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Measurement and computation of the dipole moment of globular proteins III: Chymotrypsin

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Abstract

The dipole moments of α- and γ-chymotrypsin are determined experimentally using the dielectric constant measuring method. The values thus obtained are compared with the results of the electric dichroism measurements for α-chymotrypsins by other investigators. The agreement is reasonably good, if not satisfactory. The cause of difference appears to be due to the difficulty of finding the correct internal field. The interaction between two neighboring dipoles is found to be a minor component of the local fields.

Secondly, the dipole moment of α-chymotrypsin was computed using Protein Data Bases. The dipole moment of proteins consists of two major components, the moment due to fixed surface charges and the core moment due to polar chemical bonds. The method of calculation was described in detail in previous papers. The pK shifts of polar side chains were calculated using the methods of Tanford et al. and its modification by Warshel et al. The agreement between measured and calculated dipole moments is satisfactory.

Keywords: Chymotrypsin; Dipole moment; Dielectric constant

1. Introduction

In general, the dipole moment of polymer molecules such as proteins and nucleic acids in solution can be determined either by the frequency domain dielectric constant measurement technique [1] or the time domain electric birefringence method [2]. However, the correction of the measured dipole moment for the internal field is essential for both techniques. In view of the difficulty of finding the correct internal field, neither the time domain nor frequency domain techniques is completely unequivocal.

In this report, I discuss the dipole moments of α- and γ-chymotrypsins which were determined by the dielectric constant technique and also those obtained by the electric dichroism method. The dipole moments of α-chymotrypsins obtained by previous investigators is 2.4×10^{-27} C m [3]. This value was corrected for the internal field using the following equation [4]:

$$E_i = [3\epsilon_a/(2\epsilon_a + \epsilon_i)]\, E_o \qquad (1)$$

where E_i and E_o are internal and applied electric fields, and ϵ_a, ϵ_i are the dielectric constants of external medium and that of spherical cavity. This cavity is either assumed to be vacuum or filled with dielectric material. ϵ_i was assumed to be 1 by

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previous authors, a value for vacuum, and a value of 1.6×10^{-27} C m (ca. 480 Debye unit) was obtained. The dipole moments of α-chymotrypsins determined by the present author using the frequency domain technique is about 390 D as discussed below.

2. Experimental

2.1. Measuring techniques

The electrical capacitance of chymotrypsin solutions were measured, using an impedance analyzer Hewlett-Packard 4191A, between 10 KHz and 10 MHz. The system was fully automated by the use of an on-line computer. The measurements were performed in a wide range of protein concentrations.

2.2. Materials and procedures

Both α- and γ-chymotrypsins were purchased from Boehringer Mannheim (Mannheim, Germany) and Sigma Chemical Corp. (St. Louis, MO, USA). They were used without further purification. The pH of the protein solutions was loosely controlled to about 7.0–8.5 by adding small amounts of dilute HCl or NaOH solutions. No buffer was used for pH adjustment in order to avoid the increase of sample conductivities. In this pH range, the dipole moment of α-chymotrypsin is reasonably independent of pH [3].

The dielectric constant of sample solution was determined using an impedance analyzer between 10 kHz and 10 MHz. The dielectric measurement with a sample solution was followed by measurement with a matched reference solution with its conductance adjusted to that of the sample solution. The purpose of this procedure is to facilitate the correction of measured sample capacitance for electrode polarization and other artifacts. The dielectric constant was calculated using the following formula

$$\epsilon = A(\epsilon_w - 1) \text{ where } A = (C_s - C_o)/(C_w - C_o)$$

where C_s, C_o and C_w are the capacitances of the sample cell with the samples, air, and matched salt solutions. ϵ_w is the dielectric constant of water, 78.5 at 25°C.

