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DIELECTRIC RELAXATION IN SOLID LYSOZYME

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Dielectric constant and loss factor of solid polycrystalline lysozyme have been measured as a function of frequency from 30 Hz to 3 MHz at different temperatures from -25° to 30°. A very weak and broad dispersion peak has been observed in the frequency range 10 - 100 KHz in addition to a strong dispersion at lower frequencies. Activation parameters of the former relaxation process have been obtained using the Cole-Cole modified Debye model of dielectric relaxation. Internal mobilities in the polar protein components are found to be the probable cause of relaxation.

Key words: Dielectric relaxation, Protein dynamics, Lysozyme.

Introduction

Dielectric properties of synthetic polymers in the solid state have been extensively studied and have been reviewed in many comprehensive texts [1-3]. These studies describe the mobilities in the main chain and side chains of the polymers. Dielectric studies of natural polymers, on the contrary, have generally been restricted to measurements in aqueous solutions [4-6]. Such solution state measurements have given valuable information about shapes and sizes of protein molecules, their dipole moments, the water of hydration and about the nature of the inter-molecular bondings. Solid state dielectric data in hydrated proteins collected over the last few decades has mainly concentrated on the protein-water interaction [7,8] and mechanisms of charge transfer in protein systems [9-11].

Intra-molecular dynamics in proteins which is being regarded as the key to the understanding of their biological behaviour can also be studied via solid state dielectric data. Only a few such studies have been reported [12,13] and there is a need for a systematic investigation of dielectric properties of solid proteins with reference to their internal dynamics.

Experimental

The egg-white lysozyme used in this study was obtained from the Sigma Chemical Company. It was stated to be 3 times crystallised, dialysed and lyophilised (Grade 1). The sample was estimated to have about 6% water by weight from gravimetric analysis of the sample before and after vacuum drying at 110° for one day.

All measurements were made on lysozyme powder packed to a density of 120 mg/cc in a sample cell of electrode diameter 17 mm, guard electrode diameter 18 mm and the thickness of the sample was 0.8 mm. The temperatures required were maintained by placing the sample cell in a thermostatic oven whose accuracy was ±1.5° in the temperature range of these observations.

Dielectric measurements were made using the complete dielectric measuring set of Ando Electric Company, Japan which consisted of a bridge used in combination with a null detector and an oscillator. Details of the experimental setup can be found elsewhere [14,15].

The measured quantities were the capacitance (Cx) and the conductance (Gx) of the specimen and the static relative permittivity ε' and loss factor ε" were calculated as

$$\varepsilon' = Cx/Ca \quad \text{and} \quad \varepsilon'' = Gx/\omega\, Ca$$

where Ca is the capacitance of the cell with air and ω is the angular frequency of observation. All measurements were reproducible within the 5% experimental error.

Results and Discussion

The measured values of dielectric constant and loss factor in polycrystalline lysozyme powder at three different temperatures are shown as a function of frequency in Figs. 1 and 2 respectively.

These curves are indicative of two distinct dispersions; a lower frequency strong dispersion and a weak dispersion at a higher frequency with a very broad peak. The former dispersion could not be characterised completely as the peak of this dispersion could not be observed even at the lowest obtainable frequency of our measuring set.

The weaker dispersion (to be referred to as α'-dispersion) which is centered at about 35 KHz at 0° could only be detected at temperatures below the normal freezing point of water. At higher temperatures this ∝'-dispersion is completely overmasked by the strong lower frequency dispersion. A pronounced effect of the bound water on the low frequency dielectric dispersions of hydrated proteins has been known for quite some time [10] and is considered to be associated with the electrode polarisation effects and processes of non-conductive long range charge transport.

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As is clear from the loss curves (Fig.2), the α '-loss peak shifts towards higher frequencies with the increase of temperature. Thus indicating the relaxation process to be a temperature activated rate process [16]. In order to obtain the activation parameters of the relaxation process involved in the observed dispersion, the mean correlation time (reciprocal of the angular frequency at maximum loss) was plotted as a function of reciprocal temperature on a log linear graph (Fig.3). An Arrhenius type behaviour has been observed [16]. The relaxation process is therefore described by

$$\tau_c = \tau_o \exp (E_A/RT) \quad \ldots\ldots (1)$$

where τ_c is the mean correlation time, τ_o the pre-exponential factor, E_A the activation energy, R the gas constant and T the absolute temperature. The relaxation process can further be developed in terms of a chemical rate process in the form of the following equation.

