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Electric, optical and hydrodynamic parameters of lac repressor from measurements of the electric dichroism

High permanent dipole moment associated with the protein

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Lac repressor and its tryptic core have been investigated by electro-optical methods. The reduced dichroism measured as a function of the electric field strength is not consistent with an induced dipole, but indicates the existence of a strong permanent dipole moment ($\sim 4\times10^{-27}$ C m) for the holo-repressor, which is almost independent of ion concentration and pH. A dominant contribution of a permanent dipole is also demonstrated by the shape of the dichroism rise curve. The experimental data are not consistent with a counterion polarization phenomenon and also do not indicate a major contribution from proton fluctuations. Probably the nature of the dipole is similar to that found for compounds with a tetrahedral substitution by angular residues. Other potential models involve large conformational fluctuations or inherent asymmetry of the lac repressor. Rotation time constants obtained from the dichroism decay are not consistent with a spherical shape, for either the holo- or core repressor. A simple interpretation of the data by prolate ellipsoids suggests a short diameter of 6 nm for both holo- and core repressor and long diameters of 14 and 12 nm for holo- and core repressor, respectively. Addition of the inducer isopropyl-β-D-thiogalactopyranoside leads to a change of the limit dichroism, but does not affect the rotation time constants within experimental accuracy.

1. Introduction

Because of its general interest for the control of genetic information readout, the lac repressor is among the most thoroughly studied proteins [1–3]. However, the structure of this protein has not yet been determined in molecular detail. It is known that the lac repressor is a tetramer consisting of identical subunits with molecular weights of 39000. Measurements of neutron scattering [4] and small-angle X-ray scattering [5] indicated that the shape of the protein is elongated, with the amino-terminal domains – denoted headpiece – located at the ends. From these results it has been suggested that one end of the lac repressor with two headpieces is involved in the specific complex with operator DNA [5–7] analogous to the complexes proposed for the cro and λ repressors, whereas the other is relatively distant from the DNA and thus does not contribute to the binding. A completely different model involving contacts of all four headpieces with the operator in the specific complex has been proposed from binding studies with hybrid repressors and from biochemical modification experiments [8]. Obviously more information on the lac repressor would be useful to reduce the number of possible molecular models.

A very sensitive approach for the analysis of molecular structures is the measurement of rotation time constants. Since the rotational diffusion coefficient increases essentially with the third

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power of the molecular dimensions [9], these dimensions can be evaluated with high accuracy and also small changes of conformations can be detected. The rotation may be characterized by application of short electric field pulses and measurements of the linear dichroism [10] (or birefringence). Owing to recent extensions of the time resolution and improvements in the sensitivity of electro-optical experiments [11], the rotation time constants for a protein such as the lac repressor can be determined with a rather high accuracy. This method has been used to study the lac repressor and also the core protein with the headpieces removed by chymotrypsin. The orientation data provide useful information not only about the dimensions of the lac repressor but also concerning the nature of its electric dipole. The analysis of dipole moments has been a classical method for the assignment of molecular structures, which was pioneered by Debye [12]. The method should again be useful for the characterization of large protein molecules.

2. Materials and methods

The lac repressor was isolated from an overproducing strain, which was kindly provided by Professor B. Müller-Hill (Universität Köln). The sample was purified according to the procedure described by Platt et al. [13] and proved to be homogeneous according to SDS-polyacrylamide gel electrophoresis. The core protein was prepared from the holo-repressor by the action of chymotrypsin and isolated by Sephadex G-150 chromatography according to Geissler and Weber [14]. Isopropyl-β-D-thiogalactopyranoside (IPTG) was obtained from Serva (Heidelberg, F.R.G.). The protein samples were dialysed against the appropriate buffers directly before measurements. A standard buffer, TE buffer, contained 5 mM Tris (pH 8.0) 100 μM EDTA and 100 μM 1,4-dithioerythritol with various amounts of NaCl added as specified separately. The buffers TMg1 and TMg5 contained 5 mM Tris (pH 8.0), 10 mM NaCl, 100 μM 1,4-dithioerythritol with 100 and 500 μM Mg^{2+} respectively. The buffer T9 contained 5 mM Tris (pH 9.0), 100 μM EDTA and 100 μM 1,4-dithioerythritol. The repressor concentration was 2.5 μM ($\pm$10%) tetramer units, unless specified otherwise; repressor concentrations were calculated from the absorbance values at 280 nm using an extinction coefficient of 9×10^4 M^{-1} cm^{-1} (tetramer units) [15].

