Massachusetts Institute of Technology, June, 1952. It is a pleasure to acknowledge the guidance and encouragement of Prof. F. O. Schmitt in this work.

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[1] Hall, C. E., Jakus, M. A., and Schmitt, F. O., *J. App. Phys.*, **16**, 459 (1945).

[2] Bear, R. S., *J. Am. Chem. Soc.*, **66**, 2043 (1944). Cannan, Cecily, M. M., Ph.D. Thesis, MIT, June, 1950.

[3] However, the small angle diffraction pattern of intact clam muscle shows, in addition to the paramyosin reflections, a set of reflections corresponding to the so-called "myosin" net. The latter reflections are relatively strong in the red and weak in the white portions of the muscle, an observation in accord with the fact that the white portions contain a larger proportion of paramyosin. Furthermore, it seems likely that the treatment used in preparation of purified suspensions of paramyosin fibrils (maceration in dilute salt solution and repeated centrifugation) results in the elimination of the material giving rise to the "myosin" net reflections (presumably actomyosin).

[4] Highberger, J. H., Gross, J., and Schmitt, F. O., PROC. NATL. ACAD. SCI., **37**, 286, 1951.

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# *THE INFLUENCE OF DIPOLE MOMENT FLUCTUATIONS ON THE DIELECTRIC INCREMENT OF PROTEINS IN SOLUTION**

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Proteins in solution are known to produce[1] remarkably large increments in the dielectric constant of water. These increments have been conventionally attributed to large permanent molecular electric dipole moments, which are partially oriented by an external electric field. An alternative mechanism of dielectric polarization of macromolecules containing a large number of loosely bound ions, for example, protons, is provided by the migration of the small ions within the molecule under the action of the external field. For proteins, containing a number of neutral and negatively charged basic sites, such as $-NH_2$ and $-COO^-$, to which protons are bound, this mechanism might be especially significant. Except in highly acid solutions, the number of basic sites generally exceeds the average number of protons bound to the molecule, so that there exist many possible configurations of the protons, differing little in free energy. Fluctuations in the configuration of the protons should be associated with a non-vanishing mean square electric dipole moment, even if the mean permanent moment were zero. In the present article we shall investigate

the contribution to the dielectric polarization of protein molecules arising from fluctuations in charge and configuration of mobile protons in the molecule. We shall show that the dielectric increments of many proteins can be entirely accounted for by this mechanism of polarization without postulating the existence of permanent electric moments. Fluctuations in the charge and configuration of mobile protons also make an important contribution to the force between protein molecules. A theory of intermolecular forces of this new type will be presented in a later article.

For liquid solutions of high dielectric constant, the theories of Onsager[2] and Kirkwood[3] lead to the following relation between the dielectric constant $D$ and the molar polarizations $P_j$ of the several components,

$$D = 1 + \frac{9}{2}\sum_j P_j$$

$$P_j = \frac{4\pi N}{3}\left[\alpha_j + \langle \mu_j^2 \rangle_{\text{av.}}/3kT\right] \qquad (1)$$

where $\alpha_j$ is the optical polarizability and $\langle \mu_j^2 \rangle_{\text{av.}}$ is the mean square of the dipole moment of a molecule of type $j$. Correlations in the orientation of neighboring molecules, treated in the theory of Kirkwood, will be neglected here, an approximation adequate for protein molecules in aqueous solution. For the mean square dipole moment of a protein molecule, averaged over-all configurations of its mobile protons, we shall write,

$$\langle \mu^2 \rangle_{\text{av.}} = \langle \boldsymbol{\mu} \rangle_{\text{av.}}^2 + \Delta\mu^2$$

$$\Delta\mu^2 = \langle (\boldsymbol{\mu} - \langle \boldsymbol{\mu} \rangle_{\text{av.}})^2 \rangle_{\text{av.}} \qquad (2)$$

where $\langle \boldsymbol{\mu} \rangle_{\text{av.}}$ is the mean permanent dipole moment and $\Delta\mu^2$ is the dipole moment fluctuation associated with fluctuations in charge and configuration of the mobile protons.

