Biophysical Chemistry 9 (1979) 91–95
© North-Holland Publishing Company

HIGH ELECTRIC FIELD DIELECTRIC STUDIES OF AQUEOUS MYOGLOBIN SOLUTIONS

G. PARRY JONES
Department of Physics, University of Petroleum and Minerals,
Dhahran, Saudi Arabia

and

A.E. DAVIES and M.J. VAN DER SLUIJS
School of Physical and Molecular Sciences, University College of North Wales,
Bangor, Gwynedd, United Kingdom

Received 30 July 1978

High field dielectric measurements of the Piekara factor $\Delta\epsilon/E^2$ have been carried out for a range of concentrations of horse heart myoglobin in water at 293 K. Using the literature value for the dipole moment of myoglobin and the established theory for the classical orientational dipolar non-linear effect predicts a value of $\Delta\epsilon/E^2$ one order of magnitude greater than that for water. The measured effect, however, was found to be one order of magnitude less than for water. This difference is explained as being most probably due to the existence of antiparallel molecular dipole pairs in the myoglobin solution. The possibility of a positive $\Delta\epsilon$ due to a field induced conformational change of the myoglobin cannot, however, be ruled out.

1. Introduction

While it is possible to elucidate the geometrical structure of proteins by X-ray diffraction analysis the electrical configuration of these molecules is not clear despite numerous dielectric studies over a period of thirty to forty years. These dielectric studies have revealed two or three dispersion regions for each molecule. The lowest dispersion region is generally agreed to be due to the permanent dipole moment while the higher frequency dispersions can be due to free water, bound water or individual side chains. Studies by Oncley and collaborators [1–4] of globular protein molecules showed their electric dipoles to be in the range 6×10^{-28} to 3×10^{-27} Cm and their low frequency dispersions to fall in the range 0.1 to 10 MHz. A dielectric dispersion study of myoglobin was also undertaken by Marcy and Wyman in 1942 [5].

More recently an extensive dielectric dispersion study of myoglobin in water has been reported by South and Grant [6]. In addition to rigid dipole rotation these authors also consider proton fluctuation [7], the Maxwell-Wagner effect [8,9] as applied to proteins [10], the surface conductivity theory [11], the ion mobility model [12], the ion atmosphere effect of Debye and Falkenhagen [13] and the suggestion that the water of hydration is solely responsible for the dielectric dispersion [14]. From their experimental data they came to the conclusion that all these mechanisms other than the rigid dipole rotation could be excluded excepting a possible allowance for proton fluctuation effects. These authors proceed to calculate the dipole moment of myoglobin from the experimental equation used by Oncley [2]:

$$\mu = \left(\frac{2\epsilon_0 M k T \delta}{N}\right) \sqrt{g}\, \frac{3}{n_\mathrm{p}^2 + 2}\,, \qquad (1)$$

μ is the dipole moment in Cm, ϵ_0 the permittivity of free space, M the relative molecular mass, k Boltzmann's constant, T the absolute temperature, δ the specific dielectric increment and N Avogadros number: g is the Kirkwood g factor [15], and n_p the Onsager internal refractive index of the protein [16]. Due to ignorance regarding the values of g and n_p they are both assumed to be unity by South and Grant [6]. Uncertainties about these factors plus other problems such as the value of the internal field etc undoubtedly cast doubt on the values of dipole moments calculated using this formula. Nonetheless the experimental values quoted by South and Grant [6] of 5.26×10^{-28} Cm and 5.79×10^{-28} Cm at the isoelectric point (pH ≈ 7) for whale and horse myoglobin respectively are found to be in agreement with the dipole moment as calculated from first principles (knowing the structure of myoglobin) by the same authors. The centre of the dispersion for concentrations of about 1 kg m^{-3} is about 6 MHz.

2. Non-linear dielectric effects

Non-linear dielectric effects (NDE) are recorded as small changes ($\Delta\epsilon$) in the relative electric permittivity due to large applied electric fields. The first NDE were recorded as early as 1920 by Herweg [17] in diethyl ether. The development of the subject since that time has been in large measure due to Piekara and his collaborators [18–20]. An excellent account of the basic ideas of NDE is given in "Theory of electric polarization" by Böttcher [21] and reviews are given by Kielich [22] and Parry Jones [23].

