Dielectric 1/*f* noise of proton glass on a hydrated protein surface

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From nonexponentiality of dielectric relaxation detected in hydrated lysozyme powder we evaluate the power spectrum x_ω^2 of the fluctuating dipole moment $\overline{x}^2$ of one macromolecule, due to the glass of protons migrating on the protein surface. Near room temperature, the contribution to the total dipole moment by the integrated strength of the $1/f^\alpha$ noise in the frequency range from 10^4 to 10^6 Hz, is well consistent with early Kirkwood's predictions in solution and more recent computations. The biochemical significance of the nonequilibrium region near the Vogel-Fulcher temperature $T_0 \approx 268$ K calls for further investigations.

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In a recent paper [1] we have shown that protons migrating on hydrated surface of the protein lysozyme below room temperature behave as a proton glass exhibiting frustration and disorder. This result was reached analyzing the complex dielectric susceptibility of protein powders by a temperature-frequency plot, thus confirming our previous suggestions [2] grounded on current treatments for broad distributions of dielectric relaxation times. The dielectric properties of this system have been studied in this laboratory and are already reviewed [3,4].

The powdered lysozyme samples, prepared according to the standard procedure [1], have been hydrated isopiestically in moist air at 5 °C above $h = 0.25$ g H_2O/g dry weight and below the first water monolayer where a long-range proton displacement along percolative networks was observed [3,4]. Each sample, lightly compressed, was placed in a standard cryostat inside the sample holder, made up of two plane brass electrodes in thermal contact with liquid N_2. In order to prevent charge injection, and to keep constant the hydration level, samples were contained within a seal-tight Teflon cell. The complex dielectric constant of the protein sample was evaluated by considering the equivalent circuit consisting of a composite three-layer (Teflon-sample-Teflon). After having quenched samples to about 200 K at a cooling rate of 3 K/min, using a Hewelett-Packard 4284 A bridge with the oscillator level set to 1 V, the measurements of the complex dielectric constant were collected at 70 frequencies in the range from 100 Hz to 1 MHz by heating the sample at a rate of 0.2 K/min. Typical uncertainties were about ± 1 %, and ± 0.5 K for dielectric and temperature measurements, respectively.

The purpose of this paper is to extend our previous studies by evaluating the power spectrum (PS) of the fluctuating dipole moment x_ω^2 of the same samples reported in Ref. [1] from the nonexponentiality of observed dielectric losses. The fluctuating dipole moment of a globular protein due to proton displacements among ionizable side-chains, was suggested long ago by Kirkwood (see Ref. [4]) to be comparable to the permanent dipole moment arising from charged groups, and as the source of effects relevant for enzymatic activity. Since most enzymes display a turnover of a few milliseconds, the PS of the fluctuating dipole moment in the frequency range from kHz to MHz can offer information for the catalytic event. The PS of migrating protons in this frequency range can be evaluated from dielectric data of hydrated powders only, since in aqueous solution the overlapping contributions by the rotating dipole moment of the macromolecule and by mobile counterions cannot be avoided. Our studies have been limited to only one enzyme, lysozyme, but all conclusions are supposed to have a wide validity, since the hydrated surface is similar in all water-soluble globular proteins [3].

In order to evaluate the PS of the dipole moment $x(t)$ of one protein molecule, we use the fluctuation-dissipation theorem in its classical form [5], namely,

$$x_\omega^2 = \frac{1}{n_0}\left(\frac{kT}{2\pi}\right)\left(\frac{\varepsilon_2(\omega)}{\omega}\right), \tag{1}$$

where $\varepsilon_2(\omega)$ is the imaginary part of the dielectric constant of the sample, $\omega = 2\pi f$ where f is the frequency, T is the absolute temperature, k is the Boltzmann constant, and n_0 is the number of macromolecules per unit of volume. In systems with a broad range of relaxation times centered around τ_p, in the higher frequency wing where $f > f_p = 1/\tau_p$, often $\varepsilon_2(\omega)$ monotonously decreases with increasing f according to a power law $\varepsilon_2(\omega) \approx f^{-\beta}$ with β constant inside a decade or more. It is known that β is the exponent of the *Kohlrausch-Williams-Watt* (KWW) stretched exponential function $\exp[-(t/\tau)^\beta]$ widely used in glasses [6]. Then, inside this frequency range, the noise coefficient $1/f^\alpha$ of PS is $\alpha = \beta + 1$, and both the KWW exponent $\beta \leqslant 1$, and the noise coefficient $\alpha \geqslant 1$ are different ways to describe the same scenario. For instance, when $\beta = 1$, the simple exponential Debye-like relaxor also displays the Brownian noise $1/f^2$.

