Pak. j. sci. ind. res., vol. 36,nos. 2-3, Feb. - Mar. 1993

HYDRATION INDUCED FLEXIBILITY IN CYTOCHROME-C

T.Z. Rizvi, A. Shamim* and M.A. Mirza*

Applied Chemistry Research Centre,PCSIR Laboratories Complex, Lahore 54600, Pakistan

(Received May 25, 1992; revised February 25, 1993)

Real and imaginary parts of dielectric constant have been measured in hydrated cytochrome C at various hydration levels in the frequency range 30 Hz-3 MHz at temperatures between (-40°) - (+30°).The data reveal the presence of a very weak and broad relaxation peak in the intermediate frequency range between two commonly observed Ω and α dispersions.This weaker dispersion has been attributed to Debye type relaxation of polar protein components. Considerable hydration induced flexibility has been observed in the polar components of the protein. Such studies could be beneficial towards understanding the important role of water in protein dynamics and functioning.

Key words : Cytochrome-C, Protein hydration, Dielectric relaxation.

Introduction

Dielectric behaviour of biomolecules in the solid state is of great importance in understanding the charge transport and various charge polarization processes at molecular and sub-molecular levels [1-3]. Biologically important processes like protein-water interaction and intramolecular dynamics in proteins can also be understood in terms of dielectric behaviour of hydrated protein systems [4,5]. Charge polarization and transport processes are specially important in cytochrome-C, which is an electron carrier protein that functions in electron transport from cytochrome reductase to cytochrome oxidase multienzymatic complexes. Present studies were undertaken to study the hitherto unresolved role of water in charge polarization and intra-molecular dynamical processes in cytochrome-C.

Experimental

Sample preparation. Horse heart cytochrome-C was obtained from Sigma Chemical Co. Four different hydration levels were achieved. The driest possible sample was prepared by subjecting the sample to a vacuum of the order of 10^{-5} Torr for atleast 72 hrs. at room temperature. Higher hydration levels, were achieved by subjecting the driest sample to known relative humidity values in close containers containing appropriate salt solutions. Amounts of water gained by the samples were measured gravimetrically.

Dielectric measurements. All measurements were made using Ando's complete measuring set (TRS-10T) which constituted of a bridge (TR-10C) in combination with a null detector (BDA-9) and an oscillator (WBG-9). The measured quantitites were the capacitance (Cx) and conductance (Gx) of the samples. Static relative permittivity ε and loss ε factor were calculated as

$$\varepsilon' = \frac{Cx}{Ca} \text{ and } \varepsilon'' = \frac{Gx}{\omega Ca}$$

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

Figures 1- 3 show the variation of dielectric constant with frequency at various temperatures in samples with 0.5%, 3.2% and 5% water respectively. It may be noted that these weights also include strongly bound water which could not be removed even after the evacuation procedure. The amount of this strongly bound water, as suggested by the loss of weight of the sample after evacuation at about 105C was estimated to be about 0.5% of the total weight of protein which corresponds to about 3 - 4 water molecules per protein molecule. This is in agreement with X-ray crystallographic studies of both ferro- and ferricytochrome C refined at 1.5 and 1.8°A° resolution

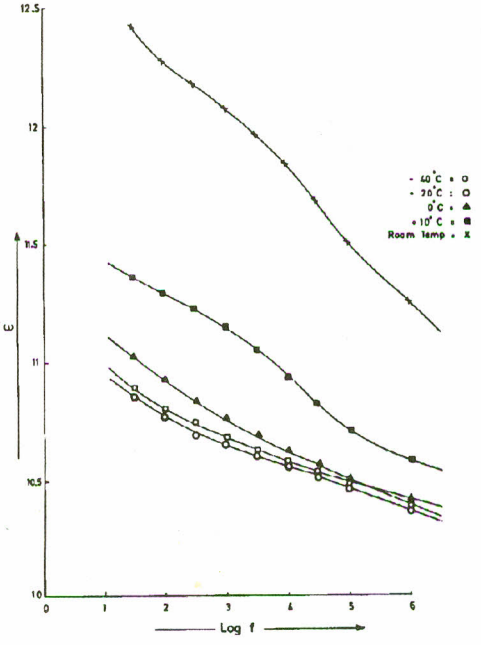

Fig.1. Dielectric constant *vs.* log f of driest possible sample (I) at different temperatures.

