Critical exponents of protonic percolation in hydrated lysozyme powders

G. Careri and A. Giansanti
Dipartimento di Fisica, Università di Roma I, Piazzale Aldo Moro 2, 00185 Roma, Italy

John A. Rupley
University Department of Biochemistry, University of Arizona, Biological Sciences West, Tucson, Arizona 85721
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The dc protonic conductivity of powders of lysozyme was calculated from capacitance data measured for the frequency range 10 KHz to 10 MHz and for varied levels of hydration. Analysis of the hydration dependence of the conductivity close to the percolation threshold gave the value for the critical exponent $t = 1.29 \pm 0.05$. This value is in close agreement with expectation from theory for a two-dimensional percolative process. These results support the idea that the threshold for hydration-induced protonic conduction (and, quite possibly, for enzymatic activity) corresponds to the formation of a percolation network of absorbed water molecules on the surface of this protein macromolecule.

Recent work has shown that powders of the protein lysozyme at low hydration display protonic conductivity[1] and that the conduction process follows the percolation model.[2] In this picture, the conductivity reflects motion of protons along threads of hydrogen-bonded water molecules adsorbed on the surface of the macromolecule, with long-range proton movement developing along with the extended network at the percolation threshold. Substantial interest has developed in the critical exponents of percolation processes.[3] Here we report the value of the critical exponent for proton percolation in hydrated powders of lysozyme. We are not aware of previous similar studies for biological materials.

We have measured the dielectric properties (capacitance and loss) of the lysozyme powders by a dielectric-gravimetric method, through the use of a composite condenser with insulated electrodes, for the frequency range 10 KHz to 10 MHz. The hydration level (h, in g of water per g of dry protein) was varied continuously under a flow of dry air. Sample weights were about 0.5 g and were determined to a precision of 10^{-4} g. Capacitance was determined to a precision of 10^{-4} pF.[1,2]

Percolation theory[3,4] predicts the critical exponents of the dc conductivity (σ). In order to compare the results obtained for the lightly hydrated powders with the theoretical models, we have calculated values of the dc conductivity as a function of hydration level [$\sigma(h)$] from the previously recorded values of the capacitance as a function of frequency and hydration.[1] The electrostatic analysis of the three-layer capacitor (glass–hydrated sample–dry air), assuming a single Maxwell-Wagner relaxation process, is straightforward. Inspection of the frequency dependence of the loss factor shows that the assumption of a single relaxation in these samples is correct for the frequency range 10–100 KHz. The precision in the estimation of σ is 0.1%. Because the absolute value is more uncertain, we have normalized σ in the presentations of Figs. 1 and 2.

Typical results of this analysis are given in Fig. 1 for capacitance data for 10, 20, and 40 KHz. As reported previously,[2] there is a percolation threshold at $h \sim 0.14$. Above this threshold the conductivity shows apparently exponential dependence on hydration, as found for other biological samples.[5]

From percolation theory,[4] above and near the threshold p_c,

$$\sigma(p) \sim (p - p_c)^t , \tag{1}$$

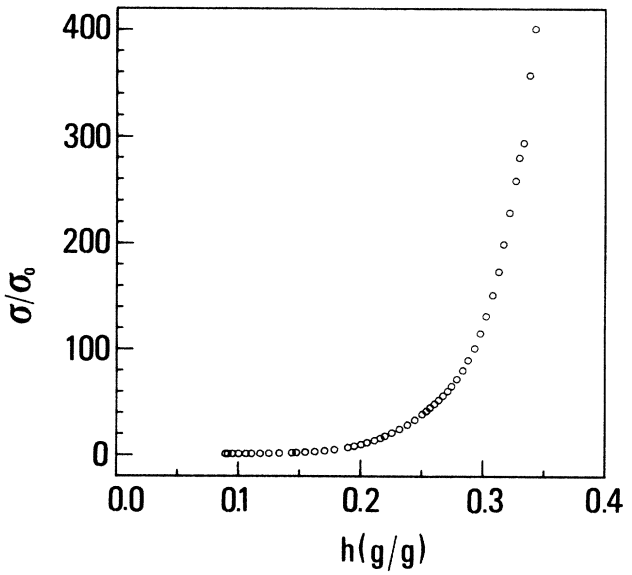

FIG. 1. Normalized dc conductivity (σ/σ_0) vs hydration level (h) of the lysozyme powder for pH 7.0, 28 °C. Hydration was with H_2O. σ_0 is the dc conductivity of the dry sample.

