosinski et al., 1987), lysozyme (Lioutas et al., 1986, 1987; Kakalis and Baianu, 1988), soy protein (Kakalis and Baianu, 1988), corn zeins (Myers-Betts and Baianu, 1987, 1990), and myofibrillar proteins (Lioutas et al., 1988). With the assumption of negligible protein activity, ^{17}O and ^{1}H NMR data for lysozyme (Lioutas et al., 1986, 1987) indicated the presence of four distinct hydration regions over the concentration range from liquids to hydrated powders. ^{17}O and ^{23}Na NMR studies of myofibrillar proteins (Lioutas et al., 1988) were recently reported, and the effects of salt concentration and ion binding on protein hydration and "water activity" were considered. The effects of protein activity in these systems were presented in a recent report (Baianu et al., 1988).

From our previous NMR studies of corn zeins, protein–protein interactions were quantitated and shown to have a large affect on the ^{1}H NMR transverse relaxation rates (Myers-Betts and Baianu, 1987, 1990). These results were consistent with the first report of the influence of protein activity on deuterium NMR relaxation rates of β-lactoglobulin A in dilute solutions with salt (Kumosinski and Pessen, 1982).

The purpose of this study is to investigate further the relationship between protein–protein interactions and water activity in corn zeins and lysozyme solutions based on ^{17}O and ^{1}H NMR relaxation measurements. Our first objective is to calculate the protein activities from NMR relaxation data with a charge interaction model and to derive theoretical water activity (a_w) curves. These curves will then be compared with the published experimental sorption isotherms. We will then compare the data from both ^{1}H and ^{17}O nuclear spin relaxation in an attempt to determine protein hydration mechanisms in relation to water sorption.

2. THEORY

Prior to 1982, NMR relaxation data were interpreted in terms of the two-state model with fast exchange (Zimmerman and Brittin, 1957; Derbyshire, 1982). This model considers two water populations, free and bound, with different relaxation rates. The observed relaxation rate is a weighted average of the two water populations that are in fast exchange on the time scale of the nuclear spin relaxation

$$R_{2obs} = P_B R_{2B} + P_F R_{2F} \quad (2)$$

where R_{2obs} (s^{-1}) is the observed relaxation rate and R_{2B} (s^{-1}) and R_{2F} (s^{-1}) are the relaxation rates of bound and free water, respectively. P_B and P_F are the percentages of these water populations. This relationship should be linear with increasing solute concentration for ideal solutions if no other contribution to relaxation exists. Furthermore, eq 2 does not take into consideration the dual motions of the hydration water in proteins, where at least two very distinct correlation times (~10 ns versus ~30 ps) are present, as found from the field dispersion of the NMR relaxation rates of water in hydrated proteins (Kakalis and Baianu, 1988). Marked deviations from linearity of the concentration dependence are often observed (Derbyshire, 1982; Lioutas et al., 1986; Kumosinski and Pessen, 1982; Kumosinski et al., 1987; Myers-Betts and Baianu, 1990). Derbyshire (1982) proposed that any nonlinearities could be due to (1) a change in the hydration of the biopolymer and/or (2) a change in the relaxation rate of bound water. However, this model does not directly take into account the interactions that occur between protein molecules in solution.

Another model considering interactions between protein molecules was proposed by Kumosinski and Pessen (1982). This model is based on the charge fluctuation theory of Kirkwood and Shumaker (1952). Briefly stated, this model considers electrostatic interactions between isoionic protein molecules; some of these interactions arise from fluctuations in the charge and charge distribution associated with fluctuations in the number and configuration of bound protons. This type of interaction has been shown to have a square root dependence on protein concentration from light-scattering studies (Timasheff et al., 1957). In the absence of salt, such fluctuations of the mobile protons cause the protein molecules to experience fluctuating electric multipole moments that lead to attractive or repulsive forces. In the presence of a moderate amount of salt (e.g. ~0.1 M NaCl), these proton fluctuations are screened out.