$$\tau_C = \frac{h}{kT} \exp \frac{(-\Delta S)}{R} \exp \frac{(\Delta H)}{RT} \quad \ldots\ldots (2)$$

where h is the Planck's constant, k the Boltzmann's constant, ΔS the molar entropy of activation for the relaxation process and ΔH the Arrhenius activation enthalpy per mole. The molar enthalpy of the relaxation process, as obtained from the slope of the line in Fig. 3, is 14.6 k cal/mole. This enthalpy value is not very different from that found in poly-γ-methyl-L-glutamate (H=14 k cal/mole) for the side chain reorientations [17]. The pre- exponential factor as obtained from the intercept of this Arrhenius plot, is approx. 7×10^{-18} s, which gives the molar entropy of activation as 2×10^{-2} cal/deg mole at temperatures around 0°. This small value of ΔS suggests that the reorientation takes place with a little cooperative interaction of the neighbouring groups.

To account for symmetrical distributions of correlation times, Cole and Cole [18] used the following modified Debye equation

$$\varepsilon^*(\omega) = \varepsilon_\infty + \frac{\varepsilon_s - \varepsilon_\infty}{1 + (i\omega\tau_c)^{1-\beta}} \quad \ldots\ldots (3)$$

where ε* is the complex dielectric constant (ε'–i ε". The real part, ε' is the dielectric constant, and the imaginary part, ε" is the dielectric loss. ε_∞ and ε_s are the high and low frequency limits of the dielectric constant, ω is the angular frequency and τ_c is the mean relaxation time. The parameter β indicates the width of the distribution of correlation times around τ_c which may range from 0 to 1. For materials with a single correlation time where β = 0, the Cole- Cole equation reduces to the usual Debye equation [19]. The graphical representation of equation 3 gives the arc of a semicircle in the complex plane with the diameter of the semicircle making an angle βπ /2 with the real axis. Intercepts of this semicircle on the real axis give the limiting values of high and low frequency static relative permittivities.

Cole-Cole plots of our data as shown in Fig. 4 indicate that there is a large distribution in correlation times. Data points,

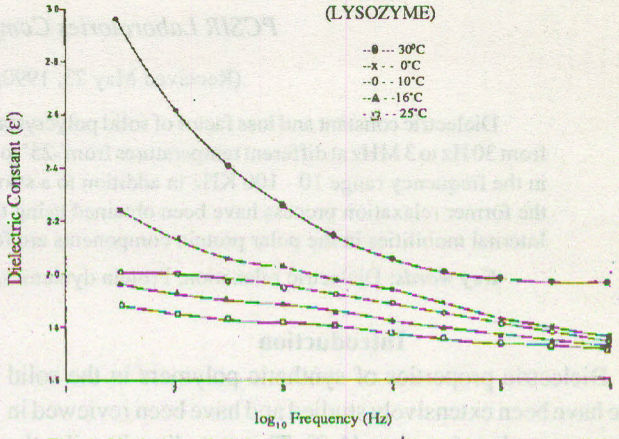

Fig. 1. Variation of dielectric constant (ε') with frequency at different temperatures.

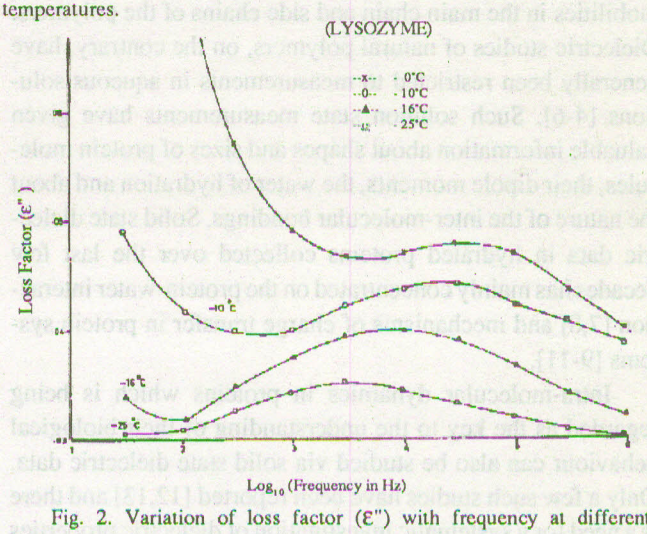

Fig. 2. Variation of loss factor (ε") with frequency at different temperature.

