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A MODEL FOR THE CALCULATION OF THE DIELECTRIC DISPERSION AND THE DIPOLE MOMENT OF GLOBULAR PROTEINS IN SOLUTION

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We describe a new procedure whereby the magnitude of the dielectric dispersion of a solution of globular protein molecules can be calculated. The protein molecule is considered to have spherical symmetry and the charged residues are thought to be situated in a medium whose dielectric constant increases continuously as a function of the distance from the centre of mass. The dipole moment of the protein in the solution is made up of two parts: the intrinsic dipole moment due to the charge distribution of the protein and the dipole moment due to polarization of the medium and the ionic cloud. When the model is applied to solutions of cytochrome *c* it is found that polarization of the medium results in a decrease in the dielectric dispersion amplitude. The mean square dipole moment calculated with the help of this method indicates that the fluctuation of the configurations cannot be responsible for the large dispersion in the megahertz region.

1. Introduction

Investigations on the dielectric relaxation of solutions of protein molecules carried out during the last 40 years have been of importance for the study of proteins [1–5]. The dispersion observed in the megahertz region is generally attributed to the permanent dipole moment of the protein. The dipole moment can be calculated from the dispersion data using the well known Onsager [6] relation.

In the last 10 years, the structure of a large number of protein molecules has been determined by means of X-ray crystallography. Consequently, we can now relate the experimentally determined dipole moment to the structural parameters of the molecule.

In this paper, we present a model for the globular proteins in which spherical symmetry in the dielectric constant of the molecules is assumed, and in which the electric fields in and around the molecules are taken into account. According to the Tanford-Kirkwood [7] theory, the globular protein molecule can be considered as a sphere with low dielectric constant, embedded in a medium with high dielectric constant. The charged residues are located near or on the surface of the sphere. It is unlikely, however, that discrete changes in the dielectric constant occur on a microscopic scale, as is implied by the Tanford-Kirkwood model. In the model described here the charged residues of the molecule are located in a medium whose dielectric constant ϵ varies as a function of the distance from the centre of mass of the molecule.

To understand the shape of the experimentally obtained titration curves, one has to assume small interaction energies between the charged residues of the polypeptide. Our model accounts for these low energies because the environment of the charged groups is assumed to be relatively polar. Recently, we have shown that this model provides an adequate description of the proton titration curve of oxidized horse heart cytochrome *c* [8].

To take account of the low water content near

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the centre of the molecule and the high water content near its edge, we consider in our model a non-polar centre and an increase in the dielectric constant with increasing distance from the centre of mass [8]. In this model, most charged residues have a highly polar environment, which is energetically favourable with respect to the solvation energies of the charges. On the assumption that there are no interactions between protein molecules (infinite dilution), an analytical expression for the electric field can be given. Once the electric field in the sphere is known, one can calculate the electric dipole moments induced in the medium. The induced dipole moments combined with the moments of the charges of the molecule give the total dipole moment of the sphere.

By averaging over all possible orientations in the external field and by averaging over all charge configurations of the protein molecule, one can calculate the mean dielectric constant of the solution.

2. Theoretical section

Consider a sphere with radius R having the polyelectrolyte (protein) at its centre. Since we only consider proteins at infinite dilution, R will have macroscopic dimensions. The sphere is subdivided into three concentric shells each of which has a different dielectric constant ϵ:

$r \leqslant a \quad \epsilon_r = \epsilon_i,$
$a \leqslant r \leqslant b \quad \epsilon_r = ar^{\sigma},$
$b \leqslant r \leqslant R \quad \epsilon_r = \epsilon_w.$

Here ϵ_i and ϵ_w represent the dielectric constant of the non-polar centre of the molecule and the polar region outside the molecule, respectively. The parameters α and σ are such that ϵ_r changes continuously at a and b. We assume that the salt ions do not penetrate into a sphere with radius c ($b < c < R$). The charges of the protein can be situated anywhere within the sphere with radius c.

We want to calculate the mean dielectric permittivity of the sphere with radius R. When only dipole terms are considered the mean polarization density $\bar{P}$ of the sphere is given by [9]:

$$\bar{P} = \langle \bar{M} \rangle / V. \tag{1}$$

where V is the volume of the sphere and the brackets $\langle\ \rangle$ denote the statistical mechanical average of the total dipole moment $\bar{M}$ of the sphere in the presence of the external field. In our case, we have to determine the average over the possible configurations of the charge (denoted by the brackets $\langle\ \rangle$) and the average over the possible orientations of the molecules in the external field (indicated by $\bar{}$). This leads to:

$$\epsilon - 1 = \frac{\langle \bar{M}_z \rangle}{\epsilon_0 E_0 V}, \tag{2}$$

where E_0 is the applied Maxwell field which points in the z direction, and ϵ_0 the permittivity of a vacuum. The net total dipole moment in the direction of the external field of a given configuration can be split into two parts:

$$\bar{M}_z = \bar{M}_z^{\Phi} + M_z^{\Psi}. \tag{3}$$

M_z^{Ψ} is the dipole moment due to the polarization of the medium in the sphere with radius R and is caused only by the external field E_0. This term is independent of the charge distribution of the molecule. $\bar{M}_z^{\Phi}$ is the dipole moment of the charges in the polyelectrolyte, including the polarization of the medium in the electric field of these charges.