The model of Kumosinski and Pessen (1982) relates the concentration dependence of the NMR relaxation rates in dilute protein solutions to the Kirkwood and Shumaker theory (1952) through the calculation of the protein activity. The activity of a protein, a_p, is related to its concentration, c_p, by the activity coefficient, γ_p, which accounts for the *nonideality* of the solution:

$$a_p = \gamma_p c_p \quad (3)$$

The activity coefficient can be expressed as a virial expansion similar to that used in osmotic pressure measurements

$$d \ln \gamma_p / dc_p = 2B_0 + 3B_2 c_p + \ldots \quad (4)$$

where the B_i quantities are the virial coefficients. Applying this relationship to zein and lysozyme NMR relaxation data (Myers-Betts and Baianu, 1990; Kumosinski and Pessen, 1982) yields

$$R_{2obs} - R_{2F} = n_H (R_{2B} - R_{2F}) c_p \exp[2B_0 c_p + 1.5 B_2 c_p^2 + B_3 c_p^3 + B_4 c_p^4] \quad (5)$$

where R_2's are transverse relaxation rates; the subscript obs stands for the measured relaxation rate of the pro-

tein solution, F stands for the R_2 of liquid water (free), B stands for the R_2 of bound water, and n_H is the hydration (gram of H_2O/gram of dry protein). Values for n_H were obtained from Table I of Asbi and Baianu (1986). The B_0 virial coefficient reflects the repulsive forces arising from the net average charge on the protein, the protein-excluded volume, and a preferential ion-binding term according to the following expression (Kumosinski et al., 1987; Arakawa and Timasheff, 1982)

$$2B_0 = [Z^2/(4m_sM_p)] + [\upsilon_p/1000] + \beta_{22} \quad (6)$$

where Z is the average charge on the protein molecule, m_s is the molarity of salt or other extraneous ions, M_p is the molecular weight of the protein, and υ_p is the average partial specific volume of the protein. The β_{22} term represents the preferential interaction of ions and water at the protein surface

$$\beta_{22} = -(\partial g_s/\partial g_p)(1/m_s) \quad (7)$$

where $(\partial g_s/\partial g_p)$ is the preferential binding term. The B_2 virial coefficient represents attractive forces caused by fluctuating multipoles. Essentially, the exponential portion of eq 5 represents the activity coefficient; therefore, determination of these virial coefficients will allow for quantitation of the protein–protein interactions, or the protein activity.

To determine the contribution of the calculated protein activity to the water activity under conditions of thermodynamic equilibrium, the following relationship should be used according to Kumosinski et al. (1988)

$$a_w = 1 - [(\text{mol bound } H_2O/\text{mol total } H_2O) \times \text{mol protein} \times a_p] \quad (8)$$

where a_w is the thermodynamic water activity derived from the change in the chemical potential and a_p is the protein activity calculated by fitting the NMR relaxation data. This relationship is calculated for a constant volume in order to retain thermodynamic equilibrium. For convenience, protein activities can be scaled such that, at $c_p = 0$, $a_w = 1$ and, at $c_p = 1$, $a_w = 0$ according to the relationship in eq 8.

3. MATERIALS AND METHODS

3.1. NMR Measurements. The ^1H NMR data for alkaline corn zein solutions (pH 11.5) were reported previously (Myers-Betts and Baianu, 1987, 1990). Sorption isotherm data for zein at 25 °C were also obtained from previous reports (Bull, 1944; Asbi and Baianu, 1986). The ^{17}O and ^1H NMR data, as well as sorption isotherm data for lysozyme at pH 7.0, were derived from the reports of Lioutas et al. (1986, 1987). Details of the NMR relaxation measurements as well as of the sorption isotherm methodologies were given in previous reports (Lioutas et al., 1986, 1987; Myers-Betts and Baianu, 1987, 1990).

