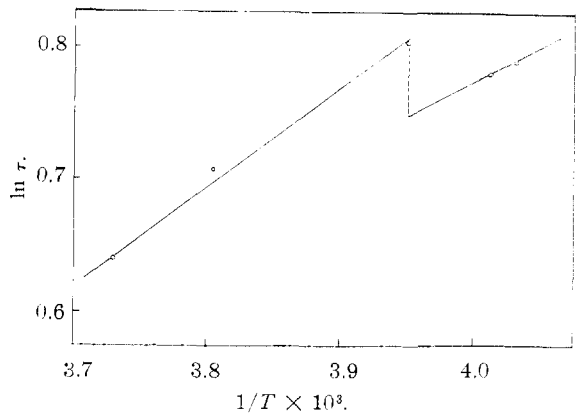

Fig. 7.—The temperature dependence of relaxation time of oxyhemoglobin crystal, ln τ *vs.* $1/T$.

entropy although the entropy decrease in the solution is small. The entropy change of the dielectric polarization may be considered to be the sum of the increase of the breaking of the directed valence bonds of solvent molecules and the decrease due to the orientation of the molecules in the direction of the field. But the orientation of the whole molecule is unlikely in the solid sample and the polarization may occur in such a way that it does not break the directed hydrogen bonds of water.

The marked difference between the thermodynamic quantities of the solid and solution supports our conclusion that the dielectric increments have different origins. However, it is unlikely that the dielectric increment of the protein solid has the same mechanism as that of ice, since the heat of activation of the protein solid is much smaller than that of ice and the entropy change very different. This fact eliminates the possibility of the polarization of hydrogen bonds by simple elastic displacement of protons in protein molecules and their environments.

TABLE II

THERMODYNAMIC QUANTITIES OF DIELECTRIC POLARIZATION OF HEMOGLOBIN

	ΔH^*, kcal.	ΔF^*, kcal.	ΔS^*, cal.
O_2Hb(crystal)	2.74	6.49	−14.3
COHb(soln.)[a]	5.56	7.80	− 7.7
Water[b]	4.05	2.30	5.67
Ice[c]	13.2	9.0	18.1

[a] S. Takashima, to be published in *Arch. Biochem. Biophys.* [b] C. P. Smyth, "Dielectric Behavior and Structure," Chap. 4, McGraw-Hill Book Co., Inc., New York, N. Y., 1955. [c] S. Glasstone, K. J. Laidler and H. Eyring, "The Theory of Rate Processes," McGraw-Hill Book Co., Inc., New York, N. Y., 1941.

These complex phenomena can only be explained by the assumption that at least two polarization mechanisms are involved in the dielectric behavior of protein solution and solid. Probably the presence of various polar groups and their coördination to water molecules makes the dielectric properties of protein molecules very complicated. However, information on the dielectric losses is not available for the protein solids. Thus, we lack the experimental background necessary for further analysis of the dispersion of the solid sample.

It is a pleasure to thank Dr. H. Yamabe of the University of Minnesota and Dr. H. P. Schwan for their advice. Also the author is indebted to Prof. H. Tamiya of the Tokyo University for his encouragement and guidance.

PHILADELPHIA, PENNA.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOPHYSICS, SCHOOL OF MEDICINE, UNIVERSITY OF PENNSYLVANIA]

Dielectric Properties of Albumins

BY SHIRO TAKASHIMA

RECEIVED DECEMBER 23, 1957

The dielectric properties of egg and human serum albumins were studied with frozen solutions between −5 and −60°. Freezing of the solution results in a decrease of dielectric increment, a finding which indicates that the dielectric polarization of these proteins is largely due to the orientational polarization. However, these frozen solutions have small but well-defined dielectric increments. The dielectric polarization at low temperature was discussed in terms of the Kirkwood theory.

Introduction

The experiments with hemoglobin, described in the preceding paper, were extended to egg and human serum albumins, whose dielectric properties in solution were investigated by Oncley, *et al.*[1] The dipole moments of egg and serum albumin are about 280 and 700 debye, respectively. However, the dielectric increment of serum albumin obtained by Bayley[2] with dry crystals is extremely small.

