thus obtained was a dark red oil consisting essentially of 2,4,6-tri-(dimethylaminomethyl)-*m*-cresol. It was soluble in cold water but only sparingly soluble in hot water. It can be distilled under a good vacuum; b. p. 200° (0.5 mm.). The crude oil was converted without distillation or purification to (VI) as follows:

A mixture of 84 g. of the above red oil and 153 g. of acetic anhydride was boiled under reflux for two and three-quarters hours and the reaction product fractionated under reduced pressure. The fraction boiling at 184–204° at 1–2 mm. was collected; yield 103 g. Upon redistillation the product came over at 194–204° (1 mm.) as a colorless oil. *Anal.* $C_{18}H_{22}O_8$: sap. value calcd., 612.8. Found: 608.

Summary

1. Phenol and *m*-cresol each react with excess formaldehyde and strongly basic non-aromatic secondary amines to take up three tertiary aminomethyl groups in the free ortho and para positions to the hydroxyl group. These free bases on acetylation with acetic anhydride split off the tertiary amino groups and form the tetra-acetates of the corresponding but hitherto unisolated trimethylolphenol or tri-methylol-*m*-cresol.

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[CONTRIBUTION FROM THE DEPARTMENT OF PHYSICAL CHEMISTRY, HARVARD MEDICAL SCHOOL]

Studies of the Dielectric Properties of Protein Solutions. III. Lactoglobulin

BY JOHN D. FERRY AND J. L. ONCLEY

From measurements of the dielectric constant of a protein solution over a wide range of frequency, a description of the protein is obtained in terms of the dielectric increment and the form of the anomalous dispersion; and deductions may be drawn concerning the electrical symmetry and the size and shape of the molecule. Studies of solutions of hemoglobin[1] and of the water-soluble proteins of normal horse serum[2]—albumins and pseudoglobulin—have been reported previously from this Laboratory. Measurements upon egg albumin,[3] insulin[4] and edestin[5] have also been carried out and will be reported in detail later. The present paper is concerned with lactoglobulin.

The crystalline lactoglobulin of Palmer[6] can be prepared in a highly purified state, and its solubility in dilute salt solutions has been found to be nearly constant and independent of the amount of saturating body.[7] Furthermore, its salting-in in dilute salt is very large, suggesting a high value of the dipole moment of the molecule.[8] It seemed of particular interest, therefore, to investigate the dielectric properties of this protein.

Being a globulin, it has a very low solubility in water, and in aqueous solutions the increase in dielectric constant over that of water is too slight to permit its measurement with much accuracy. Although it is easily soluble in dilute salt (about 0.01 *M*), solutions with conductivities corresponding to over 5×10^{-4} *M* salt cannot be measured with our present apparatus. However, solutions of lactoglobulin in 0.25 and 0.50 *M* glycine can be studied easily.

The dielectric measurements here reported were made in conjunction with the solubility measurements of Cohn, Ferry and Blanchard.[9] Two preparations of crystalline lactoglobulin (I and II) were very kindly furnished by Dr. A. H. Palmer. Portions of these crystals, after being washed with conductivity water, were rotated in double glass-capped solubility bottles at 5 or 25° for periods of from two to seven days with successive portions of 0.25 or 0.50 *M* glycine. After filtration by the usual procedure of this Laboratory,[10] the filtrates were used for solubility and for dielectric constant measurements at 0 or 25°. For analysis, the protein was coagulated by heating to 100° in the presence of *M*/4 sodium chloride and was filtered on sintered glass, washed free of glycine, dried at 105°, and weighed.

Method.—The dielectric measurements on preparation I of lactoglobulin were made exactly as described in the first papers of this series[1,2] except that the use of a General Radio type 684A oscillator[11] permitted extending the frequency range down to 12,500 cycles.

Measurements on preparation II were made

(1) Oncley, THIS JOURNAL, **60**, 1115 (1938).
(2) Ferry and Oncley, *ibid.*, **60**, 1123 (1938).
(3) Oncley, Ferry and Shack, *Ann. N. Y. Acad. Sci.*, in press.
(4) Cohn, Ferry, Livingood and Blanchard, *Science*, **90**, 183 (1939).
(5) Oncley, *J. Phys. Chem.*, in press.
(6) Palmer, *J. Biol. Chem.*, **104**, 359 (1934).
(7) Sørensen and Palmer, *Compt. rend. trav. Carlsberg*, **21**, 283 (1938).
(8) Cohn, McMeekin, Ferry and Blanchard, *J. Phys. Chem.*, **43**, 169 (1939).
(9) Cohn, Ferry and Blanchard, to be published subsequently. Solubility measurements at two temperatures, 5 and 25°, indicated a heat of solution in 0.25 and 0.50 *M* glycine of approximately −2000 calories.
(10) Ferry, Cohn and Newman, THIS JOURNAL, **60**, 1480 (1938).
(11) General Radio Company, 30 State Street, Cambridge, Mass.

with the following alterations in the experimental method.

