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LETTERS

Anomalous Dielectric Relaxation of Aqueous Protein Solutions

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Dielectric relaxation of aqueous protein solutions show many anomalous properties. For example, (i) protein solutions have higher static dielectric constant compared to that of pure water, (ii) the real part of the dielectric function (DF) exhibits a crossover with varying concentration, and (iii) the imaginary part of DF exhibits a bimodal frequency dependence. There is no coherent microscopic explanation available for any of these phenomena. Here we present the first unified, microscopic theory of the dielectric relaxation spectra of an aqueous protein solution that explains all the above anomalies, with excellent agreement with all the known experimental results.

1. Introduction

Dielectric relaxation is a popular method to probe the dynamics of protein solutions. However, several interesting and anomalous phenomena observed in the dielectric spectra have surprisingly eluded molecular explanation despite a number of studies over decades.[1−6] Three such anomalies are (i) the significantly higher static dielectric constant of aqueous protein solutions compared to that of pure water at the low-frequency region (β relaxation),[2] (ii) the interesting concentration-dependent crossover at the end of β relaxation where the real part of the dielectric function (DF) of the solution (ϵ') with higher concentration of protein dips suddenly and sharply to a value *lower* than that for a solution with smaller concentration,[4] and (iii) the strong bimodality of the imaginary part of the frequency-dependent DF[6] (ϵ'').

The anomalies described above clearly depend on protein−water interactions whose understanding can provide valuable insight into the influence of water on proteins. This has obvious importance because proteins are evolved to function in water.[7] The molecular understanding of the dielectric function of an aqueous protein solution is also essential to follow the interaction between a charged species and the protein−solvent system. The study of concentration dependence of dielectric relaxation spectra can also help in understanding the forces responsible for protein association—the latter is a competing process with protein folding and is the reason for diseases such as prion disease, cataract, amyloidoses, etc.[8]

In this Letter we present a theory of dielectric relaxation of aqueous protein solution over the whole frequency range. We use an exact relation between the frequency-dependent DF and the total dipole moment correlation function of the protein solution that is appropriate for the inhomogeneous system under consideration. The total dipole moment correlation function derives contribution from all molecules present in the system

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(the protein molecules, the water molecules in the hydration shell, and the bulk water molecules) and is described in detail in the following section. Using the theory, we have explained *all* known experimental results of systems such as whale myoglobin, equine hemoglobin, and bovine serum albumin solutions. The results are presented and discussed in the section following the theoretical formulation.

2. Theoretical Formulation

Aqueous protein solutions are multicomponent heterogeneous systems containing three distinct species—the bulk water molecules, the water molecules in the hydration shell surrounding the proteins, and the protein molecules themselves.

The frequency-dependent dielectric function ($\epsilon(\omega)$, where ω is the frequency) of such a multicomponent inhomogeneous medium can be treated by the exact relation between the dielectric function of a macroscopic sphere in a vacuum and the total time-dependent dipole moment correlation function[9−11]

$$\frac{[\epsilon(\omega)-1]}{[\epsilon(\omega)+2\,]} = \frac{4\pi\langle\vec{M}(0)\cdot\vec{M}(0)\rangle}{9Nk_{\mathrm{B}}TV}[1 - i\omega\int_0^\infty \exp(-i\omega t)\,\phi(t)\,\mathrm{d}t] \quad (2.1)$$

where $\vec{M}(t)$ is the total time-dependent dipole moment of the solution at time t. $\phi(t)$ is the normalized total dipole moment time correlation function. N is the total number of molecules present within the volume V of the protein solution at temperature T. It is important to note that the conventional dielectric measurements are performed at constant volume.[12]