Our results with frozen albumin solutions are essentially the same as those obtained with oxyhemoglobin. The dipole moments were calculated and compared with the theoretical values of Kirkwood.[3]

Experimental

Measurements were made as described in the preceding paper. Commercial egg and human serum albumins were used without purification, except that the solutions were dialyzed overnight before use. The concentration of protein was about 20–25 g. per liter.

The dielectric constants of egg and serum albumin and the anomalous dispersions are shown in Figs. 1 and 2. The dielectric increments per gram are 0.11 and 0.13 for egg and serum albumin, respectively, at −5°; the corresponding values at −20° are approximately 0.05 and 0.06. The

(1) J. L. Oncley, J. D. Ferry and J. Shack, *Ann. N. Y. Acad. Sci.*, **40**, 371 (1940); J. Ferry and J. L. Oncley, THIS JOURNAL, **60**, 1123 (1938).

(2) S. T. Bayley, *Trans. Faraday Soc.*, **47**, 5, 509 (1951).

(3) J. Kirkwood and J. B. Schumaker, *Proc. Nat. Acad. Sci.*, **40**, 371 (1940).

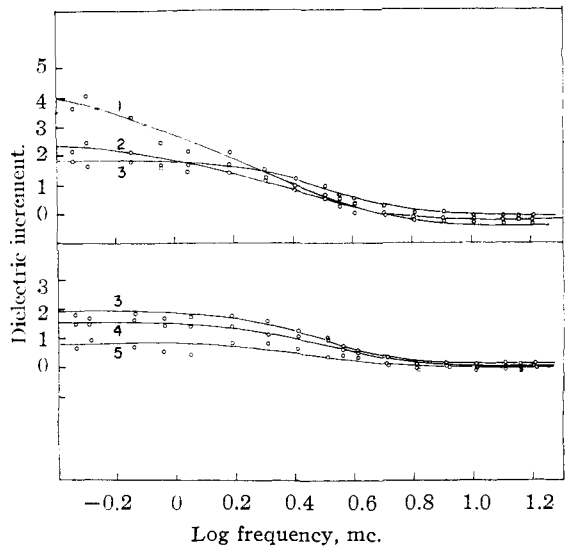

Fig. 1.—The dielectric constant of frozen solution of egg albumin. Curves from 1 to 5 were obtained at −5, −10, −30, −40 and −60°, respectively.

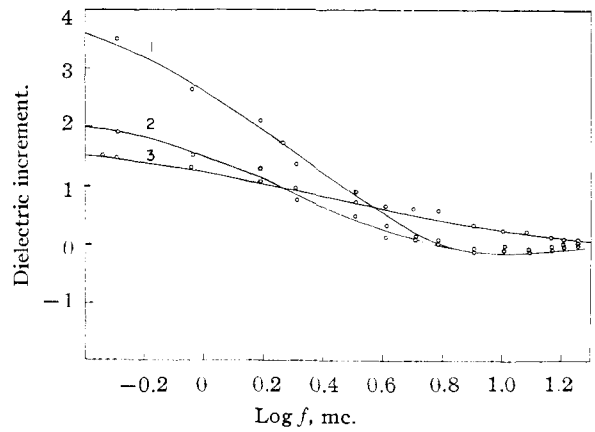

Fig. 2.—The dielectric constant of frozen serum albumin solution. Curves 1, 2 and 3 were obtained at −5, −10 and −20°, respectively.

temperature dependences of dielectric increments of both proteins are shown in Fig. 3. Figure 4 shows temperature dependence of the relaxation times of both albumins. In both cases the relaxation time decreased rapidly between −5

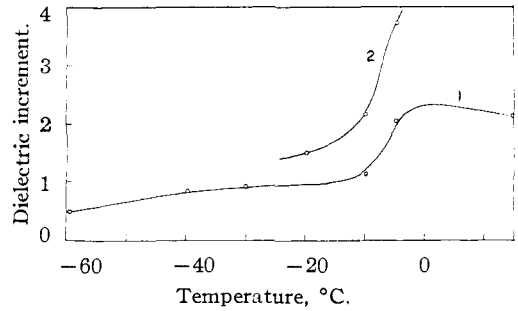

Fig. 3.—The temperature dependence of the dielectric increment of egg and serum albumins. Curve 1, egg albumin and curve 2, serum albumin.