3. Results

The dielectric constant of α-chymotrypsin solution between 30 kHz and 100 MHz is shown in Fig. 1. Because of the frequency limitation of the impedance analyzer, the measurements are truncated at 10 MHz. In order to obtain the complete dispersion curve, the following procedure was used. The point designated as ϵ_∞ (high frequency dielectric constant) was calculated using Fricke's equation [5]

$$\frac{\epsilon_\infty - \epsilon_w}{\epsilon_\infty + 2\epsilon_w} = p\frac{\epsilon_i - \epsilon_w}{\epsilon_i + 2\epsilon_w} \tag{2}$$

where ϵ_w and ϵ_i are the dielectric constants of the solvent (78.5 at 25°C) and of the protein molecule (= 5 or 6). p is volume fraction of protein. An approximate dispersion curve is drawn by computer fitting and the relaxation frequency f_r is found (f_r is the frequency corresponding to the half point of the dispersion curve). Substitute ϵ_o, ϵ_∞, f_r and a chosen value of n ($0 < n \leq 1.0$) in the Cole–Cole equation [6]

$$\epsilon = \epsilon_\infty + \frac{\Delta\epsilon\{1 + (\omega\tau)^n \cos(n\pi/2)\}}{1 + 2(\omega\tau)^n \cos(n\pi/2) + (\omega\tau)^{2n}} \tag{3}$$

where $\omega = 2\pi f$, $n = 1 - \alpha$ (α is the Cole–Cole distribution parameter), τ is the relaxation time ($= 1/2\pi f_r$). We can now calculate the dispersion curve

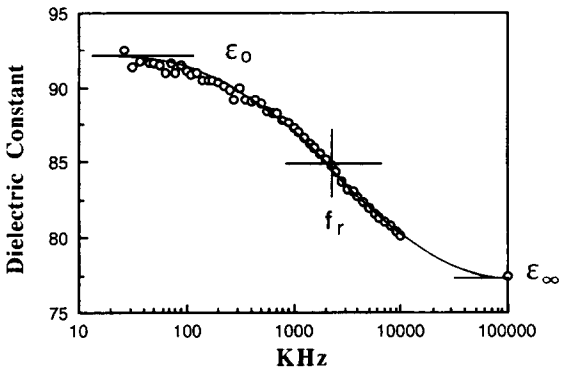

Fig. 1. The dielectric dispersion curve of α-chymotrypsin. The point ϵ_∞ is calculated by Fricke's equation and the solid line was calculated using the Cole-Cole equation. The distribution parameter n is 0.79, f_r is the relaxation frequency at which $\epsilon = (\epsilon_o - \epsilon_\infty)/2$.

more accurately. If the initial computer curve fitting is correctly done, the Cole–Cole equation should yield a dispersion curve that is identical to the initial curve. However, normally, 2–4 iterations are required to obtain a satisfactory agreement between the initial guess and the calculated dispersion curve. The proper choice of n value is important for the curve fitting and a range of 0.19–0.2 is found to be satisfactory for chymotrypsin (see Fig. 1).

Dipole moment was calculated using Oncley's equation [7]

$$\mu^2 = 9000kTM\delta/4\pi Nh \quad (4)$$

where k is the Boltzman constant, N is the Avogadro's number, M is the molecular weight. δ is the intrinsic dielectric increment, i.e., $\Delta\epsilon/c$ extrapolated at $c \rightarrow 0$. $\Delta\epsilon$ is the difference between the low and high frequency limiting dielectric constant and c is the concentration per gram per liter. h is an empirical parameter, which has been considered to represent the correction for internal fields. The numerical value of h was determined empirically using glycine and glycine peptides as calibration standards. The dipole moments obtained with these h-parameters are shown in Table 1. The dipole moment can also be calculated using Kirkwood's theory [8] for the binary mixture of polar molecules.

$$\mu^2 = 9kTP_2/4\pi N \quad (5)$$

where

$$P_2 = (2/9)1000\delta - \epsilon_w v \quad (6)$$

δ is molar increment and v is partial molal volume of the dipolar molecule and ϵ_w is the dielectric constant of the solvent. The dipole moments calculated by Kirkwood's theory are also shown in Table 1. These two equations yield similar dipole moments, however, the Kirkwood theory which has no empirical parameter seems to be more reliable than Oncley's equation.

