Study of Lipid-Protein Interaction Using Pulsed NMR

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ABSTRACT

Pulsed nuclear magnetic reasonance (NMR) measurements were made on freeze-dried emulsions containing fat and protein. It was demonstrated that this method can be used to determine the degree of lipid-protein interaction in the samples. Interaction is maximized near the isoelectric range of the protein, which supports the theory that hydrophobic interactions are predominant. Triglycerides and fatty methyl esters interact to nearly the same degree as do free fatty acids, which indicates that the carboxylate group plays a minor role in interaction. Degree of interaction increases when the emulsions are homogenized at higher pressures. There is an inverse relationship between interaction and foamability of the rehydrated emulsion suggesting that less protein is available for film formation after interaction. In addition, fat-protein interaction was shown to protect the protein from heat denaturation. These pulsed NMR measurements may represent an approach toward a better understanding of lipid-protein interactions in food systems.

INTRODUCTION

It is known that serum albumin binds small quantities of fatty acids predominately by nonpolar interactions (1). Since methyl esters are bound only slightly less strongly than free fatty acids, it appears that the carboxylate group plays only a minor role in these interactions (2). This fat-protein binding phenomenon is important since the fatty acids protect the protein against denaturation (3). More recently, free fatty acid binding to albumin was shown to be part of the mammalian lipid transport process (4).

The measurement of lipid-protein interactions by pulsed nuclear magnetic reasonance (NMR) should be possible since the spin-spin relaxation time of lipid protons is ca. 100-200 msec, while that of protons in solid or crystalline materials is much shorter (10 μsec.). Sixty μsec. after an rf pulse, the signal from a liquid vegetable oil is of considerable magnitude, whereas that from a solid is zero. Thus, a simple mixture of oil and dry protein gives a signal corresponding to the concentration of protons in the oil which is present in the sample. When oil and protein are emulsified together, some of the protein may "dissolve" in the oil at the oil-protein interface. In this case the protons in the protein should also contribute to the total NMR signal.

The objectives of this work were to demonstrate the feasibility of measuring fat-protein interactions in freeze-dried emulsions using pulsed NMR and then to relate the extent of these interactions to some behavioral properties of the emulsions.

EXPERIMENTAL PROCEDURES

Materials

Soybean and corn oils were from Best foods Inc., Union, NJ. Safflower oil was obtained from PVO International Inc., Richmond, CA. Peanut oil was a product of Standard Brands Inc., New York, NY, and butter oil was from Land O'Lakes, Minneapolis, MN. Promine D. was a product of Central Soya, Chicago, IL. Peanut grits (low fat) were obtained from the Goldkist Peanut Co., Atlanta, GA. Deuterium oxide was a product of Stohler Isotope Chemicals, Waltham, MA.

Equipment and Methods

The pulsed NMR Analyzer was the Praxis PR-103 (Praxis Corp., San Antonio, TX). This unit was equipped with a 25 mm probe. Settings were: Display FID; Response Fast; Variable Delay 60 μ sec.; Function 90°; Controls Manual. The analyzer was connected to an integrating recorder (Linear Instruments Corp., Irvine, CA., Model 282/mm).

Sample Preparation

Dry protein was mixed with water to form a 5% dispersion. This was heated with stirring for 1 hr at 30 C, then allowed to stand for 24 hr at room temperature to assure complete hydration. A 100 ml aliquot was mixed for 5 min. with 45 g of vegetable oil at high speed in a Waring Blendor. The resulting emulsion was then frozen quickly in a shallow tray using dry ice. The sample was then freeze-dried for 3 days without application of heat to the tray (Virtis Dryer, Model No. OX/145 NRBA, Virtis Co., Gardner, NY). Some samples were homogenized before drying using a Gaulin Homogenizer, Type 15M TA, Everett, MA.

Analysis by Pulsed NMR

Tared NMR tubes (25 x 150 mm) were packed to the 30 ml mark with freeze-dried emulsions and then reweighed to determine bulk density of each sample. The NMR signals were monitored for 1 min on 3 different volume segments of each tube. The average value from these three readings was then normalized by dividing it by the bulk density of the sample. For freeze-dried emulsions consisting of 90% oil with 10% protein, the percentage of fat-protein interaction is defined by the relationship:

$$\frac{\text{Normalized sample signal} - \text{Normalized oil signal}}{\text{100\% solubilized protein signal}}$$

The normalized oil signal is 90% of the oil signal divided by oil density. The solubilized protein signal is determined by preparing a 5% dispersion of protein in deuterium oxide, obtaining 3 NMR readings of different volume segments, then dividing by the bulk density to obtain the normalized signal. The signal for 100% solubilized protein is calculated by dividing the normalized signal by 0.05.