We consider a protein molecule containing $\nu$ basic groups of intrinsic charges, $e_i$, situated at points $\mathbf{R}_i$ relative to the molecule center of mass. We define a proton occupation variable $x_i$ for each basic group to be unity when that group is occupied by a proton and zero otherwise. The electric moment $\boldsymbol{\mu}$ of the molecule, its mean value $\langle \boldsymbol{\mu} \rangle_{\text{av.}}$ and fluctuation $\Delta\mu^2$ may then be expressed in the form,

$$\boldsymbol{\mu} = \sum_{i=1}^{\nu} (e_i + e x_i)\mathbf{R}_i$$

$$\langle \boldsymbol{\mu} \rangle_{\text{av.}} = \sum_{i=1}^{\nu} (e_i + e\langle x_i \rangle_{\text{av.}})\mathbf{R}_i$$

$$\Delta\mu^2 = e^2 \sum_{i,k=1}^{\nu} [\langle x_i x_k \rangle_{\text{av.}} - \langle x_i \rangle_{\text{av.}} \langle x_k \rangle_{\text{av.}}]\mathbf{R}_i \cdot \mathbf{R}_k \qquad (3)$$

where $e$ is the protonic charge, and the averages are to be taken over all distributions, $x_1, \cdots x_\nu$ of the protons among the basic sites of the molecule. It is to be remarked that neither $\langle \mu \rangle_{av.}$ nor $\Delta\mu^2$ are independent of the origin to which they are referred, even at the isoionic point, because of fluctuations in net charge. They should properly be referred to the center of drag of the molecule, which is here assumed identical with the center of mass, which is true if non-linear hydrodynamic terms are neglected.

$$\langle x_i \rangle_{av.} = \sum_{x_1 \cdots x_\nu = 0}^{1} x_i e^{\beta[A_c - W_c(x_1 \cdots x_\nu)}$$

$$\langle x_i x_k \rangle_{av.} = \sum_{x_1 \cdots x_\nu = 0}^{1} x_i x_k e^{\beta[A_c - W_c(x_1 \cdots x_\nu)}$$

$$e^{-\beta A_c} = \sum_{x_1 \cdots x_\nu = 0}^{1} e^{-\beta W(x_1 \cdots x_\nu)}$$

$$\beta = 1/kT \tag{4}$$

where $W_c(x_1 \ldots x_\nu)$ is the local free energy of proton configuration, $x_1 \ldots x_\nu$, and $A_c$ is the total configurational free energy. The pertinent mean values $\langle x_i \rangle_{av.}$ and $\langle x_i x_k \rangle_{av.}$ over-all proton configurations may be calculated by methods previously developed by Kirkwood[4] in the theory of acid-base equilibrium of ampholytes.

$$\langle x_i \rangle_{av.} = \frac{\partial \log G}{\partial \log \lambda_i}$$

$$\langle x_i x_k \rangle_{av.} = \frac{\lambda_i \lambda_k}{G} \frac{\partial^2 G}{\partial \lambda_i \partial \lambda_k}$$

$$G = \sum_{x_1 \cdots x_\nu = 0}^{1} B(x_1 \ldots x_\nu)[H^+]^{\sum_{i=1}^{\nu} x_i}$$

$$B(x_1 \ldots x_\nu) = \left[\prod_{i=1}^{\nu} \lambda_i^{x_i}\right] e^{-\alpha\left[\sum_{i=1}^{\nu} (e_i + e x_i)\right]^2/e^2}$$

$$\lambda_i = (K_i e^{+\alpha})^{-1}$$

$$\alpha = \frac{e^2}{2DbkT} \frac{1}{1 + \kappa b} \tag{5}$$

where $b$ is the radius of the protein molecule, assumed spherical, $K_i$ is the intrinsic dissociation constant of the acid conjugate to the basis group $i$, $[H^+]$ is the hydrogen ion activity, and $\kappa$ is the ionic strength parameter of the Debye-Hückel theory. If electrostatic interaction between the protons is neglected, we obtain the simple result,

$$\langle x_i \rangle_{\text{av.}} = \frac{1}{1 + K_i/[H^+]}$$

$$\langle x_i x_k \rangle_{\text{av.}} - \langle x_i \rangle_{\text{av.}} \langle x_k \rangle_{\text{av.}} = 0; \qquad i \neq k$$

$$\langle x_i^2 \rangle_{\text{av.}} - \langle x_i \rangle_{\text{av.}}^2 = \frac{1}{2 + K_i/[H^+] + [H^+]/K_i} \tag{6}$$

In this approximation, the dipole moment fluctuation becomes

$$\Delta\mu^2 = e^2 \sum_{i=1}^{\nu} \frac{R_i^2}{2 + K_i/[H^+] + [H^+]/K_i} \tag{7}$$