3.2. Data Analysis. The dependence of the observed NMR relaxation rates on protein concentrations was fit according to eq 5 with an iterative nonlinear regression program (SYSTAT version 3.1) on a Macintosh II microcomputer that employed both the Simplex and Quasi-Newton algorithms. This program minimizes the standard deviation of the experimental points from the curve, also known as the root mean square (RMS), where the RMS is defined as

$$\text{RMS} = [(R_{2calc} - R_{2obs})^2/(\text{no. data points} - \text{no. parameters})]^{1/2} \quad (9)$$

where R_{2obs} and R_{2calc} are the observed and calculated relaxation rates, respectively. The parameters allowed to vary were the virial coefficients of the protein activity.

The Simplex algorithm was initially employed due to its lack of truncation problems and high flexibility (Motulsky and Ransnas, 1987; Caceci and Cacheris, 1984; Noggle, 1985). However, because the Simplex does not estimate the standard error of each parameter, the Quasi-Newton algorithm also had to be used in order to obtain the confidence intervals for each virial coefficient.

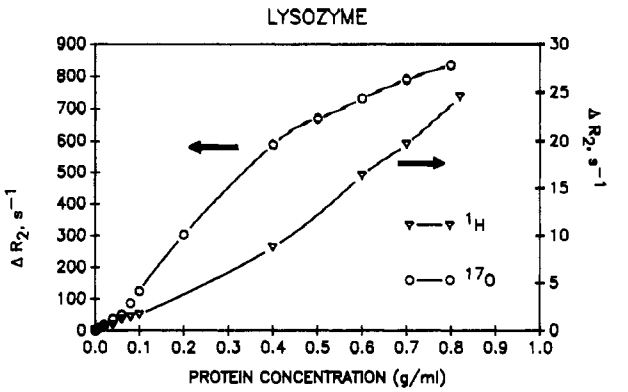

Figure 1. ^{17}O (34-MHz) and ^1H (360-MHz) NMR relaxation rates ($\Delta R_2 = R_{2obs} - R_{2F}$, s^{-1}) as a function of protein concentration for lysozyme/D_2O system at pH 7.0.

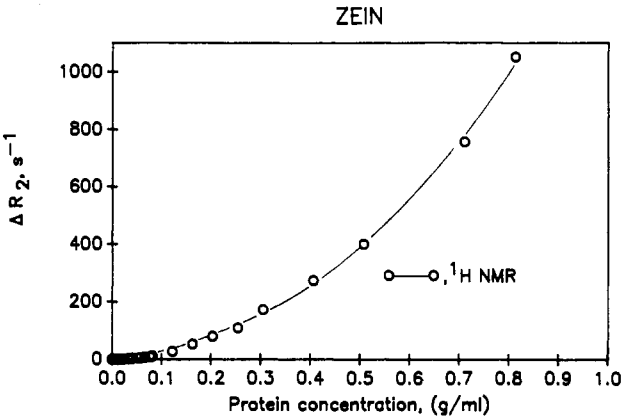

Figure 2. ^1H (10-MHz) NMR relaxation rates ($\Delta R_2 = R_{2obs} - R_{2F}$, s^{-1}) as a function of protein concentration for zein/H_2O system at pH 11.5.

4. RESULTS

4.1. Influence of Protein Activity on NMR Relaxation Rates. Figure 1 represents the ^{17}O and ^1H NMR relaxation rates ($\Delta R_2 = R_{2obs} - R_{2F}$) as a function of protein concentration for lysozyme in D_2O at pH 7.0 in the absence of salt. In both cases, the relaxation rate increases linearly with protein concentration up to approximately 10% (g/mL), indicating that the protein activity is small in this region. Above this point, the relaxation rate increases nonlinearly with protein concentration, indicating that protein activity coefficients have appreciable values.