Foaming Tests

A 10 g sample of freeze-dried emulsion was added to 99 g H_2O in a Waring Blendor. The mixture was blended at high speed for 3 min. Then it was poured carefully into a 250 ml graduated cylinder. Best results were achieved by

TABLE I

NMR Response – Oil-Protein Mixtures

Sample	Normalized NMR signal	Calculated % oil
A. Corn oil	285.0	---
B. Sodium caseinate	0	---
C. 90A/10B	256.6	90.03
D. 85A/15B	242.4	85.05

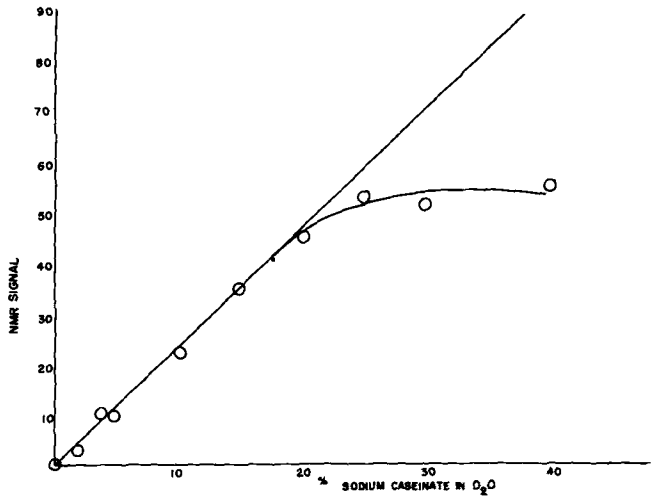

FIG. 1. Protein concentration (caseinate) in D_2O vs. NMR signal.

pouring down the side of the cylinder. Foam heights were measured in ml immediately.

RESULTS AND DISCUSSION

Preliminary tests were run to confirm that dry protein gave no NMR response. Corn oil and sodium caseinate were mixed thoroughly in a Waring Blendor. The data in Table I show that no signal was observed from the protein (at 1.1% moisture content) when it was mixed with vegetable oil

In order to measure oil-protein interactions in emulsions, it was necessary to freeze dry the samples. Free water itself does not interfere significantly since the spin-lattice relaxation time of its protons is in the range 2-3 sec. Since the rf bursts are pulsed every 0.1 sec., these protons have little chance of relaxing before the next burst occurs. Because of this rf saturation, the signal contributed by free water is very small. But when protein dissolves in water, the relaxation time of its protons is comparable to that of oil protons, and in this case the signal is of considerable magnitude. Protons from water which is bound to protein have a much shorter relaxation time than those from free water. Bound water can thus contribute to the total NMR signal.

The moisture content of the freeze-dried emulsions described here was in the range 0.5-1.1% (5). Proteins which contained as much as 1.1% water gave no NMR response. The only source of an NMR signal from the freeze-dried emulsions is the oil, or the protein which is "dissolved" in the oil at the oil-protein interface.

The physical structure of proteins provides a clue to the mechanism of fat-protein interaction in dried emulsions. Protein molecules are arranged in several conformational modes: pleated sheet, unordered, coiled helix (6). The hydrophobic residues tend to be buried on the inside of the molecule and the hydrophylic groups are on the outside. When the protein is dispersed in water, the hydrophobic sites are exposed and can come into contact with the oil. Homogenization of this mixture facilitates contact of the oil droplets with these exposed hydrophobic sites. Quick freezing retains the protein in this configuration. When water is removed by freeze drying, the protein remains in contact with the oil, and associates with it through weak hydrophobic bonds. This mechanism may explain the large NMR signal response observed with freeze-dried emulsions.

To determine the amount of protein bound to the oil in freeze-dried emulsions, it is first necessary to establish the NMR signal which protein would contribute it it were all in the solubilized or completely hydrated state. A series of levels of protein was prepared in deuterium oxide as solv-

TABLE II

Interaction of Oils with Various Proteins

Dried emulsion	% Interaction
Soybean oil – Na Caseinate (A)[a]	54.7
Butter oil – Na Caseinate (A)[a]	98.0
Corn oil – Na Caseinate (A)[a]	66.9
Safflower oil – Na Caseinate (A)[a]	14.5
Peanut oil – Na Caseinate (A)[a]	35.0
Butter oil – Na Caseinate (B)[a]	79.0
Butter oil – Na Caseinate (C)[a]	81.9; 82.6
Soybean oil – Promine D[b]	20.1
Butter oil – Promine D[b]	25.8
Butter oil – Promine D[b]	19.7
Corn oil – Promine D[b]	10.7
Safflower oil – Promine D[b]	11.4
Peanut oil – Promine D[b]	20.0
Soybean oil – Peanut grits	9.6
Butter oil – Peanut grits	11.3
Corn oil – Peanut grits	7.9
Safflower oil – Peanut grits	6.6
Peanut oil – Peanut grits	12.3

[a]Commercial sources A, B, and C.