*Centre for Solid State Physics,Punjab University, Lahore, Pakistan.

respectively [6], which show the presence of 3 water molecules buried between Asn 52, Tyr 67 and Thr-74 in both the states of cytochrome-C. Figures 4-6 show the frequency dependence of dielectric loss factor (ε"); the imaginary part of the complex dielectric constant (ε* = ε' - i ε" in the three samples.

The data reveal the presence of three distinct dielectric dispersion processes. The lowest frequency Ω -process which is strongly hydration dependent and increases rapidly with increasing hydration level at temperatures above O° (the normal freezing point of water). This was attributed to charge accumulation at electrode-sample interface as suggested by observed d^{-2} (d is the electrode separation) dependence of the measured capacitance values at lower frequencies and higher hydration values. The effect of electrode polarization on the measured impedence of a biological system has been thoroughly investigated and discussed by Schawn [7]. Such a dispersion has also been observed in many other protein samples [3].

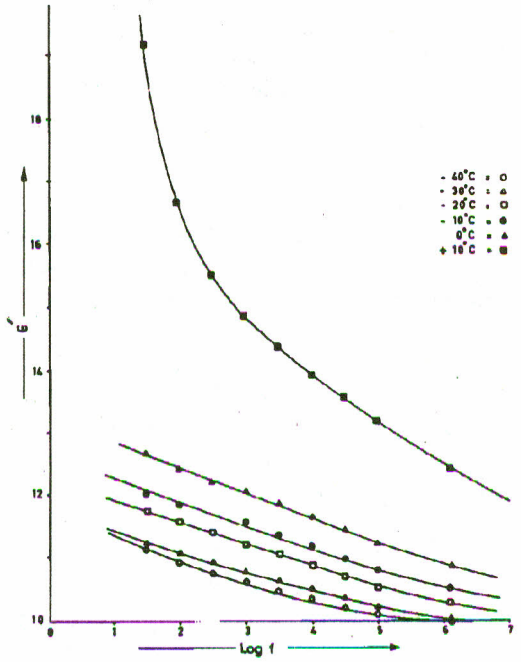

Fig. 2. Dielectric constant *vs.* log f in sample (II) of 3.2% hydration level. (At various temperatures).

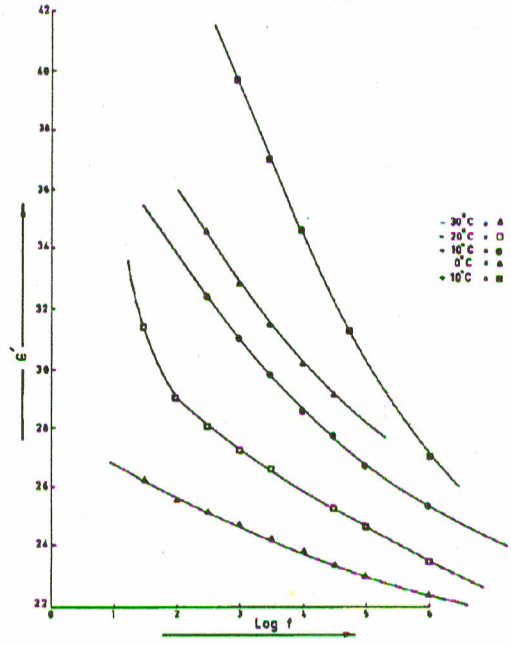

Fig. 3. Dielectric constant *vs.* log f in sample (III) of 5% hydration level, at various temperatures.

