Bioelectret state in amylase: a study by the thermally stimulated discharge current technique

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Abstract: The bioelectret state has been proposed to be a universal property of enzymes and to play an important role for the catalytic action of enzymes. In the present investigation the bioelectret state in an enzyme amylase (EC 3.2.2.1) has been studied using the thermally stimulated discharge current (TSDC) technique. The enzyme has been found to be able to manifest the bioelectret state; the TSDC spectrum is characterized by four peaks at temperatures around 170 K, 220 K, 265 K and 300 K. The 265 K peak is attributed to rotation of some polar amino acid residues, whereas bound water molecules are identified as the source of polarization storage for all other peaks. The 170 K peak is ascribed to bound water molecules directly attached to the polypeptide backbone; the 220 K peak is ascribed to a second layer of multiple hydrogen-bonded water molecules, proposed to be a chain-like structure around the polypeptide backbone, and the 300 K peak is attributed to the bound water molecules adsorbed on the surface of the enzyme matrix. The existence of several relaxation polarizations supports the Fröhlich model of longitudinal polarization waves that are proposed to play an important role for the catalytic action of the enzyme.

Keywords: bioelectret state; enzyme action; bound water; amylase

INTRODUCTION

The study of physical properties of enzymes is of special importance as it lies on the borderline where the biological and physical sciences meet. From the point of view of physics, the enormous efficiency of enzymes as bio-catalysts poses the question whether this involves certain general physical properties common to all the enzymes. The importance of electrical polarization properties of enzymes for their catalytic action has been pointed out by Frohlich[1–3] and Green[4] who suggested that there are strong connections between charge storage and conformational properties of enzymes. Change in polarization storage in enzyme macromolecules induces changes in their conformation that are of paramount importance for their catalytic action.

The state of persistent polarization, ie the bioelectret state, has been proposed to be a universal property of enzymes,[5] and continuous experimental evidence is needed in a variety of enzymes. It is in this context that a systematic study of the polarization properties of the enzyme amylase (EC 3.2.2.1) has been undertaken in our laboratory. The results of our studies on frequency/temperature dependence of dielectric constant/loss factor and a c conduction[6] have demonstrated the great influence of bound water molecules on the dielectric behaviour of amylase. Therefore, in the present investigation we have employed the thermally stimulated discharge current (TSDC) technique to study the polarization storage in amylase under varied hydration. The TSDC technique, applied to a variety of natural and synthetic biopolymers, has provided a valuable contribution to the understanding of complex behaviour of water–biomolecule systems.[7] TSDC is specially suited to our objective because: (a) it is highly sensitive, able to detect effects due to bound water at hydration levels as low as 10 mg water per gram of enzyme, and (b) it can detect processes with very long relaxation times (10^{-1} to 10^{3} s).

MATERIALS AND METHODS

The enzyme used in the present investigation was procured from Central Drug House (CDH), India, in powder form. The purity of the material was established by thin layer chromatography and column chromatography. The powder was compressed to make pellets of diameter 13 mm and thickness ranging between 0.5 and 2 mm. The pellets were provided with non-injecting silver electrodes[8] and samples with the desired degree of hydration (7–20 %) were obtained using a standard procedure.[9]

The TSDC technique consists of studying the

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thermally activated release of stored dielectric polarization. In this technique, the specimen is first polarized at room temperature (poling temperature, T_p) by applying a d c electric field (poling field, E_p) and then with field kept on, it is cooled to low temperature (usually to 100 K), so that the polarization is frozen. Now the field is removed, the sample is short circuited and is heated at a linear rate; depolarization takes place, giving rise to a discharge current in the external circuit. The general form of TSDC is

$$I(T) = A \exp[-E/kT - B \int_{T_0}^{T} \exp(E/kT) \mathrm{d}T]$$

where *I(T)* is the externally measured discharge current as a function of absolute temperature T. T_0 is the starting temperature of TSDC, E is the activation energy of dipole-rotation or trap depth of the charge carriers, k is the Boltzmann constant, while A and B are constants depending on the type of polarization and heating rate.