If there are $\nu_\alpha$ equivalent basic groups of type $\alpha$ in the molecule we may write,

$$\Delta\mu^2 = e^2 \sum_{\alpha} \frac{\nu_\alpha R_\alpha^2}{2 + K_\alpha/[H^+] + [H^+]/K_\alpha} \tag{8}$$

where $R_\alpha^2$ is the mean square distance of groups of type $\alpha$ from the molecular center of mass. A further simplification is possible, if we assume that all basic groups are uniformly distributed on the surface of the molecule, considered as a prolate ellipsoid of revolution.

$$\Delta\mu^2 = e^2 f^2 b_0^2 \sum_{\alpha} \frac{\nu_\alpha}{2 + K_\alpha/[H^+] + [H^+]/K_\alpha}$$

$$f^2 = \frac{\sigma^{4/3}}{4} \frac{(\sigma^2 + 2)\sqrt{\sigma^2 - 1} + \sigma^2(\sigma^2 + 4)\ \text{sec.}^{-1}\ \sigma}{\sigma^2\sqrt{\sigma^2 - 1} + \sigma^4\ \text{sec.}^{-1}\ \sigma} \tag{9}$$

$$\sigma = a/b \qquad b_0 = (ab^2)^{1/3}$$

where $\sigma$ is the axial ratio of the ellipsoid and $b_0$ is the radius of the equivalent sphere.

We present in table 1 calculations of dipole moment fluctuations, based on equation (9), for some representative proteins. It will be remarked that only those groups with p$K$ values nearly equal to the pH of the solution contribute significantly to $\Delta\mu^2$. At the pH values in question for the four proteins, $\beta$-lactoglobulin, ovalbumin, horse hemoglobin, and human serum albumin, only the carboxyl and histidyl groups make appreciable contributions. For each protein, two values of $\Delta\mu^2$ are presented, one for the molecule considered as a sphere, and one for the molecule considered as an ellipsoid. The values are compared with $\langle \mu^2 \rangle_{\text{av.}}^{1/2}$, calculated from Oncley's[1] measurements of the dielectric increments of the several proteins. The experimental values are subject to considerable uncertainty and will doubtless undergo revision on the basis of more precise measurements. Nevertheless, it will be observed that the calculated

values of $\Delta\mu$ are of the same magnitude as the experimental values of $\langle\mu^2\rangle_{av.}^{1/2}$. Only in the case of $\beta$-lactoglobulin does it seem to be necessary to postulate the existence of a permanent dipole moment in order to account for the observed value of $\langle\mu^2\rangle_{av.}^{1/2}$. The dielectric constant increments of ovalbumin, hemoglobin and serum albumin are all adequately accounted

TABLE 1

DIPOLE MOMENT FLUCTUATIONS WITHOUT ELECTROSTATIC INTERACTION

| PROTEIN | $\beta$-LACTOGLOBULIN | OVALBUMIN | HORSE HEMOGLOBIN | HUMAN SERUM ALBUMIN |
|---|---|---|---|---|
| Molecular weight | 37,000 | 46,000 | 68,000 | 69,000 |
| Dissociating groups per molecule[a] | | | | |
| Arginine (pK = 12.5) | 6 | 15 | 14 | 25 |
| Histidine (pK = 6.0) | 4 | 7 | 36 | 16 |
| Lysine (pK = 10.5) | 29 | 20 | 38 | 58 |
| Tyrosine (pK = 10.1) | 8 | 9 | 11 | 18 |
| Free carboxyl (pK = 4.0) | 59 | 51 | 53 | 93 |
| pH | 5.6 | 4.8 | 6.4 | 5.0 |
| $b_0$ (Å.) | 24 | 26 | 30 | 30 |
| $a/b$ | 5 | 4 | 5 | 5 |
| $\langle\mu^2\rangle_{av.}^{1/2}$ Debye units (experimental) | 700 | 260 | 480 | 380[b] |
| $\Delta\mu$ (sphere) | 170 | 320 | 390 | 430 |
| $\Delta\mu$ (ellipsoid) | 270 | 440 | 620 | 680 |

[a] G. R. Tristram, *Advances in Protein Chem.*, **5**, 84 (1949).

[b] This experimental dipole moment is for horse serum albumin. Professor J. L. Oncley has informed us that recent measurements of the dielectric increment of serum albumin lead to a value of 700 Debye units for $\langle\mu^2\rangle_{av.}^{1/2}$.

