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Biophysical Chemistry 69 (1997) 71–84

Biophysical Chemistry

Effect of central metal substitution on linear dichroism of porphyrins: evidence of out-of-plane transition moments [1]

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Received 28 March 1997; received in revised form 20 May 1997; accepted 29 May 1997

Abstract

Absorption anisotropy and emission anisotropy measurements in poly(vinyl) alcohol (PVA) films of different porphyrin derivatives are reported. Wavelength dependent absorption anisotropy in oriented PVA films, and wavelength dependent excitation spectrum of emission anisotropy of fluorescent porphyrin derivatives in isotropic PVA films indicate the presence of multiple transition moments with different well-defined orientation. Comparison of linear dichroism and orientation behavior in stretched PVA films of deuteroporphyrin III (C_{2V} symmetry) and its iron derivative reveals significant out-of-plane transition moment components. A considerable participation of out-of-plane polarized absorption components is also observed for metal derivatives of non-symmetrical protoporphyrin IX. It appears that central metal substitutions in porphyrin rings do not produce 'circular' degeneration of electronic transition moments. Instead, the presence of metal induces absorption components orthogonal to the porphyrin plane. © 1997 Elsevier Science B.V.

Keywords: ?

1. Introduction

Porphyrins are a large group of molecules of considerable biological and spectroscopic importance for many processes such as photosynthesis, oxygen transport, oxidation–reduction and electron transport. The optical spectra of these highly conjugated molecular systems have been studied extensively for many years [1–12] but still, some features are not fully understood. Their physico–chemical properties are often utilized in numerous physical and biological applications. The porphyrin's capability for optically-induced electron-transfer has been recently applied for constructing ultrafast optical switches [13] and for triggering the folding processes in cytochrome c [14]. Water-soluble cationic porphyrins bind to DNA and they are extensively used for studies of different DNA regions [15,16]. The heme molecule (i.e., iron–protoporphyrin IX) is one of the most biologically relevant porphyrin derivatives. The specific structure of the heme and of the heme pocket is a primary factor which dictates the physiological functions of oxygen carrying hemoproteins. Experimental work on myoglobins and hemoglobins

Abbreviations: PVA = poly(vinyl) alcohol

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PII S0301-4622(97)00077-X

reconstituted with hemes of varying size and symmetry show that both steric and electronic interactions of the heme with surrounding amino acids are important factors for the final protein folding and ligand binding properties [17–21]. The strong dependence of the absorption spectra on the formal oxidation and spin state of the iron or the presence of ligands make heme molecules very suitable for spectroscopic studies of the functional properties of hemoproteins. Transient absorption spectra are widely used for studying ultrafast ligand photodissociation from hemoproteins and protein conformational changes [22–26]. Recently, probing of the spectral evolution of different absorption bands allowed investigators to follow the dynamics of conformational relaxation of hemoproteins [26–29], the dynamics of ligand motion [24–30] and estimation of the CO orientation before and after photolysis [30]. Heme redox properties are correlated with the energies of charge-transfer transitions in their complexes [31,32] and they are an important factor in many redox-active proteins.

In view of the numerous spectroscopic applications, experimental and theoretical studies of the electronic structure of porphyrin derivatives are rapidly expanding. At present, transition moments orientations of chlorophyll derivatives are well known [33–36], however, the character and orientation of the electronic transition moments of heme derivatives are still controversial. This is due to the low symmetry of heme molecules, extensive overlap of different transition bands across the absorption spectrum and complete lack of fluorescence response. The very low solubility of most of the porphyrin derivatives add to the difficulty of spectroscopic studies.

The time-resolved polarized absorption spectroscopy [28,29] of photolyzed hemoproteins opens a new possibility for studying the dynamic motions of heme or whole hemoproteins and also for observing the reorientation of the photodissociated ligands inside the heme pocket. The resolution of these studies depend on the detailed knowledge of the heme transition moment orientation at the photolysis wavelength. The proposed 'circular absorber' model [37,38] for heme electronic transitions simplifies theoretical considerations, and many experimental data have been interpreted on that assumption. However, recent investigations of the linear dichroism of different symmetry porphyrin derivatives and hemes [39,40] showed that the linear type of porphyrin electronic transitions is not significantly degenerated by the metal substitutions in the center of the porphyrin ring; therefore, in many regions of the absorption spectrum the 'circular absorber' model may significantly distort data interpretations.