1. A cell constructed of two platinum cups mounted in a Lucite head was employed (Fig. 1). This had the advantages of a smaller fluid capacity, thereby requiring less solution; a smaller electrical capacity, thereby diminishing the inductance effects in the bridge and the polarization capacity correction; and complete shielding of the inner by the outer electrode, thereby allowing the use of water as a thermostat medium. The platinum cups, which were sand-blasted on the surfaces in contact with solution, were slightly tapered, and cemented to corresponding tapers on the head with Lucite cement. The cell constant, $Q(= dC/d\epsilon)$, was 5.46 $\mu\mu$fd.

2. The variable resistance in the bridge was placed in series with a 150-ohm resistance (instead of 100 ohms, as before), together with a variable capacity of about 50 $\mu\mu$fd, which was adjusted to balance the inductance of the variable resistance and hence eliminate the correction term[12] ΔC_2. At the same time, the inductance of the cell and its leads was adjusted to equal that of the standard condenser; the terms ΔC_1 and ΔC_3 accordingly cancelled, and it was unnecessary to calculate any inductance corrections.

3. The range of application was extended to low frequencies by use of a Clough-Brengle[13] type 79-U beat-frequency oscillator, range 25–15,000 cycles, and, as a detector, a General Radio Company[11] type 760 sound analyzer, range 25–7500 cycles. The Sargent radio receiver previously described was modified by the introduction of an additional condenser decade to detect frequencies down to 6300 cycles. It was possible to make satisfactory bridge balances at frequencies as low as 1000 cycles, but in practice the lower limit was 10,000 cycles, on account of difficulties discussed in the next section.

Polarization Capacity and Standardization of Low Frequency Measurements.—As described in the first paper of this series, the measured capacities are too high by an amount which increases with decreasing frequency and is attributed to polarization capacity at the electrodes. Down to 25,000 cycles, the lower limit of the original frequency range, the additional capacity ΔC_5 has been accurately represented by the expression $AG^2\nu^{-3/2}$, where A is a constant for the particular solution, G the conductance of the cell, and ν the frequency; or, since G varies but little in the frequency range where ΔC_5 appears, by the expression $B\nu^{-3/2}$. Correction for this effect was made very successfully for salt solutions and protein solutions by plotting capacities against $G^2\nu^{-3/2}$ (or simply $\nu^{-3/2}$) and determining A (or B) empirically as the slope of the resulting straight line. The dielectric increment $\Delta\epsilon$ was thus given by equation 10b, ref. 1

(12) The correction terms discussed here are those given by equations 9a to 9g, ref. 1.

(13) Clough-Brengle Co., 2815 West 19th Street, Chicago, Ill.

$$\Delta\epsilon = (C_x - B\nu^{-3/2} - C_x^0)/Q \qquad (1)$$

where C_x is the cell capacity at frequency ν with the protein or salt solution, C_x^0 the cell capacity with conductivity water, and Q the cell constant $(dC_x/d\epsilon)$, given in the first paper as 10.95.

Below 25,000 cycles, however, the plot against $\nu^{-3/2}$ often deviates from linearity, and it is no longer possible to correct for the polarization capacity effect by this simple procedure. To involve the least additional complication, a method of comparison between solutions of equal conductivities was employed. If the polarization correction is represented by $B\nu^{-3/2} + f(\nu)$, where $f(\nu)$ takes care of the departure from linearity of the $\nu^{-3/2}$ plot, and if $f(\nu)$ should prove to be dependent only on the conductivity, but not on the nature of the solute, then the difference between the corrections of two solutions of different substances or mixtures with the same conductivity should be proportional to $\nu^{-3/2}$. The two constants B and B' will in general not be the same, but their difference, ΔB, can be determined from the plot of $C_x - C_x'$ against $\nu^{-3/2}$, and the values of $\Delta\epsilon$ obtained as