Figure 2 shows the ^1H NMR relaxation rates for corn zein in H_2O at pH 11.5. Parallel studies of zein in D_2O exhibited the same behavior, suggesting that cross-relaxation has a minor contribution to the relaxation rates (data not shown). The behavior of the corn zein relaxation rates also has an initial, almost linear region (0–8%, g/mL) followed by a marked nonlinear concentration dependence.

Kumosinski and Pessen (1982) suggested that whenever protein activities are appreciable, the dependence of the NMR relaxation rates on protein concentrations should be nonlinear. The data presented here clearly have a nonlinear dependence. In this case, substitution of protein activities for protein concentrations should result in a linear relationship. Figure 3 represents such a linear relationship, with a high correlation coefficient; linear

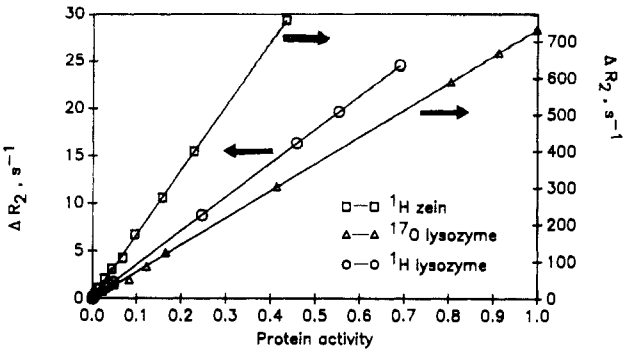

Figure 3. NMR relaxation rates ($\Delta R_2 = R_{2obs} - R_{2F}$, s^{-1}) as a function of calculated protein activities for lysozyme (△, ^{17}O; ○, ^1H) and zein (□, ^1H).

Table I. Virial Coefficients of Protein Activity Obtained from Nonlinear Regression Analysis of NMR Relaxation Data

parameter	zein $^1H^a$	lysozyme $^1H^b$	lysozyme $^{17}O^c$
$n_H(R_{2B} - R_{2F})$	62.624 ± 10.778	20.427	709.449 ± 21.852
B_0, mL/g	6.905 ± 0.758	-1.721	3.664 ± 0.159
B_2, $(mL/g)^2$	-20.547 ± 3.200	11.433	-15.013 ± 0.737
B_3, $(mL/g)^3$	32.699 ± 6.406	-23.976	26.368 ± 1.519
B_4, $(mL/g)^4$	-12.362 ± 3.048	10.823	-11.146 ± 0.715
RMS	3.515	0.043	2.441

[a] 10-MHz data from Myers-Betts and Baianu (1990). [b] 360-MHz data from Lioutas et al. (1987). [c] 34-MHz data from Lioutas et al. (1986).

regression coefficients for a least-squares fit of the NMR relaxation data against calculated protein activities have the values of $r = 0.9999$ in all three cases. The protein activities were calculated according to eq 3 and 5 using the virial coefficients (Table I) obtained from the nonlinear regression analyses.

The virial coefficients listed in Table I contain alternating positive and negative signs, indicating the presence of both repulsive (e.g., positive B_0) and attractive (e.g., negative B_2) interactions between protein molecules. The positive B_0 virial coefficient for zein (6.905 ± 0.758) is twice as large as the B_0 for the ^{17}O data of lysozyme without salt (3.664 ± 0.159). This is attributed to the presence of Na^+ ions from the NaOH required for zein solubilization. Preferential binding of Na^+ to the zein protein increases the electrostatic free energy, resulting in repulsive forces (Arakawa and Timasheff, 1982). This is reflected in the large, positive B_0 virial coefficient.

4.2. Relationship between Protein Activity and Water Activity. Calculations of the water activity were carried out based on the protein activity for each concentration and the moles of bound water according to eq 8. The molecular weight of lysozyme was 14 400 (Sigma Chemical Co.), and the average molecular weight of zein was ~44 000 (Turner et al., 1965). About 180 mol of H_2O/mol of lysozyme can be considered as bound (Lioutas et al., 1986; Kakalis and Baianu, 1988). For zein, ~290 mol of bound H_2O/mol of zein can be calculated from hydration values for C-zein [Table I of Asbi and Baianu (1986)].