The total dipole moment time correlation function is given by

$$\langle\vec{M}(t)\cdot\vec{M}(0)\rangle = \langle\vec{M}_p(t)\cdot\vec{M}_p(0)\rangle + \langle\vec{M}_p(t)\cdot\vec{M}_h(0)\rangle + \langle\vec{M}_p(t)\cdot\vec{M}_w(0)\rangle + \langle\vec{M}_h(t)\cdot\vec{M}_p(0)\rangle + \langle\vec{M}_h(t)\cdot\vec{M}_h(0)\rangle + \langle\vec{M}_h(t)\cdot\vec{M}_w(0)\rangle + \langle\vec{M}_w(t)\cdot\vec{M}_p(0)\rangle + \langle\vec{M}_w(t)\cdot\vec{M}_h(0)\rangle + \langle\vec{M}_w(t)\cdot\vec{M}_w(0)\rangle \quad (2.2)$$

Here, p stands for a protein molecule, h stands for a water molecule in the hydration shell, and w stands for the same in the bulk.

$\langle\vec{M}_{\mathrm{p}}(t)\cdot\vec{M}_{\mathrm{p}}(0)\rangle$ consists of the permanent dipole moment correlation function of the molecule arising from the permanent dipoles.[13,14] $\langle\vec{M}_{\mathrm{h}}(t)\cdot\vec{M}_{\mathrm{h}}(0)\rangle$ is bimodal and is composed of two slow time constants. It was shown recently that the slow dynamics of water in the hydration shell could arise from the dynamic exchange between the free and the bound water molecules within the hydration shell;[15] we shall discuss it later.

$\langle\vec{M}_{\mathrm{w}}(t)\cdot\vec{M}_{\mathrm{w}}(0)\rangle$ is well-characterized and is a sum of exponential and Gaussian components.[16] However, the first exponential component ($\tau = 8.32$ ps) consists of 95% weight of the total dielectric relaxation spectrum of water. The second exponential component ($\tau = 1.02$ ps) and the femtosecond Gaussian component have relatively little weight and do not contribute significantly in the frequency range below gigahertz region. In the present work we represent the bulk water relaxation by the single exponential ($\tau = 8.32$ ps).

Using the correlation function given in eq 2.2 we get the expression for the dielectric relaxation spectrum as follows

$$\frac{[\epsilon(\omega)-1]}{[\epsilon(\omega)+2]} = \frac{[\epsilon_0-1]}{[\epsilon_0+2]} - \frac{4\pi}{9NVk_{\mathrm{B}}T} i\omega\int_0^\infty \exp(-i\omega t)\,\mathrm{d}t\,[N_{\mathrm{p}}^2\mu_{\mathrm{p}}^2\, g_{\mathrm{p}}\exp(-t/\tau_{\mathrm{p}}) + N_{\mathrm{p}}N_{\mathrm{h}}\mu_{\mathrm{p}}\mu_{\mathrm{h}}g_{\mathrm{ph}}(\exp(-t/\tau_{\mathrm{p}})) + N_{\mathrm{p}}N_{\mathrm{w}}\mu_{\mathrm{p}}\mu_{\mathrm{w}}g_{\mathrm{pw}}(\exp(-t/\tau_{\mathrm{p}})) + N_{\mathrm{h}}N_{\mathrm{p}}\mu_{\mathrm{h}}\mu_{\mathrm{p}}g_{\mathrm{ph}}(\sum_{i=1,2}\Delta h_i\exp(-t/\tau_{\mathrm{hi}})) + N_{\mathrm{h}}^2\mu_{\mathrm{h}}^2g_{\mathrm{h}}(\sum_{i=1,2}\Delta h_{\mathrm{i}}\exp(-t/\tau_{\mathrm{hi}})) + N_{\mathrm{h}}N_{\mathrm{w}}\mu_{\mathrm{h}}\mu_{\mathrm{w}}g_{\mathrm{hw}}(\sum_{i=1,2}\Delta h_{\mathrm{i}}\exp(-t/\tau_{\mathrm{hi}})) + N_{\mathrm{w}}N_{\mathrm{p}}\mu_{\mathrm{w}}\mu_{\mathrm{p}}g_{\mathrm{pw}}\exp(-t/\tau_{\mathrm{w}}) + N_{\mathrm{w}}N_{\mathrm{h}}\mu_{\mathrm{w}}\mu_{\mathrm{h}}g_{\mathrm{hw}}\exp(-t/\tau_{\mathrm{w}}) + N_{\mathrm{w}}^2\mu_{\mathrm{w}}^2g_{\mathrm{w}}\exp(-t/\tau_{\mathrm{w}})] \quad (2.3)$$