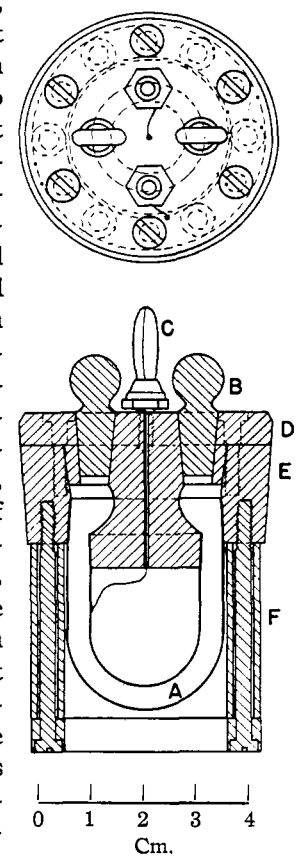

Fig. 1.—Cell for dielectric measurements: A, concentric platinum thimbles; B, 7/15 standard glass stoppers; C, General Radio plug; D, lucite top; E, lucite ring; F, brass spacers.

$$\Delta\epsilon = (C_x - C_x' - \Delta B\nu^{-3/2})/Q \qquad (2)$$

This procedure also permits neglecting the correction ΔC_4, since the latter varies only with the conductivity of the solution. Thus equations 9a to 9g of ref. 1 are no longer necessary, and equation 2 above replaces equation 10b of ref. 1.

A test of this method of correction was made by a series of measurements comparing solutions of potassium chloride, ammonium sulfate, glycine (with potassium chloride added to adjust the con-

TABLE I

DIELECTRIC INCREMENTS OF VARIOUS SOLUTIONS CALCULATED BY COMPARISON WITH KCl SOLUTIONS OF EQUAL CONDUCTIVITIES

Solution	Conductivity $\kappa \times 10^3$ Solution	Conductivity $\kappa \times 10^3$ KCl comparison	$\Delta B \times 10^3$	Dielectric increment, $\Delta\epsilon$, at various frequencies[a] ν = 0.0032	ν = 0.005	ν = 0.010	ν = 0.032	ν = 0.100	ν = 0.32	ν = 1.00	ν = 3.2
Temperature 25°											
$(NH_4)_2SO_4$	19	19	+ 2.1	−2.6	−1.1	0	0	0.2	0.2	0	0
Glycine, 0.5 M[b]	19	19	− 8.7	11.7	11.2	11.0	11.2	11.2	11.4	11.4	11.5
$(NH_4)_2SO_4$	27	27	0	−1.6	−0.4	0	0.2	0	0	0	0
Lactoglobulin in 0.5 M glycine	27	27	−21.5	22.0	22.9	22.8	22.9	22.8	Dispersion		
$(NH_4)_2SO_4$	32	32	+11.8	−8.2	−2.5	−0.2	0.2	0.2	0	0	0
Glycine, 0.5 M[b]	30	32	−15.0	7.7	9.7	11.4	11.4	11.5	11.5	11.5	11.4
Glycine, 0.5 M[b]	39	40	−29.7	−0.4	3.7	11.5	11.2	11.2	11.2	11.4	11.4
Lactoglobulin in 0.5 M glycine	40	40	−39.0	15.9	19.6	22.0	22.5	22.2	Dispersion		
Temperature 0°											
Glycine, 0.25 M	8	6	+ 5.7	5.1	5.7	6.1	6.1	6.1	6.0	6.0	5.7
Glycine, 0.5 M[c]	5	6	+ 8.4	7.5	10.6	12.6	12.8	12.8	12.6	12.4	12.4
Lactoglobulin in 0.25 M glycine	6	6	0	9.7	10.1	10.4	10.4	10.4	Dispersion		
$(NH_4)_2SO_4$	18	18	+ 1.5	0	0	0.4	0.4	0.4	0.4	0.2	0.2
Glycine, 0.5 M[c]	16	18	−11.5	11.2	11.9	12.3	12.3	12.1	12.1	12.3	12.3
Lactoglobulin in 0.5 M glycine	16	18	− 7.5	11.9	14.6	16.3	16.3	16.1	Dispersion		

[a] Calculated from the equation $\Delta\epsilon = [C_x - C_x' - \Delta B\nu^{-3/2}]Q$. [b] Wyman and McMeekin[15] give $\Delta\epsilon = 11.3$; Lindquist and Schmidt[16] $\Delta\epsilon = 11.5$. [c] Wyman and McMeekin[15] give $\Delta\epsilon = 11.9$; Lindquist and Schmidt[16] $\Delta\epsilon = 13.1$.

ductivity), and lactoglobulin in glycine.[14] Lactoglobulin is a particularly suitable protein for such a comparison, because its dispersion occurs at rather high frequencies and scarcely overlaps the polarization correction region.