As M_z^{Ψ} is independent of the possible orientations of the protein molecule in the external field and also of the charge of the molecule we can write:

$$\epsilon_\infty - 1 = M_z^{\Psi} / \epsilon_0 E_0 V, \tag{4a}$$

and

$$\Delta\epsilon = \langle \bar{M}_z^{\Phi} \rangle / \epsilon_0 E_0 V, \tag{5a}$$

where ϵ_∞ is the mean dielectric permittivity at frequencies much larger than ω_τ, with $\omega_\tau = 1/\tau$, and τ the dielectric relaxation time of the protein. $\Delta\epsilon$ is the dielectric dispersion of the protein solution. For N_p moles of non-interacting protein molecules in volume V (m^3) we can write:

$$\epsilon_\infty - 1 = (\epsilon_w - 1) - \frac{c_p N_0 V}{\epsilon_0 E_0} \left\{ (\epsilon_w - 1)\epsilon_0 E_0 - \frac{M_z^{\Psi}}{V} \right\} \tag{4b}$$

and

$$\Delta\epsilon = c_p N_0 \frac{\langle \bar{M}_z^{\Phi} \rangle}{\epsilon_0 E_0}, \tag{5b}$$

where N_0 is Avogadro's number and $c_p = N_p/V$ which is the concentration in mmol/l.

2.1. Calculation of M_z^ψ

By solving the Poisson equation one can find the potential ψ arising from the external field. Once the potential ψ is known, one can find the dipole moment arising from the polarization of the medium in a volume V [9]:

$$M_z^\psi = \epsilon_0 \iiint_V (\epsilon_r - 1) E_z^\psi \, dv = -\epsilon_0 \iiint_V (\epsilon_r - 1) \frac{\partial \psi}{\partial z} dv. \quad (6)$$

The potential ψ can be written as an expansion in Legendre polynomials. It can be shown that because of the orthogonality of the Legendre polynomials, only the dipole terms ($n = 1$) of the expansion contribute to the dipole moment, so we shall concern ourselves only with the dipole term of the potential ψ. The general solution of the Poisson equation is given by:

$$\psi(\bar{r}) = (\lambda^+ r^{\rho^+} + \lambda^- r^{\rho^-}) \cos\theta. \quad (7)$$

with

$$\begin{aligned} &r \leq a \qquad \rho^+ = 1, \quad \rho^- = -2; \\ &a < r \leq b \quad \rho^{+,-} = \tfrac{1}{2}\left\{ -(\sigma+1) \pm \sqrt{(\sigma+1)^2 + 8} \right\} \text{ (ref. 10)}; \\ &b < r \leq c \quad \rho^+ = 1, \quad \rho^- = -2; \end{aligned} \quad (8)$$

λ^+ and λ^- are determined by the boundary conditions in the various regions of our model (see the Appendix). We can generally write $\epsilon_r = \alpha r^\sigma$ (for $r \leq a$, $\sigma = 0$ and $\alpha = \epsilon_i$; for $b < r \leq c$, $\sigma = 0$ and $\alpha = \epsilon_w$). When the integration of eq. 6 is performed over a shell with inner radius x_1, outer radius x_2 and volume V_1 we have:

$$\begin{aligned} -\epsilon_0 \iiint_{V_1} (\epsilon_r - 1) \frac{\partial \psi}{\partial z} dv = &-\frac{4\pi}{3} \epsilon_0 \lambda^+ x_2^{\rho^+ + 2} \\ &\times \left\{ \left(\frac{\rho^+ + 2}{\rho^+ + 2 + \sigma} \alpha \cdot x_2^\sigma - 1 \right) - \left(\frac{x_1}{x_2} \right)^{\rho^- + 2} \right. \\ &\left. \left(\frac{\rho^+ + 2}{\rho^+ + 2 + \sigma} \alpha \cdot x_1^\sigma - 1 \right) \right\} \\ &- \frac{4\pi}{3} \epsilon_0 \lambda^- x_2^{\rho^- + 2} \left\{ \left(\frac{\rho^- + 2}{\rho^- + 2 + \sigma} \alpha \cdot x_2^\sigma - 1 \right) \right. \\ &\left. - \left(\frac{x_1}{x_2} \right)^{\rho^- + 2} \left(\frac{\rho^- + 2}{\rho^- + 2 + \sigma} \alpha \cdot x_1^\sigma - 1 \right) \right\} \end{aligned} \quad (9)$$

for x_1 and $x_2 < c$, where α, ρ^+, ρ^-, λ^+, λ^- and σ are different in the various domains of the model. It follows from the term between the second set of curly brackets in eq. 9 that if the potential varies as r^{-2} ($\sigma = 0$ and ϵ_r is constant) in a certain region (i.e., $\rho^- = -2$), this region will not contribute to the dipole moment.