Table 1
Numerical values of the h-parameter in Oncley's equation. Gly and gly-peptides were used as the calibration standards

n	h	μ of α-chymotrypsin
gly	4.0	394.6
gly-dipeptide	4.2	385.1
gly-tripeptide	4.28	381.5
gly-tetrapeptide	4.34	378.8
gly-pentapeptide	4.38	374.7
Dipole moment calculated by Kirkwood's theory		369.0

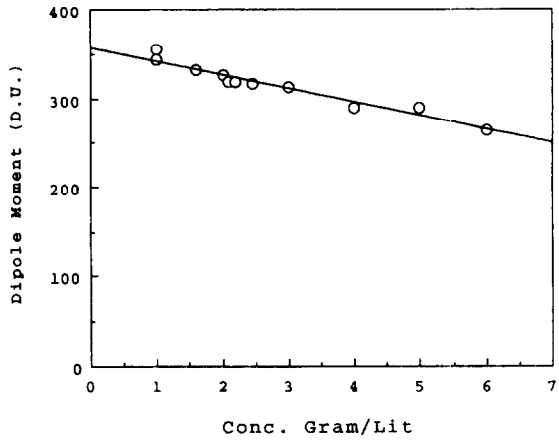

Fig. 2. Concentration dependency of the dipole moment ω of α-chymotrypsin. Intrinsic value of the dipole moment is obtained by extrapolating the plot at zero concentration by linear regression.

Fig. 2 illustrates the plot of measured dipole moment of α-chymotrypsin as a function of protein concentration. The intrinsic dipole moment is 360–370 DU determined at zero concentration by linear regression.

3.1. Analysis of data

As shown in Table 2, the difference between the dipole moments obtained by the dielectric constant and the electric dichroism methods may be partially due to the experimental errors in both measurements. However, the lack of thorough understanding of the

Table 2
Dipole moments and relaxation times of α- and γ-chymotrypsin determined by the electric birefringe method and those obtained by dielectric constant measurements

	Present work		Elec. dichroism		
	μ	τ [a]	μ	$\tau 1$	$\tau 2$
α-chymotrypsin	389 DU	55.29 ns	480 DU	29 ns	94 ns
γ-chymotrypsin	401 DU	66.32 ns	–	–	–

[a] Mean relaxation time.

local fields may be a more serious problem than random technical errors. As mentioned earlier, the dipole moments obtained by the electric dichroism technique were corrected using Eq. 1, which represents the cavity field with $\epsilon_i = 1$ defined by Onsager [4]. However, for the present case, the cavity is filled with a protein molecule and thus, a dielectric constant of 5–6 must be used for ϵ_i. This consideration will result in a decrease in the internal field and an increase in the dipole moment.

According to Onsager [4], the local field consists of two components, i.e., internal field E_i and reaction field E_r. The internal field E_i is already defined by Eq. 1. The reaction field is a local field created by the polarization of neighboring dipoles. This problem was already discussed by Onsager for gas molecules. However, there has been very little work on the general theory of reaction field for liquid states or for solutions.

Recently, Stoy [9,10] investigated the potential distribution around two particles using the bispherical coordinates. He treated two cases, i.e., (1) the axis connecting the centers of two particles is perpendicular to applied electrical field and (2) the axis is parallel to E vector (see Fig. 3). The electrical field in the vicinity of a particle 2 with the axis perpendicular to the applied field is given by the following equation

$$F_1 = E_o\left[1 + \frac{2K_1}{r^3}\left(\frac{1 - K_2/D^3}{1 - K_1K_2/D^6}\right)\right] \qquad (7)$$

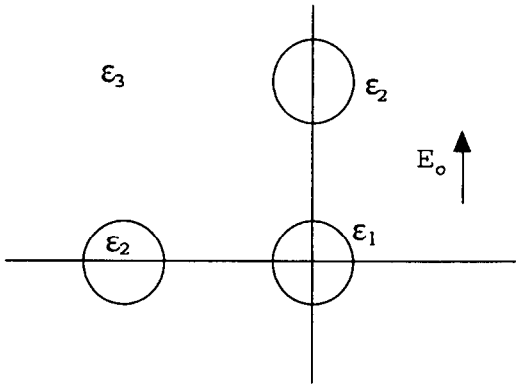

Fig. 3. Spatial arrangements of two particles. (1) The axis connecting the centers of the two particles is perpendicular to the vector of the applied field. (2) The axis parallel to E-vector.