The electro-optical experiments were performed with a pulse generator, an optical setup and a detector described previously [11]. The standard cell used for the present measurements had an optical path length of 20 mm and a distance of 7.5 mm between the Pt electrodes. Electroluminescence effects were suppressed by interference reflection filters from Schott (Mainz, F.R.G.), placed directly in front of the detector. The dichroism signals together with the field pulses were transiently stored on a 7612D digitizer from Tektronix and transferred to the computer of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH, Göttingen, for the evaluation of exponentials. Reference signals for deconvolution [11] were obtained by application of field pulses to water and measurement of birefringence by the detection circuit corresponding to that used for the dichroism experiments.

3. Results

3.1. Dichroism as a function of the electric field strength indicates a high permanent dipole moment of lac repressor

When solutions of lac repressor are exposed to electric field pulses, the absorbance of light at 280 nm polarized parallel to the electric field vector is reduced, indicating a negative dichroism of the protein (cf. fig. 1). The absorbance attains a stationary level after about 1 μs and returns to its original value, when the electric field is switched off. Experiments at the magic angle orientation [16,17] of polarized light do not reveal any changes and thus demonstrate that the observed field-induced effect cannot be attributed to a field-induced conformation change or any similar reaction, but is due to field-induced orientation of the lac repressor. This is also shown by the fact that the amplitudes $\Delta A_{\parallel}$ and $\Delta A_{\perp}$ observed with light

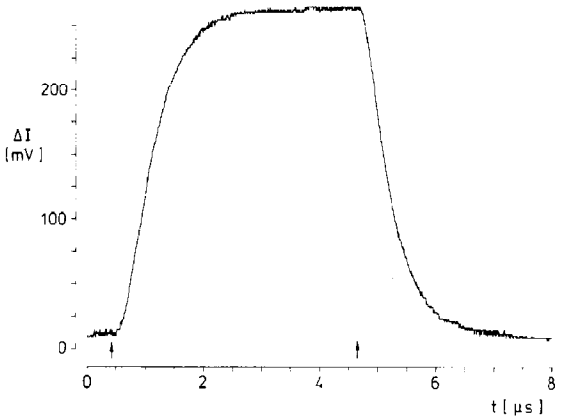

Fig. 1. Change of light intensity I as a function of time t for 2.3 μM lac repressor in TMgl buffer induced by an electric field pulse of 46.2 kV/cm. Start and termination of the field pulse are indicated by arrows (measurement at 280.4 mm; plane of polarization parallel to the field vector; 10 °C; detector rise time ~ 0.5 μs.

polarized parallel and perpendicular to the field vector, respectively, follow the relation expected for electric dichroism [10]

$$\Delta A_{\parallel} = -2\Delta A_{\perp} \qquad (1)$$

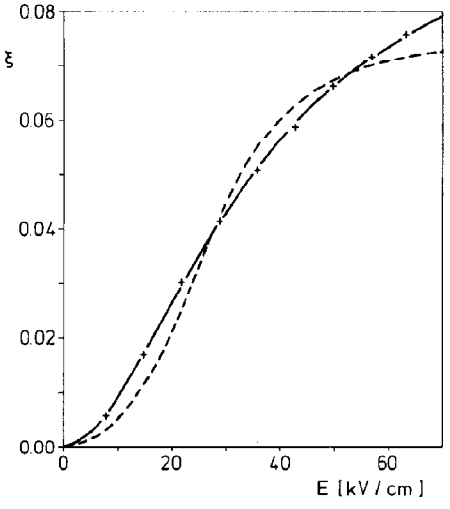

Fig. 2. Linear dichroism ξ of lac repressor as a function of the electric field strength E in TE buffer at 20 °C in the presence of 100 μM IPTG (280.4 nm). The continuous line shows a least-squares fit according to a permanent dipole mechanism with a dipole moment of 4.4×10^{-27} C m and $\xi_{\infty} = -0.12$. The dashed line shows a least-squares fit according to an induced dipole mechanism.