TABLE 2

DIPOLE MOMENT FLUCTUATIONS WITH ELECTROSTATIC INTERACTION

| PROTEIN | $\beta$-LACTOGLOBULIN | OVALBUMIN | HEMOGLOBIN | SERUM ALBUMIN |
|---|---|---|---|---|
| pH | 5.6 | 4.8 | 6.4 | 5.0 |
| $\Delta\mu$ (sphere) | 360 | 330 | 300 | 320 |
| $\Delta\mu$ (ellipsoid) | 560 | 460 | 480 | 510 |

TABLE 3

EFFECT OF pH ON THE DIPOLE MOMENT FLUCTUATION OF OVALBUMIN

| pH | 2.0 | 4.0 | 4.8 | 6.0 | 8.0 | 10.0 |
|---|---|---|---|---|---|---|
| $\Delta\mu$ | 449 | 390 | 330 | 224 | 163 | 5 |

for on a semiquantitative basis, by the calculated values of $\Delta\mu^2$ arising from fluctuations in charge and configuration of the mobile protons.

The effect of electrostatic interaction between the protons has been calculated numerically at zero ionic strength with the use of equation (5) in its complete form, for the four proteins. The results are presented in table 2. It will be remarked that $\Delta\mu$ is closer to the experimental value of $\langle\mu^2\rangle_{av.}^{1/2}$ for $\beta$-lactoglobulin than without interaction. Although the

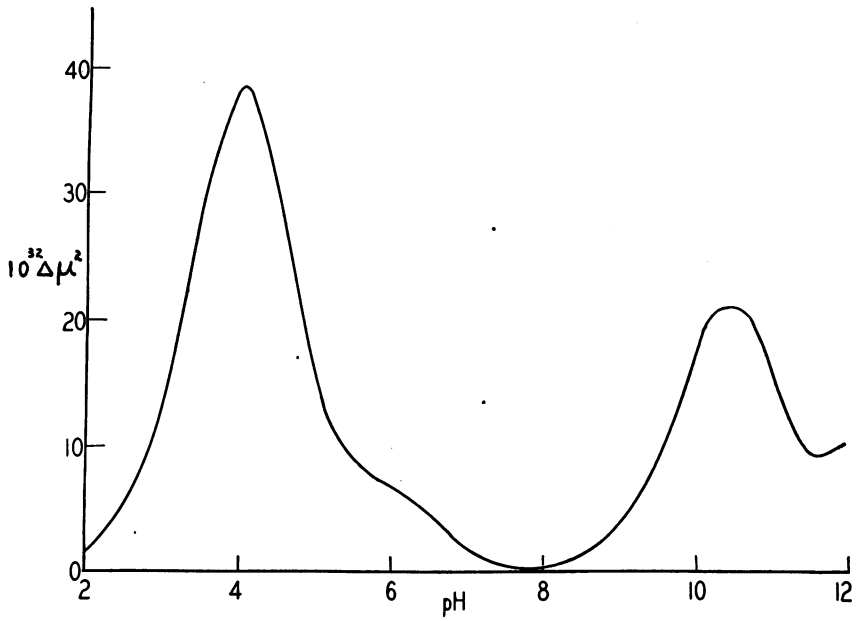


FIGURE 1.

The effect of pH on the dipole moment fluctuation of ovalbumin without electrostatic interaction.

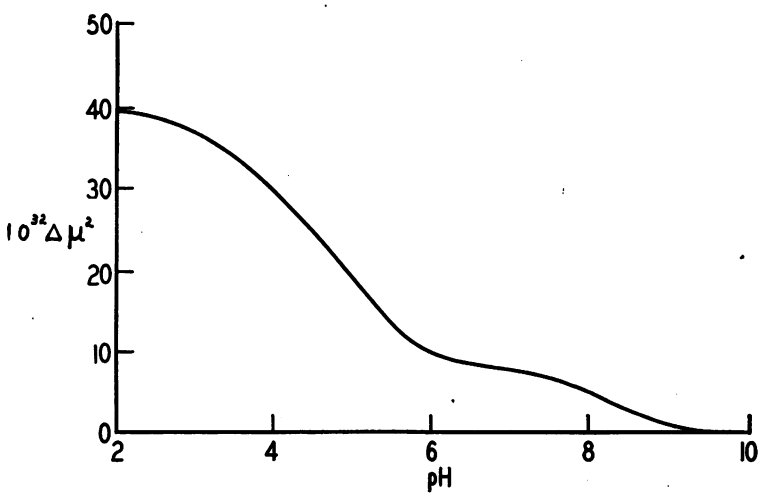


FIGURE 2.