In the 'classical' NDE as predicted by Debye [24] $\Delta\epsilon$ is negative due to the polarization of the dipoles in the large field. A similar competition between the field and thermal agitation for non-polar but anisotropic molecules will give rise to a positive $\Delta\epsilon$. Yet another type of effect is due to the change in the dipole moment of the molecule itself giving a positive $\Delta\epsilon$. This change in the dipole moment has been attributed to conformational changes [25], ionization of hydrogen bonds [26] etc.

Following Böttcher [21] the classical NDE based on the Onsager equation is given by

$$\frac{\Delta\epsilon}{E^2} = -\left\{\frac{[\epsilon(n^2+2)]^2}{(2\epsilon^2+n^4)(2\epsilon+n^2)^2}\right\}\frac{\mu^4}{45(kT)^3}\frac{NR_s}{\epsilon_0}, \qquad (2)$$

ϵ is the low frequency relative permittivity and n the refractive index of the liquid, μ is the effective molecular moment in Cm, k the Boltzmann constant, T the absolute temperature, N the number of dipoles per unit volume (m^3), ϵ_0 the permittivity of free space and R_s the non-linear field effect correlation factor defined by Piekara and Kielich [27]. The units of $\Delta\epsilon/E^2$ are $m^2\ V^{-2}$. These authors also define a further quantity R_p which is essentially the same as the Kirkwood 'g' factor mentioned previously. If the energy of molecular interaction is zero then $R_p = R_s = 1$. Considering the simple case of dipole pairwise coupling R_p and R_s are given by

$$R_p = 1 \pm L, \qquad R_s = 1 - 2(1 - 3L/y) + (5L \pm 4)L, \qquad (3,4)$$

where $L = L(y)$ is the Langevin function and $y = W/kT$ the energy of dipole coupling of two molecules in units of kT. The upper sign corresponds to a tendency for the molecules to be in parallel alignment while the lower sign corresponds to antiparallel alignment. Piekara and Kielich [28] show that $R_s \approx R_p^3$ and hence $g \approx \sqrt[3]{R_s}$.

3. Experimental

The horse heart myoglobin was supplied by BDH Chemicals Ltd. The water was doubly distilled and deionized having a conductivity of 10^{-4} S m^{-1} or less. The solution concentrations were in the range 0.4 to 3 kg m^{-3} and with associated conductivities of 0.5×10^{-3} S m^{-1} to 3×10^{-3} S m^{-1} respectively. All solutions were sonicated briefly before the measurements.

The non-linear dielectric measuremenets were carried out using a computing counter system which has been previously described [29,30]. Oscillation frequencies in the range 6 to 8 MHz were used. The pulse width at half height was 15 μs and the maximum field amplitude of the order of 10^7 V m^{-1}. The pH of the solutions was measured using an EIL model 38A pH meter. The temperature of the measurements (293 K) was kept constant to ±0.1 K.

4. Results

The NDE was measured as a function of concentration. All plots of $\Delta\epsilon$ against E^2 were found to be linear within experimental accuracy. A plot of $\Delta\epsilon$ against E^2 for a 0.57 kg m^{-3} solution of myoglobin in water is

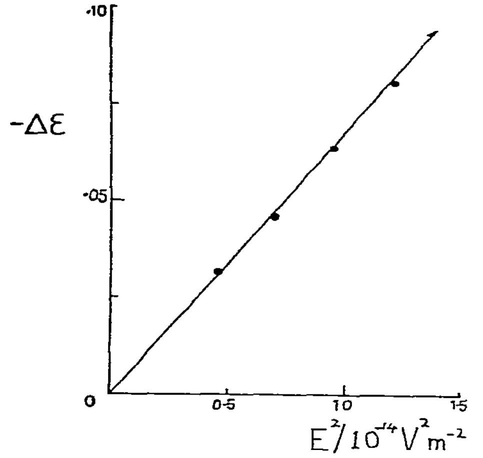

Fig. 1. Plot of $-\Delta\epsilon$ against $E^2/10^{-14}$ V^2 m^{-2} for myoglobin in water at a concentration of 0.57 kg m^{-3} and a temperature of 293 K.

