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Dielectric spectroscopy study of myoglobin in glycerol–water mixtures

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ABSTRACT

Due to the interest in protein dynamics, there are numerous dielectric relaxation studies of proteins in water and in glass-forming aqueous solvents such as glycerol–water mixtures. In the regime of low frequencies, the inevitable dc-conductivity of such systems limits the resolution of dynamics that are slow compared with the solvent relaxation. Solutions of myoglobin in glycerol/water mixtures of various compositions are measured by dielectric spectroscopy in the frequency range from 10 mHz to 10 MHz. The resolution of low frequency modes is improved by two approaches: electrical 'cleaning' and the analysis of the derivative of the real component of permittivity, which shows no direct signature of dc-conductivity. Effects of internal interfacial polarization are also addressed by measuring the same solvents in confinement as well as mixed with glass beads. We find two processes, the structural relaxation of the solvent and the slower rotational mode of the protein, with no indication at even lower frequencies of a dielectric signature of fluctuations associated with protein dynamics.

1. Introduction

The dynamics of solvated proteins is a field that has attracted considerable attention in recent years. In many cases, one is interested in investigating the protein in its native state, which usually requires water within the solvent because proteins function only in an aqueous environment and need a certain hydration level (≈0.3 g water per gram protein) for full biological function [1–3]. This notion suggests that conformational fluctuations of the protein are important for its function, and that the solvent impacts these fluctuations [1,4–10]. Solvent mixtures can lead to highly viscous behavior and glass forming capabilities of the samples while retaining the aqueous character required by the protein. In such materials, practically all modes of motion involve the displacement of charge, implying that these are amenable to detection by dielectric relaxation techniques [11–18]. On the one hand, dielectric spectroscopy is advantageous regarding the range of frequencies or time scales that can be observed with a single technique. On the other hand, dc-conductivity resulting from mobile charges can dominate the dielectric loss signal, $\varepsilon''(\omega)$, at low frequencies, potentially obscuring other signatures of slow dynamics. In a typical protein/solvent system, one can expect to observe the dynamics of the solvent, potentially modified by the presence of the protein, fluctuations within the protein, contributions from the hydration layer, rotational motion of the protein, as well as internal interfacial polarization and electrode polarization [19–21]. A considerable challenge is the identification and assignment of the distinct loss peaks that are observed. It is worth noting that the designation of the various peaks is not uniform. With some analogy to the phenomenology of supercooled liquids, the solvent dynamics can be referred to as α-process, while the hydration layer dynamics is designated as a (statistically independent) β-relaxation [1,5,9]. Other sources use the term γ-process for the bulk solvent dynamics, β-process for the protein tumbling motion, and δ-processes for the features occurring at frequencies intermediate between the γ and β positions [15,18,22].

In this present report, broadband dielectric spectroscopy (BDS) [23] is employed as an investigative tool to study the slow dynamics of solvated myoglobin. Glycerol–water mixtures of varying contents have been adopted as the solvent, with glycerol serving mainly the purpose of preventing the formation of ice and thus stabilizing the native structure of the protein even at low temperatures [24]. The study focuses on the low frequency regime in which the dielectric loss, $\varepsilon''(\omega)$, is dominated by dc-conductivity. We explore approaches to overcome the issues related to dc-conductivity, namely electrical 'cleaning' [25] and analyzing the real part of permittivity, $\varepsilon'(\omega)$, as this quantity is not directly affected by dc-conductivity. The possibility of internal interfacial polarization is also assessed by measuring the solvent in the presence of interfaces with silica. Apart from the structural relaxation process of the solvent, only one additional clear process is found at lower frequencies, which we interpret as the rotational motion of the entire protein molecule. A very small δ-process, usually considered originating from hydration water fluctuations, may be present between the frequency positions of the protein rotation and solvent relaxation processes.