Figures 4–6 represent the water activity curves calculated from the protein activities with eq 8, which were then compared with the sorption isotherms of lysozyme and corn zein, respectively (Lioutas et al., 1987; Asbi and Baianu, 1986; Bull, 1944). The water activity values derived from the ^1H NMR relaxation data via protein activity (eq 5) look very similar for lysozyme and zein.

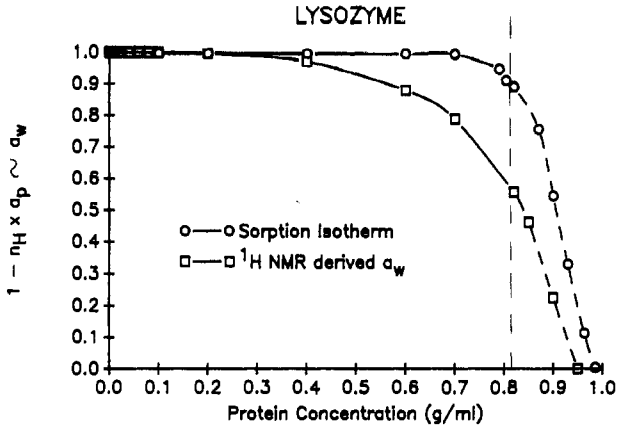

Figure 4. Water activity curve for lysozyme calculated from ^1H NMR relaxation data according to eqs 4 and 5. Sorption isotherm data from Lioutas et al. (1987). Dashed line represents calculated RVP values in the region of thermodynamic nonequilibrium.

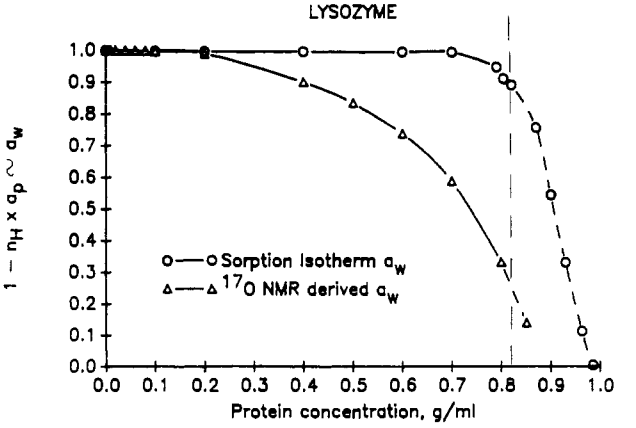

Figure 5. Water activity curve for lysozyme calculated from ^{17}O NMR relaxation data according to eqs 4 and 5. Sorption isotherm data from Lioutas et al. (1987). Dashed line represents calculated RVP values in the region of possible thermodynamic nonequilibrium.

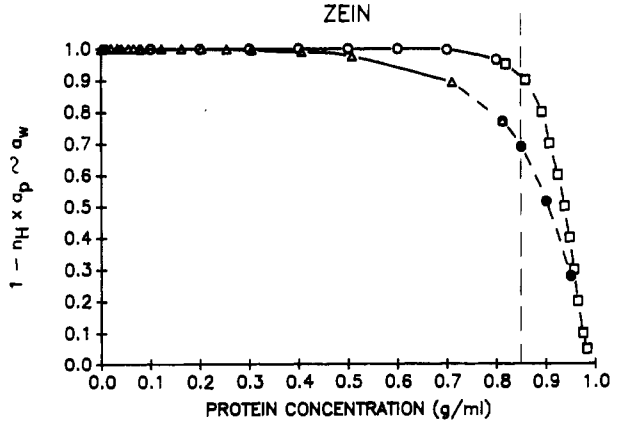

Figure 6. Water activity curve for corn zein calculated from ^1H NMR relaxation data according to eqs 4 and 5. Sorption isotherm data from Bull (1944). Open squares represent computer-generated values with a water activity algorithm. Dashed line represents calculated RVP values in the region of possible thermodynamic nonequilibrium. Open triangles represent ^1H NMR derived a_w.

