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Proton tunneling in hydrated biological tissues near 200 K

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We measure the protonic conductivity in water clusters adsorbed on intact samples of viable biological samples (corn embryo and endosperm, *Artemia* cysts, and *Typha* pollen) below room temperature. In the low-temperature region, the conductivity increases with temperature as exp T^6, in agreement with prediction by the theory of dissipative quantum tunneling. We detect the onset of this effect near 180 K, where a glass transition in the hydrated protein matrix is known to take place. Above 220 K other transitions are superimposed onto this simple behavior.

1. Introduction

Previous work from this laboratory has shown that hydrated lysozyme powders exhibit dielectric behavior in the megacycle range due to protonic conductivity, and that this behavior can be described in the framework of percolation theory [1–3]. Long-range proton displacement appears only above the critical hydration for percolation h_c (h in g H_2O/g sample dry weight), when two-dimensional motion takes place on fluctuating clusters of hydrogen-bonded water molecules adsorbed on the protein surface. The critical hydration required for the onset of enzymatic activity was found to coincide with the critical hydration for percolation near $h_c = 0.15$ [2,3]. More recently, this room-temperature study has been extended towards more complex biological systems, such as patches of the plasma membrane (purple membrane) of *Halobacterium halobium* [4], *Artemia* cysts [5], corn seeds [6], and a unifying review has been presented [7].

A low-temperature study of the dielectric conductivity in hydrated lysozyme powders has very recently been accomplished [8] and it is particularly relevant for this paper. The main aim of that study was to provide evidence for proton quantum tunneling in water clusters adsorbed on the protein matrix. This evidence was obtained from the temperature dependence of the d.c. protonic conductivity, which was found to increase as exp T^6, in agreement with prediction by the theory of dissipative quantum tunneling [9]. However, as an additional result, the existence of two transition temperatures become apparent. The first one was detected as the onset of protonic conductivity near $T = 180$ K (T_{g1}) and it has been associated with a glass transition previously found in several hydrated proteins around this temperature. A second transition has been observed near 210 K (T_{g2}) as a knee in plots of conductivity data vs temperature [8]. Since the transition at T_{g1} enhanced and that at T_{g2} reduced proton conductivity, this behavior can be understood if T_{g1} is assigned to melting of

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hydrogen-bonded networks of the hydrated protein, and T_{g2} to relaxation of polar side chains.

The aim of this paper is to investigate the low-temperature conductivity of intact viable biosystems already studied at room temperature [5,6] in order to detect the likely presence of proton tunneling. Moreover, this investigation has been extended to include samples of partially hydrated intact pollen (*Typha latifolia*) grains.

2. Materials and methods

Dry seeds, pollen, and anhydrobiotic microscopic animals like *Artemia* were chosen as model systems to use in this study primarily because their hydration properties are well known, and because of their availability in large quantities. Hand-dissected corn (*Zea mays* L.) embryo and endosperm were ground to produce pellets whose diameter d was $0.5 < d < 1$ mm. Samples of *Typha* pollen were a gift from Professor A.C. Leopold (Boyce-Thompson Institute, Ithaca, NY). Samples of *Artemia* cysts were a gift from Professor J.S. Clegg (Bodega Marine Laboratory, University of California at Davis).

Samples were hydrated using the isopiestic method. The dielectric apparatus has been previously described [1], as well as the procedure to evaluate the d.c. conductivity [3,4]. In this work, the insulated electrodes of the capacitor contain two layers, on being the teflon sample holder and the other being the sample itself. This capacitor is enclosed in a cryostat which is cooled to near 170 K at a rate of 3 K min^{-1}. Dielectric data in the frequency range 10 kHz–10 MHz were recorded with increasing temperature at a rate of about 1 K min^{-1}. In order to evaluate the dielectric parameters, only the low-frequency region (10–100 kHz) was used to avoid Debye relaxations observed in the dielectric loss factor above 100 kHz (data not shown).

3. Results

Samples at several different values of water content were tested and the relevant information is listed in table 1.