Measurements were made, as usual, at frequencies spaced at approximately equal intervals logarithmically, ten to a decade. From the capacity values (C_x) of every ammonium sulfate, glycine, or lactoglobulin solution were subtracted, point by point, the capacities (C_x') of a potassium chloride solution of the same conductivity. The plot of $C_x - C_x'$ against $\nu^{-3/2}$ was much more nearly linear than that of C_x or C_x'. The slope of their plot, ΔB, was taken in the frequency region 0.01–0.03 megacycles, and values of $\Delta\epsilon$ calculated by equation 2. For the ammonium sulfate, $\Delta\epsilon$ should be zero, and for glycine it should be independent of frequency over the entire frequency range; for lactoglobulin it should be independent of frequency up to 0.1 megacycle, where dispersion begins.

The data in Table I show that, above 0.01 megacycle, these requirements are fulfilled within 0.4 unit, but below that frequency there are in some cases marked deviations. In the measurements on lactoglobulin, therefore, only data above 0.01 megacycle are reported.

(14) We are indebted to Mr. Marshall Melin for making some of these measurements.

Experimental Results

Measurements were made in 0.25 M glycine and 0.50 M glycine,[17] on Preparation I at 25° and on Preparation II at 25 and 0°. From the capacity values (C_x) of every lactoglobulin solution were subtracted the capacities (C_x') of the corresponding glycine solvent, made up with a content of potassium chloride to give approximately the same conductivity. Values of $\Delta\epsilon$ were then calculated according to equation 2.

Low-Frequency Dielectric Increments.—The low-frequency dielectric increment, $\Delta\epsilon_0$, was taken as the average of ten values of $\Delta\epsilon$ from 0.010 to 0.080 megacycle, inclusive. Table II presents values of $\Delta\epsilon_0$ and of $\Delta\epsilon_0/g$, the increment per gram per liter.

As in the case of serum pseudoglobulin,[2] the increment $\Delta\epsilon_0$ is not proportional to the concentra-

(15) Wyman and McMeekin, THIS JOURNAL, **55**, 915 (1933).

(16) Lindquist and Schmidt, *Compt. rend. lab. Carlsberg*, **22**, 307 (1938).

(17) A few measurements were made on aqueous solutions of Preparation I, of concentrations about 1 g./liter at 25°. The average value of $\Delta\epsilon_0/g$ was 1.3 ±0.4, indistinguishable from the values obtained in glycine solutions.

TABLE II

LOW-FREQUENCY DIELECTRIC INCREMENTS OF LACTOGLOBULIN SOLUTIONS

Prepn.	Concn. lactoglobulin, g./liter	pH	Conductivity, $\kappa \times 10^6$ Solution	Conductivity, $\kappa \times 10^6$ Solvent comparison	$\Delta\epsilon_0$	$\Delta\epsilon_0/g$
	Temp. 25°; Glycine concn. 0.25 *M*					
I	1.09	..	10	..	1.5	1.39
I	1.20	..	11	..	1.8	1.49
II	2.63	..	12	12	3.8	1.43
I	2.72	5.66	13	..	3.8	1.39
I	2.99	5.62	14	..	4.2	1.41
II	3.28	..	13	12	4.7	1.43
II	3.40	..	19	12	5.1	1.50
	Temp. 0°; Glycine concn. 0.25 *M*					
II	2.51	..	7	8	4.3	1.71
II	2.69	..	6	8	4.4	1.63
	Temp. 25°; Glycine concn. 0.50 *M*					
I	1.71	..	16	..	2.6	1.50
I	3.48	..	22	..	5.2	1.49
I	3.55	..	19	..	5.2	1.45
II	7.05	5.60	23	19	9.1	1.29
II	7.86	..	40	39	11.0	1.40
I	8.70	5.71	31	..	11.2	1.29
I	8.88	5.67	25	..	11.7	1.32
II	9.09	5.54	26	30	11.6	1.28
	Temp. 0°; Glycine concn. 0.50 *M*					
II	2.20	..	9	9	3.8	1.75
II	2.20	..	16	16	4.2	1.89
II	2.89	..	9	9	5.0	1.74
II	4.62	..	11	9	7.5	1.63
II	5.49	..	12	9	8.7	1.58
II	6.18	5.62	11	9	9.4	1.53
II	6.60	5.60	13	16	10.4	1.57
II	7.14	5.56	12	9	10.3	1.44
II	7.21	..	14	16	11.0	1.53
II	7.70	..	14	16	11.3	1.47

tion. It can be expressed by the following interpolation equations

$$\begin{aligned} &\text{at } 25°: \quad \Delta\epsilon_0 = 1.51\, g - 0.025\, g^2 \\ &\text{at } 0°: \quad \Delta\epsilon_0 = 1.84\, g - 0.047\, g^2 \end{aligned} \qquad (3)$$

In Fig. 2, the curves are calculated from equations (3), while the points give the experimental values. The values of $\Delta\epsilon_0/g$ thus extrapolated to infinite dilution, 1.51 at 25° and 1.84 at 0°, surpass those found for all other proteins so far studied, indicating that lactoglobulin is a highly polar molecule. The corresponding figures per mole (taking the molecular weight as 40,000) are 60,500 and 73,600.