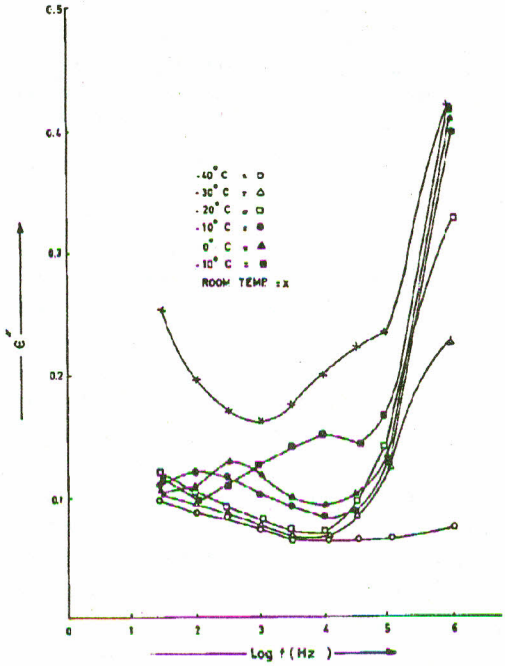

Fig. 4. Loss factor *vs* log f driest possible sample (I) (0.5%H_2O), at various temperatures.

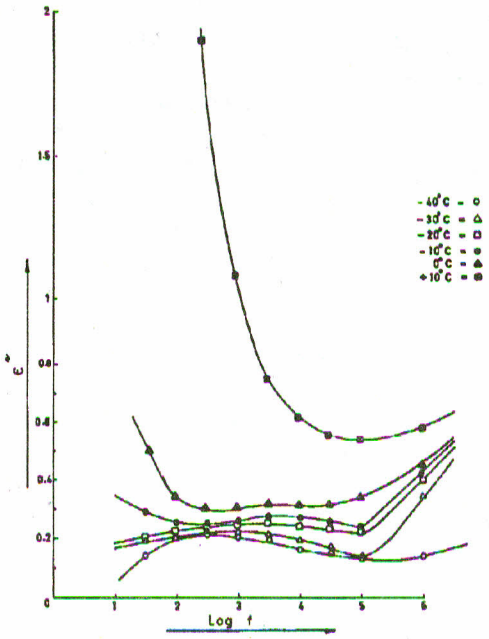

Fig. 5. Loss factor *vs* log f of sample (II) of 3.2% hydration level at various temperatures.

Another strong dispersion which is generally termed as α– dispersion in most proteins and polypeptides is also discernible at highest observed frequencies. As no peaks of these two dispersions could be observed in the observable frequency range of our measuring set at all desired and attainable temperatures, further analysis of these dispersions, regarding details about the strength of the dispersions and their exact activation parameters was not pursued.

A very broad temperature activated dispersion peak centred at around 100 Hz - 30 KHz was observed, at intermediate frequencies. This will be referred to α' as dispersion and is attributed to Debye type relaxation [8] of polar protein components. It may be noted that the cytochrome-C molecule contains 104 peptide residues, out of which 65 have polar side chains. Peptide units of all the residues are also polar. There are many internal degrees of freedom within the conformed structure of proteins and almost all portions of a molecule are capable of hindered internal motions with a large variation in the time scales. All the polar species with motional frequencies corresponding to the dielectric measurement frequency are capable of causing a Debye type relaxation peak in the frequency dependence curve of dielectric loss factor. Dispersions of this magnitude and frequency range have also been observed for solid films of poly (methyl-L- glutamate) (PMLG) and poly (benzyl-L-glutamate) (PBLG) [9,10] and were ascribed to vibrational motions of the side chains, complicated with some sort of main chain segmental mobilities in case of PBLG. It may be noted that this dispersion peak being very weak could only be observed at very low hydration values where the relaxation due to the Ω dispersions is also very small. The dielectric loss increases tremendously with the increase of hydration due the accompanied rapid increase in the Ω dispersion. At hydration values equal to or greater than 5% the Ω dispersion becomes strong enough to completely overmask the effect of the weak α' dispersion. This effect of disappearance of the α' peak at 5% hydration value may be noted in Fig. 6.