[b]Promine D was Lot 1363 (all other emulsion prepared using Lot 9962).

TABLE III

NMR Interactions at Various pH Values[a]

Safflower oil		Butter oil	
pH	% Interaction	pH	% Interaction
6.0	24.2	1.9	3.3
6.4	23.0	6.0	98.0
8.1	15.0	8.1	18.5
9.0	10.9	9.0	8.2

[a]The oil in 90% of the dried emulsion; the protein is sodium caseinate.

TABLE IV

Interaction of Sodium Caseinate with Various Lipids

Lipid	% Interaction
1. Triolein	24.7
2. Oleic acid	24.2
3. Methyl oleate	16.4
4. Methyl linoleate	15.6
5. Oleyl oleate	14.4
6. n-Octadecene	10.4
7. Methyl palmitate	7.4
8. Methyl elaidate	1.9
9. Oleyl alcohol	0.4

TABLE V

Effect of Homogenization Pressure on Interaction

Homogenization pressure (PSI)	% Interaction	
	Series 1	Series 2
1000	6.9	2.9
2000	---	7.2
3000	10.4	12.4
4000	---	11.1
5000	21.7	15.8
6000	---	14.8
7000	18.5	17.3

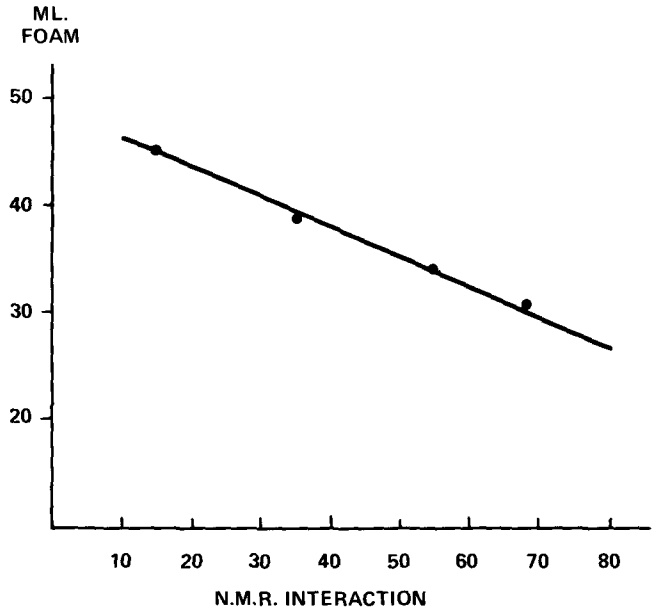

FIG. 2. Interactions vs. foam heights of reconstituted, whipped emulsions.

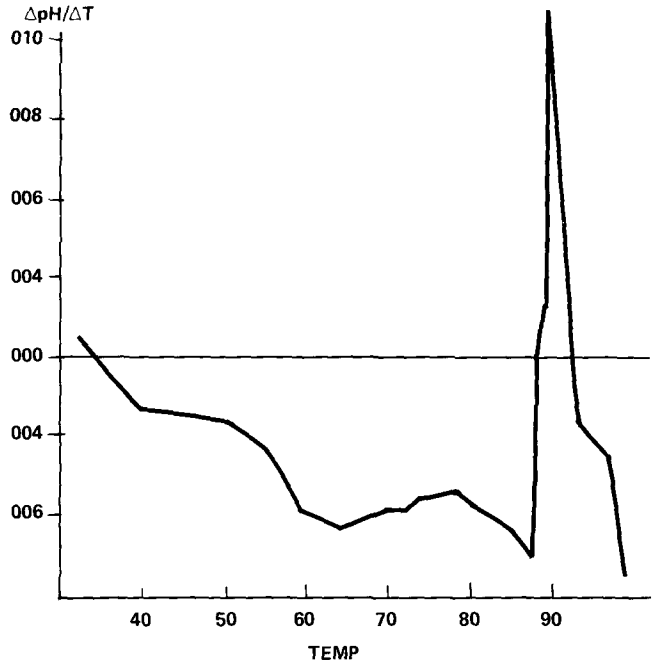

FIG. 3. Plot of Δ ph/Δ T vs. T for a sodium caseinate dispersion.

ent. At all concentrations up to the saturation level (ca. 20%), the plot of NMR signal vs. concentration is linear (Fig. 1). D_2O was used because deuterium gives no NMR response, so that when a protein is dispersed in D_2O, the protein is responsible for the entire signal. The signal for "pure" protein, completely solubilized, can be estimated.