Table 3
The internal field in a spherical particle in the presence of another particle. ϵ_1 and ϵ_2 are assumed to be 6 and ϵ_3 is 78.5

(A) Transverse field				
R_i/D	r/D	F_2	E_i	E_{random} [a]
0.40	0.600	1.1356	1.6407	1.4552
0.33	0.666	1.0565	1.5264	1.4473
0.285	0.714	1.0287	1.4863	1.4456
				1.4444 [b]

(B) Parallel field			
R_i/D	r/D	F_2	E_i
0.40	0.600	0.7506	1.0844
0.33	0.666	0.8923	1.2892
0.285	0.714	0.9442	1.3642

[a] E_{random} is calculated by $(2E_{\text{trans}} + E_{\text{long}})/3$.
[b] Calculated by Eq. 1.

where r is the distance from the center of particle 1. K_1 and K_2 are given by

$$K_1 = \frac{\epsilon_1 - \epsilon_3}{\epsilon_1 + 2\epsilon_3}R_1^3 \quad K_2 = \frac{\epsilon_2 - \epsilon 3}{\epsilon_1 + 2\epsilon_3}R_2^3 \qquad (8)$$

where ϵ_1, ϵ_2 and ϵ_3 are the dielectric constants of particle 1 and 2 and the medium surrounding particles. R_1 and R_2 are the radii of particles 1 and 2. D is the distance between two centers. Likewise, we can obtain a similar equation for a longitudinal field in the vicinity of particle 2.

$$F_2 = E_o\left[1 + \frac{K_1}{r^3}\left(\frac{1 + 2K_2/D^3}{1 - 4K_1K_2/D^6}\right)\right] \qquad (9)$$

In the present analysis, two protein molecules 1 and 2 are identical. Therefore $K_1 = K_2$. Using either Eq. 7 or 9, the internal field can now be calculated by

$$E_i = \left[3\epsilon_a/(2\epsilon_a + \epsilon_i)\right]F_2 \text{ or } F_1 \qquad (10)$$

Table 3 shows the results of this calculation. The table indicates that the transverse coupling creates a larger internal field than the longitudinal coupling and that the average internal field approaches the value of Eq. 1 as the distance between two particles increases. This clearly indicates that Eq. 1 represents the internal field in a single particle without an electrostatic interaction between neighboring molecules. The result also indicates that the interparticle interaction produces a relatively small in-

crease in the internal field. This indicates either that inter-particle dipole interactions are not a predominant factor for determining the magnitude of local fields or that Stoy's theory underestimates the reaction field by limiting the interparticle interaction to two particle problem.

A recent calculation of internal field by the present author using ellipsoidal particles surrounded by a thin hydration shell demonstrates that the internal field depends markedly on the dielectric constant of hydration shell. The detail of this calculation will be published elsewhere.

3.2. Calculation of the dipole moment using the protein data base

With the advent of the X-ray crystallography and the availability of the protein data base, the calculation of the dipole moments of globular proteins became possible to perform with considerable accuracies [3,11–16]. The dipole moment of proteins consists of two major components, the one is the core moment due to the vector sum of chemical bond moments and the other is the moment due to fixed surface charges.

3.2.1. Dipole moment due to fixed surface charges.

Positively charged amino acid residues used for the computation are lysine, arginine and histidine in side chains and N-terminals. On the other hand, negatively charged residues are Aspartic acid, glutamic acid, tyrosine and C-terminals. The pK shift of the ionizable amino acid residues in proteins was calculated using the theory by Tanford and Kirkwood [17] and the modification by Warshel and Russell [18]. The detail of this computation was given by previous papers [3,15,16]. The reconstituted titration curve using calculated pK values does not agree satisfactorily with the experimental result [19]. However, calculated dipole moments do not critically depend on the choice of the pK values of polar residues.