and −20°. The negative temperature dependence of τ may be due to the phase shift or to a transition of polarization mechanism. However, as can be seen from the figure,

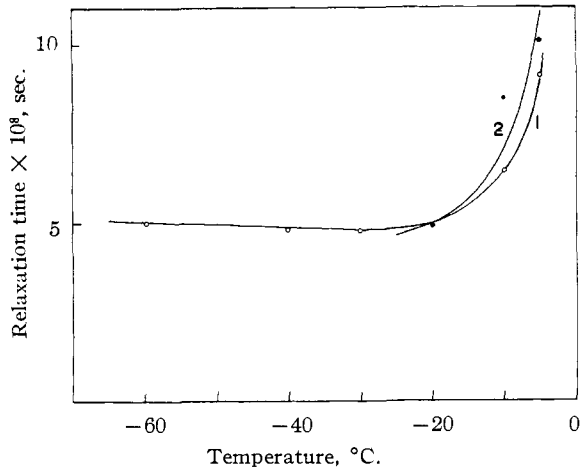

Fig. 4.—The temperature dependence of the relaxation time of egg and serum albumin. Curve 1, egg albumin and curve 2, serum albumin.

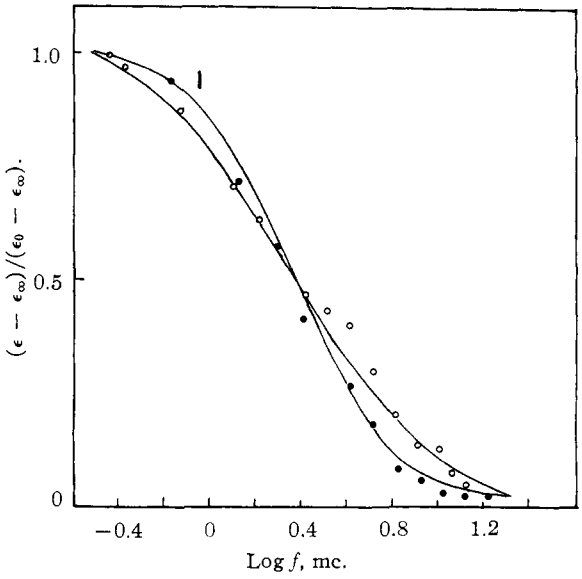

Fig. 5.—The normalized dispersion curve of serum albumin. Curve 1 is the Debye dispersion curve, closed circles at −5° and open circles at −20°.

the relaxation time of egg albumin reaches a constant value below −20°.

The normalized dispersion curves of egg albumin, shown in Fig. 5, were compared with Debye's dispersion curve. The dispersion at −5° fits the Debye curve markedly well, but the curve at −20° deviates considerably from it.

Discussion

The results observed on albumin are essentially the same as those for oxyhemoglobin which were reported in the preceding paper. The dielectric increment of serum albumin solution decreases on freezing, a change which seems to be due to the loss of the freedom of rotation of the whole molecule. The relaxation time has a similar temperature dependence. However, neither albumin shows a sharp order–disorder transition; the dielectric increment values decrease gradually on freezing and approach asymptotically to a lower plateau. This was observed markedly in the case of egg albumin. As is shown in Fig. 3, the freezing of the

solution does not give rise to a marked decrease in the dielectric increment; on the contrary the value at $-5°$ is larger than that of 25°.

The dipole moments were calculated as described in the preceding paper, and listed in Table I. It is assumed that the axial ratio of both proteins is 5:1. As can be seen from the table, the dipole moment of egg albumin in the frozen state is of the same order of magnitude as that of the solution; however, the dipole moment of serum albumin drops considerably on freezing. The results on egg albumin may be interpreted in two ways: (1) the molecule has the same freedom of orientation in ice as in solution; or (2) the dielectric polarization of this protein is entirely due to the sorbed water or to mobile proton fluctuation in solution as well as in ice. The asymptotic decrease of the dipole moment below $-10°$ to a lower value favors explanation (1). However the dimensions of the molecule and its shape indicate that orientation of the whole molecule in ice will require a large heat of activation, since the orientation might accompany the breaking of the directed hydrogen bonds of ice. However, the temperature dependence of the relaxation time which is shown in Fig. 4 gives the heat of activation as about 1 kcal., which indicates that orientation of the whole molecule in ice may not be the case.

