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Low-resolution ^{1}H spin–spin relaxation of *n*-decane/water emulsions stabilized by β-casein

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Abstract

A low-resolution ^{1}H NMR relaxometry study on the dynamics of an *n*-decane/water emulsion stabilized by β-casein is presented. Spin–spin (transverse) relaxation time constants (T_2) were used to assess relative mobilities of emulsion components, by a selective deuteration procedure. Data analysis allowed the emulsion investigated to be described by a heterogeneous collection of dynamically distinct populations. A major population of *n*-decane molecules presented an average mobility that very nearly approached that of pure solvent, which is compatible with its occurrence in the emulsion continuous microphase. β-Casein molecules displayed a prevalent population with significantly decreased mobility as compared to the free protein in solution, which is in accordance with the protein location at the oil/water interface. Also, a major H_2O population with significantly lower average T_2 as compared to the pure liquid was detected and has been assigned to interfacial water.

Keywords: β-Casein; Emulsion; T_2; Relaxation; Lipid–water interface

1. Introduction

Complex interfacial events are involved in milk capability to generate derivatives of biotechnological and commercial interest [1]. Protein–lipid self-assembled structures, such as emulsions, are a most prominent aspect in this respect. A proper understanding of their dynamics is of relevance in order to be able to control and modulate desired product properties [1–4].

Caseins are the main emulsifying milk protein components to be considered in these instances. In the form of surface active soluble caseinates, they guarantee good stability for emulsions, partly due to their resistance to heat and capacity to incorporate water [5]. From a fundamental point of view, such systems comprise very interesting colloidal systems to investigate, relative to questions concerning details of mechanisms of interactions.

Caseins comprise the predominant (80%) milk protein fraction. They present a highly hydrophobic character, being constituted by α, β and κ caseins, and characterized by an isoelectric point of 4.6 and a molecular weight within a 19–25 kDa range. Caseins are conjugated proteins, with phosphate groups sterified to serine residues, which is a relevant aspect regarding their calcium binding properties. A very typical micellar structure results from such interactions, which responds for the protein ability to carry highly insoluble calcium phosphate in the liquid form [6,7]. This constitutes a biochemical detail that plays a most relevant physiological role in terms of milk nutritional properties [8–11].

Caseins present high proline content and no disulfide bonds, which leads them to adopt a less packed spatial conformation. Their 3D structure and surface charge distribution is still controversial in the literature [12–15]. They tend to be less thermolabile than most globular proteins, possibly due to their less organized structure. Due to a significant exposure of hydrophobic residues, they present low affinity for water, which leads them to adopt a characteristic micellar form of organization in aqueous media [6,7].

β-Casein, one of casein fractions, has 209 amino acid residues, of which 35 prolines and no cystein, and a molecular

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mass of 24 kDa. It has a strong amphiphylic character, represented by a highly negatively charged N-terminal, due to the close location of the five serine phosphates, and a hydrophobic C-terminal end. Such an attribute provides the protein with an efficient surfactant role in the oil/water interface. In this respect, molecular modeling work clearly indicated β-casein affinity for the interface, with the macromolecular dipole defining the protein orientation relative to the interface, eventually leading to a less extended conformation in the dielectrically discontinuous environment [13–15]. Modeling and experimental work has also indicated that phosphoserine residues are a requisite for β-casein stabilization at the water/oil interface [15,16]. That is confirmed by ^{31}P NMR results, where broadening of ^{31}P casein peaks in spectra of triacylglycerol/water emulsions was taken as evidence of casein phosphoserine residue interactions with lipids at the interface [17]. β-Casein is also less sensitive to calcium precipitation and may stand polymerization under certain circumstances [18,19], which renders them of further interest in colloidal science.

NMR spectroscopy can be relevant in the investigation of conformational features of interfacially active proteins, such as casein. The technique has been applied in several conformational studies of caseins, casein peptides and casein micelles (see for instance ref. [20] and refs. therein]. Specifically concerning β-casein, a 25 residue long N-terminal β-casein peptide was investigated by NMR and the dependence of the peptide structure on the nature of the cation present was pointed out [21]. ^{1}H NMR has been also used to investigate interactions between β-casein micelles and other relevant species, such as surfactants [22]. Significant changes were found both in the surfactant micellization properties and in the protein secondary micellar structure, as a result of β-casein/SDS (sodium dodecylsulfate) interaction [22]. An indication that β-casein micelles are more dynamic and less compact than the κ-casein micelles is also reported by means of ^{1}HNMR technique [23].