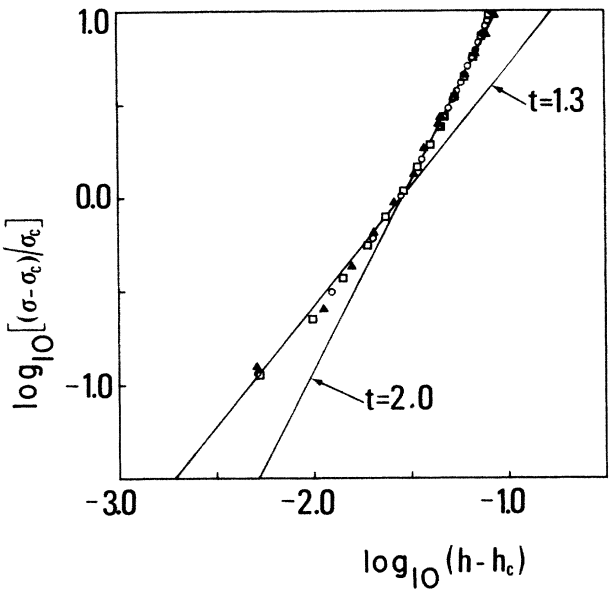

FIG. 2. Hydration dependence of the dc conductivity of lysozyme powders of pH 7.0 near the percolation threshold for the three samples of Table I, plotted according to Eq. (2). Symbols: ○, native lysozyme hydrated with H_2O; □, native lysozyme hydrated with D_2O; ▲, 1:1 complex of lysozyme with $(GlcNAc)_4$, hydrated with H_2O. The lines are drawn for values given by theory (Ref. 3) for the critical exponent for two-dimensional percolation ($t=1.30$) and three-dimensional percolation ($t=2.00$).

where p is the probability of site occupancy by the conducting species and t is the critical exponent for dc conductivity. The exponent t depends on the dimensionality of the system. In order to estimate the critical exponent from dc conductivity values, such as those displayed in Fig. 1, we can write for the experimental system, because values of h are directly proportional to the site occupancy probability,

$$\sigma(p)-\sigma(p_c)\sim(h-h_c)^t . \quad (2)$$

In order to remove the nearly negligible contribution of nonpercolative processes and systematic errors in the evaluation of capacitor geometry to the total conductivity, the value of the dc conductivity at the percolation threshold $\sigma(p_c)$ must be subtracted from $\sigma(p)$. The value of h_c has been determined previously.[2] Table I gives the results of this analysis for three samples: native lysozyme hydrated with H_2O or D_2O, and the complex of lysozyme with an oligosaccharide substrate [the tetrasaccharide of *N*-acetylglucosamine $(GlcNAc)_4$] of the enzyme. Figure 2 shows data for the same samples described in Table I.

Considering first the range of hydration levels closest to the percolation threshold, the value for the critical exponent $t=1.29$ (Table I), is in close agreement with the range of estimates of t, 1.1–1.3, obtained from theoretical and experimental studies on two-dimensional percolation.[3,4,6] A previous and different[2] analysis of the dielectric data for lysozyme independently reached the same conclusion about the dimensionality of the percolation, from the close agreement between the measured value of h_c (Table I) and the prediction from theory for a surface process.[4] As described previously,[2] the dielectric response at hydration levels near h_c reflects protonic conduction over pathlengths of the order of the diameter of a single macromolecule. For hydration levels far from h_c, the critical exponent, $t=2.08$, is characteristic of three-dimensional percolation.[3,4] It is possible that intermolecular water bridges are established at higher hydration, leading to long three-dimensional conduction pathways through the hydrated powder layer. A similar transition from two- to three-dimensional percolation has been observed for mixtures of conducting and nonconducting polymers upon varying the sample thickness.[6] Alternatively, Eq. (2) may fail far from h_c.

The theoretical values of the critical exponent t of Eqs. (1) and (2) are derived for very large or infinite lattices. In view of the consistency between the previous analysis of the capacitance data and the independent analysis of the conductivity given here, the number of elements participating in the conduction process in the protein powder appears to be sufficient to mimic a very large system.

As expected for a percolative process, which is controlled by the fractional occupancy, there is no deuterium isotope effect on h_c or t (Table I). Complexation of lysozyme with a substrate, the tetrasaccharide of *N*-acetylglucosamine, increases h_c by nearly 50% (Table I).

TABLE I. Measurements are for pH 7 and 28 °C. The values of h_c are from graphical analyses of capacitance data (Ref. 2). The values of t were obtained from fitting the conductivity values to Eq. (2), for ranges of h near and far from h_c, giving the values t_1 and t_2, respectively.

Conditions	h_c	t_1	t_2
Native lysozyme, hydrated with H_2O	0.142±0.014	1.30+0.09	2.20 +0.19
Native lysozyme, hydrated with D_2O	0.157±0.005	1.24±0.08	2.107+0.008
1:1 complex with $(GlcNAc)_4$, hydrated with H_2O	0.219+0.004	1.34+0.11	1.94 +0.12
	average	1.29±0.05	2.08 ±0.13

The value of t found for the saccharide complex suggests that the protonic conduction remains a surface process. This observation is in agreement with the suggestion[1] that preferred paths of proton movement pass through the active site; substrate would be expected to block these without changing the surface character of the percolation. We call the attention to the fact that for the saccharide complex the threshold for proton percolation coincides with the onset of catalytic properties.[7] Our results support the suggestion that the water-induced onset of enzymatic activity in this protein system corresponds to a percolation threshold for proton conduction.

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