The reduced dichroism ξ is calculated – using the isotropic absorbance A – according to

$$\xi = \frac{\Delta A_{\parallel} - \Delta A_{\perp}}{A} \qquad (2)$$

ξ increases with the electric field strength E as shown in fig. 2. Least-squares fits demonstrate that these experimental data can be represented with high accuracy by an orientation mechanism corresponding to a permanent dipole and are not consistent with an induced dipole mechanism [10]. Since the observed function $\xi = f(E)$ is completely different from that expected for an induced dipole mechanism, the orientation of lac repressor induced by electric field pulses clearly cannot be attributed to any standard induced dipole. This is in contrast to expectation, because lac repressor is usually regarded to be a symmetrical tetramer, which should not be associated with a permanent dipole moment.

The problem raised by these data appears to be similar to that encountered for DNA, where strong permanent dipoles were observed, although the double helix is clearly symmetrical [18]. In the case of DNA, the dipole moments are strongly dependent upon the salt concentration. Thus, the field-induced orientation of lac repressor was measured at different salt concentrations and also different ion compositions. The measurements revealed some variation of the dipole moment (cf. table 1), but these variations are rather small, especially when compared to the strong dependence observed for DNA double helices. These results demonstrate that the nature of the dipole is different in the cases of DNA and lac repressor.

Since the dipole of the lac repressor may be due to fluctuations of its protonation [19], the dipole moment may depend on pH. However, measurements at pH 9.0 did not reveal any major change of the dipole moment compared to those observed at pH 8.0 (cf. table 1).

Evaluation of the experimental data by an orientation function also provides values for the electric dichroism in the limit of complete orientation ξ_{∞}. As shown in table 1, these values are relatively small, but can still be useful to indicate conformation changes. An increase in salt concentration leads to some increase of the ξ_{∞} val-

Table 1

Electro-optical parameters of lac repressor

ξ_∞, reduced dichroism extrapolated to complete orientation; μ_p, permanent dipole moment in units of 10^{-27} C m; τ, rotation time constant; t, temperature; − and + IPTG indicate the absence and presence of 100 μM IPTG, respectively. τ values given in parentheses are scaled to 20 °C by the scaling factor η/T.

Buffer	248 nm				280 nm				Decay		t (°C)
	−IPTG		+IPTG		−IPTG		+IPTG		−IPTG+IPTG		
	ξ_∞	μ_p	ξ_∞	μ_p	ξ_∞	μ_p	ξ_∞	μ_p	τ (ns)	τ (ns)	
TE	−0.10	4.5	–	–	−0.16	4.3	−0.12	4.4	139	133	20
TE+5 mM NaCl	–	–	–	–	−0.19	4.0	−0.16	3.8	133	129	20
TE+10 mM NaCl	−0.11	4.0	−0.10	4.0	−0.19	3.6	−0.18	3.3	130	129	20
TMg1	−0.15	4.1	−0.09	4.2	−0.18	4.2	−0.15	3.8	165	156	10
									(122)	(116)	(20)
TMg5	−0.17	4.4	−0.10	4.6	−0.19	4.2	−0.15	4.0	151	157	10
									(112)	(116)	(20)
T9					−0.17	4.5	−0.14	4.2	173	168	10
									(128)	(124)	(20)
T9+5 mM NaCl					−0.18	3.5	−0.14	3.4	170	170	10
									(126)	(126)	(20)

ues, whereas addition of the inducer IPTG induces some decrease in ξ_∞. The dipole moments remain almost unaffected by addition of IPTG.

3.2. The dichroism decay

After termination of the electric field pulses, the protein molecules are converted from a partial alignment to a random orientation by rotational diffusion. The dichroism decay process observed for the lac repressor can be described with high accuracy by a single relaxation time constant τ_d, which is related to the rotational diffusion coefficient(s). The rotation relaxation time constant can be measured with rather high accuracy and thus may be used for some tests on the structure of lac repressor in solution. Since the lac repressor is a tetramer, the protein may undergo some association equilibrium, which should be reflected by a concentration dependence of its rotation time constant. However, measurements in the concentration range from 0.16 to 4.6 μM did not show any variation of τ_d within the experimental accuracy (cf. fig. 3).