Proteins appear to interact to different degrees with various oils. Table II illustrates some of these differences All of the sample are freeze-dried emulsions which contain 90% oil with 10% protein. Butter oil interacts with sodium caseinate to a greater degree than do any of the other oils tested. Butter oil and peanut oil both appear to interact as strongly with soy isolate (Promine D) as does soybean oil. Peanut oil shows a slightly greater interaction with peanut grits than do the other oils tested. When duplicate emulsions are prepared using the same batch of protein and oil, interaction values obtained are quite reproducible. When different lots of the same protein are used with a single oil, however, the interactions may be more variable.

The pH of the emulsion before freeze drying affects the amount of interaction after drying (Table III). Interaction is maximized near the isoelectric range of the protein as the case of sodium caseinate-safflower oil or sodium caseinate-butter oil emulsions show. This result would be expected if the predominant bonding force is nonpolar in character.

The effect of autoxidation on interaction was also investigated using sodium caseinate, corn oil, and safflower oil. Oil samples were incubated at 60 C, tested for hydroperoxide content at regular intervals (7), and aliquots held in frozen storage (-17 C) until used. Aliquots were taken until ca. 80 meq/kg peroxide value (PV) had been reached. When interactions were measured on freeze-dried emulsions, a maximum was observed in the range 30-40 meq/kg, and these interactions decreased sharply above this PV range. The same thing occurred both with corn and safflower oil. These experiments were repeated several times with consistent results. At present there is no obvious explanation for these observations.

Room temperature storage tests were also run on freeze-dried emulsions, following the change in interaction with time. In the case of safflower oil-sodium caseinate, using either fresh or autoxidized oil, there was no significant change after one month. But the values for the sample containing oxidized oil (PV 36 meq/kg) were almost twice those for the fresh sample.

Previous workers found that oleic acid interacts with proteins more strongly than do any of the other straight chain fatty acids (8). Interactions with sodium caseinate were determined for a series of fatty derivatives (Table IV). Oleic acid and triolein give the same degree of interaction, whereas the methyl esters, fatty alcohols and wax esters show low reactivity. The contrast between methyl oleate and methyl elaidate is striking. This indicates that the configuration of the oil molecule is important in determining its degree of interaction.

It was demonstrated that the amount of interaction is also dependent upon the degree of homogenization used to prepare the samples. Several 90:10 corn oil/sodium caseinate emulsions were homogenized using a Gaulin Homogenizer at 500 PSI on the second stage and at a series of pressures on the first stage. After these samples had been freeze-dried, interactions were measured with results as shown in Table V. It appears that as homogenization pressure is raised to produce smaller average oil droplet sizes which provides more interfacial area, the extent of interaction is increased. Others have observed this same effect, but have indicated that the type of equipment used, e.g., pressure homogenizer vs. Waring Blendor, results in different degrees of interaction at the same average oil droplet size (9).

RELATIONSHIP OF INTERACTION TO PROPERTIES OF DRIED EMULSIONS

Foamability

Four samples of oil-protein freeze-dried emulsions (90:10) were chosen with NMR interactions ranging from 14-67%. Foam heights were measured on the rehydrated, whipped emulsions as shown in Figure 2. There was an inverse linear correlation between interaction and foam height with these samples. This indicates that the greater the interaction, the less protein is available as a film former.

Heat Denaturation

Another physical effect of oil/protein interaction is on the thermal transition temperature of the protein, or simply heat denaturation. A method was devised by Bull (10)for

TABLE VI

Transition Temperatures

Samples	% Interaction	Transition temp
Sodium caseinate	---	90
Corn oil-sodium caseinate 90:10 (Freeze-dried)	66.9	97
Promine D	---	76
Soybean oil-promine D 90:10 (Freeze-dried)	20.1	82

determining this transition. It involves measuring the change in pH of a protein dispersion as the temperature is programmed upward in a linear fashion. When $\Delta pH/\Delta T$ is plotted vs. temperature (Fig. 3), a sharp peak appears at the the transition temperature. When this technique was applied to freeze-dried emulsions, a significant increase was observed in this transition temperature (Table VI), indicating that the protein is protected from heat denaturation by virtue of its interaction with the oil.

The results reported in this paper are of a preliminary nature. Much more remains to be done to establish the relationship of lipid-protein interaction to the properties of emulsions. It is encouraging to note that the results obtained here by pulsed NMR parallel those observed by Goodman using partition analysis (8). It is believed that these measurements represent a lead toward a better understanding of lipid-protein interactions in food systems.

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