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The electric dipole moment of DNA-binding HU protein calculated by the use of an NMR database

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Abstract

Electric birefringence measurements indicated the presence of a large permanent dipole moment in HU protein–DNA complex. In order to substantiate this observation, numerical computation of the dipole moment of HU protein homodimer was carried out by using NMR protein databases. The dipole moments of globular proteins have hitherto been calculated with X-ray databases and NMR data have never been used before. The advantages of NMR databases are: (a) NMR data are obtained, unlike X-ray databases, using protein solutions. Accordingly, this method eliminates the bothersome question as to the possible alteration of the protein structure due to the transition from the crystalline state to the solution state. This question is particularly important for proteins such as HU protein which has some degree of internal flexibility; (b) the three-dimensional coordinates of hydrogen atoms in protein molecules can be determined with a sufficient resolution and this enables the N–H as well as C=O bond moments to be calculated. Since the NMR database of HU protein from *Bacillus stearothermophilus* consists of 25 models, the surface charge as well as the core dipole moments were computed for each of these structures. The results of these calculations show that the net permanent dipole moments of HU protein homodimer is approximately 500–530 D (1 D = 3.33×10^{-30} Cm) at pH 7.5 and 600–630 D at the isoelectric point (pH 10.5). These permanent dipole moments are unusually large for a small protein of the size of 19.5 kDa. Nevertheless, the result of numerical calculations is compatible with the electro-optical observation, confirming a very large dipole moment in this protein.

Keywords: HU protein; Reversing-pulse electric birefringence; Electric dipole moment; NMR protein database; HU–DNA complex

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1. Introduction

The HU protein plays an important role of compact packaging of the high molecular weight DNA in prokaryotic cells, and its specific function as well as the structure have drawn attentions not only from biochemical but also from physicochemical points of view [1,2]. The interaction of HU proteins from various origins with DNA has been studied in the crystalline state as well as in solutions by a variety of methods. Yamaoka and colleagues [3,4] utilized the electric linear dichroism and reversing-pulse electric birefringence (RPEB) techniques to elucidate the higher-order structure and the electric properties of DNA and related macromolecules in solutions. A mutant HU from *Escherichia coli,* so-called super HU (sHU) species, in which the 53rd asparagine of HU-2 species is substituted by a positively charged arginine [5], is used for the present measurement. Sonicated and well-fractionated DNA fragments of various chain lengths, the sHU–DNA complexes in solutions were studied by the electrooptic methods. A preliminary report [6] shows that the RPEB signal of an sHU–DNA complex in solution exhibits a distinct minimum in the reverse process at an optimal mass ratio, $(\mathrm{sHU/DNA})_{\mathrm{mass}}$, of 5, whereas the signal of the DNA alone shows no such minimum [7].

As the introduction, a typical example is shown in Fig. 1 (the detailed electrooptic study of the HU–DNA protein system will be reported elsewhere). The absence of a minimum in the reverse process is a strong indication that the anti-parallel double-stranded DNA possesses no permanent electric dipole moment, though DNA molecule is oriented easily with a large induced electric dipole moment mostly due to counterion polarization [8–12]. The appearance of a marked minimum for the sHU–DNA complex is attributed to the permanent dipole moment, as theories predict [9,10]. The observed dipole moment is most likely to originate from the sHU species bound to DNA. In order to construct a tertiary structure like a super helix of the sHU–DNA complex from the electrooptic data, the magnitude and direction of electric moments must be known for an isolated sHU subunit and also for the dimer. The purpose