However, the ^{17}O-derived activity curve for lysozyme without salt shows a much larger deviation from the sorption isotherm. In the initial regions of high water activity (at low protein concentrations), the calculated a_w values are very close to the sorption isotherms (RVP curves) in all

three cases. This is the region of "low" protein concentration where the protein activity coefficient is negligible. For the ^{1}H-derived water activity curve this region occurs from 0 to 0.4 g/mL of protein. In the case of the ^{17}O-derived water activity curve for lysozyme, this region occurs from 0 to 0.2 g/mL of protein. As protein concentrations increase, the calculated a_w values deviate from the sorption isotherms more markedly.

5. DISCUSSION

The results presented in Figures 4–6 show that there are major, intriguing differences between the water activity curves calculated from NMR relaxation data and those obtained from sorption equilibration (RVP measurements). Slade and Levine (1987) have stated that "treatments based on equilibrium thermodynamics (e.g. water activity) of very dilute solutions fail, due to non-equilibrium conditions that exist for pragmatic timescales and conditions". However, Franks (1982) has stated that "for systems with $a_w > 0.90$, the activity as determined experimentally, is an equilibrium quantity of thermodynamic significance." In reference to Figures 4–6, one could question whether or not the sorption isotherm data at high protein concentrations (for RVP $\ll 0.90$) were actually obtained for thermodynamic equilibrium states, especially since protein conformational changes and a tightly packed matrix restrict the translational diffusion of water molecules. This would imply that when very slow equilibration processes occur on the time scale of the methodology employed (i.e., vapor equilibration at high solute concentrations), the RVP would no longer be a satisfactory measure of "water activity". In view of Franks' (1982) statement, the sorption isotherm data in Figures 4–6 have been depicted with dashed lines in the region of possible thermodynamic nonequilibrium (RVP < 0.90). For zein this occurs at approximately 86% protein and at approximately 81% protein for lysozyme. The NMR-derived a_w curves are also depicted with dashed lines starting at the protein concentrations corresponding to RVP < 0.90 in the sorption isotherm data. The dashed line for the NMR-derived a_w curves represents the values obtained from applying the protein activity model to the NMR relaxation data. If these protein systems are not in thermodynamic equilibrium at RVP < 0.90, then a different model should be applied to obtain a_w values from the NMR relaxation data in the high-concentration region. However, in the region of thermodynamic equilibrium (solid lines in Figures 4–6), there are still major differences between the a_w values obtained with the two techniques. Several reasons can be invoked to explain these differences.

A first possible explanation would be that the protein activity is being overestimated, resulting in a lower calculated a_w value for each protein concentration according to eq 8. This would have to originate from a possible, but yet undiscovered, omission in the basic Kirkwood and Shumaker (1952) theory. We do not have sufficient cause to accept this as a valid explanation. The Kirkwood and Shumaker (1952) theory has been successfully applied to explain anomalous hydration and molecular weight values obtained by techniques such as light scattering (Timasheff and Kronman, 1959; Timasheff and Coleman, 1960), sedimentation equilibrium (Schachman, 1959), and osmometry (Tanford, 1961), which were derived when the data was analyzed without taking into account protein activity. The data obtained by these techniques can be influenced by charge fluctuations and charge–charge interactions between protein molecules, as can be NMR relaxation rates.