To clarify this situation, we have analyzed the linear dichroism data of metal-free and metal-substituted symmetrical and non-symmetrical porphyrins in this report. The linear dichroism of free and metal-substituted porphyrins indicate the presence of out-of-plane polarized absorption components induced by the central metal atoms. To properly interpret the orientational behavior of porphyrins in oriented systems, we have expanded our comparative study of linear dichroism of symmetrical (D_{4h}, C_{2v}) and non-symmetrical porphyrins to the investigation of excitation emission anisotropy of their fluorescent derivatives. Linear dichroism and emission anisotropy confirm the linear nature and well-defined orientation of the electronic transition moments across the heme absorption spectra. They also allow one to estimate the contributions of the out-of-plane polarized absorption induced by the central metal-atom.

2. Basic theory

2.1. Absorption anisotropy (K)

Absorption anisotropy (K) in oriented system is defined as [40–42]

$$K = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}} = \frac{3}{2}\frac{A_{\parallel}}{A} - \frac{1}{2} \qquad (1)$$

where $A_{\parallel}$ and $A_{\perp}$ denote light absorbance parallel and perpendicular to the direction of the sample orientation. $A = A_{\parallel} + 2A_{\perp}$ is the total absorbance. The absorption anisotropy K is related to the reduced linear dichroism by $LD^r = 3(A_{\parallel} - A_{\perp})/(A_{\parallel} + 2A_{\perp}) = 3K$ and to the dichroic ratio by $R_d = A_{\parallel}/A_{\perp} = (2K + 1)/(1 - K)$.

In the x, y, z molecular coordinate system, the orientation of molecules is characterized by the orientation tensor $\{\cos Zi \cos Zj\}$ ($i, j = x, y, z$) [41], where cos Zi denotes the direction cosine of the

angle between the i-th molecular axis and the stretching direction (orientation).

For a planar molecule, where transition moments lie in the molecular plane, the absorption anisotropy K can be expressed as [40]:

$$K = \frac{1}{3}\mathrm{LD}^{r} = S_{zz}\cos^2\varphi + S_{yy}\sin^2\varphi \qquad (2)$$

where S_{ii} are the Soupe molecular orientation parameters [43] and ϕ is the angle between the absorption transition moment and the main orientation axis of the molecule. For high symmetry planar molecules (D_{4h}) the absorption anisotropy becomes [40]:

$$K = \frac{1}{2}S_{zz} + \frac{1}{2}S_{yy} \qquad (3)$$

According to Eq. (3), the apparent absorption anisotropy is independent of the wavelength of observation and is a function only of the two orientation parameters S_{zz} and S_{yy}. In this case, the linear dichroism response is equivalent to the response of any uniaxially-oriented assembly of planar molecules with the transition moments equally distributed in the molecular plane, proposed by the 'circular absorber' model [37,38].

2.2. Fluorescence anisotropy (r)

For fluorescent molecules in the system with cylindrical symmetry, emission anisotropy can be defined as [44–47]:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \qquad (4)$$

where $I_{\parallel}$ and $I_{\perp}$ are the components of fluorescence intensity polarized parallel and perpendicular to the polarization of the excitation light vector. The term $I_{\parallel} + 2I_{\perp}$ represents the total fluorescence intensity. In general, in the absence of depolarization factors (rotational diffusion or excitation energy migration) r gives a direct measure of the angle β between absorption and emission transition moments in the molecules [46–49]. For well-separated transitions, fluorescence anisotropy can be expressed as:

$$r = \frac{2}{5}\left(\frac{3}{2}\cos^2\beta - \frac{1}{2}\right) \qquad (5)$$

A general property of fluorescent molecules, for which the equilibrated lowest excited states and consequent emission spectrum are independent of the excitation wavelength, makes it possible to resolve polarization of the overlapping absorption bands [50,51]. In this case, for overlapping transitions (of which porphyrins are good examples) fluorescence anisotropy can be expressed in terms of weighted sums of the anisotropies belonging to the different contributing transitions:

$$r = \Sigma_i f_i r_i = \frac{2}{5}\left(\frac{3}{2}\Sigma_i f_i \cos^2\beta_i - \frac{1}{2}\right) \qquad (6)$$

where β_i is the angle between the i-th absorption and the emission transition moments in the molecule, and f_i is the normalized contribution (probability) of the i-th transition moment, to the total absorption at the excitation wavelength.