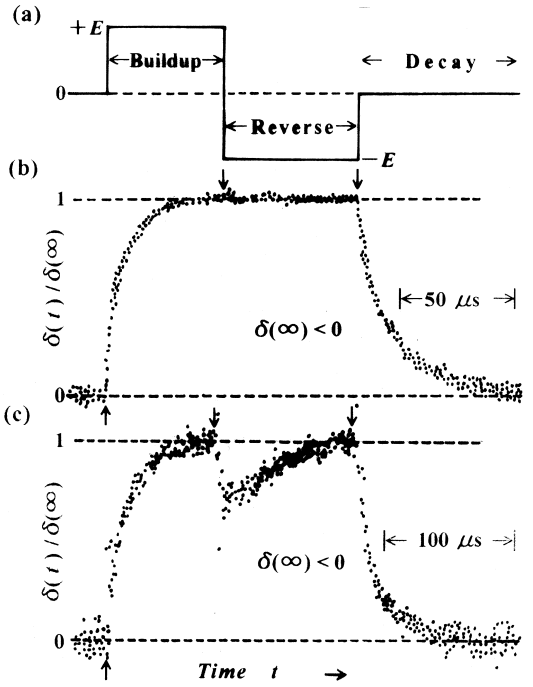

Fig. 1. RPEB signals of DNA (b) and sHU–DNA complex (c), and the schematic applied pulse field (a). The ordinate is the optical phase retardation at time t, $\delta(t)$, normalized by the steady-state value, $\delta(\infty)$, which is $-2.0°$ in (b) and $-0.23°$ in (c). The applied field strength in kV/cm: 3.94 (b) and 3.69 (c). The solution condition: 1 mM Tris acetate buffer (pH = 7.5) containing 0.27 mM $MgCl_2$. The concentration of DNA in μg: 46 (b) and 37 (c). The base pair of sonicated DNA is 320. The mixing ratio (sHU/DNA) mass corresponds to 40 sHU dimers per DNA fragment.

of this research is to find the large dipole moment in HU protein and its direction by numerical calculations instead of dielectric constant measurements, which may prove to be exceedingly difficult with HU protein solution. Moreover the measurement will not provide us with the direction of the dipole moment (Fig. 1).

HU protein from *Bacillus stearothermophilus* (Bst) consists of two homotypic subunits A and B having identical amino acid sequence and compositions. Each subunit consists of a head and a β-hairpin tail or arm [13]. The structure of the head part is reasonably stable and rigid, while the configuration of β-hairpin arm is variable in solution. The NMR study by Vis et al. [14,15] detected 25 distinguishable conformations for the

HU protein. In view of the marked internal flexibility of β-hairpin region, the dipole moment of HU protein is most likely to vary from one model to another. In the present work, two methods were used for the calculation. With the first method, the dipole moment of the subunits A and B were calculated separately and the overall dipole moment of the homodimer was obtained by vectorial summation (designated as A + B). With the second method, the dimeric HU protein was used for the calculation (designated as AB). The calculation was repeated for all of the 25 structures and the results are averaged. As will be shown, the results of both calculations are similar to each other and confirm the presence of a large permanent dipole moment in HU protein.

The advantages of the NMR method are twofold. The first one is the ability of this method, unlike the X-ray crystallography, to detect the coordinates of hydrogen atoms. NMR databases document the coordinates of all the hydrogen atoms with sufficient resolutions; hence, the group dipole moments of H–N bonds can be computed in addition to those of C=O bonds. This enables a more accurate information to be obtained as to the magnitude of core dipole moment.

Previous calculations, which are based on the X-ray diffraction method, ignore the H–N bond moment because of the lack of coordinates of the H atoms in databases. As will be discussed later, the net H–N bond moments in HU protein was found to be significant and cannot be ignored without causing a considerable error for the estimate of core dipole moment. Another important feature of this technique is that the determination of protein structures by NMR is carried out in solution rather than in the crystalline state. The question that persisted for many years is whether protein molecules maintain the same or, at least, similar conformations in solution as in the crystalline state. The results of previous computations of the dipole moments of globular proteins, using X-ray databases, were found to be in reasonable agreement with the measured dipole moments for many proteins studied so far. Under these circumstances, it can perhaps be claimed that the conformation of a protein in solution is similar to that in crystal. However, for the HU protein which has considerable flexibility in solution, the conformation in solution may not necessarily be the same as in crystals. As shown in Fig. 2, the head or rigid body region of HU protein is reasonably stable even in solution. However, β-arms seem to have sufficient flexibility to affect the overall conformation of homodimeric HU protein molecule in solution and cause variation in the magnitude of dipole moment.

