Short Communication

The Dipole Moment of Myoglobin

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Protein molecules normally carry a complex assembly of charges that give rise to a large dipole moment, which is reflected in high values of permittivity (dielectric constant) and in the well defined dielectric dispersions observed for protein solutions (Onley, 1942; Schwan & Pennock, 1969; Grant, South, Takashima & Ichimura, 1971). It has been common practice to calculate a value for the dipole moment of a protein molecule in water from dielectric measurements on the aqueous solution. This has been done by assuming a semi-empirical relationship between dipole moment (μ) and the specific increment (δ), and has yielded values of μ that have become accepted as the effective value of the dipole moment in water. However, although this approach has been useful for comparing the dielectric behaviour of protein molecules, the values of μ so obtained have been of limited use as structural parameters, a fact that has tended to diminish the importance of dielectric studies in biophysics and biochemistry. They would be correspondingly enhanced if one could be confident that the dipole moment obtained from permittivity measurements represented the true structural dipole moment. Now that the crystal structure is known for several proteins that are also amenable to dielectric investigation it is possible to make a comparison between calculated and measured dipole moments. The purpose of this paper is to report the conclusions obtained for one protein (myoglobin).

Aqueous solutions of sperm-whale myoglobin and horse myoglobin were prepared according to standard procedures (Akeson & Theorell, 1960) by using the Seravac Laboratories (Maidenhead, Berks., U.K.) product. A series of concentrations up to about 20 g/l was prepared for both proteins, and samples were dialysed before use. The pH was varied by adding KCl or KOH. Concentrations were determined spectrophotometrically. The dielectric measurements were made between 200 kHz and 100 MHz by use of Wayne Kerr Bridges (South, 1970; Jordan & Grant, 1970). The dielectric results

were analysed by fitting various dispersion functions (Grant *et al.* 1971; South, 1970) and minimizing the root-mean-square deviation. This procedure yielded the specific increment, from which the dipole moment was calculated in C·m from the expression:

$$\mu = \left(\frac{\epsilon_0 M k T \delta}{2\pi N_A} \right)^{1/2} \quad (1)$$

where M is the protein molecular weight, N_A is Avogadro's number, ϵ_0 is the permittivity of free space, k is Boltzmann's constant and T is the absolute temperature. Eqn. (1) was originally derived on semi-empirical grounds (Onley, 1942), but recent work (South, 1970) has shown that the evidence for its validity is stronger than might originally have been contemplated. Fig. 1 shows the experimental values of μ obtained by the above procedure expressed in the more familiar Debye units. The range of pH values that was experimentally accessible was, unfortunately, limited by the maximum conductivity that could be tolerated by the dielectric measuring apparatus.

The dipole moment of the myoglobin molecule was calculated from the structural co-ordinates (kindly supplied by Dr J. C. Kendrew) as follows.

In general the dipole moment of a protein molecule in aqueous solution fluctuates with time, as protons move to and from the ionizable sites on the molecular surface. The calculation may therefore be divided into two parts, first, the permanent (or average) dipole moment, and second, the mean-square fluctuation dipole moment. The kinetic considerations of such a system have been discussed previously (Scheider, 1965).

The permanent dipole moment may itself be subdivided into two parts, the contribution from the charged surface groups and the contribution from the remainder, or the core, of the protein.

The surface charges reside on the ionizable amino acids, on the terminal carboxyl and amino groups of the peptide chain, and on the iron atom and the two propionic acid groups of the haem moiety.

Fig. 1. Calculated and experimental dipole moments of myoglobin. Experimental details are given in the text. (a) Whale myoglobin; (b) horse myoglobin. —, Surface charges only; ---, fluctuation dipole only; ----, surface charges + core dipole; - - - - , surface charges + fluctuation dipole; ———, surface charges + fluctuation dipole + core dipole; ●, experimental values.

Special consideration must be given to the histidine residues.

Evidence from titration studies shows that approximately six of the 12 histidine residues in myoglobin are in equilibrium with H^+ ions and have a pK of 6.6. The other six appear to be buried in the fabric of the protein and are not available for titration in the native protein. Studies on the chemical modification of the histidine residues with bromoacetate show that between seven and eight histidine residues are accessible to the reagent. These have been identified in the sequence of amino acids and presumably include the six titratable histidine residues. On the basis of these studies and by making reference to the atomic model of myoglobin we have assumed that the following seven histidine residues are in equilibrium with H^+ ions: A10, B5, CD6, EF4, FG3, G14 and G17 (the nomenclature is that proposed by Kendrew *et al.* 1962). These residues, when ionized, thus contribute to the dipole moment of myoglobin.

The probability, P , that such ionizable sites are charged depends on the pH of the solution and on the pK of the group concerned. The average charge on a group is eP (where e is the charge on an electron) and if the position vector of the group with respect to the centre of mass is r , then the contribution of

the group to the permanent dipole moment is ePr . These contributions have been summed over all the ionizable sites and the results are shown in Fig. 1.

The contribution to the permanent dipole moment from the core of the protein is not so easily quantified, but has been estimated by using the result of Wada (1959) that the dipole moment per amino acid residue along the length of an α -helix is 3.5D. This has been summed vectorially along the peptide chain and its effect on the permanent dipole moment is illustrated in Fig. 1. It may be seen that the core dipole is a minor term.

A calculation has also been made of the root-mean-square fluctuation dipole of the molecule, which is a measure of how far the fluctuating dipole deviates from its average value. It was first pointed out by Kirkwood & Shumaker (1952) that a dielectric dispersion may result from such fluctuations, even if the average dipolar moment is zero. It was further shown by them and by South (1970) that the fluctuation dipole is also a simple function of pH and the pK values of the groups. The contribution from the fluctuating dipole is shown in Fig. 1 with the contributions from the permanent dipole. The total dipole moment, indicated by the solid line in Fig. 1, is obtained by summing the effect of all these contributions.

Having regard to the complexity of the system under investigation, the agreement between theory and experiment is very satisfactory. For example, the calculations are successful in predicting that the dipole moment of horse myoglobin is about 20D greater than that of whale myoglobin in the isoelectric range, owing to 19 of the 153 residues being different. The curves in Fig. 1 might at first sight seem to suggest that best agreement is obtained in some cases by ignoring the contribution to the dipole moment from the core dipole. Alternatively, Schlecht (1969) ignored the presence of both the core dipole and the fluctuation dipole when considering the dipole moment of the uncharged molecule only. However, all these factors must be present and therefore must be accounted for in a full analysis. Additional contributions may arise from the counterions adjacent to the surface charges and from the bound water. This preliminary analysis could also be improved upon by considering the C-C, C-N and C-O bonds in the amino acid side chains, although many of these contributions to the dipole moment would tend to cancel because of the helical nature of the peptide chain. Finally, there are the refinements necessary to eqn. (1). Preliminary investigation (G. P. South & E. H. Grant, unpublished work) suggests that these and other considerations can be accounted for, including the effect on the dipole moment of uncertainty in the co-ordinates. A root-mean-square error of

1 Å in the co-ordinates would lead to an error in μ of the order of 30D.

The important conclusion from the present work is that dielectric studies can be used to provide values of the dipole moment of a protein molecule that agree well with those calculated from structural considerations. It is hoped that this agreement can be improved still further by refining the dielectric theory of polar liquids and by including other subsidiary factors in the calculation of the dipole moment.

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