A number of expressions have been suggested for calculating activation energy E.[10] Knowing the activation energy, the relaxation time τ can be calculated as

$$\tau = \tau_0 \exp(E/kT)$$

where the pre-exponential factor τ_0, for peak temperature T_m and heating rate b, is given by

$$\tau_0 = [kT^2/bE] \exp(-E/kT)$$

The discharge current is measured with the help of a sensitive electrometer, and when plotted as a function of temperature shows a number of peaks characteristic of the mechanism by which the polarization storage takes place. An analysis of the TSDC spectrum can be made by varying various poling parameters, and hence the sources for polarization storage and related phenomena may be studied. A standard experimental apparatus[11] was used in the present investigation to measure the TSD currents in the temperature range 100–320 K. The measurements were made in the range of poling field 1–4 kV cm^{-1}, poling temperature 295–320 K, thickness of the sample 0.5–2 mm, and degree of hydration (defined as the ratio of the weight of the sorbed water to the weight of the dried sample) 7–20 %.

RESULTS

A representative TSDC spectrum of amylase is shown in Fig 1. The various poling parameters were as follows: (i) poling field 2 kV cm^{-1}, (ii) poling temperature 300 K, (iii) heating rate 2.5 ± 0.3 K min^{-1}, (iv) sample thickness 1 mm, and (v) degree of hydration 9 %. The spectrum is characterized by peaks at temperatures around 170 K, 220 K and 265 K, and a very sharp peak of large magnitude at around 300 K. The 'activation energy' and 'relaxation-time' values corresponding to these peaks are tabulated in Table 1.

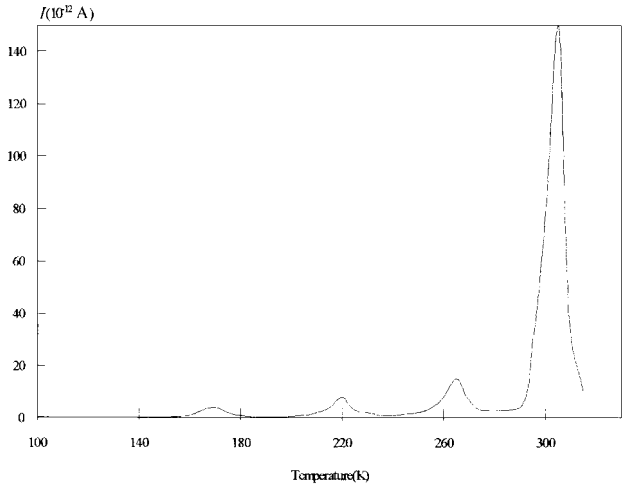

Figure 1. TSDC spectrum of amylase (E_p=2 kV, T_p=300 K, h=9 %).

To study the nature of these peaks and hence to identify the possible sources of polarization storage, the analysis of the TSDC spectrum is attempted by systematically varying the poling parameters and the degree of hydration as discussed below.

The TSDC spectra were recorded for four poling field strengths (1, 2, 3 and 4 kV cm^{-1}) keeping all other parameters the same. It is observed that with the increasing poling field the 170 K, 220 K and 265 K peaks show a linear increase in peak magnitude and area under the curve: however, a saturation effect is observed for the 300 K peak, as can be inferred from Fig 2. The TSDC spectra recorded for various poling temperatures show that the change in poling temperature has no effect on peak magnitude or peak temperature for the 170 K, 200 K and 265 K peaks, but an increase in peak magnitude and a shift in peak temperature towards the lower side is observed for the 300 K peak, as shown in Fig 3.

The hydration has a pronounced effect on the TSDC spectrum of amylase. An increase in peak magnitude and a shift in peak temperature towards the lower side are observed for the 170 K, 220 K and 300 K peaks, as is evident from Figs 4–6. This observation suggests that the polarization corresponding to 170 K, 220 K and 300 K peaks is directly related to bound water present in the system. The 265 K peak does not shift with increasing hydration; however, a small increase in peak magnitude is observed. This peak is masked by 300 K at higher hydration.

Table 1. Activation energy and relaxation time corresponding to various TSDC peaks of amylase

Peak temperature (K)	*Activation energy (eV)*	τ_0	τ (s)
170	0.4	7.50×10^{-12}	2.5605
220	0.8	3.21×10^{-18}	2.1444
265	1.0	7.92×10^{-19}	2.4888
300	2.0	3.35×10^{-33}	1.5948