The static and frequency-dependent dielectric function is obtained using eq 2.3.

Note that the theory *does not use any adjustable parameter*. However, the protein solutions being a complicated dynamic syatem, a large number of parameters are necessary as input in the theory (see Table 1). The number of protein molecules is calculated from the molecular volume of the protein and the total volume occupied by the protein molecules known for a given concentration of solution. The number of water molecules in the hydration shell are calculated from the thickness of the hydration shell. The number of bulk water molecules are calculated from the residual volume.

τ_{p} and τ_{w} are taken from the literature data. The two slow time constants of the dynamics of water in the hydration shell τ_{hi} and their relative weights (Δh_i) are outcome of dynamic exchange of water in the hydration shell, slow motion of water on the protein surface, smaller diffusion constant of water near the protein, and slow diffusion of water from bulk to hydration shell and vice versa.[15,17−18] If one considers only the dynamic exchange, then we obtain two time constants in the range 30−50 ps and 10−20 ns, depending on the strength of the hydrogen bond.[15] However, the other two factors mentioned above shall introduce slower time constants. Recent studies on the rotation of structural water in a protein indicate that the rotational correlation time of water in bovine pancreatic trypsin inhibitor is about 45 ns, which is quite slow. The environment of hydrogen bond donors around the rotating water molecule is indicated as responsible for the slow motion of water in the protein.[19] In the present study we have taken the first time constant (τ_{h1}) as equal to that observed in the δ relaxation and the second slower time constant (τ_{h2}) as 35 ns—with more weight to the slower time constant.

The Kirkwood correlation factor for protein in solution (g_{p}) is as usual taken as unity.[2] The correlation factor for water (g_{w}) is well-known, equal to 2.8 at room temperature, and the correlation factor for water in the hydration shell (g_{h}) is assumed to be equal to g_{w}. The correlation factors for cross-terms (g_{ph}, g_{hw}, and g_{pw}) are assumed to be equal to the geometric mean of the individual correlation factors.

We tabulated all the required parameters in the Table 1 for aqueous *whale myoglobin solution, bovine serum albumin solution,* and the *equine hemoglobin solution*. In the limit of zero protein concentration, the theory recovers the static dielectric constant of pure water ($\epsilon_0 = 78$ at 298.15 K), as expected. We have varied different parameters appearing in the theory for a reasonable range around the values used in the present work to check the sensitivity of the dielectric constant at different frequency ranges (see Table 2 in the Supporting Information). It is found that the theoretical results are not very

TABLE 1: Parameters Used in the Present Theory for Whale Myoglobin (MYG), Bovine Serum Albumin (BSA), and Equine Hemoglobion (HEM) Solutions at 298.15 K[a]

parameter	magnitude	parameter	magnitude
μ_p (MYG)	110 D	τ_{h2} (MYG)	35 ns ($\Delta h2 = 0.8$)
μ_p (BSA)	380 D	τ_{h2} (BSA)	35 ns ($\Delta h2 = 0.8$)
μ_p (HEM)	320 D	τ_{h2} (HEM)	35 ns ($\Delta h2 = 0.8$)
τ_p (MYG)	74 ns	μ_w	1.84 D
τ_p (BSA)	75 ns	μ_h	1.84 D
τ_p (HEM)	84 ns	g_w	2.8
τ_{h1} (MYG)	40 ps ($\Delta h1 = 0.2$)	g_h	2.8
τ_{h1} (BSA)	50 ps ($\Delta h1 = 0.2$)	g_p	1.0
τ_{h1} (HEM)	100 ps ($\Delta h1 = 0.2$)	τ_w	8.32 ps

[a] The details of the choice of parameters are given in the Supporting Information.