shown in fig. 1. The $\Delta\epsilon$ due to Joule heating has been subtracted [31] but no allowance has been made for any ionic effects. The dependence of the NDE on concentration is given in fig. 2. The full circles correspond to the data after correction for Joule heating. The value of $\Delta\epsilon/E^2$ rapidly becomes more negative with increasing concentration. Over the concentration range the value doubles from that of water of -0.8×10^{-15} m^2 V^{-2} at 293 K [32]. Comparison of this plot with fig. 5 of ref. [32] shows the data to be virtually the same as those from water to which trace amounts (a few ppm) of NaCl had been added. It would appear, therefore, that the dominating effect in this case is due to conductivity, the mechanism of which has been discussed previously [32]. Bearing this in mind measurements on water to which trace amounts of HCl had been added to adjust the conductivity to be the same as the particular myoglobin solution were carried out. These sets of measurements i.e. on the myoglobin solution and on the water of the same conductivity were carried out successively and under as identical conditions as possible. Subtraction of the water value from the myoglobin solution value gave the open circles of fig. 2.

While the error in the absolute value of $\Delta\epsilon/E^2$ in each case is about 15% [32] the relative error in the two corresponding values is from 3 to 5%. As a result, the error in the difference (open circles) at say 1 kg m^{-3} is of the order of $\pm 0.07 \times 10^{-15}$ m^2 V^{-2}. Since the difference is very small the percentage error is very large. Nonetheless, approximate limits can be set on the difference value e.g. at 1 kg m^{-3} the value of $\Delta\epsilon/E^2$ is likely to be between -0.01 and -0.05×10^{-15} m^2 V^{-2} the most probable value being -0.03×10^{-15} m^2 V^{-2}.

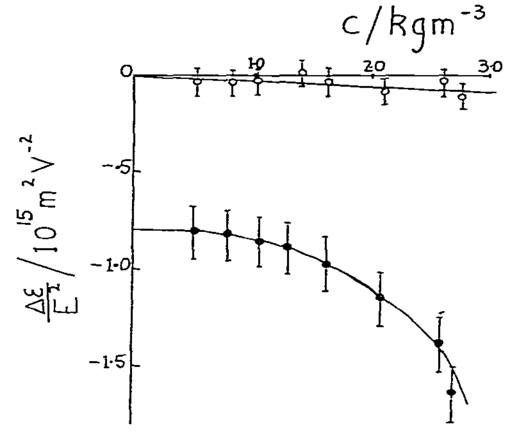

Fig. 2. Plot of $\Delta\epsilon/E^2/10^{-15}$ m^2 V^{-2} against concentration C/kg m^{-3} of myoglobin in water at 293 K. Full circles correspond to measured values. Open circles correspond to values for myoglobin solution less those due to water of the same conductivity in each case.

Since the measurements of the NDE were carried out in the middle of the dipolar dispersion region [6] the contribution of the dipole to the measured value has been reduced by about 60%. As a result, the estimated value of $\Delta\epsilon/E^2$ for the myoglobin at 1 kg m^{-3} becomes -0.06×10^{-15} m^2 V^{-2}. The corrections due to the atomic and nuclear and electronic contributions have been assumed negligible in the context of this estimate.

The pH of the solution was found to vary with concentration becoming more acidic with increasing concentration. Dilute solutions were found to have a pH close to the isoelectric pH of 7. Typically the pH for solutions of 3 kg m^{-3} was about 5. The more concentrated solutions were dialysed against distilled water in an attempt to remove ionic impurities. This method was found to be of limited application.

5. Discussion

Inspecting the data given by the closed circles of fig. 2 (i.e. the results for the myoglobin solution) and bearing in mind the effect of conductivity on the measured $\Delta\epsilon/E^2$ for water it is clear that for the range of concentrations considered here the contribution of the myoglobin is much less than that of the water.