2. Materials and methods

Myoglobin (Mb) from equine skeletal muscle (>95%, essentially salt-free, lyophilized powder), glycerol (99.5+%, spectrophotometric grade), and water (HPLC grade), were purchased from Sigma-Aldrich

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(St. Louis, MO) and used as received. Different solvent mixtures are identified by their weight percentages of glycerol (x) and water (y). The protein samples were prepared at various hydration levels h, with h being the solvent to protein weight ratio. Note that we adopt the common term 'hydration level' instead of the more accurate designation 'solvation level', without implying that a persistent layer of pure water surrounds the protein. Samples are generated by mixing the required amount of protein powder with the solvent, using a magnetic stirrer for approximately 3 h. To study confinement effects, Vycor (corning glass, 4 nm nominal pore diameter) porous glass was used as well as SiO_2 glass beads of different sizes (30–50 μm, 18901, Polysciences, Inc.).

The impedance measurements were performed using a Solartron SI-1260 gain-phase analyzer and a DM-1360 transimpedance amplifier [26]. The range of frequencies covered was from 10 mHz to 10 MHz with frequencies spaced at 8 points per decade. The capacitor cell holds disks of 20 mm and 16 mm in diameter for the lower and upper electrodes, respectively. A schematic outline of the sealed cell with adjustable electrode separation d is provided in Fig. 1. Sample thicknesses of $d = 300$ μm and $d = 750$ μm were realized. For each individual measurement, the sample is allowed to equilibrate at the target temperature prior to an isothermal (±0.2 K) frequency scan. Temperatures were controlled and measured by a Novocontrol Quatro liquid nitrogen setup.

The quantity of interest for a dielectric measurement is the frequency dependent complex dielectric permittivity $\varepsilon^*(\omega)$ given by

$$\varepsilon^*(\omega) = \varepsilon'(\omega) - i\varepsilon''(\omega), \tag{1}$$

where $\omega = 2\pi\nu$, $\varepsilon'(\omega)$ is the real component of permittivity related to how much energy from an external electric field can be stored in the material, and $\varepsilon''(\omega)$ is the loss factor that quantifies how much energy is lost irreversibly in the material when an external electric field is applied. Quantitative results are obtained by fitting the experimental data with the empirical Havriliak–Negami (HN) function [27],

$$\varepsilon^*_{\mathrm{HN}}(\omega) = \varepsilon_\infty + \sum_k \frac{\Delta\varepsilon}{[1 + (i\omega\tau_{\mathrm{HN}})^\alpha]^\gamma} + \frac{\sigma_{\mathrm{dc}}}{i\omega\varepsilon_0}, \tag{2}$$

where the final term accounts for the level of dc-conductivity in terms of σ_{dc}, which dominates $\varepsilon''(\omega)$ at low frequencies. The constant ε_0 represents the dielectric permittivity of vacuum. The different variables include the dielectric constant at high frequencies (ε_∞) as well as the dielectric strength ($\Delta\varepsilon$), the relaxation time (τ_{HN}), and the shape parameters (α, γ with $0 < \alpha, \alpha\gamma \leq 1$) of a particular relaxation process. The summation extends over the number of different processes expected in the dielectric response, and the subscripts 'k' for the parameters $\Delta\varepsilon$, τ_{HN}, α, and γ are omitted in Eq. (2).

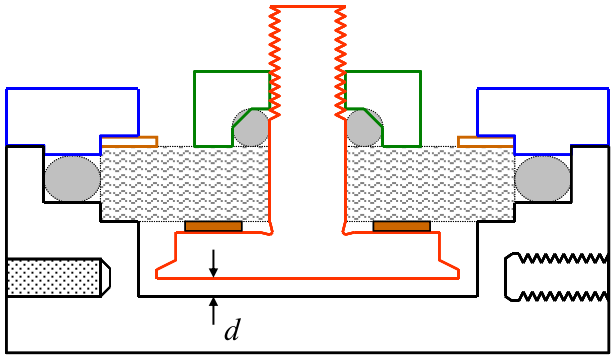

Fig. 1. Schematic diagram of the dielectric cell. The top electrode is held by a sapphire window (22 mm ∅ × 4 mm) with 4 mm center hole, pressed in place by a wavy copper washer. The larger o-ring (Kalrez 4079, K# 32015) seals against the polished outer sapphire surface. The active surface area of the capacitor is a 16 mm ∅ disk, the electrode separation is adjustable between $d = 0.1$ mm and $d = 1.5$ mm via a precision steel washer between upper electrode and sapphire plate. The stainless steel cell body acts as lower electrode.