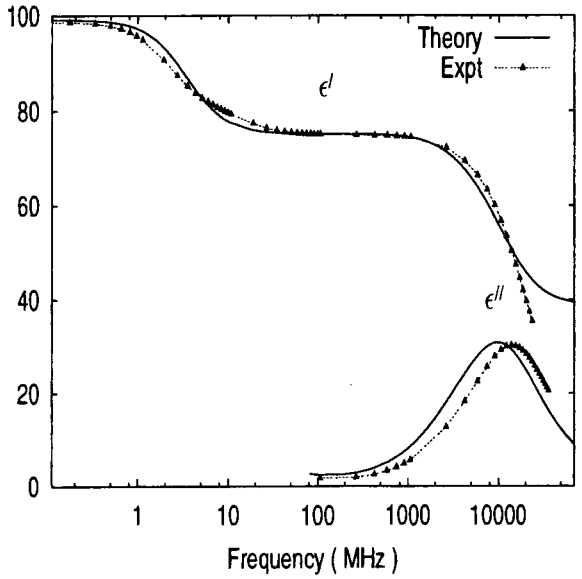

Figure 1. Real (ϵ') and the imaginary part (ϵ'') of the dielectric function of aqueous myoglobin solution (concentration is 170 mg/mL) calculated from the present theory (indicated by the solid line) and that from the experiment (ref 3 in the text) (indicated by the dotted line with solid triangles) at 298.15 K.

sensitive to the choice of parameters, except for the value of the dipole moment of the water molecule. In the following section we present and discuss the numerical results.

3. Numerical Results and Discussions

In Figure 1 we compare the theoretical and experimental plots of the ϵ' and ϵ'' of the aqueous myoglobin solution. The theory for the first time successfully explains the anomalous enhancement of the dielectric constant (DC) over the bulk value of water. However, this increase is a result of rather delicate balance between several terms involved in the $\langle \vec{M}(0)\cdot\vec{M}(t)\rangle$. While the contribution of the bulk water to the total moment fluctuation, $\langle M(0)^2\rangle$, decreases, the cross-correlation terms, particularly those between the water molecules in the bulk and in the hydration shell surrounding the protein, increases, leading to the overall increase in the value of $\langle M(0)^2\rangle$ (see Table 3 in the Supporting Information).

Therefore, we conclude that the heterogeneity, combined with the cross-correlation, is responsible for the anomalous enhancement. This theoretical result is supported by the recent structural data in protein solutions, which indicates that the correlations between the water molecules in the protein solution extend *beyond* the hydration shell.[20] This molecular explanation is simple and transparent.

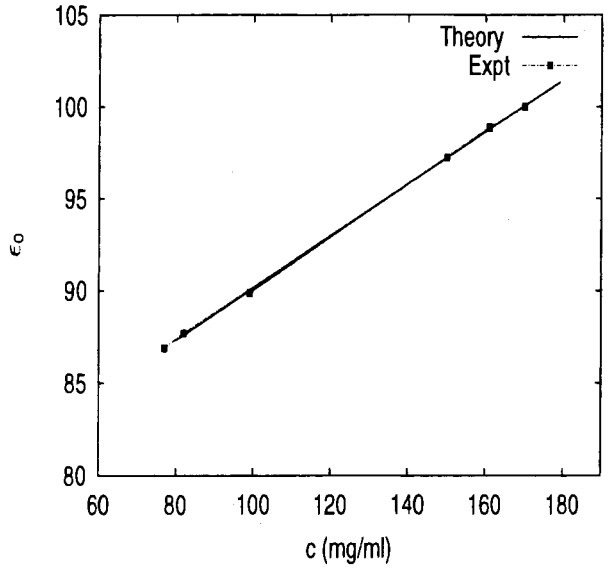

Figure 2. Concentration dependence of the static dielectric constant of the aqueous myoglobin solution calculated from the present theory (indicated by the solid line) and that obtained from the experiment (refs 3 and 4 in the text) (indicated by the line with solid squares) at 298.15 K.