The open circles of fig. 2 obtained by subtraction of the water data from those of myoglobin solutions of the same conductivity will effectively correspond to the myoglobin contribution to the NDE with certain limitations and corrections. The first and obvious point to be considered is the loss of water signal for the solution due to the volume occupied by the myoglobin. Since the concentrations used are of the order of 1 kg m^{-3} this correction will be 0.1% at most and is negligible. Subtraction of the water value assumes that is is not modified (in the solution) by the presence of the myoglobin, i.e., there are no significant solute solvent interactions. This subject has been considered in detail by South and Grant [6].

Water in the neighbourhood of the myoglobin molecules will experience an electric field due to its dipole and any surface charges on the protein, giving rise to saturation, i.e., removal of their contribution to the NDE. South and Grant [6] conclude that this effect is not large and would be negligible in terms of the volume of water involved at the low concentration used here. The maximum possible volume of the hydrated myoglobin molecules using the structured water approach of O'Konski [33] is given by South and Grant [6] as 7.5 l per m^3 of solution at a concentration of 1 kg m^{-3}. This would reduce the NDE contribution from the water (in the myoglobin solution) by 0.006×10^{-15} m^2 V^{-2} a figure which is an order of magnitude less than the calculated value for myoglobin. As a result it would appear that the value for myoglobin derived as described above is reasonably correct.

Using eq. (2) the value of $\Delta\epsilon/E^2$ due to myoglobin can be estimated. All calculations are taken for the case of a concentration of 1 kg m^{-1}. From the data of South and Grant [6] for aqueous myoglobin solutions the dielectric increment is 0.15 and hence ϵ for the solution assuming ϵ for water to be 80.1 as previously [32] will be 80.25. The dielectric decrement is given by the same authors as 0.04 and hence n^2 will be 1.77 less 0.04 or 1.73. Using these values the quantity in the braces in eq. (2) which is essentially the local field factor becomes 23.7 compared to 24.7 for water itself. Assuming μ to be 5.79×10^{-28} Cm as given by South and Grant [6], $T = 293$ K, the molecular weight of myoglobin as 17 000, $R_s = 1$ (no solute-solute interactions), the predicted value of $\Delta\epsilon/E^2$ becomes -3.7×10^{-15} m^2 V^{-2}. This compares with the value for water of -0.8×10^{-15} m^2 V^{-2} and the measured value for the myoglobin solutions of some two orders of magnitude less than this.

It is obvious, therefore that the data cannot be explained simply in terms of the classical NDE with no intermolecular interactions involving the myoglobin. The possibility of other effects such as structured water, ion atmosphere, Schwarz ion mobility and Maxwell-Wagner effects have been discounted by South and Grant [6]. These authors considered these effects up to conductivities of 4×10^{-2} S m^{-1} which is an order of magnitude higher than the most conducting solutions used in this study.

Returning to eq. (2) the product of the local field factor and R_s must be of the order of 0.01 to explain the experimental data (assuming the value of μ as taken from the literature to be correct). If it is further assumed that the local field factor is substantially correct then the data give a value of R_s of the order of 0.01. Taking the case of pair interactions as mentioned in section 2 and remembering that $R_p \approx \sqrt[3]{R_s}$ a value of R_p of about 0.2 is obtained. This value of R_p or the Kirkwood 'g' factor of 0.2 implies that the myoglobin molecules are arranged in antiparallel pairs hence reducing their effective dipole moment. As pointed out previously the Oncley [2] equation assumes $g = 1$ and hence the experimentally determined value of μ which South and Grant [6] quote will be affected; it will not, however, affect their theoretical value.

One other possibility needs to be considered namely, the presence of possible positive $\Delta\epsilon$ effects for myoglobin which are partially masking the classical negative effect. Since the myoglobin molecule is essentially spherical the contribution of positive effects due to anisotropy of polarizability would be small. The other source of positive effects, conformational changes is much more likely and cannot be ruled out as a possibility here.

6. Conclusions

Due to the relatively small difference between the measured values for myoglobin solutions and for water the NDE for myoglobin could not be accurately determined. However, the very large discrepancy between the predicted value for the classical NDE effect and the experimental value gives some interesting insights into possible mechanisms involving the myoglobin molecule. It would appear that the most likely explanation for the anomaly is a strong antiparallel alignment of myoglobin dipole pairs. The possibility that the dipole moments given in the literature are incorrect cannot of course be ruled out and any explanation of the results could conceivably partially involve a reduction of the dipole moment, but it is unlikely that the discrepancy could be completely accounted for in this way.