In order to obtain some information about the activation parameters of the observed α' disperion, relaxation times of the dispersion process were calculated as the reciprocal of frequency of maximum loss from the dielectric curves and plotted as a function of reciprocal temperature. The temperature dependence of relaxation time can be used to estimate the activation parameters assuming the dielectric relaxation to be a rate process whereby the rotating unit moves between two equilibrium positions separated by a potential barrier. According to this picture the relaxation time will be a measure of the number of times per second that such a process will occur. According to Eyring [11] the correlation time of the reorientational process is given by the following relation.

$$\tau_c = \frac{h}{kT} \exp\left(\frac{\Delta F}{RT}\right); \qquad (1)$$

h → Plank's constant.

k → Boltzmann's contant.

where Δ F is the free energy of activation. R the gas constant and T the absolute temperature. As Δ F = Δ H - T. Δ S where Δ H is the molar enthalpy of activation and Δ S the molar entropy of activation for the relaxation process, equation 1 can be written as

$$\tau_c = \frac{h}{kT} \exp\left(-\frac{\Delta S}{R}\right) \exp\left(\frac{\Delta H}{RT}\right) \qquad (2)$$

It is obvious since the variation of entropy with temperature is much less than the exponential term, a plot of log against reciprocal temperature (the Arrhenius plot) produces an approximate straight line whose slope provides Δ H and intercept Δ S.

Arrhenius plots of only two samples with low hydration values are shown in Fig. 7 where the peaks are well separated from other dispersions. Slopes of these plots show an inverse relation between the anthalpy of activation and the hydration level of the sample. Enthalpy of the process decrease τ_c from 28.5 k cal/mole at 0.5% hydration to about 9.1 k cal/mole at 3.2% hydration. This shows that the hindering barriers for the molecular reorientations get smaller with the increase of hydration level which can be understood in terms of the plasticising action of the bound water. Possibility of the bound water acting as a plasticizer to facilitate protein flexibility has also been discussd by Bone and Pethig [4].

The values of molar entropies at 300 K, as obtained from the Y-intercepts of the Arrhenius plots, are -2.97 cal/deg mole for 3.2% hydrated sample and 61 cal/deg mole for 0.5% hydrated sample. Since ΔS is related to the number of all possible activated structures, the -ve entropy value for higher

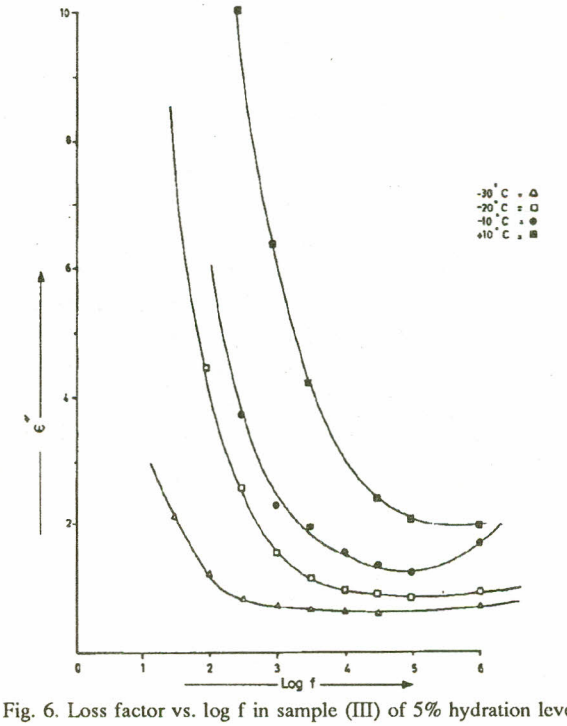

Fig. 6. Loss factor vs. log f in sample (III) of 5% hydration level at various temperatures.