A second (and more likely) explanation is based on the effect of the restricted diffusion of water molecules. Since sorption isotherms describe the retention of water molecules by macromolecules or solid substrates, presumably under conditions of vapor equilibrium, long-range translational diffusion of water molecules between the surface and the environment is the limiting process. This is a relatively slow process that has the vapor pressure gradient as a driving force. NMR relaxation, on the other hand, is particularly sensitive to fast, short-range translational or rotational diffusion of water molecules and to the exchange processes between water populations. The latter are fast on the NMR time scale. In other words, $\tau_{exch} > 1/R_{2B}$, where τ_{exch}^{-1} is the exchange rate between free and bound water populations and R_{2B} (s^{-1}) is the relaxation rate of bound water. Essentially, the two techniques can be affected differently by two distinct, basic processes: primarily, the translational diffusion in RVP measurements and mostly the effects of rotational motions in NMR relaxation measurements.

In addition to the above explanation, the implicit assumptions made in deriving eq 8 may not be valid at high solute concentrations (e.g., ≥70%). One such assumption is that *all* contributions to NMR relaxation and protein activity are also reflected in water binding and, therefore, "water activity". However, if proton fluctuations, in the absence of salt, would affect only the NMR relaxation rates and would *not* significantly affect water binding, then eq 8 would need modification. For example, in the case of an enzyme, it has been postulated (Zauhar and Morgan, 1985) that a cleft present at the active site creates a region of relatively high electrostatic potential that extends far into the solvent. This has a dynamic effect on the solvent (e.g., water) molecules, which does not involve binding of water. This dynamic effect can be propagated throughout several "water layers" or "shells". In the case of ^{17}O NMR relaxation, which is primarily dominated by the interaction of the electric field gradients with the quadrupole moment at the oxygen nucleus, an enhancement of the relaxation would be observed. The relaxation rates would be increased by very rapid changes of the electrical field gradient at the ^{17}O nucleus induced by proton fluctuations at the protein surface that average out on the time scale of the water exchange between the bound and free populations.

A second (apparent) discrepancy in Figures 4 and 5 that needs considering is the significant difference between the NMR-derived a_w curves for ^{1}H and ^{17}O data of lysozyme. The calculated a_w values for the ^{17}O-derived curve begin to decrease more rapidly at a lower protein concentration than for the ^{1}H-derived curve. This can be addressed by considering the mechanisms contributing to the NMR relaxation for the two nuclei. For protein solutions in D_2O, the major relaxation mechanism contributing to ^{17}O relaxation is quadrupolar, that is the interaction of the quadrupole moment of the ^{17}O nucleus with the fluctuating electric field gradients at the nucleus. ^{17}O NMR is not affected by either cross-relaxation or chemical exchange between protons or deuterons, two mechanisms that can significantly affect ^{1}H NMR relaxation. ^{17}O has been shown to be the best nucleus for probing protein hydration by NMR since it monitors directly the molecular motions of water (Halle et al., 1981; Kakalis and Baianu, 1988).

^{1}H NMR, on the other hand, may also be monitoring protons from groups at the surface of the proteins that would be affected by protein conformational changes. The shape of the sorption isotherms will also be influenced

by structural and conformational changes that the protein or protein matrix may undergo at different levels of hydration, especially under nonequilibrium conditions. This could be one of the reasons why, in Figure 4, the ^1H NMR derived a_w curve more closely resembles the sorption isotherm than does the ^{17}O-derived a_w curve in Figure 5. Such changes in the protein matrix would also influence the translational diffusion of water molecules.

Kumosinski et al. (1988) have employed NMR relaxation techniques to investigate the interesting possibility of using proteins as bacterial growth inhibitors through the suppression of "water activity". However, our results presented here have shown a discrepancy between water activity as derived from NMR relaxation data and isotherms determined by vapor pressure equilibration techniques. Our results have shown that the NMR relaxation rates are strongly influenced by the protein activity. To determine whether such protein–protein interactions also affect translational diffusion of water, other techniques will be needed. Among these are pulsed field gradient NMR techniques that can be used to determine the self-diffusion coefficients of water (Stejskal and Tanner, 1965; Callaghan et al., 1979). However, the results for complex systems such as wheat grains are highly dependent on the choice of diffusion model (e.g., one-, two-, or three-dimensional diffusion) and also do not seem to agree with RVP measurements if a one-dimensional model were adopted (Callaghan et al., 1979).