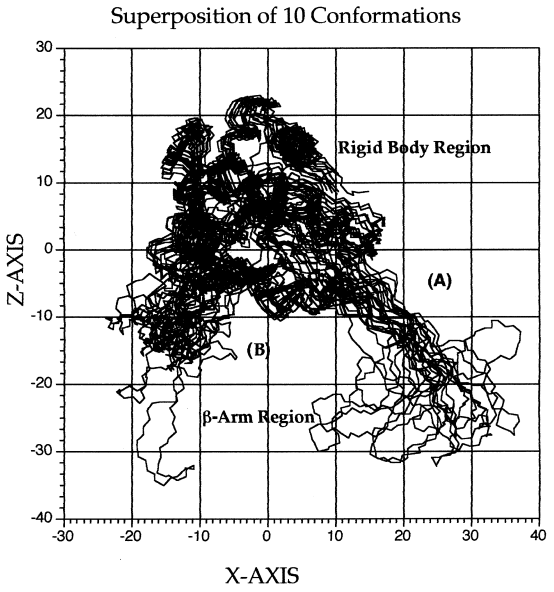

Fig. 2. The configuration of the homodimeric HU protein projected on the x–z plane. Ten randomly chosen homodimers are superimposed for this plotting. Note the considerable flexibility's in β-arms of A and B subunits.

2. Method of computation

2.1. Data acquisition

The database of HU protein was obtained directly, through the Internet, from the Protein Data Bank, Brookhaven National Laboratory (Web site: http//www. pdb.BNL. Gov/currently at Rutgers University, Web site: http//www. resb.org/pdb/Abola et al. [16], Bernstein et al. [17] (PDB ID Code '1HUE', Authors: H. Vis, M. Mariani, C.E. Wilson, R. Kaptein, R. Boelens [16,17]). The database consists of 25 models, each

having a size of approximately 1.5–1.6 Mbytes. After transferring the entire database to Microsoft Words, the remarks in the database were eliminated. The database was again transferred to Microsoft Excel, Version 5. 'Text Transport Wizard' was utilized in order to transform the data from a text format to a column format. The data were then transferred to Delta Plot for the graphic presentations in the two- and/or three-dimensional spaces. Two separate programs were written for the calculation of pK shifts and for the computation of surface charge dipole moments. The calculation of core dipole moments was performed, exclusively, by using Microsoft Excel.

2.2. Surface charge dipole moment and the pK shifts

The magnitude of dipole moments of the globular proteins is due, predominantly, to the asymmetric distribution of fixed surface charges. Thus, the calculation of surface charge dipole moment reduces to finding the center of positive and negative charges. The coordinates of charges are well documented in Protein Databases and finding the positive and negative charge centers can be done, if the effective charge of each polar site is accurately known, with the iteration technique discussed before [18]. It is well known that the pK values of amino acids are altered when they are incorporated in protein molecules. The cause of these shifts is mainly due to electrostatic interactions among polar residues distributed in the entire molecule. The method of the calculation of pK shifts was developed by Tanford and Kirkwood [19] and was modified by Warshel and Russell [20].

Although it had been known that the pK shifts had relatively small effects on the magnitude of calculated dipole moments for many proteins, the neglect of pK shifts was found, recently, to cause a considerable error for some highly charged small proteins (unpublished results). These results indicate that the extent of the error due to the neglect of pK shifts in proteins should not be underestimated.

In view of this observation, it was decided to calculate the pK shifts of each charged site and use them for the calculation of the surface charge dipole moments. In the following, the method of these calculations is summarized briefly.

3. Tanford–Kirkwood theory [19]

For HU protein, the pK shifts are calculated for arginine, lysine, aspartic acid and glutamic acid residues. In addition, N-terminals must be included in this calculation (the coordinates of C-terminals are not documented in the database). The pK shifts of amino acid residues in a protein molecule depend not only on the local environment but also on the charge distribution in the entire molecule. At the pH chosen for the calculation, particularly at the isoelectric point, most of the amino acid residues are partially ionized. This, in turn, creates numerous charge configurations necessitating the calculation repeated for all these charge configurations.