In the shell between c and R (volume V_2) we have to take the ions into account. The charge density $\rho(\bar{r})$ can be found by applying Boltzmann's law and approximating for small energies [11]:

$$\rho(\bar{r}) = -\sum_i c_i q_i^2 \psi / kT = -\frac{\kappa^2}{\epsilon} \psi. \quad (10)$$

The term $\Sigma_i \, c_i q_i$ corresponds to the net charge density of the ionic cloud, and equals $-Q/V_2$, where Q is the net charge of the protein. By taking the limit of infinite dilution ($V_2 \to \infty$) the net charge density of the ions approaches zero.

In eq. 10, $\kappa = 5.024\sqrt{I/kT}$ nm^{-1} and $I = 1/2 e^2 \Sigma_i \, c_i q_i^2$ which is the ionic strength (mol/l). The net charge of the ions is now excluded from the summation in eq. 10. Outside the protein the potential $\psi(\bar{r})$ consists of two terms, one from the external field ($-E_0 r \cos\theta$) and one from the varying dielectric constant which is due to the external field. The last term is dependent on the ionic strength. According to Kirkwood [12], the dipole term of $\psi(\bar{r})$ for ions without an intrinsic dipole moment can be written:

$$\psi(\bar{r}) = -E_0 r \cos\theta + \lambda^- r^{-2} e^{-\kappa r} (1 + \kappa r) \cos\theta. \quad (11)$$

The z component of the dipole moment of the ionic cloud in the direction of the external field is now given by:

$$\begin{aligned} \iiint_{V_2} \rho(\bar{r}) r \cos\theta \, dv = &-4\pi \epsilon_0 \epsilon_w \lambda^- \\ &\times \{ e^{-\kappa c} (1 + \kappa c + \kappa^2 c^2/3) - e^{-\kappa R} (1 + \kappa R + \kappa^2 R^2/3) \} \end{aligned} \quad (12)$$

The polarization of the dielectric medium outside the protein is given by:

$$\begin{aligned} -\epsilon_0 \iiint_{V_2} (\epsilon_r - 1) \frac{\partial \psi}{\partial z} dv = &\\ &\frac{4\pi}{3} \epsilon_0 (\epsilon_w - 1) \lambda^- \{ e^{-\kappa c} (1 + \kappa c) - e^{-\kappa R} (1 + \kappa R) \} \\ &+ \frac{4\pi}{3} (\epsilon_w - 1) E_0 (R^3 - c^3). \end{aligned} \quad (13)$$

The contribution of the volume V_2 of the shell between c and R to the medium polarization M_z^{ψ} is given by the sum of eqs. 12 and 13:

$$-\frac{4\pi}{3}\epsilon_0\lambda^-\left[e^{-\kappa c}\{\epsilon_w(2+2\kappa c+\kappa^2c^2)+(1+\kappa c)\}\right.$$
$$\left.-e^{-\kappa R}\{\epsilon_w(2+2\kappa R+\kappa^2R^2)+(1+\kappa R)\}\right]$$
$$+\frac{4\pi}{3}(\epsilon_w-1)E_0(R^3-c^3). \quad (14)$$

Using eqs. 6–14, ϵ_∞ in eq. 4b can be calculated.

2.2. Calculation of $\bar{M}_z^{\phi}$

The dipole term of the potential ϕ_l due to a charge q_l inside the molecule on the z-axis can generally be written:

$$\phi_l(\vec{r})=(\lambda_l^+ r^{\rho^+}+\lambda_l^- r^{\rho^-})\cos\theta. \quad (15)$$

Here λ_l^+, ρ^+, λ_l^- and ρ^- are again different in the various regions, ρ^+ and ρ^- are defined as in eq. 8; λ_l^+ and λ_l^- include the homogeneous as well as the inhomogeneous terms [8].

Outside the molecule we have to take the ions into account, which results in

$$\phi_l(\vec{r})=\lambda_l^- r^{-2}e^{-\kappa r}(1+\kappa r)\cos\theta. \quad (16)$$

The dipole moment $\vec{M}^{\phi}$ caused by a charge q_l at $\vec{\xi}_l$ relative to the centre of mass can be split into two parts:

the dipole moment of the charge: $\vec{M}_l=q_l\vec{\xi}_l$;

the dipole moment caused by the polarization of the medium due to the charge q_l: $\vec{M}_2=-\epsilon_0\int\int\int_V(\epsilon_r-1)\nabla\phi\,dv$. This term can be evaluated with the help of eq. 9.