The fact that the dielectric increment per gram of pseudoglobulin, at 25°, also falls off with increasing concentration suggests that the decrease is due to electrostatic interaction between these molecules of high polarity. Comparison of the coefficients of g^2 in equations 3 is in qualitative agreement with the calculations of Fuoss[18] for the decrease of polarization through electrostatic interaction; the decrease should be greater for molecules of greater dipole moment and at lower temperatures.

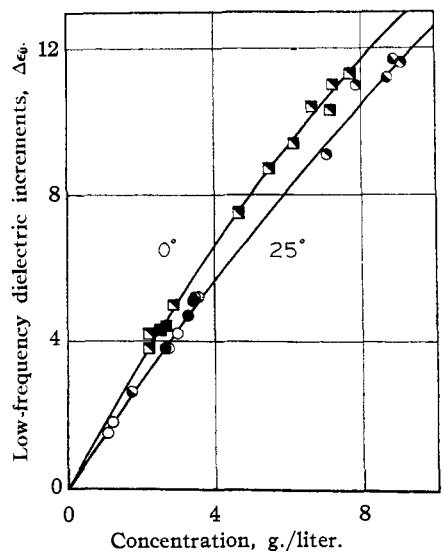

Fig. 2.—Low-frequency dielectric increments of lactoglobulin solutions: curves from equations 3; points as follows: Preparation I, 25°, ○, 0.25 *M* glycine; ◐, 0.50 *M* glycine. Preparation II, 25°, ●, 0.25 *M* glycine; ◑, 0.50 *M* glycine; 0°, ■, 0.25 *M* glycine; ◪, 0.50 *M* glycine.

Dispersion.—Measurements did not extend to quite high enough frequencies to estimate the high-frequency dielectric increments, $\Delta\epsilon_\infty$. Since, however, $\Delta\epsilon_0$ was so large, the relative contribution of $\Delta\epsilon_\infty$ to the total increment $\Delta\epsilon_0 - \Delta\epsilon_\infty$ was slight, and for the purposes of analyzing the dispersion $\Delta\epsilon_\infty$ was estimated by assuming the volume occupied by the protein to have a high-frequency dielectric constant of unity, so that

$$\Delta\epsilon_\infty = -(\epsilon^0 - 1)\bar{v}\, g/1000 \qquad (4)$$

where ϵ^0 is the dielectric constant of the solvent, and $\bar{v}$ the apparent specific volume, taken as 0.75. For the eleven runs which included measurements over the whole frequency range, values of $(\Delta\epsilon - \Delta\epsilon_\infty)/(\Delta\epsilon_0 - \Delta\epsilon_\infty)$ were calculated at each frequency. The deviations occurring in a single run can be conveniently described by plotting these values against the logarithms of the frequencies and finding the separation along the log ν axis of two curves of the form of the unbroken curves of

(18) Fuoss, THIS JOURNAL, 56, 1031 (1934).