In Figure 2, we compare the theoretical prediction of the concentration (c) dependence of the static DC with the experimental results on myoglobin. The agreement is excellent. The observed linear concentration dependence is a consequence of a c^2 dependence in the numerator but a linear dependence in the denominator of the right-hand side of eq 2.3.

The most interesting result of the present study is the concentration dependence of dielectric relaxation. This is shown in Figure 3 where we compared the calculated ϵ' of aqueous myoglobin solution with experimental result for three different concentrations. The agreement between theory and experiment is excellent. The theory successfully reproduces the experimentally observed dramatic crossover in the concentration dependence and offers the following explanation.

As the protein concentration increases, the bulk water concentration decreases, and the faster time scale of relaxation (due to the bulk water) is progressively replaced by slower time scales of relaxation. Thus, the population of relaxation times shifts from the high-frequency peak to the lower frequency region. This behavior is beautifully depicted in the Figure 4 where we have plotted the theoretical and the experimental results for the ϵ'' of aqueous myoglobin solution. These plots clearly show the presence of a low-frequency peak in addition to the peak usually observed for water in the high-frequency region (gigahertz range).

The bimodality described above is both stronger and completely distinct from that observed in the δ dispersion, also universally observed for aqueous proteins and DNA solutions. The latter is much weaker and is observed in the plateau region of the real part of DF (ϵ') in Figure 1 and arises for different reasons.[15]

That the anomalies discussed above are indeed generic to protein solutions and not limited only to myoglobin solution is demonstrated in the Figures 5 and 6 in the Supporting Information. In these figures we have compared the theoretical results for the ϵ' of bovine serum albumin and the concentration dependence of the ϵ' of equine hemoglobin with those from the experiment.[21,22] Both systems show the same behavior as found that for myoglobin.

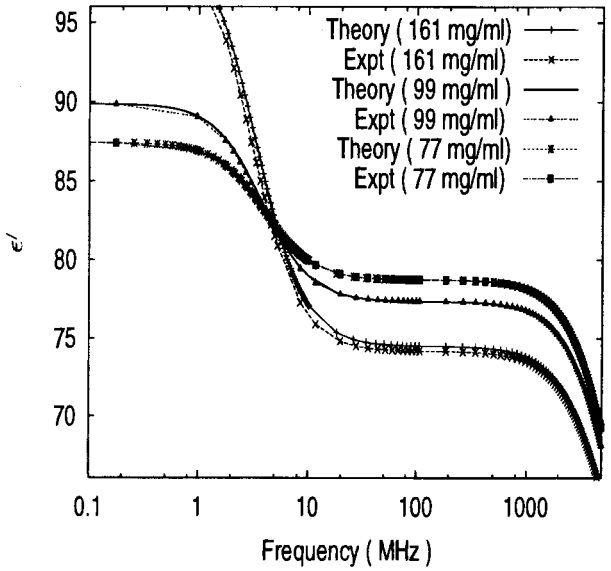

Figure 3. Concentration dependence of the real part of the complex permittivity of aqueous myoglobin solution calculated from the present theory and those from the experiment (ref 4 in the text) at 293.15 K. The line with (+) corresponds to the theoretical plot with concentration 161 mg/mL, the line with (×) corresponds to the experimental plot with concentration 161 mg/mL, the solid line corresponds to the theoretical plot with concentration 99 mg/mL, the line with (▲) corresponds to the experimental plot with concentration 99 mg/mL, the line with (*) corresponds to the theoretical plot with concentration 77 mg/mL, and the line with (■) corresponds to the experimental plot with concentration 77 mg/mL.