We now introduce the superscript i to indicate the configuration of the charges on the molecule [10]. For a system of m charges in configuration i the total dipole moment in the z-direction is:

$$M_z^{\phi,i}=\sum_{l=1}^{m}\left\{-\epsilon_0\int\int\int_V(\epsilon_r-1)\frac{\partial\phi_l^i}{\partial z}dv+q_l^i\xi_l\right\}\cos\theta_l \quad (17)$$

where θ_l is the angle between the $\vec{\xi}_l$ vector and the z-axis. The average over all possible orientations can be found as follows:

$$\bar{M}_z^{\phi,i}=\frac{\int_0^{\pi}\int_0^{2\pi}M_z^{\phi,i}e^{-W^i/kT}\sin\beta\,d\alpha\,d\beta}{\int_0^{\pi}\int_0^{2\pi}e^{-W^i/kT}\sin\beta\,d\alpha\,d\beta}. \quad (18)$$

W^i is the energy of the configuration (i) of the charges is the external field. W^i is generally much smaller than kT, so we can write:

$$e^{-W^i/kT}=1-W^i/kT, \quad (19)$$

α and β are two angles by means of which all possible orientations of the molecule in the external field which differ in W^i can be described. β transforms the system $A(x,y,z)$ to $A'(x',y',z')$ through rotation round the x-axis ($0\leq\beta\leq\pi$). α transforms $A'(x',y',z')$ to $A''(x'',y'',z'')$ through rotation round the z'-axis ($0\leq\alpha\leq2\pi$). A'' is the system that is fixed to the molecule. The energy of a molecule with m charges $q_k(k=1\approx m)$ at locations $\vec{\xi}_k$ in the potential ψ due to external field (eq. 7) is given by:

$$W^i=\sum_{k=1}^{m}q_k^i\psi(\vec{\xi}^k) \quad (20)$$

with

$$\psi(\vec{\xi}_k)=(\lambda^+\xi_k^{\rho^+}+\lambda^-\xi_k^{\rho^-})\cos\theta_k. \quad (21)$$

In the system A'' $\cos\theta_k$ (and $\cos\theta_l$) can be expressed thus:

$$\cos\theta_k=\frac{\sin\alpha\sin\beta\xi_{x,k}''-\cos\alpha\sin\beta\xi_{y,k}''+\cos\beta\xi_{z,k}''}{\xi_k} \quad (22)$$

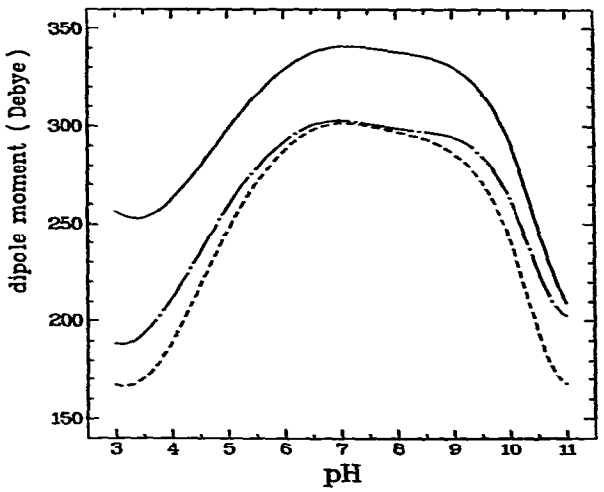

Fig. 1. Mean and mean square dipole moment as a function of pH. (———) $\sqrt{\langle\mu^2\rangle}$, (------) $|\langle\mu_p\rangle|$, (·-·-·) $\sqrt{\langle\mu_p^2\rangle}$.

where $\zeta''_{x,k}$, $\zeta''_{y,k}$ and $\zeta''_{z,k}$ are the Cartesian coordinates relative to the centre of mass of the charge q_k in the molecule-fixed system A'', and ζ_k the distance of the charge from the centre of mass. In W^i and $M_z^{\phi,i}$ only $\cos\theta_k$ and $\cos\theta_l$ are dependent on α and β. The integration of eq. 18 can now be readily performed. Considering the following terms:

$$\int_0^{\pi}\int_0^{2\pi}\cos\theta_l \sin\beta \,d\alpha\, d\beta = 0$$

$$\int_0^{\pi}\int_0^{2\pi}\cos\theta_l \cos\theta_k \sin\beta \,d\alpha\, d\beta =$$