TABLE III

DISPERSION OF LACTOGLOBULIN SOLUTIONS

ν, Frequency in megacycles	$(\Delta\epsilon - \Delta\epsilon_0)/(\Delta\epsilon_0 - \Delta\epsilon_\infty)$													
	Temperature 25°								Temp. 0°					
	Glycine concn. 0.25 M,				Glycine concn. 0.50 M				Glycine concn. 0.50 M					
	1	2	3	Av.	1	2	3	Av.	1	2	3	4	5	Av.
0.010	1.01			1.01		1.00	0.99	1.00	0.98	1.00	1.00	1.00	1.00	1.00
.0125	1.01	0.99	0.98	0.99	0.98	1.00	0.99	0.99	1.01	1.01	1.00	1.00	1.00	1.00
.0160	1.00	1.00	0.99	1.00	0.99	1.00	1.00	1.00	1.01	1.01	1.00	1.00	1.00	1.00
.0200	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.01	1.00	1.00	1.01	1.00	1.00
.0250	1.00	1.00	1.00	1.00	1.01	1.00	1.00	1.00	1.01	1.00	1.00	1.01	1.00	1.00
.032	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.01	1.00	1.00
.040	0.99	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
.050	0.99	1.00	1.01	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
.063	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.99	0.99	1.00	0.99	1.00	0.99
.080	0.99	1.00	0.99	0.99	1.00	1.00	0.99	1.00	.98	.99	0.99	.99	0.99	.99
.100	1.00	0.98	.99	.99	0.99	0.99	.98	0.99	.98	.99	.99	.98	.98	.98
.125	0.99	.98	.99	.99	.99	.99	.98	.99	.95	.98	.98	.97	.97	.97
.160	.98	.98	.99	.98	.99	.99	.98	.99	.95	.97	.97	.96	.97	.96
.200	.98	.98	.99	.98	.99	.98	.98	.98	.93	.95	.95	.94	.96	.95
.250	.97	.98	.98	.98	.98	.97	.97	.97	.91	.93	.93	.92	.93	.92
.320	.97	.96	.97	.97	.97	.96	.95	.96	.87	.88	.89	.88	.90	.88
.400	.95	.95	.94	.95	.95	.94	.93	.94	.83	.83	.84	.82	.84	.83
.500	.96	.92	.94	.94	.96	.92	.89	.93	.77	.77	.77	.75	.79	.77
.630	.94	.91	.91	.92	.93	.88	.86	.89	.71	.71	.70	.68	.70	.70
.800	.90	.87	.85	.87	.91	.83	.81	.85	.63	.61	.62	.58	.60	.61
1.00	.85	.83	.83	.84	.85	.79	.75	.80	.53	.53	.50	.50	.50	.51
1.25	.82	.79	.80	.80	.76	.72	.68	.72	.42	.43	.40	.41	.41	.41
1.60	.73	.71	.67	.70	.67	.61	.59	.62	.31	.31	.29	.30	.30	.30
2.00	.61	.59	.59	.60	.59	.52	.50	.54	.24	.23	.23	.23	.23	.23
2.50	.50	.50	.49	.50	.52	.44	.44	.47	.15	.16	.14	.15	.13	.15
3.20	.46			.46		.30	.31	.30	.09	.10	.10	.09	.10	.10
4.00	.33			.33		.22	.22	.22	.06	.06	.05	.05	.05	.05
5.00	.28			.28		.14		.14	.02	.05	.03	.02	.02	.03

Fig. 3, so placed as to contain between them all the points with $(\Delta\epsilon - \Delta\epsilon_\infty)/(\Delta\epsilon_0 - \Delta\epsilon_\infty)$ between 0.95 and 0.05. For a typical run such a pair of curves gives mid-point frequencies (*i. e.*, where $(\Delta\epsilon - \Delta\epsilon_\infty)/(\Delta\epsilon_0 - \Delta\epsilon_\infty) = 0.5$) differing by about ± 5% from their mean, ν_m. At a given temperature and glycine concentration, the values of ν_m for the several runs did not vary in any regular manner with the lactoglobulin concentration over the range employed (up to 7.7 g./liter). Accordingly the results at each frequency were averaged, as follows: at 25° in 0.25 M glycine, three runs, with values of ν_m varying by ±10%; at 25° in 0.50 M glycine, three runs, ν_m varying by ±20%; at 0° in 0.50 M glycine, five runs, ν_m varying by ±7%. The averages so obtained are given in Table III.

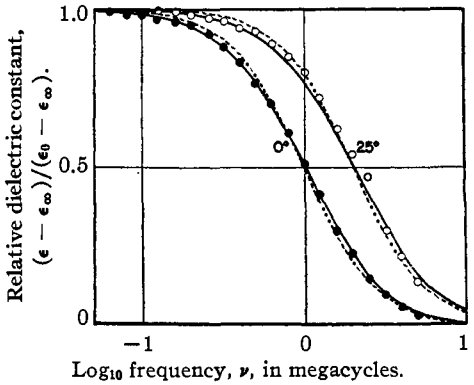

Fig. 3.—Dielectric dispersion of lactoglobulin dissolved in 0.5 M glycine, O, 25°; ●, 0°. Continuous lines, calculated from two relaxation times for an elongated ellipsoid, $(\tau_0)_{H_2O,\,25°} = 4.7 \times 10^{-8}$ sec., $a/b = 4$, $\theta = 63°$; broken lines, calculated from one relaxation time, $\tau_{H_2O,\,25°} = 7.5 \times 10^{-8}$ sec.