The possibility of a positive NDE masking the negative effect cannot be overruled. A positive effect if present would be due to field induced conformational changes of the myoglobin molecules and would be of important biological significance. Further studies of this system are indicated.

Acknowledgement

This work was supported by an SRC grant. One of us AED wishes to express her gratitude to the SRC for a research studentship held during the course of the work.

References

[1] J.L. Oncley, J. Am. Chem. Soc. 60 (1978) 1115.
[2] J.L. Oncley, in: Proteins amino acids and peptides, eds. F.J. Cohn and J.T. Edsall (Reinhold, New York) p. 543.
[3] J.L. Oncley, J.D. Ferry and J. Shack, Cold Spring Harbour Symp. Quant. Biol. 6 (1938) 21.
[4] J.L. Oncley, J.D. Ferry and J. Shack, Ann. N.Y. Acad. Sci. 40 (1940) 371.
[5] H.O. Marcy and J. Wyman Jr., J. Am. Chem. Soc. 64 (1942) 638.
[6] G.P. South and E.H. Grant, Proc. R. Soc. Lond. A. 328 (1972) 371.
[7] J.G. Kirkwood and J.B. Shumaker, Proc. Nat. Acad. Sci. U.S.A. 38 (1952) 855.
[8] J.C. Maxwell, A treatise on electricity and magnetism (Oxford University Press, 1892).
[9] K. Wagner, Ann. Phys. 40 (1913) 817.
[10] H.P. Schwan, Adv. Biol. Med. Phys. 5 (1959) 147.
[11] G. Schwarz, J. Phys. Chem. 66 (1962) 2636.
[12] C.T. O'Konski, J. Chem. Phys. 23 (1955) 1559.
[13] P. Debye and H. Falkenhagen, Phys. Z. 29 (1928) 121, 401.
[14] B. Jacobson, J. Am. Chem. Soc. 77 (1955) 2919.
[15] J.G. Kirkwood, J. Chem. Phys. 7 (1939) 911.
[16] L. Onsager, J. Am. Chem. Soc. 58 (1936) 1486.
[17] J. Herweg, Z. Physik 3 (1920) 36.
[18] A. Piekara and B. Piekara, Compt. rend. 203 (1936) 852.
[19] A. Piekara, S. Kielich and A. Chełkowski, Arch. Sci. 12 (1959) 59.
[20] A. Piekara, J. Chem. Phys. 36 (1972) 2145.
[21] C.J.F. Böttcher, Theory of electric polarization (Second Edition) Vol. 1, Ch. 7 (Elsevier Scientific Publishing Company, Amsterdam, 1973).
[22] S. Kielich, in: Dielectric and related molecular processes, Vol. 1, ed. Mansel Davies (Specialist Periodical Reports) (The Chemical Society, London, 1972). p. 192.
[23] G. Parry Jones, in: Dielectric and related molecular processes, Vol. 2, ed. Mansel Davies (Specialist Periodical Reports) (The Chemical Society, London, 1975) p. 198.
[24] A. Debye, Polar molecules, Ch. 6 (Dover Publications, Inc., New York, 1929).
[25] A. Piekara and A. Chełkowski, J. Chem. Phys. 25 (1956) 428.
[26] J. Małecki, J. Chem. Phys. 43 (1965) 1351.
[27] A. Piekara and S. Kielich, J. Chem. Phys. 29 (1958) 1297.
[28] A. Piekara and S. Kielich, J. Phys. Rad. 18 (1957) 490.
[29] P.A. Bradley and G. Parry Jones, J. Phys. E. 7 (1974) 449.
[30] A.E. Davies, M.J. van der Sluijs and G. Parry Jones, J. Phys. E. 11 (1978) 737.
[31] G. Parry Jones and T. Kurpkowski, J.C.S. Faraday II 70 (1974) 862.
[32] A.E. Davies, M.J. van der Sluijs, G. Parry Jones and M. Davies, J.C.S. Faraday II 74 (1978) 571.
[33] C.T. O'Konski, J. Phys. Chem. 64 (1960) 605.