$$\frac{1}{3}\frac{1}{\zeta_l\zeta_k}\{\zeta''_{x,l}\cdot\zeta''_{x,k}+\zeta''_{y,l}\cdot\zeta''_{y,k}+\zeta''_{z,l}\cdot\zeta''_{z,k}\}$$

$$\int_0^{\pi}\int_0^{2\pi}\sin\beta \,d\alpha\, d\beta = 4\pi. \qquad (23)$$

we find:

$$\bar{M}_z^{\phi,i} = -\sum_{l=1}^{m}\left\{-\epsilon\int\int\int_v(\epsilon_r-1)\frac{\partial\phi_l^i}{\partial z}dv + q_l^i\zeta_l\right\} \times \sum_{k=1}^{m}\frac{q_k^i}{kT}(\lambda^+\zeta_k^{\rho^+}+\lambda^-\zeta_k^{\rho^-}) \times \frac{1}{3\zeta_l\zeta_k}\{\zeta''_{x,l}\cdot\zeta''_{x,k}+\zeta''_{y,l}\cdot\zeta''_{y,k}+\zeta''_{z,l}\cdot\zeta''_{z,k}\}. \qquad (24)$$

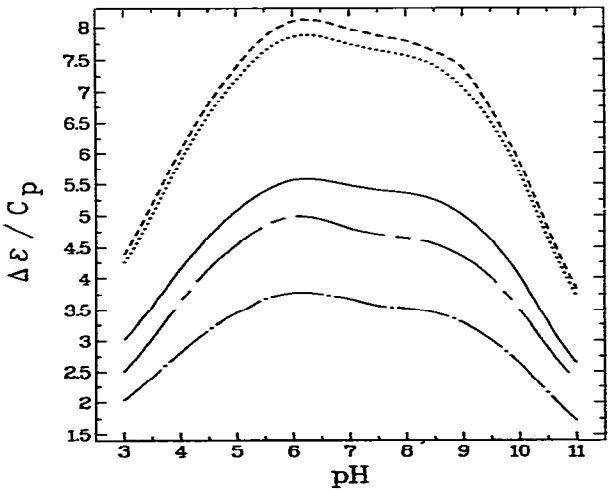

Fig. 2. Calculated dielectric dispersion per mol/l as a function of pH of horse heart cytochrome *c* at ionic strength 0. Curves: 1 (———), eq. 29; 2 (------), eq. 30; 3 (·····), $a=b=200$ nm (eq. 5b); 4 (-·-·-), $a=b=50$ nm (eq. 5b); 5 (-———-), $a=50$ nm, $b=170$ nm (Eq. 5b).

The average over all the possible configurations of the charges is given by [8,10]:

$$\langle\bar{M}_z^{\phi}\rangle = \frac{\sum_{\nu=0}^{m} a^{\nu}\sum^{*}_{i}\bar{M}_z^{\phi,i}K_{\nu}^{i}}{\sum_{\nu=0}^{m} a^{\nu}\sum^{*}_{i}K_{\nu}^{i}}. \qquad (25)$$

where a represents the activity of H^+ and the weight factors K_{ν}^{i} are given by the Boltzmann factors $K_{\nu}^{i}=e^{-\beta\Delta F^i}$. ΔF^i represents the standard free energy change for the reaction in which ν protons are coupled onto the proton-free form P of the protein to give the specific configuration i:

$$K_{\nu}^{i}: \mathrm{P}+\nu\mathrm{H}^+ \rightleftharpoons (\mathrm{PH}_{\nu})^i.$$

The asterisk in eq. 25 indicates that we sum over any configuration that involves ν protons (so the two summations may not be interchanged). The intrinsic dipole moment is the moment caused by the dipoles on the backbone of the protein molecule taking into account its mutual interaction. The interactions with the direct environment in the protein molecule are neglected.

2.3. Calculations

We first focus on the intrinsic dipole moment $\vec{\mu}$, without considering the polarization of the medium.

In polypeptides it is not only the charged amino acids which contribute to the total moment of the molecule, but the moments of the peptide backbone have to be taken into account as well. Each peptide bond has a dipole moment of about 3.5 debye which is directed from the O atom to the C′ atom of the peptide backbone [13,14]. We calculated the vector sum of all these dipoles in horse heart cytochrome *c*. The coordinates of the titratable groups were modified data from tuna heart cytochrome *c* [8,15,16]. We obtained:

$$|\vec{\mu}_b| = 95.3 \text{ debye}. \qquad (26)$$

The dipole moment of the charged residues is given by:

$$\vec{\mu}_p = \sum_{l=1}^{m} q_l\vec{\zeta}_l \quad \text{and} \quad \mu_p^2 = \sum_{k,l} q_k q_l \vec{\zeta}_l\vec{\zeta}_k. \qquad (27)$$

$|\langle\vec{\mu}_p\rangle|$ and $\langle\mu_p^2\rangle$ can be calculated by replacing $\overline{M}_z^{\phi,i}$ in eq. 25 by $\vec{\mu}_p$ and μ_p^2, respectively. The 500 most probable configurations have been used to determine the average values [19,20].