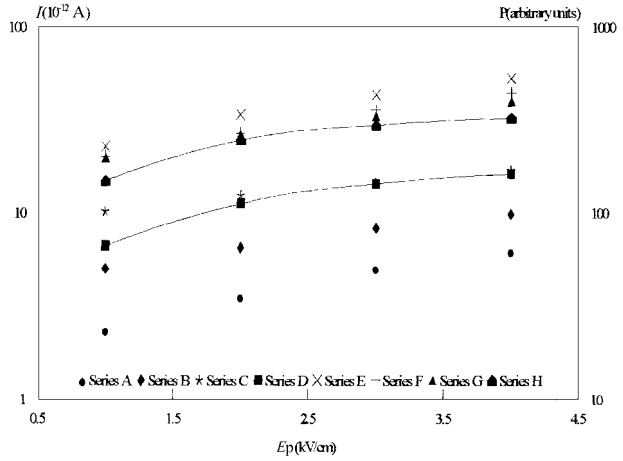

Figure 2. Effect of poling field on peak magnitude and stored polarization. (Series A, B, C: peak magnitude (*I*) for 170K, 220K and 265K peaks, respectively. Series E, F, G: stored polarization (*P*) for 170K, 220K and 265K peaks, respectively. Series D, H: peak magnitude (*I*) and stored polarization (*P*) for 300K peak, on Y_2 axis.).

DISCUSSION

The observed effects of poling field and poling temperature on the TSDC spectrum suggest that the 170K, 200K and 265K peaks are due to dipolar reorientation, while the 300K peak is due to space charge.[10,12–14] The 170K peak has been observed in almost all the hydrated biomaterials studied so far, and has been attributed to the reorientation of bound water molecules, probably attached to polar residues or to the peptide bonds.[10,15–20] These bound water molecules correspond to 'structured' water deeply located inside the enzyme matrix and are able to present dipolar rotation. The activation energy is found to be around 0.4eV. For pure water, a peak at about 160K is reported[16] and the corresponding activation energy is found to be around 0.3eV. The higher value of activation energy in the case of amylase agrees with the suggestion that the peak is due to water molecules that are bound structurally in the enzyme matrix. Similar results have been observed in some other hydrated systems.[21–24]

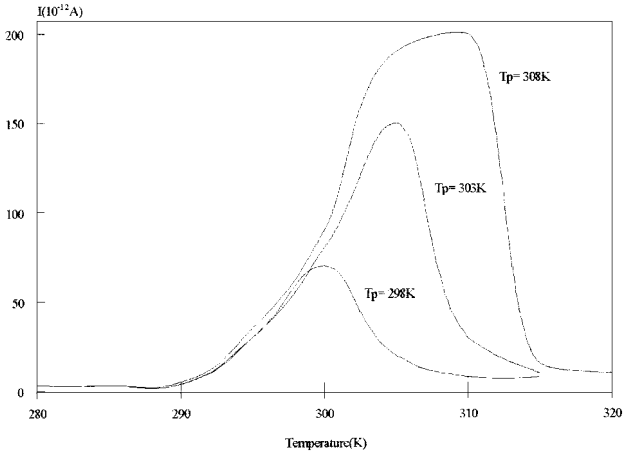

Figure 3. Effect of polarizing temperature on peak magnitude of 300K peak.

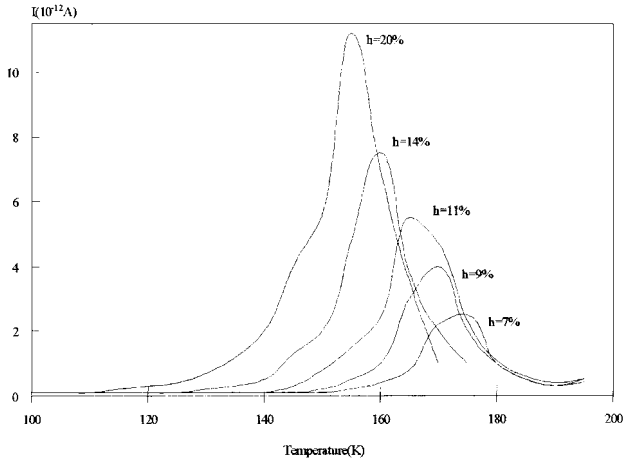

Figure 4. Effect of hydration on peak magnitude of 170K peak.

The 220K peak has also been observed in many hydrated biomaterials,[10,17–19] and could be attributed to a second layer of bound water molecules. A peak at 180K (along with the 160K peak) has been observed in the TSDC spectrum of pure water.[17] In fact, water in the macromolecules may be found in several states.[16,17,25,26] The peak occurring at around 220K may be attributed to the multiple hydrogen-bonded water molecules, the dipoles of which can rotate only by breaking several bonds. This is supported by the observed higher value of activation energy (around 0.8eV), which is double the activation energy value for the 170K peak.