In fig. 1, the reduced conductivity σ/σ_0 (h,T) is plotted vs temperature T, for some typical samples. At the lowest temperatures investigated here,

Table 1

Parameters of investigated samples, defined and discussed in the text

Sample	Tissue	h (g/g)	σ_0 ($\times 10^{-9}$) (Ω^{-1} m^{-1})	tan α ($\times 10^{-15}$) (K^{-6})
Emb 0.05	Embryos	0.05 ± 0.02	7.0 ± 0.2	7.0 ± 0.1
Emb 0.18	Embryos	0.18 ± 0.02	9.6 ± 0.3	19.1 ± 0.2
Emb 0.10	Embryos	0.10 ± 0.02	12.1 ± 0.4	11.7 ± 0.1
Emb 0.12	Embryos	0.12 ± 0.02	12.2 ± 0.4	14.4 ± 0.1
Emb 0.13	Embryos	0.13 ± 0.02	7.3 ± 0.3	10.4 ± 0.9
Emb 0.17	Embryos	0.17 ± 0.02	7.1 ± 0.2	15.3 ± 0.2
Emb 0.30	Embryos	0.30 ± 0.02	8.7 ± 0.3	20.3 ± 0.4
Art 0.20	Cysts	0.20 ± 0.02	10.1 ± 0.4	16.0 ± 0.1
Art 0.22	Cysts	0.22 ± 0.02	9.5 ± 0.4	14.3 ± 0.1
Art 0.49	Cysts	0.49 ± 0.02	12.3 ± 0.5	20.4 ± 0.2
Art 0.39	Cysts	0.39 ± 0.02	12.4 ± 0.5	20.7 ± 0.3
Art 0.11	Cysts	0.11 ± 0.02	12.5 ± 0.5	5.2 ± 0.1
Art 0.26	Cysts	0.26 ± 0.02	15.8 ± 0.5	20.0 ± 0.4
Endo 0.18	Endosperm	0.18 ± 0.02	10.3 ± 0.4	20.1 ± 0.2
Endo 0.23	Endosperm	0.23 ± 0.02	14.0 ± 0.5	22.8 ± 0.4
Typha 0.52	Pollen	0.52 ± 0.02	8.7 ± 0.3	18.6 ± 0.1
Typha 0.09	Pollen	0.09 ± 0.02	8.0 ± 0.3	7.4 ± 0.1

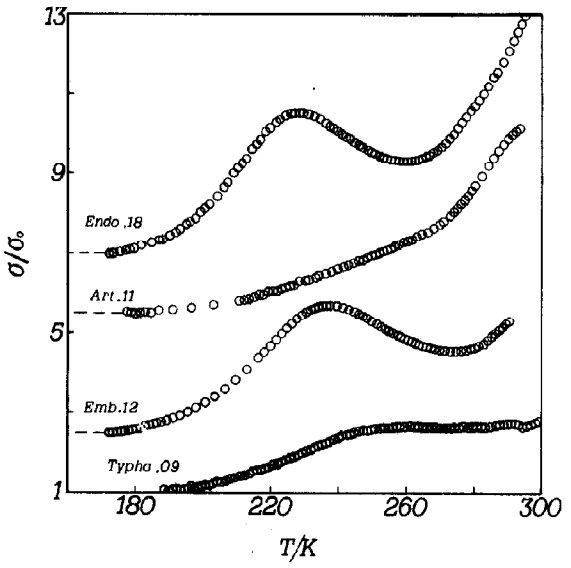

Fig. 1. Reduced protonic conductivity σ/σ_0 vs absolute temperature T for some hydrated samples reported in table 1, and discussed in the text. For the sake of convenience, σ/σ_0 data have been shifted along the vertical axis by adding 1.5 to Emb 0.12, 4.5 to Art 0.11, and 6.0 to Endo 0.18.