The total mean square dipole moment can be expressed as:

$$\langle\mu^2\rangle=\langle(\vec{\mu}_p+\vec{\mu}_b)^2\rangle=\langle\mu_p^2\rangle+\mu_b^2+2\vec{\mu}_b\langle\vec{\mu}_p\rangle. \quad (28)$$

In fig. 1, $\sqrt{\langle\mu^2\rangle}$, $|\langle\mu_p\rangle|$ and $\sqrt{\langle\mu_p^2\rangle}$ are given as a function of pH.

We shall now compare the dielectric dispersion that can be calculated according to eqs. 5b and 15–25 with the formulas that are commonly used to relate the charge distribution of a molecule to the dielectric dispersion of a solution of these molecules. For gases, the Debye [17] formula relates the mean dipole moment to the dielectric dispersion:

$$\Delta\epsilon_1=\frac{\langle\mu^2\rangle}{3kT}\frac{c_pN_0}{\epsilon_0}. \quad (29)$$

For dilute solutions the dipole can be considered to be embedded in a sphere with dielectric constant ϵ_i inside and ϵ_w outside. The cavity field works on the dipole, and the dispersion can therefore be expressed thus:

$$\Delta\epsilon_2=\frac{\langle\mu^2\rangle}{3kT}\frac{c_pN_0}{\epsilon_0}\frac{3\epsilon_w}{2\epsilon_w+\epsilon_i}. \quad (30)$$

In fig. 2, $\Delta\epsilon_1$ and $\Delta\epsilon_2$ per mmol/l are given as a function of pH for a solution of horse heart cytochrome c. For ϵ_i and ϵ_w the values 5 and 78.5, respectively, were chosen. Curves 1 and 2 are calculated according to eqs. 29 and 30. Curves 3–5 in fig. 2 are calculated, the polarization of the medium being taken into account (eqs. 5b and 15–25). The ionic strength is 0 and the 500 most probable configurations are considered. For curve 3, $a=b=200$ nm and for curve 4, $a=b=50$ nm. $a=50$ nm and $b=170$ nm gave best fit to the proton-titration curve of oxidized horse heart cytochrome c [8]. Curve 5 (fig. 2) is calculated according to these values.

Fig. 3 shows the influence of the ionic strength on the dielectric dispersion. Curves 1, 2 and 3 give the dispersion per mmol/l at ionic strengths 0, 0.05 and 0.15 mol/l, respectively. Curve 4 has been calculated by determining the weight factors K_μ^i at ionic strength 0 mol/l, and calculating $\overline{M}_z^{\phi,i}$ at ionic strength 0.15 mol/l.

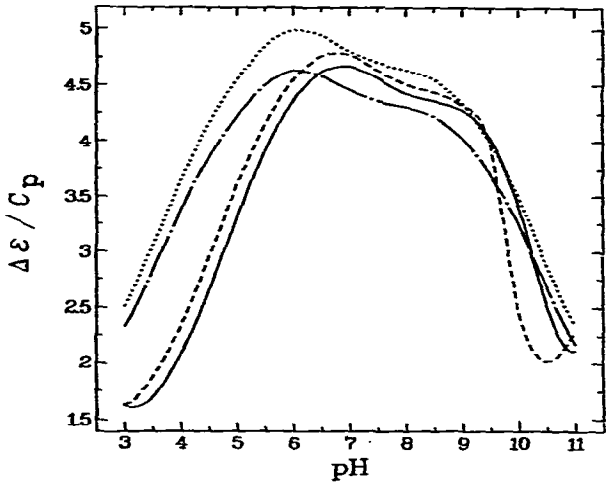

Fig. 3. Influence of the ionic strength on the dielectric dispersion amplitude per unit of concentration of horse heart cytochrome c as calculated by eq. 25, using $a=50$ nm and $b=170$ nm. Curves: 1 (· · · · · ·), $I=0$ mol/l; 2 (------), $I=0.05$ mol/l; 3 (———), $I=0.15$ mol/l; 4 (·—·—·), $I=0/0.15$ mol/l (see text).

3. Discussion

From fig. 1 two important conclusions can be drawn:

the dipole moment of the backbone does not greatly influence the total intrinsic moment (at most 25% at pH 2.5), although its absolute value is rather large (95.3 debye);

the differences between $\sqrt{\langle\mu_p^2\rangle}$ and $|\langle\mu_p\rangle|$ is rather small. Kirkwood and Shumaker [18] calculated the proton fluctuation term that is defined by:

$$\Delta\mu^2=\langle\mu^2\rangle-\langle\mu\rangle^2. \quad (31)$$

On the basis of statistical mechanical considerations and assuming a uniform distribution of basic groups on the surface of the molecule, they found values for $\Delta\mu^2$ which had the same order of magni-

tude as $\langle\mu\rangle^2$. From the calculations presented here we can conclude that the proton fluctuation term is too small to explain the large dielectric dispersion of most globular proteins in the megahertz region.