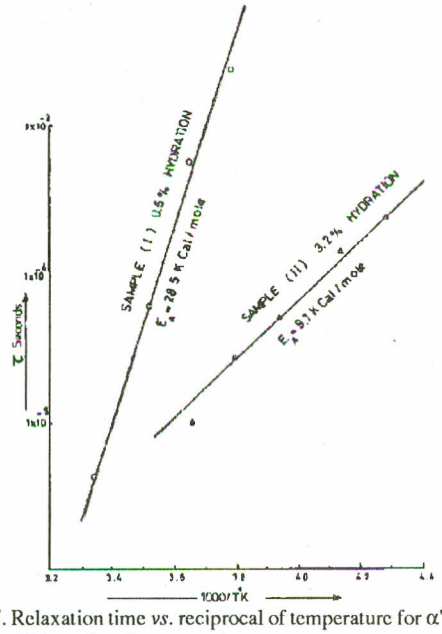

Fig. 7. Relaxation time *vs.* reciprocal of temperature for α' relaxation in samples I and II on a log-linear graph.

hydrated sample indicate the presence of slightly more ordered activated state than the normal state. This can be attributed to the availability of more water molecules to form ordered hydrogen bonded structures. A positive molar entropy in case of less hydrated sample, on the other hand, reflects the more disordered activated state. The free energy of activation is

TABLE 1. ACTIVATION PARAMETERS FOR DIPOLAR ORIENTATION AT 300 K.

Sample	H(K cal. /mole)	S(K cal. /deg mole)	F(K cal. /mole)
I	28.5	6.1×10^{-2}	10.1
II	9.1	-2.9×10^{-3}	10.0

positive for both the samples as required by the theory of rate processes and is a pproximately of the same value for both the samples. The activation parameters of the relaxation process for both the samples are summarised in Table 1.

Recently a similar dispersion referred to as α_2 – dispersion has also been reported in lysozyme and some homopolypeptides [5] which was attributed to the vibrational motion of the polypeptide backbone. Most properties of the dispersion data presented here, such as frequencies of maximum loss, strength of dispersion and hydration dependence of the strength and fequency of the dispersion are found to be very similar to those of the newly reported α_2 – dispersion. The low molar enthalpy values obtained here (Table 1), however, suggest that the major contribution to the observed relaxation comes from the mobilities of the polar side chain components which are expected to experience activation barriers of comparable magnitudes [10]. Backbone mobility of the protein, which is suggested to be the main cause of disperson [5] however, is considered to be less probable here as this would require much higher anthalpy barrier to account for the steric hindrances. No activation parameters are available in the referred studies for comparison to say definitely if the dispersion observed here is the same as that reported in literature [5].

The broad and shallow peak of the dispersion clearly shows a large distribution in relaxation times which depicts the variety of environments and hindering barriers for the polar protein components as should be expected for a complicated heterogeneous system like that of a hydrated protein.

Acknowledgement. The authors thankfully acknowledge the cooperation and helpful comments of Dr. M. Akbar Saeed, Head Applied Chemistry Research Centre, PCSIR Laboratories, Lahore and Professor M. Suleman, Director, Centre for Solid State Physics, Punjab University, Lahore.

References

1. D.D. Eley, N.C. Lockhart and C.N. Richardson, J.C.S. Faraday Trans. **I, 75**, 323 (1979).
2. J. Eden, P.R.C. Gascoyne and R. Pething, J.C.S. Faraday Trans. **I, 76**, 426 (1980).
3. Ronald Pethig, Ferroelectrics, **86**, 31 (1988).
4. S. Bone and R. Pethig, J. Mol. Biol., **181**, 323 (1985).
5. J.J. Hawkes and R. Pethig, Biochimica et Biophisica Acta, **952**, 27 (1988).
6. T. Takano, R. Dickerson, J. Mol. Biol., **153**, 79, 95 (1981).
7. H.P. Schwan, *Determination of Biological Impedences Physical Techniques in Biological Research*, W.L. Nastuk (ed.) (Academic Press, N.Y., 1963), Vol. VI.
8. P. Debye, *Polar Molecules* (Dover Publication, New York, 1929).
9. A. Tanaka and Y. Ishida, J. Polym. Sci. Polym. Phys., **11**, 1117 (1973).
10. R. Pethig, *Dielectric and Electronic Properties of Biologi cal Materials* (J. Wiley and Sons, Chichester, 1979).
11. S. Glasstone, K.J. Laidler and H. Eyring, *The Theory of Rate Processes* (McGraw-Hill, New York, 1941). pp. 341-351.