Relaxometric and diffusional NMR techniques have also been of great assistance in the field. For instance, low-resolution ^{1}H NMR relaxometry has been applied in studies concerning reconstitution processes of milk powder, allowing to discriminate between water populations with distinct dynamics in the rehydration process [24,25]. Also, a gradient ^{1}H NMR diffusional technique has been employed to determine water self-diffusion coefficients in casein matrixes as a function of casein and fat content [26].

Other nuclei have also been used as probes for the NMR investigation of casein systems. For instance, interactions between caseinate phosphoserine residues with lipids have been investigated by ^{31}P NMR [17]. Those between β-casein and phospholipid liposomes were approached by solid-state ^{2}H NMR using selectively deuterated phospholipids [27]. The protein ability to locate at the interface has been verified and it has been found that casein can interact both with charged DMPG and with neutral, zwitterionic DMPC liposomes. [27].

In the present work, a study of a water-in-oil emulsion stabilized by β-casein, with a non-polar phase represented by *n*-decane, was undertaken. Although obviously not employed in food emulsions, hydrocarbons are frequently used in model studies of these systems [28–32]. Our study focused on the relaxation behavior of the emulsion components, as reflected in the spin–spin relaxation parameter, T_2, in low-resolution nuclear magnetic resonance (LR-NMR) experiments. A selective deuteration procedure was employed for this purpose, which allowed relative mobilities of the emulsion components to be differentially assessed.

Time domain low-resolution NMR relaxometry has been most usually applied to assess liquid fat content in industrial applications, as well as to estimate the proportion of non-frozen water in frozen biological samples [33,34]. The technique is versatile and can be extended to many applications such as in the assessment of differential mobilities of self-assembled species in complex systems, as shown in the present work.

1.1. Time domain low-resolution ^{1}H NMR

In low-resolution (low field) ^{1}H NMR, an average ^{1}H signal is produced which reflect the overall sample response to the magnetic field. As should be clear, the analysis of such a low-resolution signal does not allow any inferences relative to particular molecular sites or molecular structure to be made.

However, relevant information regarding system overall dynamics can be obtained through the analysis of the relaxation of the magnetic signal to investigate how this average signal has its relaxation pattern altered in terms of changes in sample dynamic heterogeneity, in the course of an experimental work.

The loss of energy by a nucleus after irradiation in an NMR experiment can be described by two main relaxation mechanisms, namely, spin-lattice (or longitudinal) relaxation, T_1, and spin–spin (or transverse) relaxation, T_2 [35,36]. Spin–spin relaxation is generated by the loss of coherence in the *xy* plane due to mutual exchange of spin energies. That can arise from the energy exchange between spins from the sample as well as from other contributions, such as those related to instrument field inhomogeneity, which are not of particular interest in most chemical research. The CPMG (Carr, Purcell, Meiboom and Gill) [37,38] spin-echo pulse sequence allows the measurement of spin–spin relaxation T_2 due to loss of magnetization from the sample only, making contributions from instrumental source negligible.

The CPMG pulse sequence is shown below:

$$(\pi/2)_x - \tau - \pi_y - 2\tau - \pi_y - 2\tau - \pi_y - \cdots$$

A true T_2 devoid of any other contribution except that from the sample spin energy exchange is the time constant of the exponential that describes the envelope of the echo decay in a CPMG experiment. A dynamically heterogeneous sample can have its echo decay analyzed according to a multiple exponential function:

$$M = M_0[K_1\,\mathrm{e}^{-\tau/T_2(1)} + K_2\,\mathrm{e}^{-\tau/T_2(2)} + K_3\,\mathrm{e}^{-\tau/T_2(3)} + \cdots + K_n\,\mathrm{e}^{-\tau/T_2(n)}]$$

from which distinct T_2 values, corresponding to dynamically different populations within the sample, can be calculated.