3.3. Calculation of the dipole moment

3.3.1. Dipole moment due to fixed surface charges.

The three dimensional coordinates of amino acid residues of α-chymotrypsin were obtained from the Brookhaven Protein Databank (Brookhaven, NY, USA) [20]. The x-component of the dipole moment

Table 4
The calculated dipole moment of α-chymotrypsin at isoelectric point (pH 8.1–8.5)

(1) Fixed charge dipole moment

	μ_x	μ_y	μ_z	$\mu_{(charge)}$	$\Delta\mu$ [a]
(A)	246.08	281.37	60.99	378.74	88.47
(B)	268.83	297.79	80.47	397.02	98.99

(2) Core dipole moment

	μ_x	μ_y	μ_z	$\mu_{(core)}$
	30.541	−4.95	−6.486	31.612

(3) Net dipole moment

	μ_x	μ_y	μ_z	$\mu_{(net)}$	$\mu_{(obs)}$
(A)	273.31	276.63	61.31	393.63	389.13
(B)	295.57	293.08	77.34	423.37	
(C)				479 (pH 8.3)	
				461 (pH 7.0)	
				374 (pH 5.7)	

(A) pK's were calculated using the Tanford method.
(B) pK's were calculated using the Warshel's modifed theory.
(C) Numerical calculation by Antosiewicz et al. [3].
[a] Root mean square dipole moment.

produced by a group of positive and negative charges is defined by the following equation

$$\mu_x = \Sigma n_j \cdot e \cdot (X^+ - X^-) \quad (11)$$

where e is the elementary charge and X^+ and X^- are the X-coordinates of positive and negative charge centers and are defined by

$$X_+ = \Sigma\{L_{j+} \cdot X_j\} \quad X_- = \Sigma\{L_{j-} \cdot X_j\} \quad (12)$$

Likewise, Y_+, Y_-, Z_+, Z_- are given by

$$Y_+ = \Sigma\{L_{j+} \cdot Y_j\} \quad Y_- = \Sigma\{L_{j-} \cdot Y_j\} \quad (13)$$

$$Z_+ = \Sigma\{L_{j+} . Z_j\} \quad Z_- = \Sigma\{L_{j-} . Z_j\} \quad (14)$$

The coordinates X_j, Y_j and Z_j are found in the protein data base. In these equation, L_j is given by the Henderson-Hasselbach equation, i.e., $L_{j+} = 1/(1 + B)$ for Lys, Arg, His and NH_2 terminals, and $L_{j-} = B/(1 + B)$ for Asp, Glu, Tyr and COOH terminals, where $B = 10^{pH - pK}$. Other components, μ_y and μ_z can also be computed using the same method. The pK values are calculated by the method discussed earlier. All of the computations were carried out at the isoelectric point where the effective positive and negative charges are equal.

$$\Sigma n_{j+} \cdot L_{j+} = \Sigma n_{j-} \cdot L_{j-} \quad (15)$$

where n_j is the number of jth amino acid residues.

The Henderson factors L_{j+} and L_{j-} represent the effective charge of amino acid residues, in other words, the Henderson factor represents the fraction of protonated or unprotonated residues. For some proteins, fractional ionization generates a large number of different charge configurations. In the case of chymotrypsin, however, the number of charge con-