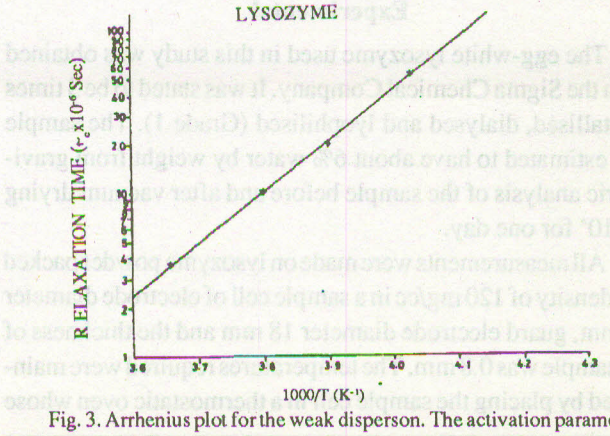

Fig. 3. Arrhenius plot for the weak disperson. The activation parameters are discussed in text.

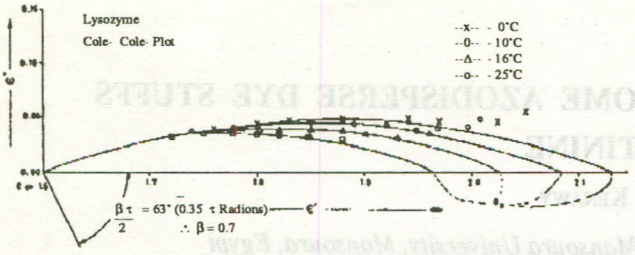

Fig. 4. Cole-Cole plots of lysozyme data at different temperatures (solid lines are best fits of semi-circular arcs for the ∝'-dispersion.) (Lower frequency points much higher the theoretical curve due to the lower frequency strong dispersion are excluded from the fit).

for which the strong lower frequency dispersion process was the dominant source of relaxation, were fitted to the semicircular curves given by equation 3. All low frequency data points for which the strong lower frequency dispersion process was the dominant source of relaxation were excluded from the fit. The diameter of the best fitted semicircles made an angle of 63 degrees (0.35 radians) with the real axis, which gave the value of the width parameter β as high as 0.7. No notable change in the width parameter of the distribution of correlation times with the change of temperature has bee observed in the temperature range of our observations.

A slight increase in the strength of dispersion ($\varepsilon_s - \varepsilon_\infty$ has been found with the increase in temperature indicating an increase in intramolecular mobilities of the polar protein components with the increase of temperature. Following the theories of Debye and Onsager, it can be shown that (20 -21).

$$\frac{(\varepsilon_s - \varepsilon_\infty)\ (\varepsilon_s + \varepsilon_\infty)}{\varepsilon_s\ (\varepsilon_\infty + 2)^2} = \frac{N < m >^2}{9\varepsilon_0 kT} \quad \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

where $<m>^2$ is the mean square of the total dipole moment.

From equation 4 $N<m>^2$ was found to vary from 2.19×10^{-32}. C^2/m at -25° to $3.41 \times 10^{-32} C^2/m$ at 0°. Lysozyme is composed of 129 residues and has a molecular weight of 14600, so that for our sample, the concentration of peptide units is $6.38 \times 10^{26}/m^3$. The values obtained for $N<m>^2$ would then correspond to an effective dipole moment per peptide unit of 0.73×10^{-29} c.m. at 0° which decreases to 0.59×10^{-29} c.m. at -25°. These values of dipole moments are about half the value of the estimated dipole moment of a peptide group (1.2×10^{-29} c.m.) [20] and therefore could have been resulted from some sort of limited relaxation of the peptide moments in the polypeptide backbones.

Out of 129 residues of lysozyme 80 are polar. Considering the observed dispersion to be the result of the dipolar relaxation of the polar side chain components of the protein, the dipole moment per polar side chain is estimated to range from 0.93×10^{-29} c.m. at 0° to 0.74×10^{-29} c.m. at -25°. These values are consistent with the average dipole moment of a polar amino acid side chain (as estimated from the considerations [20]. The low value of the mean activation energy, a wide spread in the relaxation times and an increase in dispersion strength with the increase in temperature also supports this view point. There is also a possibility of some sort of correlation motion of the main chain and the polar side chains as found in poly- γ - benzyl glutamate [20]. This would however require a higher value of activation energy.

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