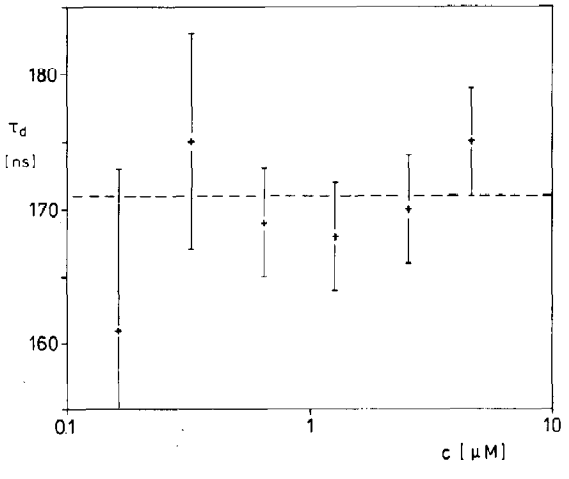

Fig. 3. Dichroism decay time constant τ_d of lac repressor as a function of the repressor concentration c (10 °C, TE buffer).

The temperature dependence of the lac repressor structure has been tested by measurements from 2 to 20 °C. In this range the observed decrease in τ_d with increasing temperature is simply due to the decrease in water viscosity η as revealed by application of the scaling factor η/T (where T is the absolute temperature; cf. table 2). Some change of the lac repressor structure is observed upon addition of Mg^{2+}: the reduction of the rotation time constant from about 130 ns in the absence of Mg^{2+} to about 117 ns in its presence (mean value at Mg^{2+} concentrations of 100

Table 2

Rotation time constants of lac repressor from dichroism decay at different temperatures in the absence and presence of 100 μM IPTG (buffer TE + 5 mM NaCl)

Temperature (°C)	−IPTG		+IPTG	
	τ_d (ns)	$\tau_d \cdot T/\eta$ (10^{-2} s^2 m K kg^{-1})	τ_d (ns)	$\tau_d \cdot T/\eta$ (10^{-2} s^2 m K kg^{-1})
2	228	3.75	218	3.59
10	172	3.73	162	3.51
20	132	3.86	127	3.71

and 500 μM; τ_d values given for 20 °C) indicates a clear decrease in the hydrodynamic dimensions (cf. table 1). From these results it is not possible to conclude whether the effect of Mg^{2+} is due to specific binding or to a general shielding of electrostatic repulsions. In any case, the data obtained in the buffers without Mg^{2+} apparently reflect a slightly denatured conformation of lac repressor, whereas the lac repressor in buffers TMg1 and TMg5 is likely to represent the native conformation. The lac repressor is considered to be native in the presence of Mg^{2+} simply because of the relatively large Mg^{2+} concentration in standard native environments.

Since the inducer IPTG decreases the stability constant for the interaction of lac repressor with its operator by 3 orders of magnitude [3], conformation changes induced by IPTG are of particular interest. In spite of this large affinity change, the rotation time constant τ_d is hardly affected by addition of IPTG (cf. table 1). Thus, the change induced by IPTG binding is restricted to the 'internal' structure of lac repressor and does not affect its 'external' dimensions to any great extent.

3.3. The dichroism rise

Independent information on the orientation mechanism may be obtained from the dichroism rise curves observed upon application of field pulses. In contrast to the dichroism decay of the lac repressor, the rise curves cannot be described by single exponentials. As shown in fig. 4, the initial slope of the dichroism rise is zero. This phenomenon can be described by a sum of two

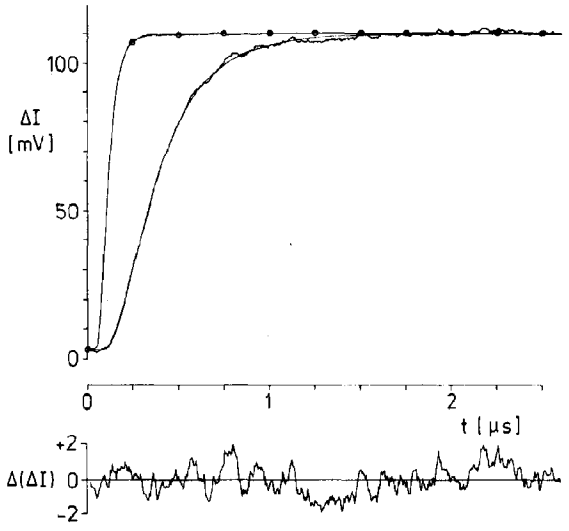

Fig. 4. Dichroism rise curve for lac repressor in TMg1 buffer at 10 °C induced by a field pulse of 40.5 kV/cm. The experimental data (average of 10 subsequent pulse experiments, wavelength 280.4 nm) and the least-squares fit with two time constants ($\tau_1 = 70$ ns, $\tau = 230$ ns) can hardly be distinguished; the difference between the experimental curve and the fit is shown in the lower part of the diagram. The curve marked by small circles shows the reference curve used for deconvolution.