In the case of the 265K peak, the source of polarization storage may be the rotation of some polar side-groups of amylase macromolecules. As suggested by the increase in peak magnitude with increasing hydration, the addition of water may increase the rotational mobility of side-groups responsible for the 265K peak. A complex band in the temperature range 230–260K has been reported for the protein casein, for which the possible source of polarization is suggested to be rotation of some side-groups affected by the bound water molecules.[10] It is noteworthy that at the same temperature, ie around 265K, a peak is obtained in the

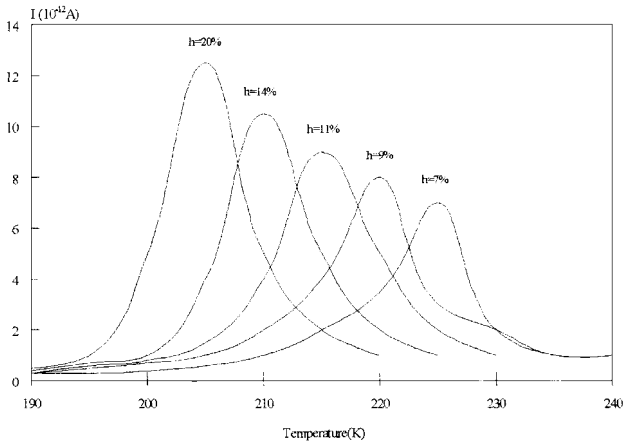

Figure 5. Effect of hydration on peak magnitude of 220K peak.

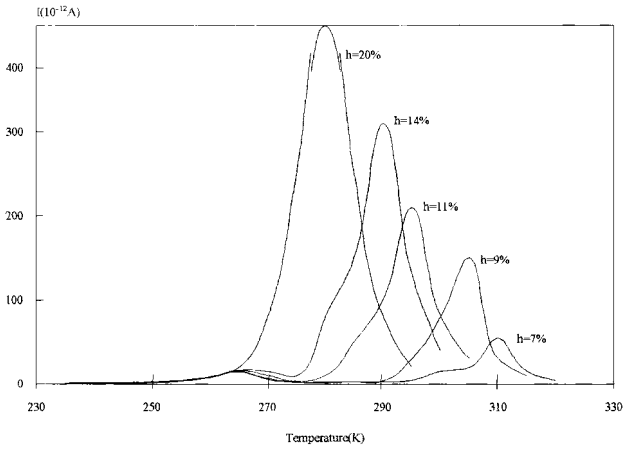

Figure 6. Effect of hydration on peak magnitude of 300 K peak.

TSDC spectrum of glycine,[27] one of the active-site components of amylase; even the activation energy values are comparable in both cases, being around 1 eV. Thus, in the case of amylase also, it could be concluded that the rotation of some amino acid residue or associated side-group in the peptide linkage is responsible for the observed peak around 265 K.

The 300 K peak could be attributed to the water molecules bound at the surface of the macromolecules.[21] These are the molecules most remote from the polypeptide chain and are very weakly bound; they could be considered to have the usual water properties, namely they will freeze at low temperature. They may undergo transition from the bound to the free state very easily and be easily removed by dehydration.[16,17] They may be the possible source for polarization storage through Maxwell–Wagner type space-charge polarization, giving rise to a peak around 300 K in the TSDC spectrum of amylase. The observed very high activation energy value (around 2 eV) supports the above suggestion.

The results of the present investigation support the Fröhlich[3] model of longitudinal polarization waves that are proposed to play a fundamental role in the catalytic action of enzymes. From very general theoretical considerations, Fröhlich concluded that, (i) enzymes should have highly polar metastable states, and (ii) these should be capable of dipole vibrations, because the excitation of the highly polar metastable state is coupled with excitation of dipole oscillations. Because the enzyme conformation is largely dominated by the interaction of its charged groups with the surrounding water molecules, such polarization induces the conformational changes that are of paramount importance for the catalytic action of enzymes. In the case of amylase, four relaxation polarizations are observed with long enough relaxation time (about 2 s) to support Fröhlich's suggestion of polar metastable states, ie the enzyme may sustain polarization waves. The presence of calcium ions is found to be necessary for the catalytic action of amylase, which may induce an electric field of high enough magnitude to induce the bioelectret state in the enzyme.

CONCLUSIONS

The enzyme amylase has been found to manifest the bioelectret state. The observed polarization storage may be attributed to the rotation of polar amino acid residues or associated side-groups, and bound water molecules present in three different states in the amylase macromolecule. Four relaxation polarizations are observed, with long enough relaxation times to sustain the Fröhlich waves that are proposed to play a fundamental role in the catalytic action of enzymes.

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