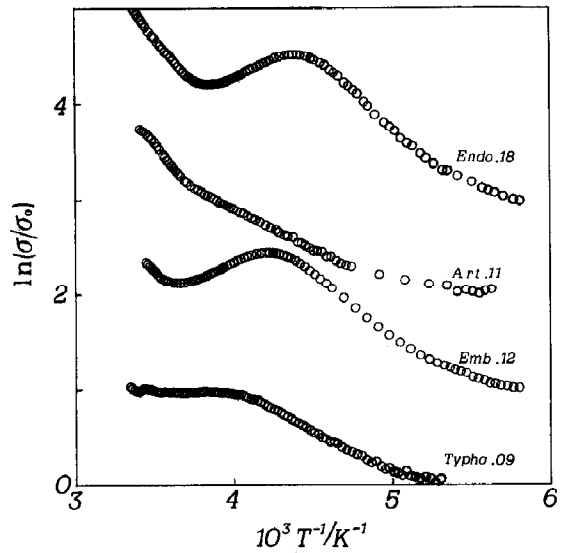

Fig. 2. Natural logarithm of conductivity data shown in fig. 1 plotted vs reciprocal absolute temperature $1/T$. For the sake of convenience, ln σ/σ_0 data have been shifted along the vertical axis by adding 1.0 to Emb 0.12, 2.0 to Art 0.11, and 3.0 to Endo 0.18.

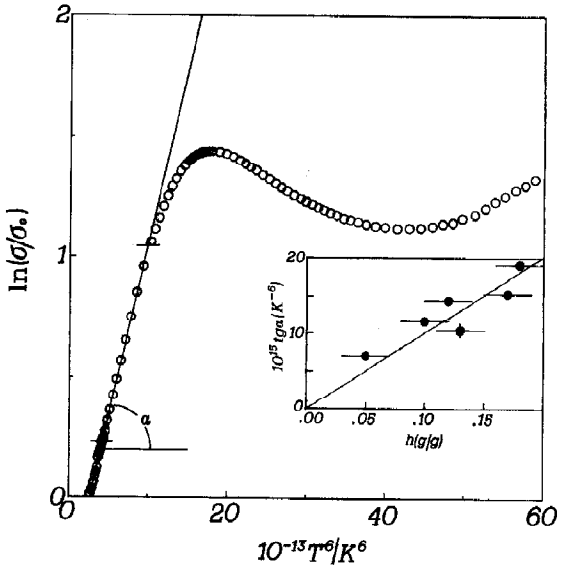

Fig. 3. Natural logarithm of conductivity data shown in fig. 1 for sample Emb 0.12, plotted vs the sixth power of the absolute temperature T^6 in the low-temperature range. Solid lines are best fit through data within vertical bars. In the inset, the slope tan α (tg α) of the straight lines (see fig. 6) focusing on $(T_{g1}, \sigma_1/\sigma_0)$ is reported vs hydration level h.

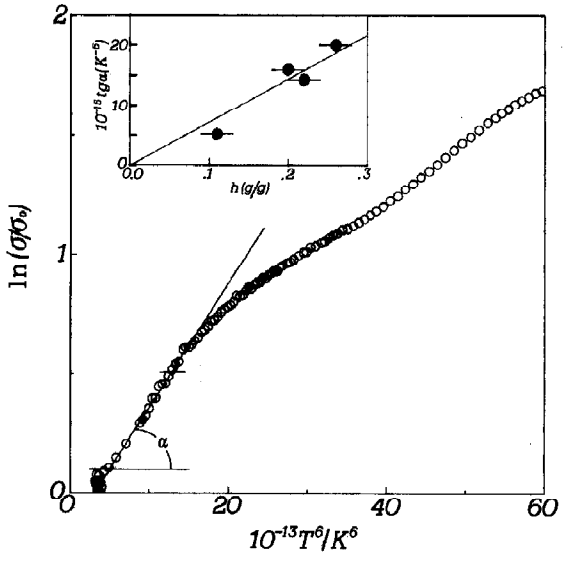

Fig. 4. Same as fig. 3 but for sample Art 0.11.