The major source of the pK shifts, i.e. the electrostatic interaction free energy, which was first computed by Tanford and Kirkwood is given by Eqs. (1) and (2).

$$\Delta G = \Sigma 2.303 \chi_n kT(\mathrm{p}K - \mathrm{p}K_n) \tag{1}$$

where χ_n is the occupation number, i.e. 0 for protonated site and 1 for unprotonated site. pK_n is the intrinsic dissociation constant. The electrostatic free energy of proton binding is given by

$$\Delta G_e = (e^2/2b) \sum_{k=1}^{n} \sum_{l=1}^{n} \xi_k \xi_k (\mathrm{A}_{k_l} - \mathrm{B}_{k_l}) - (e^2/2a) \sum^{n} \sum^{n} \xi_k \xi_l \mathrm{C}_{k_l} \tag{2}$$

where 'a' is the radius of protein including the thin layer of solvent surrounding it and 'b' is the radius of the protein core. ξ_k and ξ_l are the charges on the kth and lth sites. They are either $+1$ or -1 and e is the elementary charge. A_{kl} represents the energy required for charging polar residues in an unbounded medium with a dielectric constant ε_j. B_{kl} is the modification of the energy due to the assumption that proteins being

a bounded cavity of dielectric constant ε_i. Finally, C_{kl} represents the interaction energy among counterions in the solvent. The full expressions of A_{kl}, B_{kl} and C_{kl}, which are very cumbersome to reproduce, are found in the original paper (see Tanford and Kirkwood [19]).

The original theory by Kirkwood and Tanford was modified by Warshel and Russell by adding a self energy term [20] (see also Antosiewicz and Pörschke [21] who may be the first ones to incorporate the electrostatic effects in this calculation).

$$\Delta G_t = \Delta G_e + \Sigma x_k(\xi_k + \gamma_k)\frac{e^2}{2\sigma_k}\left(\frac{1}{\varepsilon_i} - \frac{1}{\varepsilon_0}\right) - \Sigma x_k \frac{e^2(\xi_k^2 - \gamma_k^2)}{2b} B_{kk} \quad (3)$$

where ΔG_e is given by Eq. (2), $x_k = \kappa_a$ where κ is the Debye screening constant and 'a' is given in Eq. (2) $e\xi_k$ and $e\gamma_k$ are the charges of protonated and unprotonated sites. σ_k is the radius of the kth ion to which a value of 2 Å is assigned. ε_i and ε_o are the dielectric constants of protein core and water, respectively. B_{kk} is the Legendre polynomial with the angle θ_{kl} being zero. Since this term approaches infinity as the angle θ_{kl} reduces to zero, the evaluation of this term is not straightforward. Another difficulty of using Eq. (3) is the choice of ε_i, the dielectric constant of protein core. Traditionally, the dielectric constant of protein core is assumed to be very small, i.e. 5–6. This small value, however, is not compatible with the Warshel–Russell theory and a much larger number has to be assigned in order to obtain a reasonable agreement with acid–base titration curves Antosiewicz and Pörschke [21], using a value as high as 50, found a reasonable pK shift which is compatible with the measured acid–base titration curve. The present authors varied the angle θ from 0.1 to 0.001° and found the value of ε_i that is needed to produce a compatible pK shift, to approach a limiting value of approximately 60. This value turned out to be similar to the one used by Antosiewicz and Pörschke [21]. The mean pK shifts, averaged for all the models (1–25), for lysine, arginine, aspartic acid, glutamic acid and N-terminal in the subunits A and B are shown in Table 1.

4. Method of dipole moment calculation [18]

4.1. Surface charge dipole moment

The x, y, and z components of the dipole moment of HU protein subunits A and B are calculated separately and the total dipole moment is reconstituted, by using Eq. (4):

$$\mu^2 = \mu_x^2 + \mu_y^2 + \mu_z^2 \quad (4)$$

The x component, e.g. of the surface charge dipole moment is defined by:

$$\mu_x = \Sigma n_j \cdot e \cdot (X^+ - X^-) \quad (5)$$

and also by two similar equations for the y and z-components. Here n_j is the number of surface charge of the jth amino acid, e is the elementary charge, and X^+ and X^- are the weighted x-coordinates of positive and negative charge centers.