The mean mid-point frequencies, ν_m, derived from these average values were 2.5 megacycles at 25° in 0.25 M glycine, 2.2 megacycles at 25° in 0.50 M glycine and 1.05 megacycles at 0° in 0.50 M glycine. Now the interpretation of dispersion in terms of rotation of dipoles in a viscous medium gives for the critical frequency of a spherical molecule

$$\nu_c = RT/6\pi\eta V$$

Where V is the molecular volume and η the viscosity of the solvent. For a sphere, $\nu_c = \nu_m$,

and for any other shape the ratio ν_c/ν_m should be a function of the molecular shape only. Hence for comparison the above values of ν_m may be corrected to water at 25° by the equation $(\nu_m)_{25°,w} = \nu_m(\eta/\eta_{25°,w})(298/T)$. The viscosities relative to water at 25° of the three solvents were 1.04, 1.08, and 2.12, respectively, so that the values of $(\nu_m)_{25°,w}$ become 2.4, 2.6, and 2.4, respectively—in very satisfactory agreement.

Discussion

Dipole Moments.—The dipole moment may be estimated from the total dielectric increment by the empirical equation[1]

$$\mu = \alpha\sqrt{M(\Delta\epsilon_0/g - \Delta\epsilon_\infty/g)} \qquad (5)$$

where M is the molecular weight, and α is 2.9 at 25° and 2.8 at 0°, when μ is given in Debye units. Taking $M = 40{,}000$ and $\Delta\epsilon_\infty/g = -0.07$ at 25° and -0.08 at 0°, the dipole moment is calculated to be 730 Debye units at 25° and 770 at 0°. This difference may be due to a difference in the charge patterns at the two temperatures, arising from differences in dissociation constants of the groups dissociating in the neutral range—such as imidazole or terminal amino groups.[19] The dipole moment of lactoglobulin is somewhat greater than that of hemoglobin. This may be correlated with the effect of glycine upon the solubilities of the two proteins, as will be discussed in a subsequent paper.[9] Although the dielectric increment of lactoglobulin surpasses that of serum pseudoglobulin, and of edestin, these substances have higher dipole moments by virtue of their greater molecular weight.

Relaxation Times.—The inverse proportionality of mid-point frequency to the ratio of absolute temperature and relative viscosity as observed for lactoglobulin, supports the interpretation of anomalous dispersion in terms of orientation of dipoles in a viscous medium. According to this interpretation, we should be able to analyze the dispersion curve in terms of the size, shape and dipole angle of the lactoglobulin molecule. The difference between the size so obtained and that obtained from ultracentrifugal and diffusion studies can be explained by hydration of the molecule. Figure 3 shows the observed dispersion curves in the various solvents. The continuous curves are calculated on the basis of the two relaxation times[5] of an elongated ellipsoidal molecule with $a/b = 4$, $(\tau_0)_{H_2O,\,25°} = 4.7 \times 10^{-8}$ sec., and $\theta = 63°$; where a/b is the ratio of major to minor axes of the ellipsoid, $(\tau_0)_{H_2O,\,25°}$ is the relaxation time reduced to water at 25° for a spherical molecule with identical molecular weight, and θ is the angle between the major axis of the ellipsoid and the electric moment vector, called the dipole angle. The broken curves are calculated from the simple Debye expression for a single relaxation time, $\tau_{H_2O,\,25°} = 7.5 \times 10^{-8}$ sec. Both sets of curves fit the points reasonably well; however, the continuous curves fit somewhat better, especially the one at 0°. Since the measurements at 0° are more reliable (as evidenced by the smaller variations among the runs averaged) because of (1) the lower conductivity of the solutions at this temperature, and (2) the location of the dispersion at lower frequencies, we favor the interpretation based on the two relaxation times of an elongated ellipsoidal molecule.

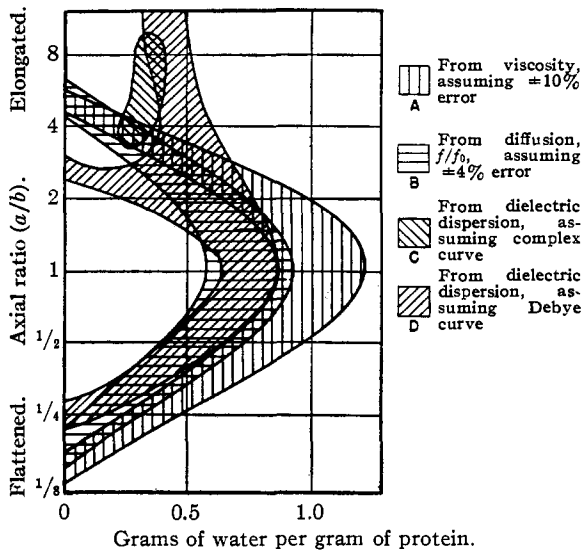

Fig. 4.—Asymmetry and hydration of lactoglobulin, calculated from viscosity, diffusion and dielectric dispersion.