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Effect of the Acyl Chain Length of Phosphatidylcholines on Their Dynamic States and Emulsion Stability

Kazuhiro Chiba and Masahiro Tada*

Laboratory of Bio-organic Chemistry, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183, Japan

The relationship between the emulsion stability of phosphatidylcholines (PCs) and their headgroup motion of varied acyl chain lengths was investigated by ^{31}P and ^{13}C NMR. The acyl chains of PCs at glycerol *sn*-2 were shortened by the acylation of lysophosphatidylcholine (LPC) with C_2–C_{10} acids, and PCs with short glycerol *sn*-1 and *sn*-2 chains were also synthesized by the acylation of glycerophosphorylcholine (GPC) with C_6–C_{10} acids. Their physical properties and emulsion stability were compared with those of an equimolar mixture of egg PC and LPC, and similarities of the headgroup motion and emulsion stability were found between some short-*sn*-2-chained PCs and the mixture of PC and LPC. The results suggested that the headgroup motion correlated with the interfacial absorptivities of PCs and with their emulsion stability.

Phospholipids are widespread in nature as an important component of biomembranes. Phosphatidylcholine (PC) is one of common phospholipids, and it is also important as a naturally occurring emulsifier. In addition, lysophosphatidylchloline (LPC), although it exists as a minor component, has diverse functions such as the interaction with PC (Howell et al., 1973; Mandersloot et al., 1975; Inoue, 1977; Morris et al., 1980) and formation of a stable emulsion. The fatty acid composition and configuration of PC varies on its origin (Kuksis and Marai, 1967), and we are interested in the composition of fatty acid moieties in PC and their behavior in emulsion and in membranes. A number of reports have appeared on the carbon relaxation time measurements and thermal analysis of PC concerned with its constitution or length of acyl chains (Mason et al., 1981a; Burns et al., 1983). On the other hand, the line width of ^{31}P NMR has been employed to characterize the headgroup motion of phospholipids (Berden et al., 1974), and a marked difference has been found between the signals of PC and LPC (Wu et al., 1984). Moreover, the phosphorus signals are influenced by the states of phospholipids such as lamella, liposome, and emulsion (Smith and Ekiel, 1984).

Previously, we investigated the headgroup motion of emulsified PC and LPC by ^{31}P NMR and T_2^* relaxation times of the glycerol and choline carbons and found that the emulsion stability correlated well with the headgroup motional properties of phospholipids (Chiba and Tada, 1989). In a simple emulsion system composed of water, *n*-decane, and egg PC, a significant broadened signal of phosphorus was observed and the emulsion was unstable. It was suggested that PC was prone to aggregate to form lamella in emulsion. LPC formed a stable emulsion in comparison with PC; however, emulsion breaking occurred when the headgroup motion of LPC had much motional freedom. On the other hand, an equimolar mixture of PC and LPC had adequate freedom in emulsion, and the emulsion became more stable. As the acyl chain at glycerol *sn*-2 is replaced by a proton, the headgroup of LPC can rotate rapidly. Consequently, in the mixture of PC and LPC, it is suggested that the headgroup of PC also has motional freedom by the interaction of LPC and that they formed a stable emulsion.

Thus, we expected that the headgroup motion of PC also increased by replacement of acyl chain at glycerol *sn*-2 with short-chained one and that a stable emulsion could be obtained by using the short-*sn*-2-chained PC as an emulsifier. In this paper, we discuss a relationship between headgroup motion and emulsion stability comparing physical properties of short-chained PC at glyc-

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