TABLE I

THE COMPARISON OF THE CALCULATION AND OBSERVED DIPOLE MOMENT OF EGG AND SERUM ALBUMINS

Temp., °C.	μ_{obs} Egg alb.	μ_{obs} Serum alb.	μ_{cal} Egg alb.	μ_{cal} Serum alb.
− 5	270	360	330	320
−10	210	300	(sphere)	(sphere)
−20	..	220	460	510
−30	180		(ellipsoid)	(ellipsoid)
−40	150			
−60	120			
Soln.(25°)	250	700		

As in the case of hemoglobin, the dipole moments calculated from the data on the albumins are not far from the theoretical values of Kirkwood; see Table I. This strongly suggests that proton fluctuation is a possible mechanism for the dielectric polarization of these proteins.

Bayley[2] who worked with dry albumin crystals observed a very small dielectric constant, but he also found that the presence of excess moisture increased the dielectric constant and dielectric loss. However, as mentioned in the preceding paper, it is obvious that the polarization which was observed in the present experiment cannot be attributed merely to the polarization of sorbed water. The polarization may be due partly to the dielectric polarization of the sorbed water itself, but there is the possibility that water increases the dissociation of polar groups and thus may enhance the fluctuation of protons.

The quantitative interpretation of the anomalous dispersion of the solid sample requires further study, particularly to explain the shift of the dispersion curve and its shape between -20 and $-30°$.

The author is indebted to Dr. H. Yamabe of the University of Minnesota, Department of Mathematics, and to Dr. H. P. Schwan for the helpful discussions.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF SYDNEY]

The Resolution of the Quinquedentate Cobalt(III) Complexes with Ethylenediaminetetraacetic Acid

BY FRANCIS P. DWYER[1] AND FRANCIS L. GARVAN[1]

RECEIVED APRIL 21, 1958

The resolution of the quinquedentate cobalt(III) complexes with ethylenediaminetetraacetic acid with nitro, chloro and bromo groups occupying the sixth coördination position has been effected with optically active *cis*-dinitrobis-(ethylenediamine)-cobalt(III) chloride. Treatment of the active bromo and chloro complexes with mercury(II) nitrate solution or solid silver oxide caused a quantitative transformation to the sexadentate complex with complete retention of configuration. When the optically active sexadentate complex was treated with concentrated hydrochloric acid, it was converted to the quinquedentate complex in which a chlorine is attached to the central metal ion, with some retention of configuration. On reaction with ethylenediamine, all of the complexes exchanged to give tris-(ethylenediamine)-cobalt(III) ion. Only the nitro complex yielded an inactive product.

Introduction

The sexadentate function of ethylenediaminetetraacetic acid, (H_4Y), in the anion, (ethylenediaminetetraacetato)-cobaltate(III),[2] has been established by the infrared studies of Busch and Bailar.[3] The complex ion also has been separated into the optical forms.[3-5] Quinquedentate Co(III) complexes in which one carboxyl group of the organic moiety is not attached to the metal have been obtained as the acid salts $[Co(HY)X]^-$, (X = Br, NO_2) by Schwarzenbach,[6] who reported that when the sexadentate anion, $[Co(Y)]^-$, was

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(2) It is considered desirable with organic molecules capable of multidentate chelate function that the nomenclature applied to the metal complex should distinguish clearly between groups or atoms that are coördinated and those that are unattached, especially when the stereochemistry of the complex has been established. The name, potassium (ethylenediaminetetraacetato)-cobaltate(III) implies coördination of all carboxyl groups; whereas potassium chloro-(ethylenediaminetriacetatoacetic acid)-cobaltate(III), implies coördination of the chlorine and three of the carboxyl groups, with the fourth carboxyl group unattached to the octahedral sphere of the complex.

(3) D. H. Busch and J. C. Bailar, THIS JOURNAL, **75**, 4574 (1953).

(4) F. P. Dwyer, E. C. Gyarfas and D. P. Mellor, *J. Phys. Chem.*, **59**, 296 (1955).

(5) F. P. Dwyer and F. L. Garvan, "Inorganic Syntheses," H. Rochow, Ed., Vol. VI, in publication.

(6) G. Schwarzenbach, *Helv. Chim. Acta*, **32**, 839 (1949).