In Fig. 4 we have recorded values for the hydration (calculated from the apparent size) and the shape of the lactoglobulin molecule as obtained not only from dielectric dispersion data, but also from diffusion and viscosity data, as recorded by Svedberg and Pedersen[20] and by Polson.[21] The small "island," area C, is obtained on the basis of two relaxation times, while a single relaxation time gives results falling within area D. Area A gives the results from viscosity measurements, and area

(19) Cannan, *Cold Spring Harbor Symp. Quant. Biol.*, **6**, 1 (1938).

(20) Svedberg and Pedersen, "The Ultracentrifuge," Oxford University Press, London, 1940.

(21) Polson, *Kolloid. Z.*, **88**, 51 (1939).

B from diffusion measurements. It would appear that a ratio of about 4 for a/b, and a hydration of about 0.3 g. of water per g. of protein would be in good agreement with all the available data.

Summary

1. The effect of polarization capacity upon the measurements of dielectric constants of conducting solutions has been further investigated.

2. The dielectric constants of solutions of lactoglobulin in 0.25 and 0.50 M glycine have been measured at 0 and 25° over a frequency range of 10,000 to 5,000,000 cycles.

3. The low-frequency dielectric increments per gram per liter, extrapolated to zero concentration of lactoglobulin, are in both solvents 1.51 at 25°, and 1.84 at 0°. These figures diminish with increasing concentration.

4. The dipole moments are estimated to be 730 Debye units at 25° and 770 at 0°.

5. The mean mid-point frequency is approximately proportional to the ratio of absolute temperature and relative viscosity of the solvent.

6. The dispersion curves have been analyzed and the results compared with those obtained from ultracentrifuge, diffusion and viscosity measurements. All these data can be interpreted in terms of an elongated ellipsoidal molecule of axial ratio (a/b) 4 and hydration 0.3 g. of water per gram of protein.

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The Crystal Structure of Iodic Acid

BY MAX T. ROGERS AND LINDSAY HELMHOLZ

Introduction

Although reports have been published on the crystal structures of all the alkali iodates, no completely satisfactory structure determination of any iodate has been made. The isomorphous crystals cesium iodate, rubidium iodate, potassium iodate, and ammonium iodate are pseudocubic and are said to give no powder lines indicating any deviation from the ideal perovskite structure assigned to them.[1,2,3] This places a regular octahedron of six oxygen atoms around each iodine atom with the distance I–O = 2.23 Å. and gives the alkali atom a coördination number of twelve. Sodium iodate, which is orthorhombic, has been assigned a deformed anti-perovskite structure the alkali atom having a coördination number of six and the iodine atom twelve. Lithium iodate is hexagonal with a reported structure based on hexagonal closest packing,[4] in which octahedra of oxygen atoms surround both lithium and iodine atoms, the IO_6 octahedra sharing corners and the LiO_6 octahedra sharing faces.

Potassium iodate and the isomorphous rubidium, cesium and ammonium compounds are known to be monoclinic hemihedral from crystallographic and piezoelectric measurements and the assignment of a simple cubic structure to them on the basis of powder pictures is unsatisfactory since the intensities of the lines are rather insensitive to the oxygen parameters and could be roughly accounted for by an essentially incorrect structure. The deviations from the ideal perovskite structure may also be appreciable for sodium iodate since the observed axial ratios 0.903:1:0.636 differ considerably from those of a cubic perovskite structure in that orientation, *viz.*, 1:1:0.707. The oxygen parameter in lithium iodate is not closely enough known to give the oxygen positions with any certainty. Hence none of the structure determintions can be accepted as showing what the configuration of the iodate group is.

One might expect on chemical grounds and from the correlation of Raman spectra in crystals and solution[5] that discrete iodate groups or simple polymers would exist in the crystal rather than regular IO_6 octahedra with shared corners. The observed I–O distance (2.23 to 2.33 Å.) in these crystals seems rather large in view of the value 1.93 Å. observed in KIO_2F_2 and $(NH_4)_2H_3IO_6$,[6]

(1) V. M. Goldschmidt, "Geochem. Vert. Gesetze der Elemente," VII and VIII.

(2) W. H. Zachariasen, *Skrifter Norske Videnskapsakad. Oslo I, Mat. Natur. Klasse*, 1928, No. 4.

(3) J. Garrido, *Anales soc. espan. fis. quim.*, **30**, 811 (1932).

(4) W. H. Zachariasen and F. A. Barta, *Phys. Rev.*, **37**, 1628 (1931).

(5) James Hibben, "The Raman Effect and Its Chemical Applications," A. C. S. Monograph, 1939, p. 378.

(6) L. Helmholz and M. T. Rogers, THIS JOURNAL, **62**, 1537 (1940).