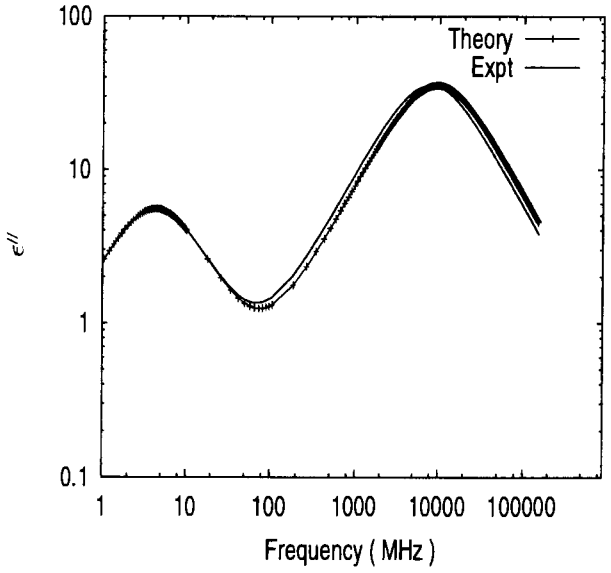

Figure 4. Imaginary part of the dielectric function of the aqueous myoglobin solution (concentration is 60 mg/mL) calculated from the theory (indicated by the line with +) and that from the experiment (indicated by the solid line) (ref 6 in the text) at 293.15 K.

Since protein association is a synergic phenomena with increasing protein concentration in solution, we have checked whether aggregation is substantial in the concentration range considered in the present work, and the conclusion was negative. For example, the dimerization of myoglobin starts above 150 mg/mL concentration.[23] So, we can exclude the protein association as a possible reason for the observed crossover.

An aqueous protein solution has a rich dynamical behavior having a wide spectrum of relaxation times.[24] It is well-known that both fast and slow relaxation processes are involved in many fundamental chemical and biological processes.[25−28] It might be fruitful to use solvation dynamics experiments to probe these diverse time scales.[27,28] Because of the heterogeneity of a protein solution, the observed time-dependent response should depend on the position of the probe. This can provide information about the local dynamics of the system. Recently nonlinear spectroscopic techniques have been used to study solvation dynamics in protein solutions, which showed that both slow and fast time scales are present in protein solution.[29] This is in agreement with the theory presented here. The present study can also be useful in understanding electron-transfer reactions in protein solutions.[30]

We note in conclusion that the present work appears to be the first detailed theoretical study of the dielectric relaxation of protein solutions and explains many hitherto unexplained anomalous properties. The success of the theory comes partly from the use of the correct expression at constant volume (eq 2.1) and partly from the proper description of the inhomogeneity and the static and dynamic correlations present in the solution. The decisive role of the cross-correlation terms in enhancing the value of the static DC opens the door for using it as a probe of the properties of water not only in proteins but also in other organized assemblies. The present theory can be used for such a purpose.

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Supporting Information Available: Details of the choice of parameters presented in Table 1 and used in the present calculation and detailed analysis of the sensitivity of the theoretical result to the choice of different parameters and the analysis of the relative contributions of the relaxation terms in eq 2.3 and their role in concentration crossover; average variation of the static dielectric constant of different frequency regions in the dielectric function with the variation of parameters in the theory (Table 2) and magnitudes of the different components contributing to the static dielectric constant of the myoglobin solution at solution at 298.15 K (Table 3); comparison of theoretical results of ϵ' of bovine serum albumin and concentration dependence of ϵ' of equine hemoglobin with experiment (Figures 5, 6) and plots of different static terms of eq 2.3 (R and N) versus concentration (9 pages). See any current masthead page for ordering and access instructions.

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