In order to reduce the amount of dc-conductivity, the technique of 'electrical cleaning' [25] was utilized. In this approach, a sufficiently high dc field (10 kV/cm) was applied to the sample at ambient temperature for a certain period of time (approx. 3 h), until a reduced time invariant current through the sample was reached. Then the sample was cooled with the field applied to the lower target temperature of the measurement. This produces a state in which ions are immobilized for a time in which several measurements can be made with reduced dc-conductivity.

3. Results

In the following, samples are designated as MbhGxWy, where h is the hydration level and x and y are the weight percentages for glycerol and water, respectively. The absence of the 'Mb' prefix indicates the solvent without the protein. Typical dielectric loss spectra for a series of temperatures are shown for two myoglobin samples in Fig. 2, with the samples having the same hydration level ($h = 1$) but subject to different solvents, Mb1G67W33 and Mb1G38W62. The features common to all of these spectra are the asymmetrically broadened relaxation peaks and the considerable level of dc-conductivity, with a more or less pronounced shoulder indicating a further process at intermediate frequencies. The highest frequency process in each case is near the loss peak of the respective pure solvent, but additional modes seen here at intermediate frequencies remain absent in the pure solvent spectra. The goal of the data analysis will be to discern processes that are associated with protein dynamics, especially those obscured by the dc-conductivity signal. The solvent with about twice the water content, G38W62, has a viscosity at ambient temperature that is much below than that of the G67W33 case, resulting in easier sample preparation and better mixing behavior with Mb. Because an even higher water content would result in the formation of ice even at low hydration levels [28], G38W62 was the highest water fraction used. All combinations of solvent composition and hydration level realized in this study are represented in Fig. 3. Although each sample has been measured over a range of temperatures, only the $T = 210$ K case is shown in this graph. Fig. 3a depicts loss curves of all samples based upon the G38W62 solvent, including the solvent itself, and mixtures with myoglobin at hydration levels $h = 1$ and $h = 2$. As a result of ice formation, the loss spectra change qualitatively as a matter of hydration level. For the pure solvent, G38W62, the lower frequency process is due to ice, while the smaller peak at higher frequencies originates from the residual solvent. A similar situation is seen for Mb2G38W62, albeit with different relative amplitudes, and the formation of ice is effectively suppressed only for the lower hydration level case, Mb1G38W62. Note that solvent and ice peaks are subject to different activation behaviors, so that the relative peak positions will reverse with temperature, as detailed by Hayashi et al. [28]. Results for solvents with higher glycerol content are compiled in Fig. 3b, which includes the G67W33 solvent, mixtures with myoglobin at hydration levels of $h = 1$ and $h = 10$, as well as Mb in pure glycerol at $h = 100$. It can be observed that the solvent peak for G67W33 is shifted to lower frequencies as the amount of protein is increased. The peak for Mb100G100W0 is even slower because the plasticizing effect of water is absent in this pure solvent.

The effect of electrical 'cleaning' is demonstrated for the Mb1G38W62 sample at temperatures between 175 K and 210 K in Fig. 4. The obvious effect of exposing the sample to a 10 kV/cm field at high ion mobility is the reduction of the level of dc-conductivity by several orders of magnitude, while the higher frequency peak remains practically unchanged. As a consequence of the lowered σ_{dc} value, a peak at intermediate frequencies becomes apparent, positioned about three decades left of the faster process.