Table 1
The pK shifts of amino acid residues[a]

	Intrinsic pK	Shifted pK in subunit A	Shifted pK in subunit B
Asp	3.86	3.56	3.49
Glu	4.25	4.812	4.78
Lys	10.53	10.83	10.84
Arg	12.48	13.28	13.26
N-Term	9.21 (*Met*)	9.06	9.09

[a] Each of 25 models exhibited a slightly different pK shift.

X^+ and X^- are defined by the following equations.

$$X^+ = \Sigma\{L_j^+ \cdot X_j\} \text{ and } X^- = \Sigma\{L_j^- \cdot X_j\} \quad (6)$$

Likewise

$$Y^+ = \Sigma\{L_j^+ \cdot Y_j\} \text{ and } Y^- = \Sigma\{L_j^- \cdot Y_j\} \quad (7)$$

$$Z^+ = \Sigma\{L_j^+ \cdot Z_j\} \text{ and } Z^- = \Sigma\{L_j^- \cdot Z_j\} \quad (8)$$

where X_j, Y_j and Z_j are the coordinates of surface charges, which can be found in the database. The weighting factors L_j^+ and L_j^- are defined by the Henderson–Hasselbalch equation, i.e.

$$L_j^+ = M/(1+M) \text{ for Asp and Glu} \quad (9)$$

$$L_j^- = 1/(1+M) \text{ for Lys, Arg, and N-Terminal} \quad (10)$$

where $M = 10^{\mathrm{pH}-\mathrm{p}K}$ and pK is the corrected value as discussed above.

The dipole moment of HU protein can be calculated for the homodimer directly. Also it can be calculated for each subunit A and B separately and the computed dipole moments can be added vectorially. The calculation can be done by the use of Eq. (5) at the isoelectric point (pH 10.5 for HU protein) where

$$\Sigma n_j L_j^+ = \Sigma n_j L_j^- \quad (11)$$

In the present work, the computations were also performed at pH 7.5 where the electro-optic measurements were carried out. For these calculations, Eq. (12) instead of Eq. (5) was used.

$$\mu_x = \Sigma n_j e \Delta X \left\{ \frac{2\sigma^+\sigma^-}{\sigma^+ + \sigma^-} \right\} \quad (12)$$

where $\Delta X = X^+ - X^-$, and σ^+ and σ^- are the effective or weighted numbers of positive and negative charges, i.e.

$$\sigma^+ = \Sigma n_j^+ L_j^+ \text{ and } \sigma^- = \Sigma n_j^- L_j^- \quad (13)$$

4.2. Core dipole moments

Of polar groups in proteins, the bonds contributing a large dipole moment are C=O and H–N bonds. As mentioned before, the coordinates of H atoms were not documented in the X-ray databases because of the inability of the X-ray technique to locate H atoms due to the low electron density. In the previous calculations of the dipole moments of proteins, the H–N bond moments were neglected assuming that the net H–N bond is negligibly small [22].

For the present calculation, the database was obtained by the NMR measurement and the coordinates of H atoms were determined with sufficient accuracy. The net core dipole moments were calculated by adding the group moments of C=O and H–N bonds vectorially. Fig. 3 illustrates the backbone chain of the subunit B of HU protein including C=O and H–N bonds. By and large, the dipole moment vectors of C=O bonds appear to be parallel to those of H–N bonds, particularly in the helix regions, indicating that the bond moments of H–N and C=O groups are additive to one another. Table 2 shows the net group moment due to C=O bonds alone and those of C=O and H–N bonds together. This table clearly demonstrates that the calculated core moment with C=O bonds alone is much smaller than the sum of the C=O and H–N moments. In other words neglect of the H–N bond moments causes a considerable error in the estimate of core moment. However, the magnitude of the core unit is still much smaller than that of the surface charge moment and, because of this reason, the error due to the neglect of H–N bond moment is still minor, if not negligible. The additivity of C=O and H–N bond moments is similar, albeit to a much lesser extent, to the case of helical polyamino acids, in which C=O and H–N bonds are conjugated by an intramolecular hydrogen bond so as to produce one-dimensional array of C=O$\cdots$H–N groups creating a very large dipole moment of several thousand D [1 Debye unit (D) = 3.33×10^{-30} Cm] for a long helical polyamino acids [23,24]. In globular proteins, the one-dimensional array of group moments