Figure 1 shows the PS evaluated on the basis of expression (1) at different temperatures for one of the hydrated lysozyme samples already reported in our previous work [1]. From this figure we see that at constant temperature the PS follows a power-law with a constant exponent α, in a fre-

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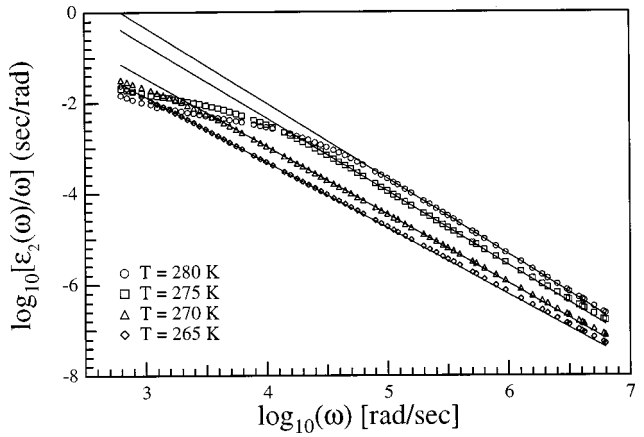

FIG. 1. The contribution $\{\log_{10}[\varepsilon''(\omega)/\omega]\}$ to the power spectrum, see Eq. (1), plotted versus $\log_{10}(\omega)$ at different temperatures (as indicated) in the case of the hydrated sample of lysozyme powder at $h=0.28 g_{H_2O}/g_{dry}$ reported in Table I of Ref. [1]. Error bars are inside symbols. The high-frequency noise coefficient α is evaluated as the slope of the full straight line in a frequency range of about 1 decade just above the relaxation frequency.

quency range of one or more decades. From previous dielectric studies [3,4] of this protein powder we know that $\varepsilon_2(\omega)$, and therefore α, of one sample must be understood as the simple average of the same quantity for one single macromolecule of that sample, namely for the purpose of these studies one can neglect the electric interactions among nearby macromolecules. Values of α evaluated by the aforementioned method are shown as a function of the temperature in Fig. 2 for the 3 samples previously reported in Ref. [1]. Inspection of Fig. 2 reveals that small differences in the hydration level or in the pH value of the samples only slightly affect α. The observed α values agree with those of several systems displaying a broad spectrum of relaxation times reported in the literature [7].

From data such as those shown in Fig. 1 we have evaluated the integrated strength $\overline{x^2_\Delta}$ of the fluctuating dipole moment PS in the frequency range Δ from 10^4 to 10^6 Hz. This

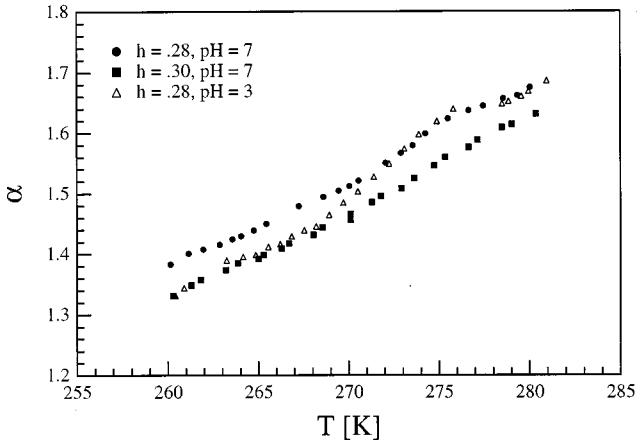

FIG. 2. The high-frequency noise coefficient α plotted as a function of temperature T (K) for the three hydrated samples previously reported in Table I of Ref. [1]. Error bars are inside symbols.

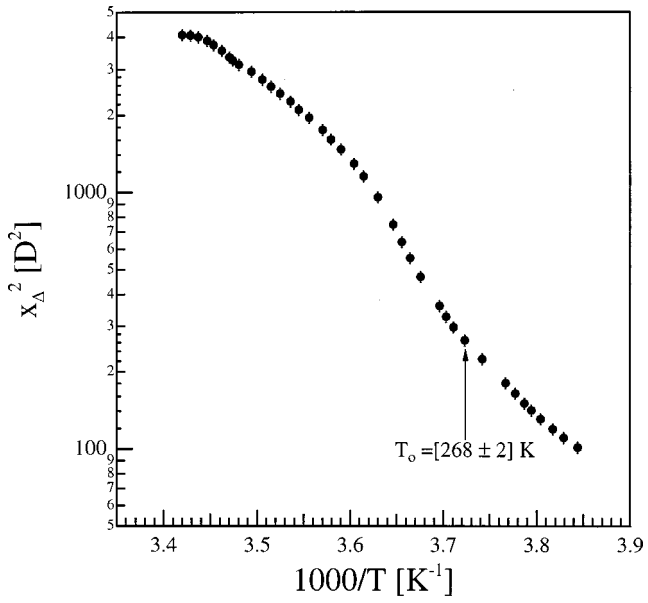

FIG. 3. The integrated strength $\overline{x^2_\Delta}$ of the fluctuating dipole moment in the frequency range $\Delta=[10^4;10^6]$ Hz for the same hydrated sample of lysozyme reported in Fig. 1. Error bars are inside symbols. Note the change of the slope near the VF temperature T_0 evaluated in Ref. [1].