exponentials with the amplitudes determined by their time constants τ_1 and τ_2 according to

$$\Delta I = \Delta I_\infty \left(1 + \frac{\tau_1}{\tau_2 - \tau_1} e^{-t/\tau_1} - \frac{\tau_2}{\tau_2 - \tau_1} e^{-t/\tau_2}\right), \quad (3)$$

where ΔI and ΔI_∞ are the changes of light intensity at time t and time ∞, respectively. Eq. 3 is also valid for a convolution of a polarization process described by a time constant τ_1 with a rotation process described by a time constant τ_2, provided that the polarization process is not associated with any change in optical parameters [20]. The dichroism rise curves found for lac repressor could be represented by eq. 3 with high accuracy over the entire range of electric field strengths used in the present investigation. The time constants evaluated according to eq. 3 turned out to be independent of the lac repressor concentration, but proved to be strongly dependent on the electric field strength. An example for a set of experi-

mental data is given in fig. 5, where τ_2 is given as a linear function of the electric field strength E. A reasonable correlation is also found, when $1/\tau_2$ is plotted as a function of E^2. By linear regression of τ_2 as a function of E the time constant at zero field strength is $\tau_2(0) = 500$ ns. A corresponding linear regression for the time constant τ_1 provides a value $\tau_1(0) = 180$ ns (both $\tau_1(0)$ and $\tau_2(0)$ given for buffer TMg1 at 10 °C). Similar data were obtained for other buffers (and also for other temperatures, with appropriate scaling by the factor η/T). For the purpose of the present analysis the observed variation of the time constants τ_1 and τ_2 with the electric field strength E is simply taken as an empirical fact. A quantitative interpretation remains for future investigations.

The time constants $\tau_1(0)$ and $\tau_2(0)$ extrapolated to zero field strength may now be compared with predictions for standard orientation mechanisms. According to Benoit [21] the rise of optical anisotropy due to permanent dipoles should follow the relation

$$\frac{\Delta I}{\Delta I_\infty} = 1 - \frac{3P/Q}{2((P/Q)+1)} e^{-2\theta t} + \frac{(P/Q)-2}{2((P/Q)+1)} e^{-6\theta t} \quad (4)$$

with $P = \mu_p^2/k^2T^2$ and $Q = \Delta\alpha/kT$, where μ_p is the permanent dipole moment, $\Delta\alpha$ the electrical polarizability, kT the thermal energy and θ the rotational diffusion coefficient. This relation is valid in the limit of low field strengths and can be simplified for the case $P/Q \gg 1$ to

$$\frac{\Delta I}{\Delta I_\infty} = 1 - \tfrac{3}{2} e^{-2\theta t} + \tfrac{1}{2} e^{-6\theta t} \quad (5)$$

which is equivalent to eq. 3 with $\tau_1 = 1/(2\theta)$ and $\tau_2 = 1/(6\theta)$. Since the decay time constant is given in the same context by $\tau_d = 1/(6\theta)$, τ_2 should be equivalent to τ_d, whereas τ_1 should be equivalent to $3\tau_d$. Obviously, the experimental results are consistent with the model in the limit of $P/Q \gg 1$ and are clearly not consistent with the case $P/Q \ll 1$. Thus, the conclusion is confirmed that the orientation of lac repressor is caused by a permanent dipole moment.

Another test for the assignment of the permanent dipole moment mechanism has been performed by measurements at different buffer concentrations. If the dipole were to arise by proton polarization, the time constants τ_1 and τ_2 according to eq. 3 would fulfill eq. 4 purely by accident, because the protolytic reaction occurs at a rate consistent with eq. 4. Since the time constants for simple proton transfer reactions are expected to depend on the buffer concentration, this possibility may be examined by taking measurements at different buffer concentrations. Experimental data obtained at constant salt concentration but variable Tris concentration from 0.5 to 8.6 mM (pH 8; ionic strength kept constant by variation of NaCl concentration) did not reveal any variation of τ_1 (and τ_2) within experimental accuracy. These results support the conclusion that the dipole of the lac repressor is not the result of a field-induced polarization of protonation.