Debye [17] was the first to derive a relationship between the dipole moment of a molecule and the dielectric dispersion of a gas consisting of such molecules (eq. 29). For dilute solutions eq. 30 was used, to take account of the cavity field that works on the dipole. This equation is often used to relate the dielectric dispersion of a solution of protein molecules to the dipole moment of the protein [4]. The dispersions calculated according to eqs. 29 and 30 are given in fig. 2 (curves 1 and 2) as a function of pH. In eqs. 29 and 30 the polarization of the medium is not taken into account (only the Langevin factor results in the factor $3\epsilon_w/(2\epsilon_w+\epsilon_i)$ in eq. 30). In our model we do take account of the polarization of the medium (eq. 17, first term). To demonstrate the influence of the medium polarization we consider two simple models. We consider a molecule represented by a sphere with radius a, and dielectric constant ϵ_i inside and ϵ_w outside. At a the dielectric constant changes discontinuously from ϵ_i to ϵ_w.

For our first model a charge is situated at ζ inside the sphere ($0<\zeta<a$). We can write for the dielectric dispersion of a solution of these molecules at concentration c_p (using eq. 25):

$$\Delta\epsilon=\frac{\langle\mu^2\rangle}{3kT}\frac{c_pN_0}{\epsilon_0}\left(1-\frac{\epsilon_i-1}{2\epsilon_w+\epsilon_i}\right)\frac{3\epsilon_w}{2\epsilon_w+\epsilon_i} \tag{32}$$

with $\mu=q\zeta$. If $\epsilon_i=5$ and $\epsilon_w=78.5$, then the medium polarization which is given by the term $(\epsilon_i-1)/(2\epsilon_w+\epsilon_i)$ is roughly equal to 0.025, which is small compared to the contribution of the moment of the charge itself. The factor $3\epsilon_w/(2\epsilon_w+\epsilon_i)$ is due to the cavity field.

For our second model we consider the charge q situated at ζ outside the sphere. We find:

$$\Delta\epsilon=\frac{\langle\mu^2\rangle}{3kT}\frac{c_pN_0}{\epsilon_0}\left[1-(\epsilon_i-1)(a/\zeta)^3\frac{1}{2\epsilon_w+\epsilon_i}-(\epsilon_w-1)\frac{1}{3\epsilon_w}\{1-(a/\zeta)^3\}\right] \tag{33}$$

The polarization of the medium is now given by the second and the third term in the square brackets. For $a=50$ nm and $\zeta=100$ nm these terms are 0.003 and 0.29, respectively. This shows that the polarization of the medium can make an important contribution.

ϵ_∞ is the same for both models and is given by:

$$\epsilon_\infty=c_pN_0\frac{4\pi}{3}\left\{(\epsilon_i-1)a^3\frac{3\epsilon_w}{2\epsilon_w+\epsilon_i}-(\epsilon_w-1)a^3\right\}+\epsilon_w. \tag{34}$$

For $a=50$ nm we find $\epsilon_\infty=78.48$ ($c_p=1$ mmol/l).

The pararmeters $\epsilon_i=5$ and $\epsilon_w=78.5$ are chosen in accordance with the Tanford-Kirkwood model [21]. Experimental evidence shows that the low interaction energies between the iron atom and the residues in the interior of a cytochrome c molecule can only be understood by assigning large values to the effective dielectric constant. Comparison of the values obtained by Rees [22] for the dielectric constant in the case of various lysine residues with the values we calculated according to the presented model justifies the choice of the above-mentioned parameters. Results of recent calculations show an effective dielectric constant $\epsilon_i=2$ for a protein molecule with non-polar atoms in its interior region [23]. The difference between $\epsilon_i=5$ and the $\epsilon_i=2$ does not have any marked influence on our results.

The largest number of charged groups were not in the interior of the protein molecule but were in the more peripheral regions where ϵ increases with radius, and these groups contribute to the effective dielectric constant to a much greater extent than do the groups in the interior region [8].