Table 2
Comparison of the core dipole moments calculated in Debye units by the use of C=O alone and those calculated by the use of C=O as well as N–H bonds

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Subunit	Bond	μ_x	μ_y	μ_z	μ
A	C=O	15.09	−30.09	−1.95	33.72
	N–H	10.34	−18.92	−2.42	
	CO + NH	25.44	−49.01	−4.38	55.40
B	C=O	4.92	3.62	−33.17	33.73
	N–H	−2.66	2.12	−20.97	
	CO + NH	2.263	5.74	−54.15	54.50
A + B	C=O	20.19	−26.46	−35.13	48.33
	N–H	7.68	−16.80	−23.39	
	CO + NH	27.70	−43.27	−58.53	77.84

C=O···H–N does not exist or exists only locally in the region of short helices.

5. Results and discussion

5.1. Surface charge and core dipole moments for subunits A and B

The structure of HU protein was found to be unusual because of the marked internal flexibility of the binding arms (see Fig. 2). As stated by White et al. [25], several residues in the β-hairpin (or binding arm) regions are not visible in the X-ray photographs. This fact may indicate certain degrees of flexibility in the arm region even in the crystalline state and, moreover, marked flexibility of this protein in solution as shown in Fig. 2. As a result, the structure of HU protein in solution cannot be defined uniquely with one structure. Vis et al. [14] detected as many as 25 conformations and presented all of them individually in the database. Under these circumstances, the final result is obtained as an algebraic mean of these 25 calculated dipole moments.

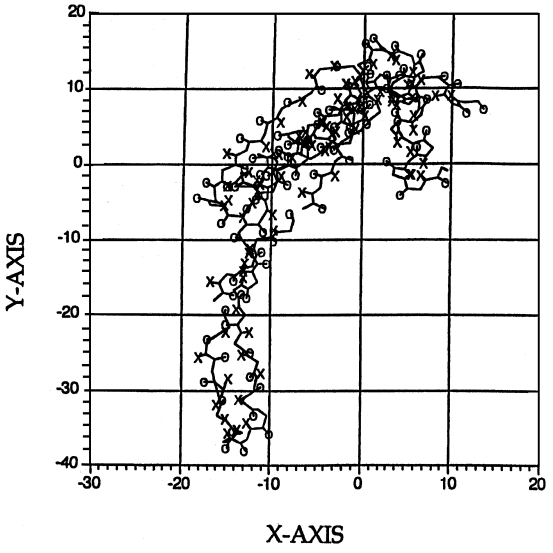

Fig. 3. The backbone chain of the B subunit of HU protein including C=O and H–N side chains. Crosses represent the hydrogen atoms in H–N bonds and circles represent the oxygen atoms in C=O bonds. Data taken from model 8.

5.2. Separate calculation for subunits A and B (A + B)

The calculation of surface charge dipole moment of subunits A and B, performed at the isoelectric point of 10.5 was repeated at pH 7.5 for each conformation (1–25). The computation includes the magnitude of the dipole moments, the RMS values and the coordinates of the positive and negative charge centers as indicated by the arrows in Fig. 4. This diagram also shows the distribution of positive (crosses) and negative (circles) surface charges. It is worth while to point out that the dipole vectors in the subunits A and B are additive (the angle between two dipole vectors is approx. 70°) producing a large net dipole moment of approx. 500 D. A net surface charge dipole moment of this magnitude is unusual for a small protein of the size of HU protein (a molecular weight of 19.5 kDa) (see Table 3). The finding that the dipole moments in the subunits are additive to each other is also uncommon. Usually, for proteins having subunits such as hemoglobin, the dipole moments in subunits tend to be antiparallel and cancel one another, at least partially, resulting in a relatively small net dipole moment [26].