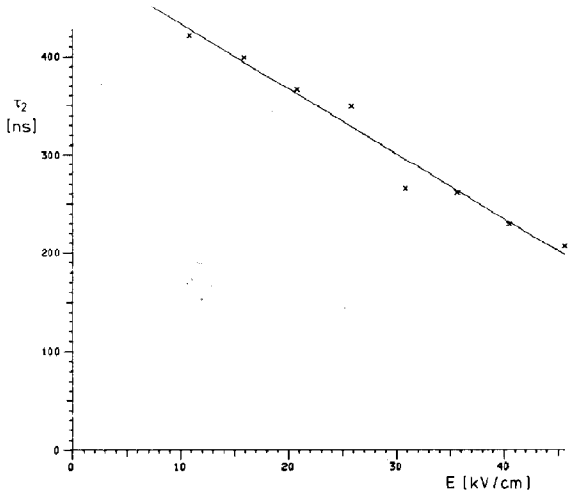

Fig. 5. Time constant τ_2 obtained from dichroism rise curves according to eq. 3 as a function of the electric field strength E (TMg1 buffer, 10 °C).

3.4. The lac repressor core

As demonstrated by Geissler and Weber [14] the DNA-binding domains of lac repressor can be removed selectively by proteolytic enzymes. The core repressor obtained by limited proteolysis has been analysed using the procedures described above. Since the experimental data are quite simi-

Table 3

Electro-optical parameters of the core repressor

Definitions as in table 1.

Buffer	248 nm				280 nm				Decay		t (°C)
	−IPTG		+IPTG		−IPTG		+IPTG		−IPTG	+IPTG	
	ξ_∞	μ_p	ξ_∞	μ_p	ξ_∞	μ_p	ξ_∞	μ_p	τ (ns)	τ (ns)	
TE	−0.30	3.3	−0.26	3.2	−0.30	3.3	−0.26	3.2	85	83	20
TE + 10 mM NaCl	−0.21	3.9	−0.17	3.9	−0.26	3.7	−0.24	3.4	114	111	10
									(84)	(82)	(20)
TMg5	−0.25	3.5	−0.19	3.5	−0.25	3.4	−0.23	3.1	113	110	10
									(84)	(81)	(20)

lar in principle to those obtained for the holo-repressor, the experiments are not discussed in detail, but the results are simply compiled in table 3.

As found for the holo-repressor, the core repressor is aligned by electric field pulses due to a permanent dipole moment. The dipole moment of the core is not much lower than that of the holo-repressor (cf. table 3), whereas the limit dichroism is clearly higher for the core than for the holo-repressor. As should be expected the decay time constant decreases when the DNA-binding domains are removed. Addition of IPTG does not change the rotation time constants of the core, but induces a reduction of the limit dichroism, particularly at 248 nm.

4. Discussion

4.1. The dipole moment

Usually the electro-optical analysis of proteins has been restricted to low electric field strengths. In this range the electro-optical amplitudes are a function of the square of the electric field strength for both permanent and induced dipoles [10]. Thus, amplitudes obtained at low field strengths cannot be used to distinguish between orientation mechanisms. In the present investigation the measurements were extended to high electric field strengths up to 65 kV/cm, which provides a much larger part of the orientation function than was accessible previously. This extension is sufficient for a clear decision concerning the orientation mechanism from the amplitudes and also for an evaluation of dipole moments or polarizabilities. By this approach dipole moments can be evaluated directly, whereas dielectric measurements can only be used to evaluate the root of the average of the squared dipole moments, which in addition requires the use of an empirical, imprecisely known parameter [22]. The existence of a permanent dipole moment for the lac repressor is demonstrated not only by the dichroism amplitudes, but also by the shape of the dichroism rise curves. In the past, conclusions on the existence of permanent dipoles for various proteins were usually based only on the shape of the electro-optical rise curves. However, an analysis on this basis does not exclude the possibility of a slow polarizability. Thus, the combined investigation of both rise curves and amplitudes up to high field strengths performed in the present investigation clearly provides more conclusive results.

The unexpected finding of a permanent dipole moment for the lac repressor raises a problem, because this protein is usually regarded as a symmetric tetramer with the subunits in a planar arrangement. According to the dichroism amplitudes the dipole moment is quite substantial, which implies conclusions on the structure of the lac repressor. However, before any conclusion as to the structure is discussed, various models should be compared, which could be used to explain a permanent dipole moment of a macromolecule.