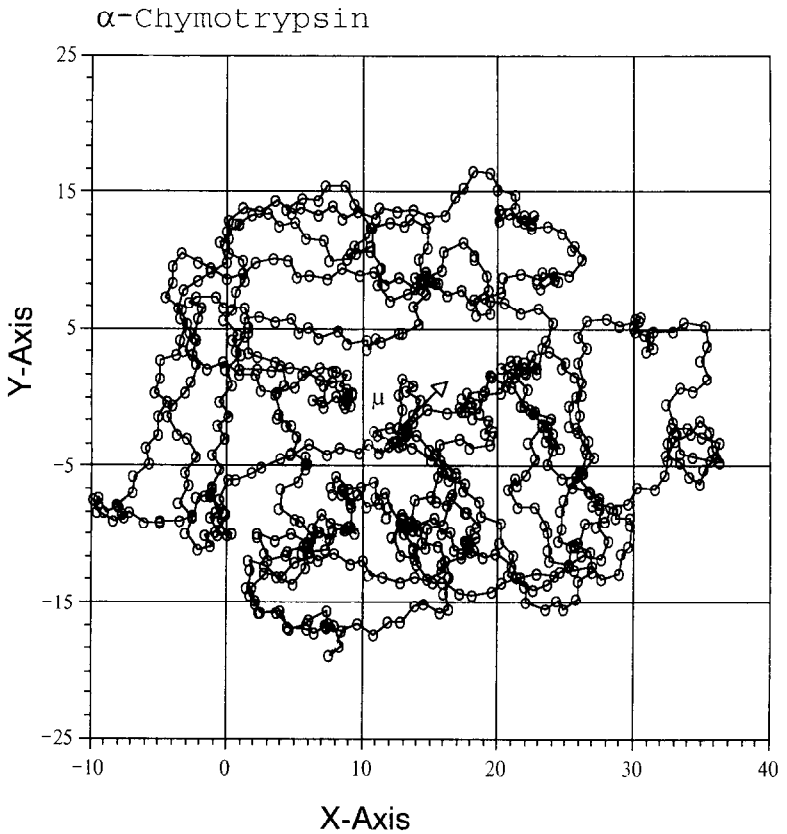

Fig. 4. The 2D plot of the chain configuration of α-chymotrypsin in the X–Y plane. Circles represent C, C_A and N atoms in the backbone chain. Side chains are not plotted. The arrow is the dipole moment vector. The distance between the positive and negative charge centers is 4.19 Å. The scale of both the X- and the Y-axes are in Å.

figurations would be only 364. Thus the calculation of dipole moment can be done fairly quickly.

3.4. Core dipole moment

The major contribution to the core moment is the bond moment of carbonyl groups (2.7 DU) [21]. The 3D coordinates of C and O in carbonyl groups are well documented in the database. In addition, the moment of N–H bond (1.31 DU) cannot be ignored. However, the coordinates of H atoms in N–H bonds is not documented in the database because of the nature of the X-ray crystallography. Thus, only the vectorial sum of C=O bond moments was computed in this work. It was found that the core moment is much smaller than the fixed surface charge dipole moment indicating that the orientation of C=O bonds is also nearly random.

3.5. The results of the computation

(a) The dipole moments of α-chymotrypsin calculated using the method discussed earlier are summarized in Table 3. The calculated dipole moments are in good agreement with the measured value, i.e., ca. 400 D vs. 369 D. It must be pointed out, however, that the result of the presented calculations is not necessarily in good agreement with those calculated by Antosiewicz et al. (see Table 4). The reason for the difference is still unknown.

Fig. 4 shows the 2D plot of α-chymotrypsin in X–Y plane. The arrow in this figure shows the vector of the fixed surface charge dipole moment. The angle between two dipole components is found to be 61.8° (core dipole moment is not shown in this figure).

4. Discussion

The aim of the experimental part of this work is the comparison of the dipole moments of chymotrypsin obtained by the frequency domain dielectric constant measurement with those obtained by the time domain electric dichroism technique. It appears that the difference between these results is due to the lack of a thorough understanding of local fields. In this paper, the effect of inter-particle interaction is investigated using the theory derived by Stoy. However, it was found that inter-particle interaction does not alter markedly the magnitude of internal field in individual particles.

Onsager's local field theory [4] which has been used for small polar molecules may not be suitable for charged polyions. The calculation by O'Konski et al. [22] using ellipsoidal particles as a model may be a better presentation of the internal field for polymer molecules. The calculation by the present author which is based on an ellipsoidal particle surrounded by a thin hydration shell will be discussed elsewhere.

For the computation of the dipole moment using X-ray data, the calculation of the pK shifts of charged groups is necessary. In this work, Tanford's theory and its modification by Warshel et al. were used. The calculated dipole moments are in good agreement with experimental results.

As discussed, calculation of the dipole moment of globular proteins is no longer prohibitively difficult because of the availability of Protein Data Bases. The dipole moment of polymers depends sensitively on the configuration of the molecule. In other words, the magnitude of the dipole moment and the change thereof, can be used as a parameter of the conformation change of the globular proteins.

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