frequency range is well covered by the $1/f^\alpha$ power law, and it is of biochemical interest for lysozyme, where the sequence of catalytic events requires a proton displacement to be concerted with conformational events faster than 10^{-4} s in the enzyme-substrate complex [8]. Here, for the purpose of the above evaluation, we have not considered the picoseconds relaxation observed in hydrated Lysozyme powders [9], which concerns the NH$\cdots$OC hydrogen bond of the peptide backbone, since this contribution to $\epsilon_2(\omega)/\omega$ is negligible in comparison with values reported in Fig. 1.

By assuming the lysozyme macromolecule in the microcrystalline powder to be a sphere of 20 Å radius, close packed in the capacitor cell, and neglecting local-field effects, from Fig. 1 we have evaluated $\overline{x^2_\Delta}$ to be about $4000D^2$ near 290 K. This value can be compared with the mean total dipole moment $M=184.2D$ of the charged groups and with $\langle M^2\rangle - \langle M\rangle^2 = \overline{x^2} \approx 4090D^2$, the square of the total fluctuating moment, of the same protein in aqueous solution at pH=7 computed [10] by molecular dynamics (MD) in the time range shorter than 1 ns, thus neglecting the lower frequency contribution considered in this paper. In this MD computation [10], formal changes to pH=7.0 were assigned to ionizable residues, all rotation of the protein was removed and the protein was assumed to be a sphere. An earlier static computation [11] of the known three-dimensional (3D) crystalline structure of the same protein at the same pH gave a mean total moment $M=111D$ and 68% of random distributions with dipole moment greater than the mean value. A later computation [11] at isoelectric pH, namely when effective positive and negative changes are equal, gave $M=134D$ for the fixed changes and $45D$ for the rms fluctuations by all configurations of protons on possible binding sites. These computations [10,11,12] are self-consistent

among themselves and with the early predictions by Kirkwood and co-workers [13,14] in aqueous solution, and even with the findings of this work, pointing out that the magnitude of dipole moment from fluctuations of the proton distribution on the protein surface is close to the mean value of the total dipole moment of the globular protein arising from the 3D distribution of charged groups. We believe that in this paper we have detected the experimental value of these proton fluctuations in the case of a nearly dry protein, where the rms of the dipole moment involved is found about one proton charge times the protein diameter. Notice, however, that at variance with aqueous solutions, in hydrated powders in the pH region here investigated, the proton redistribution on sites of nearly equal free energy arises from Lys^+ residues only [4].

The previous discussion was limited to the room-temperature region, where the proton glass behaves as a ''liquid'' of independent particles, as it was assumed in the above-mentioned computations and in the current theories of the dielectric properties of proteins (see Ref. [4]). However, as shown in Fig. 3, $\overline{x}_\Delta^2$ is strongly reduced by lowering the temperature, and approaching the Vogel-Fulcher (VF) temperature T_0^1 a new behavior of potential biochemical interest must be expected. Perhaps the glass nonequilibrium near T_0 can be the reason for the reduced enzymatic activity observed in the lysozyme-substrate complex [15]. This possibility may be studied in complexes following the method used in this paper for the native protein.

In conclusion, from the nonexponentiality exponent β measured on the high-frequency side of the dielectric absorption peak of a protein powder, we have evaluated the exponent α exhibited by the $1/f^\alpha$ noise of the mean-square dipole moment fluctuation spectrum of a globular protein, arising from the fluctuating distribution of protons on the hydrated surface. The value of the proton contribution to the total dipole moment of one macromolecule evaluated in the frequency range 10^4 to 10^6 Hz at room temperature is quite consistent with original predictions by Kirkwood and with more recent computations, while the nonergodicity near the freezing temperature of this proton glass calls for further biochemical studies. The dielectric noise due to the fluctuating proton distribution on ionizable side chains of the protein surface is a many-bodies phenomenon of a primarily physical nature and of wide biochemical interest. In this paper we have shown an experimental approach from the side of poorly hydrated proteins.

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