4.1.1. Polarization of an ion atmosphere

Macromolecules bearing charged residues at-

tract counterions in the form of an 'ion atmosphere'. Electric field pulses may induce a distortion of this ion atmosphere [23–25], which gives rise to a dipole moment. Usually the polarization of an ion atmosphere is reflected by an induced dipole mechanism. However, the polarization process can be more complex and may simulate the existence of a permanent dipole. A well-known example is the DNA double helix, which appears to be associated with an unusually high dipole moment. The data obtained for DNA have been described by a saturating induced dipole mechanism [18]. A recent investigation of the polarization process demonstrated that the permanent dipole regime of DNA is characterized by a special polarization mechanism, which is explained by biased dissociation of counterions [20]. Examination of the experimental data shows that the polarization mechanism of DNA is not comparable to that of the lac repressor. In the case of the lac repressor, eq. 4 is fulfilled in the limit of low electric field strength, whereas the time constants τ_1 and τ_2 evaluated according to eq. 3 for DNA are not related to each other as expected from eq. 4. Further evidence against a strong contribution from ion atmosphere polarization to the repressor dipole comes from the fact that the repressor dipole does not show any strong dependence on the ion concentration. In this context it should also be mentioned that the time constants τ_1 and τ_2 of the dichroism rise curves remain unaffected within experimental accuracy upon variation of the ionic strength, addition of Mg^{2+}, variation of pH and of the buffer concentration. These results provide evidence against mechanisms with a low rate of polarization [26].

4.1.2. Proton fluctuations

Another mechanism to explain dipole moments is related to ion atmosphere polarization, but involves protons as ligands, which are attached to proton acceptor sites. Proteins bear a large number of proton acceptor sites, including amino, guanidino and carboxyl groups. Any asymmetric distribution of charged residues, due to protonation or deprotonation, causes a dipole moment. In addition to (or instead of) such a 'standard' dipole, which results from the equilibrium distribution of protonation, there may be also a 'fluctuation' dipole, which is due to fluctuations of protonation. Dipole moments due to proton fluctuation were first discussed and calculated by Kirkwood and Shumaker [19]. According to these authors a dipole may arise from statistical fluctuations of protonation, even when the stationary distribution of charges is symmetric. Due to these fluctuations the distribution of charges at any individual protein molecule is asymmetric with a particular probability, which can be calculated. Obviously, fluctuations will be more effective around pK values of the protonation sites. Exact calculations of dipole moments due to proton fluctuation require knowledge of pK values and coordinates of protonation sites [27]. Experimental information on fluctuation dipoles may be obtained from measurements at different pH values. As emphasized by Kirkwood and Shumaker [19], the fluctuation dipole should decrease with increasing pH values. Usually, fluctuation dipoles have been considered as an explanation of dielectric effects, which are dependent on the square of the dipole moment [22] and thus are particularly sensitive to fluctuations. The main part of the experimental effects evaluated in the present investigation are linearly dependent on the dipole moment and thus are not as sensitive to fluctuations. Furthermore, the rather small dependence of the observed dipole moment on pH argues against a strong contribution from proton fluctuations. Unfortunately, the measurements could not be extended over a larger pH range, because the lac repressor precipitated at pH 7 and is no longer likely to be native at pH 10. For a final conclusion on the fluctuation dipole, information on pK values and coordinates of all protonation sites of the lac repressor would be required. Nevertheless, as long as this information is not accessible, the present results clearly support the conclusion that the dipole moment of lac repressor is not due to proton fluctuations.

4.1.4. Alternative models

Since the models discussed above appear to be hardly compatible with the experimental results obtained for the lac repressor, the possibility has to be examined that the lac repressor is associated

with an 'inherent' permanent dipole. According to the results of Steitz and co-workers [5] the lac repressor is usually considered as a symmetric tetramer with the subunits arranged in a plane. Because of its symmetry, this structure should not be associated with any inherent permanent dipole moment. A corresponding argument holds for a tetrahedral arrangement of subunits, which may be used to explain the recently observed binding of lac repressor to two operator sites, which are located in close proximity on the same DNA molecule. As described by Krämer et al. [28], this mode of binding requires separation of DNA-binding sites by an integral number of helix turns and is associated with strong bending of the double helix. A tetrahedral arrangement of lac repressor subunits would locate the headpieces in an orientation which fits smoothly into the grooves of the double helix segments without distortion of the protein. In the context of this discussion a tetrahedral symmetry is useful as an example, because some molecules with a symmetrical tetrahedral substitution are known to be associated with a permanent dipole moment, although simple tetramers are not expected to be associated with a permanent dipole. For example, pentaerythritol ($C(CH_2OH)_4$) has a dipole moment of 2 Debye, which is attributed to the fact that the group moments of (CH_2OH) are not aligned in the same direction as the C–C bonds [29]. This appears to be a general phenomenon for tetrahedral substitution by ligands with more than a single atom and with angular connections [30]. Similar effects may be expected for large proteins such as the lac repressor.