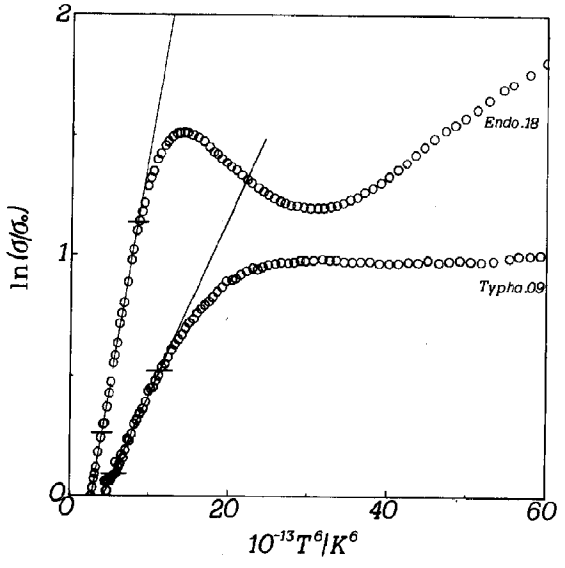

Fig. 5. Same as fig. 3 but for two samples indicated.

the conductivity σ_0 was found to be temperature independent, and is likely to be electronic in nature. This limiting low value of σ_0 is affected by a systematic error of about 10% due to lack of reproducibility of capacitor geometry in different runs. In fig. 2, the reduced conductivity σ/σ_0 is plotted vs $1/T$ for the samples indicated. In figs 3–5 the reduced conductivity data, $\ln(\sigma/\sigma_0)$, are plotted vs T^6 in order to show the linear behavior in a limited temperature range, near and above the lowest temperature here measured. In fig. 6 the linear extrapolation to find a common focus near (T_{g1}, σ_1) is shown. A linear increase of the slope $\tan\alpha$ (see inset in figs 3 and 4) was detected with increasing hydration. A similar linear extrapolation (not shown) for the samples of *Artemia* cysts gave $T_{g1} = 186 \pm 16$ K and $(\sigma_1/\sigma_0) = 1.3 \pm 0.4$. Broadly speaking, all the reported results are similar to those obtained in lysozyme powders [8] under the same experimental conditions.

Following the procedure of ref. 1, we have obtained evidence that *T. latifolia* grains are protonic conductors in the frequency range in the present study (data not shown).

4. Discussion

As shown in fig. 2, at the highest temperatures investigated, the conductivity follows Arrhenius' law, with an activation energy ranging from 4 to 7 kcal/mol, as expected for thermal hopping of protons controlled by rotation of water molecules adsorbed on the protein matrix. This picture assumes that the proton number density is temperature independent. This last assumption is frequently made in order to describe the electric conductivity of biopolymers [10], and of ice [11], where extrinsic charge carriers are believed to be produced with an energy lower than the dissociation energy of a water molecule. Thus, we suggest that at temperatures above about 270 K the rate process is controlled by a thermally activated hopping of H_3O^+ defects over a rotational energy barrier which is temperature independent, in agreement with current models [12] for proton transfer in H_2O networks. Instead, below about 270 K, the Arrhenius plot reveals an increasing contribution by other processes, probably proton tunneling, and even a glass transition superim-

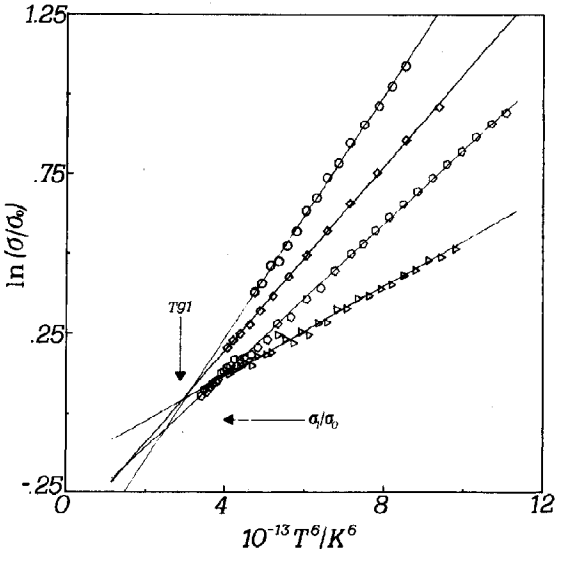

Fig. 6. Same as fig. 3, but for corn embryo samples at different hydration level. $h = 0.10$ (pentagons), $h = 0.18$ (circles), $h = 0.12$ (squares), $h = 0.05$ (triangles). Solid lines are best fit through data. From this plot we have evaluated $T_{g1} = 175 \pm 15$ K, and $\sigma_1/\sigma_0 = 1.0 \pm 0.2$.

posed near the temperature T_{g2}. These last two processes will be further considered below.