In fig. 2 (curves 3 and 4) the dispersions of cytochrome c are given for $a=b=200$ nm ($\epsilon_\infty=77.06$) and $a=b=50$ nm ($\epsilon_\infty=78.48$). Curve 5 of fig. 2 gives the dispersion as a function of pH for the values of the parameters we actually use for our model of cytochrome c; i.e., $a=50$ nm and $b=170$ nm. The value for ϵ_∞ is 78.14 in this case. As could be expected, the values for $\Delta\epsilon$ and ϵ_∞ with $a=50$ nm and $b=170$ nm lie between the results for $a=b=50$ nm and $a=b=200$ nm. For the model of the protein solution discussed here the polarization of the medium compensates the intrinsic dipole moment, thus reducing the total dipole moment. Particularly when the charges are

situated in a polar medium this effect can reduce the dispersion by 50%.

These calculations yield an important result: we can say that by considering a model where the charged residues of the polypeptide are situated in a polar environment, the effective dipole moment will be much smaller than could be expected from earlier models, except the model of Debye which gives a result similar to ours.

Fig. 3 shows the influence of the ionic strength. Curves 1, 2 and 3 are results of calculations at ionic strengths of 0, 0.05 and 0.15 mol/l, respectively. The difference between curves 1 and 4 is due to the ionic strength dependence of $\overline{M}_{-}^{\phi}$, because for curve 4 the configurations and interaction energies at ionic strength 0 are used. It is clear from the difference between curves 3 and 4 that a large part of the ionic strength dependence of the dielectric permittivity is due to changes in K_{ν}^{i}. From fig. 3 it can be seen that between pH 3 and 9 the dielectric dispersion decreases with increasing ionic strength. At higher pH there is no general tendency. The values of ϵ_{∞} differ less than 0.1% between ionic strengths 0 and 0.15. It should be noted that our model implies that the charge density of the ions responds instantaneously to the rotation of the protein molecules.

We have used a value of 10000 nm for R (for explanation, see the first part of section 2). As can be seen from eq. 14, this is justified for the case of infite dilution as long as the ionic strength is not much lower than 0.01.

It is important to note at this point that we do not take into consideration the interactions between protein molecules. At larger concentrations there will of course be deviations from the linear relationship due to interactions of proteins. Experimental evidence [4,5], however, suggests that in proteins such as haemoglobin and myoglobin there is a linear relationship between $\Delta\epsilon$ and c_p up to at least 10 mmol/l.

Appendix

We show here how the pre-factors (λ^+ and λ^-) can be determined in the various domains by applying the boundary conditions. The potential $\psi(\bar{r})$ can be written according to eqs. 7 and 11:

$$\psi(\bar{r}) = \lambda_{\mathrm{I}}^{+} r\cos\theta \quad r \leqslant a$$

$$\psi(\bar{r}) = \left(\lambda_{\mathrm{II}}^{+} r^{\rho^+} + \lambda_{\mathrm{II}}^{-} r^{\rho^-}\right)\cos\theta \quad a \leqslant r \leqslant b$$

$$\psi(\bar{r}) = \left(\lambda_{\mathrm{III}}^{+} r + \lambda_{\mathrm{III}}^{-}/r^2\right)\cos\theta \quad b \leqslant r \leqslant c$$

$$\psi(\bar{r}) = \left\{-E_0 r + \lambda_{\mathrm{IV}}^{-} r^{-2} e^{-\kappa r}(1+\kappa r)\right\}\cos\theta \quad c \leqslant r$$

The introduction of the boundary conditions at a, b and c gives:

$$\begin{pmatrix} \lambda_{\mathrm{II}}^{+} \\ \lambda_{\mathrm{II}}^{-} \end{pmatrix} = O_{\mathrm{I}}^{a} \begin{pmatrix} \lambda_{\mathrm{I}}^{+} \\ 0 \end{pmatrix}$$

$$\begin{pmatrix} \lambda_{\mathrm{II}}^{+} \\ \lambda_{\mathrm{II}}^{-} \end{pmatrix} = O_{\mathrm{I}}^{b} \begin{pmatrix} \lambda_{\mathrm{III}}^{+} \\ \lambda_{\mathrm{III}}^{-} \end{pmatrix}$$

$$\lambda_{\mathrm{III}}^{+} = \lambda_{\mathrm{III}}^{-}\beta - E_0$$

and

$$\lambda_{\mathrm{IV}}^{-} = \frac{c^3 e^{c\kappa}}{-\frac{(\kappa c)^2}{e}}\left(\lambda_{\mathrm{III}}^{+} + E_0\right),$$

where

$$\beta = c^{-3}\frac{-\frac{(\kappa c)^2}{3}}{1+\kappa c+\frac{(\kappa c)^2}{3}}$$

and

$$O_{\mathrm{I}}^{x} = \frac{1}{\rho^+ - \rho^-}\begin{pmatrix} (1-\rho^-)x^{1-\rho^+} & -(\rho^- +2)x^{-(\rho^+ +2)} \\ -(1-\rho^+)x^{1-\rho^-} & (\rho^+ +2)x^{-(\rho^- +2)} \end{pmatrix}$$

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