The effect of pH on the dipole moment fluctuation of ovalbumin with electrostatic interaction.

influence of interaction is by no means negligible, it does not affect the magnitude of the dipole moment fluctuation. Electrostatic interaction causes configurations of low net charge to be favored and is most important at values of pH departing from the isoionic point.

From equations (3) and (4), we obtain the following expression for the mean dipole moment, with the neglect of electrostatic interaction between the protons,

$$\langle \boldsymbol{\mu} \rangle_{\text{av.}} = \boldsymbol{\mu}_0 + \sum_{i=1}^{\nu} \frac{e\mathbf{R}_i}{1 + K_i/[H^+]}$$

$$\boldsymbol{\mu}_0 = \sum_{i=1}^{\nu} e_i \mathbf{R}_i \qquad (10)$$

where $\boldsymbol{\mu}_0$ is the permanent dipole moment of the fixed charges of the molecule, exclusive of that of the protons. Unless each type of basic group is distributed on the surface of the molecule with a center of symmetry, $\boldsymbol{\mu}_0$ does not vanish and $\langle \boldsymbol{\mu} \rangle_{\text{av.}}$ cannot vanish at all pH. Indeed at high values of pH, $\langle \boldsymbol{\mu} \rangle_{\text{av.}}$ approaches $\boldsymbol{\mu}_0$. If $\boldsymbol{\mu}_0$ is opposite in sign to the second terms of equation (10), as would be expected to be the case if all basic groups are either neutral or negatively charged, the absolute magnitude of $\langle \boldsymbol{\mu} \rangle_{\text{av.}}$ would be a monotonically increasing function of pH. The dipole moment fluctuation, $\Delta\mu^2$, on the other hand, possesses maxima at pH values coincident with the p$K$ of the conjugate acids and minima at intermediate values, as illustrated by figure 1, for ovalbumin. Away from the isoionic point, the effect of electrostatic interaction suppresses the maxima and minima in $\Delta\mu^2$, as illustrated for ovalbumin in table 3 and figure 2. It will be observed that $\Delta\mu^2$ is a monotonically decreasing function of pH, diminishing from a high value at pH 2 to a value effectively zero at pH 10. The experimental data relating to the effect of pH on the dielectric increment of Shutt[5] and of Oncley, Bateman, Pecher and Melin[1] are too fragmentary and contradictory to justify an attempt at quantitative interpretation by means of the present theory. Since the effect of pH provides an important criterion for determining the relative importance of the contributions of $\langle \boldsymbol{\mu} \rangle_{\text{av.}}^2$ and $\Delta\mu^2$ to the dielectric increment, additional experimental data would be highly desirable.

The theory which has been presented here relates only to the dielectric increments of proteins under the action of static electric fields. A theory of dielectric dispersion and absorption by the proposed mechanism of fluctuations in proton configuration remains to be developed. The relaxation time spectrum of an ellipsoidal molecule will be determined not only by external rotatory diffusion but also by the diffusion of the mobile protons on the surface of the molecule. This means that the structural interpretation of dielectric dispersion will be considerably more com-

plicated than for a molecule possessing a permanent dipole moment without fluctuation. Qualitative considerations, however, lead to the conclusion that internal relaxation times of the order of magnitude of those observed are not inconsistent with the proposed mechanism. For example, for a sphere of radius $b$, on the surface of which protons possess a translatory diffusion constant $D$, a relaxation time of the order of magnitude of $b^2/D$ should control the distribution of protons. With $b$ equal to 30 Å., the radius of a sphere of volume equal to that of a serum albumin molecule and with $D$ equal to $10^{-5}$ cm.$^2$/sec., a relaxation time of the order of $10^{-8}$ sec. is obtained, which agrees in order of magnitude with those observed for representative proteins.

According to the present theory, the structural significance of the dielectric increments of proteins is subject to rather drastic reinterpretation. While many proteins doubtless possess permanent electric dipole moments of considerable magnitude, they cannot be determined unambiguously from measurements of the dielectric increment, since the dipole moment fluctuation, arising from the mobile proton distribution has been shown to make a contribution to the increments, which is equivalent to that of a permanent moment of large magnitude. The influence of fluctuations in the charge and configuration of mobile protons on the forces between protein molecules, which is of greater significance than the effect on the dielectric increment, will be treated in a later article.

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