A general theory of quantum tunneling out of a metastable interacting state with an environment at temperature T has been proposed by Grabert et al. [9] (GWH theory), with the finding that for damping of arbitrary strength, the tunneling decay rate always matches smoothly with the Arrhenius factor at a crossover temperature, and that heat enhances the tunneling probability at $T = 0$ K by a factor $\exp[A(T)]$. For undamped systems $A(T)$ is exponentially small, whereas for a dissipative system $A(T)$ grows algebraically with temperature. Of particular interest here is the case of tunneling centers in solids, where $A(T)$ increases proportionally with T^n at low temperatures, with $n = 4$ or 6. To this end, in figs 3–6 we have plotted the natural logarithm of the conductivity data vs T^6, and find that a remarkably simple description can be offered as follows. In this plot, the low-temperature data can be fitted by straight lines originating around $T = T_{g1}$ and $\sigma \approx \sigma_1$. Fig. 6 shows the procedure followed to detect the focus (T_{g1}, σ_1) by linear extrapolation of the data. At low hydration the linear dependence on hydration of these slopes (see inset of figs 3 and 4) can be described in the GWH theoretical frame by stating that damping responsible for tunneling enhancement must originate in the first hydration shell of the H_3O^+ defect. We have analyzed our data using different values for n, but only $n = 4$ gave results comparable with $n = 6$ shown above, as predicted by GWH theory [9].

Although the linear dependence of $\ln \sigma$ on T^6 required by the GWH theory is certainly fulfilled, this theory requires that proton tunneling starts at $T = 0$ K. We believe that this apparent contradiction between GWH theory and our data can easily be overcome by suggesting that H_3O^+ defects are free to tunnel across a rotational energy barrier only above the protein glass transition temperature which is known to occur near T_{g1} [13,14], because the adsorbed water clusters behave as a supercooled fluid and can support defect motion. The region near and above this glass transition is shown in fig. 6 for corn embryo, and the mean value of T_{g1} of the data set, including corn embryo and *Artemia* cysts, is found to be 176 ± 13 K. This value is in very good agreement with that of 182 ± 2 K determined in lysozyme powders [8], considering the intrinsic non-reproducibility of the glass transition, the greater complexity of the samples investigated here, and the fact that no corrections have been applied to reduce the error in σ_0.

A second glass transition has been detected by several authors at T_{g2}, and in our related paper on lysozyme powder we found $T_{g2} = 203 \pm 5$ K [8]. Here, we are faced with a more complex behavior, namely, this transition is nearly absent in *Artemia* cysts and *Typha* pollen at low hydration, while it appears close to 220 K in corn embryo and endosperm (see fig. 1). At higher hydrations and temperatures other transitions have been observed and all these features will be fully discussed elsewhere.

In conclusion, in the temperature range from 180 to 220 K, the proton conductivity of four different intact biological tissues displays the same behavior as that already observed in hydrated powders of lysozyme [8]. This suggests that the same protonic rate process must take place in water clusters adsorbed on proteins of all samples thus far investigated, since the presence of adsorbed water clusters has been detected in high-frequency dielectric studies in lysozyme [15], and in a great variety of biological tissues [16]. This process is here identified as dissipative quantum tunneling because the unusual temperature dependence displayed by the measured protonic conductivity is in very good agreement with the temperature enhancement predicted by theory [9]. Moreover, our data show that the onset of the proton conductivity detected near 180 K in hydrated powders of lysozyme [8] occurs within about the same temperature range in intact biological tissues, in spite of quite different environments, probably because the onset is associated with the glass transition occurring in the hydrated proteins.

Acknowledgements

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