The total dipole moment is defined as the vectorial sum of surface charge and core dipole moments. The angle between the charge and core dipole moments is calculated by the following equation,

Table 3
The dipole moments and dipolar angles of small globular proteins [22]

Name	MW	Dipole moment (D.U.)	Dipolar angles (degree)
Myoglobin	17000	170	48°
Cytochrome *c*	13000	235	138°
Lysozyme	14300	130	92°
Ribonuclease	14000	280	85°

$$\mu_t^2 = \mu_s^2 + \mu_c^2 + 2\mu_s\mu_c\cos\theta \quad (14)$$

where μ_t, μ_s and μ_c are the total, surface charge and core dipole moments, θ is the angle between the surface and core dipole moments. As shown in Table 4, the angle θ is unusually small indicating that the two dipole vectors are virtually parallel to each other. The angle θ is usually much larger in other proteins and often greater than 90°; in other words, there occurs partial cancellation of the two components (see Table 3) [22]. The calculation was repeated for all of the 25 conformations and the mean dipole moments are tabulated in Table 4. As shown, the average total dipole moment was found to be 530 D.

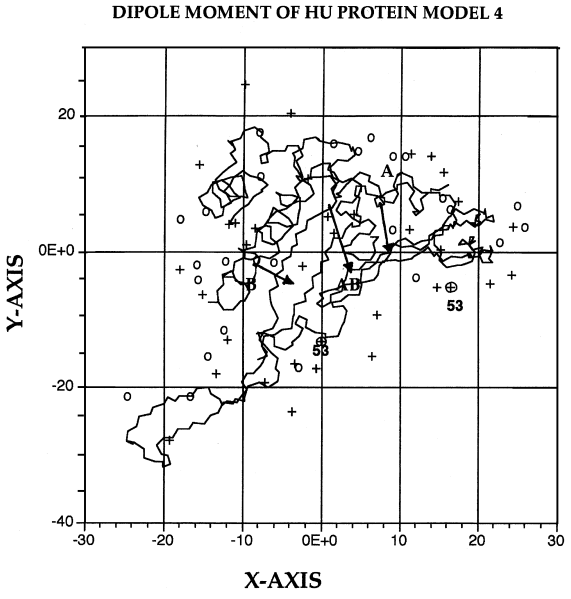

Fig. 4. The configuration of HU protein projected on the *x*–*y* plane. The three arrows show the magnitudes and orientations of the permanent dipole moments of subunits A (right) and B (left), and the homodimer (center). Crosses and circles indicate the co-ordinates of positive and negative surface charges. The crosses with circles designated as '53' indicate the arginine residue at the 53rd positions in A and B subunits. Data taken from model 4.

5.3. Direct calculation of the surface charge dipole moment of the homodimer (AB)

The dipole moment of Bst HU protein, a homodimer of subunits A and B, was computed directly, by using the databases of all the 25 homodimers. The calculation of pK shifts for polar amino acid groups in the dimer is very time-consuming because of the presence of a large number of charged residues in an HU molecule (AB), namely, 24 lysine, 16 arginine, 20 glutamic acid and 10 aspartic acid residues. Under these circumstances, it was decided to average the pK values of the amino acid residues in each A and B subunit and the mean value was used for the calculation of the dipole moment of dimers AB. The calculation of surface charge dipole moment of dimers, particularly at the isoelectric point, was also time-consuming. In this case, more than 700×10^3 iterations were required because of the very large number of charge configurations due to partial ionizations of the polar sites. The results are given in Table 4 together with the core dipole moments. Table 4 demonstrates that the results of the direct calculation of dipole moment for the homodimer (AB) are similar to those obtained by the vectorial summation of the dipole moments of the subunits A and B (531 D vs. 503 D). The dipole moment vectors of HU protein are illustrated in Fig. 5, i.e. a three-dimensional presentation of the HU protein with dipole vectors for subunits A and B and another vector for the homodimer (AB).