A permanent dipole moment may also arise by fluctuations of the conformation. If one or two subunits of the protein are in some conformational state different from the rest of the subunits, the whole subunit assembly is asymmetric and may be associated with a substantial dipole moment. Finally, an inherent asymmetry of the lac repressor cannot be ruled out completely, as long as its structure has not been solved by X-ray analysis at a minimal resolution. Some data reported in the literature [6] suggest the existence of an asymmetric structure of the lac repressor, although the evidence in favour of asymmetry remains rather weak. However, other repressor molecules are clearly known to be asymmetric [31,32] and thus are likely to be associated with a permanent dipole moment. Electro-optical measurements demonstrate the existence of a large permanent dipole for one of these repressors (in preparation). A large permanent dipole moment should lead to substantial attractive interactions with highly polarizable molecules like DNA. This type of interaction provides another explanation for unspecific binding of repressor molecules to DNA. Due to the strong distance dependence of dipole-induced dipole interactions, however, the attraction falls off abruptly with increasing distance. Another consequence of the high dipole moment should be strong self-interactions of lac repressor molecules. The absence of self-interactions at concentrations around 1 μM is probably due to shielding of excess charges by counterions under the usual conditions.

4.2. Molecular dimensions from rotation time constants

Owing to the strong dependence of rotational diffusion coefficients on molecular dimensions, the rotation time constants comprise valuable information on the molecular structure. However, due to the potential existence of several time constants for objects of unknown shape, the interpretation is not straightforward [9]. Nevertheless, it should be useful to analyse the experimental rotation time constants in terms of a simple prolate ellipsoid. For such a model a second exponential may be expected, which reflects rotation along the transverse axis. The absence of a corresponding exponential in the experimental decay curves may be explained by optical and/or electrical isotropy with respect to rotation along this axis. The dimensions of the prolate ellipsoid are evaluated as follows: From known molecular weights and a specific volume [5] of 0.73 cm^3/g, the radii of equivalent spheres may be calculated for both holo- and core repressor (35.6 and 33.5 Å, respectively). These values are increased, in order to account for the existence of a hydration layer (40 and 38 Å, respectively, estimated from an average hydration of 0.34 g H_2O/g protein [9]). From the

ratios of experimental time constants (117 and 83 ns, at 20°C) to those calculated for the spheres (66.5 and 57 ns), the axial ratios of the corresponding prolate ellipsoids [9] may be evaluated (2.35 and 1.95). Finally, these values may be used to calculate the long semi-axis a and short semi-axis b of prolates corresponding to the holo-repressor ($a = 71$ Å, $b = 30$ Å) and core repressor ($a = 59$ Å, $b = 30$ Å). These parameters are in satisfactory agreement with those evaluated by Charlier et al. [4] and McKay et al. [5]. According to all these data, the lac repressor does not have a spherical shape. Furthermore, the DNA-binding domains are clearly exposed at the exterior of the protein. The present measurements also indicate that addition of IPTG does not induce a major change in the lac repressor dimensions. However, the change of the limit dichroism induced by IPTG indicates some internal conformation change. Probably the internal conformation change also affects the relative orientation of the DNA-binding domains, although the overall hydrodynamic dimensions remain unchanged upon re-orientation.

Some additional information on the structure of the protein is provided by the values of the limit dichroism ξ_∞. The relatively large negative values found for the core repressor indicate a preferential perpendicular orientation of aromatic residues with respect to the permanent dipole vector. The less negative values found for the holo-repressor indicate that the aromatic residues of the headpeace do not have the same preferential alignment as the core residues, but are aligned to a large degree parallel to the permanent dipole vector. The differences in limit dichroism observed at 248 and 280 nm may be useful for separation of the contributions from tyrosine and tryptophan residues. Since the differences are relatively small, however, these results have not been evaluated quantitatively.

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