Also tabulated in Table 4 are the results of dipole moment calculation at the isoelectric point,

Table 4
The dipole moment of HU protein calculated for A and B subunits and for AB homodimers in Debye units[a]

Dipole moments		μ_x	μ_y	μ_z	μ	θ
pH = 7.5						
Surface charge	(A)	−11.03	−252.19	−98.17	270.85	
	(B)	120.08	−7.59	−256.33	283.16	
Surface charge	(A + B)	109.04	−259.78	−354.50	452.83	
Core	(A + B)	27.70	−43.27	−58.53	77.88	
Total	(A + B)	136.74	−303.25	−413.04	530.78	≈ 0
Surface charge	(AB)	143.56	−235.85	−323.15	425.04	
Core	(AB)	27.70	−43.27	−58.53	77.88	
Total	(AB)	171.26	−279.12	−381.68	502.91	≈ 0
RMS = 100.58						
pH 10.5 (isoelectric point)						
Surface charge	(AB)	168.76	−316.34	−418.07	550.76	
Core	(AB)	27.70	−43.27	−58.53	77.88	
Total	(AB)	196.46	−359.61	−476.60	628.53	≈ 3.1°
RMS = 236.7						

[a] The RMS is associated with the variation of the dipole moment for different structures of HU protein in solution.

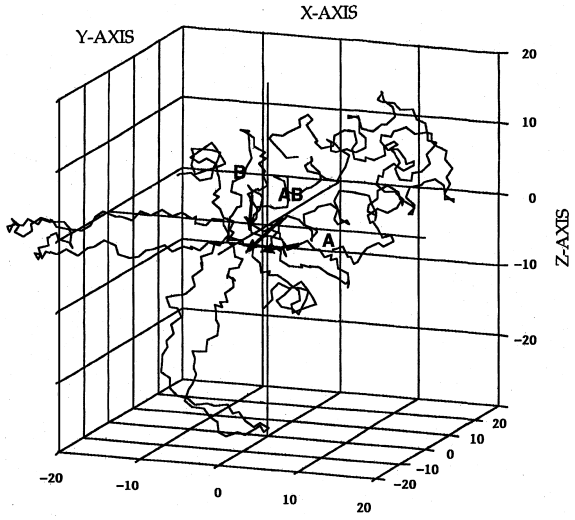

Fig. 5. Three-dimensional presentation of the HU protein. The dipole vectors designated A and B are the dipole moments for subunits A and B and AB is the net dipole moment for the homodimer AB. Data taken from model 4.

i.e. pH 10.5. The magnitude of calculated dipole moment of HU protein is considerably larger at the isoelectric point than at pH 7.5 (629 D vs. 503 D).

6. Concluding remarks

In this work, calculation of the dipole moment of HU protein was performed with two different methods, both of which confirm the presence of a large net permanent dipole moment. These calculations demonstate that the dipole moment of the subunits A and B are additive to each other, entailing a large dipole moment of approximately 500–530 D at pH 7.5 and 630 D at isoelectric point of pH 10.5 per homodimer. This is an unusually large dipole moment for a small protein of this size (19.5 kDa). Thus the results of numerical computation seems to be in agreement with the electrooptic observation, confirming the presence of a large permanent dipole moment in HU protein. This result may have an important implication for the understanding of the interaction of HU protein with DNA molecule as discussed below.

HU protein is known to form a complex with DNA molecule which is wrapped around by the two symmetry-related β-hairpin arms of the dimer.

The nature of binding is known to be nonspecific [1]. By looking at Fig. 4, one can detect a cluster of positive charges in the region of contact

between the β-hairpin of both subunits and the negatively charged DNA surface. One can thus envisage an attractive force between positive charges of HU protein and negative charges of DNA molecule providing a large binding energy. In addition, the net dipole moment of HU protein directed toward the negative charge cluster of DNA molecule thus contributing, because of the favorable orientation, an additional stabilizing force by 'dipole–charge' interaction. Thus, we can postulate that the two forces, i.e. charge–charge and dipole–charge interactions constitute the major stabilizing force for the HU protein and DNA molecule complex. As discussed above, the dipole moment of homodimers is considerably larger at isoelectric point (pH 10.5) than at pH 7.5. In view of this result, we can further postulate that HU protein–DNA complex may be more stable at the isoelectric point than at neutral pH where electrooptic measurements were performed. This hypothesis seems to be supported qualitatively by the observations by Yamaoka et al. (data to be published) that the replacement of the 53rd arginine residue in each subunit with asparagine (see Fig. 4) reduces the stability of the complex. The reduction of stability is due, presumably, to the loss of two positive charges in the region near the binding site of HU protein with DNA molecule.

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