For a molecule whose transition moments are equally distributed around the center of the molecule as required by the 'circular absorber' model, a simple average gives $\langle f_i \cos^2\beta_i \rangle = 1/2$ and the expected emission anisotropy would be $r = 0.1$ independently of the wavelength of excitation. This value is much lower than value of $r = 0.4$ expected for single transitions parallel to the emission dipole. Therefore, wavelength dependent fluorescence excitation anisotropy gives an additional independent information about absorption and emission transition moment distribution. Moreover, fluorescence anisotropies with values significantly higher or smaller than 0.1 immediately reveal the linear character of transition moments.

3. Material and methods

Deuteroporphyrin III and iron–deuteroporphyrin III were prepared according to literature procedures [40].

3.1. Film preparation

Poly(vinyl) alcohol (PVA) films were prepared from 15% aqueous solution. The various porphyrin

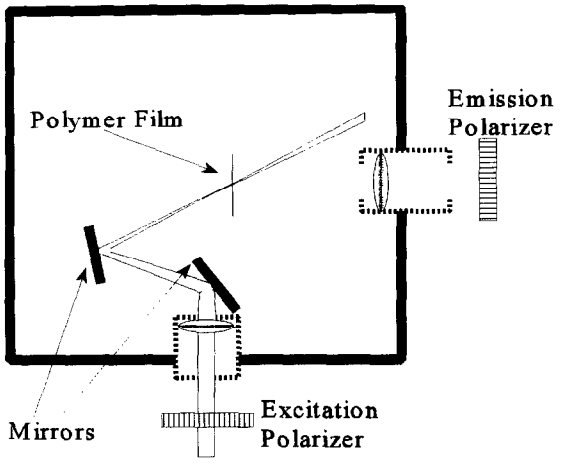

Fig. 1. Schematic view of SLM sample compartment showing adaptation of the right-angle configuration to front-face excitation of polymer films.

derivatives, if necessary, were dissolved in a minimum amount of 0.1 N NaOH and added to the prewarmed PVA solution as previously described [39,40]. PVA films used for fluorescence anisotropy measurements contained lower concentration of porphyrin and were prepared and stretched in the conditions previously described [39,40].

3.2. Experimental methods

Linear dichroism was measured by placing a Glan Polarizer in front of the sample inside the AVIV 14DS spectrophotometer. The absorption of the sample was corrected for a baseline obtained with the blank PVA films, then corrected for the film absorption calibrated for the thickness.

Polarized fluorescence measurements of different porphyrin derivatives in PVA films were performed in a specially adapted SLM module as shown in Fig. 1. In place of the regular square geometry adapter, we introduced two mirrors in the excitation line, so as to form a front face excitation of the film mounted in a special holder in the center of the SLM module. To avoid any depolarization effects in the film surfaces, the film was placed orthogonally to the direction of observation. Correction factors in the range of 550–750 nm were obtained by measuring methanol solution of rhodamine B in a 0.2-mm cuvette mounted in place of the film. Rhodamine B at 25°C in non-viscose methanol has a very short rotational correlation time and can be used as the zero anisotropy standard for steady-state emission. The alignment of this special setup was additionally controlled and corrected if necessary by using PVA films with 1,6-diphenylhexatriene (DPH) or 4-dimethylamino-4′-nitrostilbene (DNS) of known fluorescence polarizations [52,53]. Polarized fluorescence measurements of some porphyrin derivatives in viscose solvent such as Kodak oil were measured using an SLM 8000 fluorometer in a standard 1-cm cuvette with right angle square geometry optics.

4. Results

4.1. The chemical structures

Fig. 2 shows the structure of all studied compounds.

4.2. Linear dichroism

The parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) absorption components in 5-fold stretched PVA films of: $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin, and $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-porphyrin) tetra-*p*-tosylate salt; of deuteroprotoporphyrin III, protohemin III and protoporphyrin IX, hemin, Zn– and

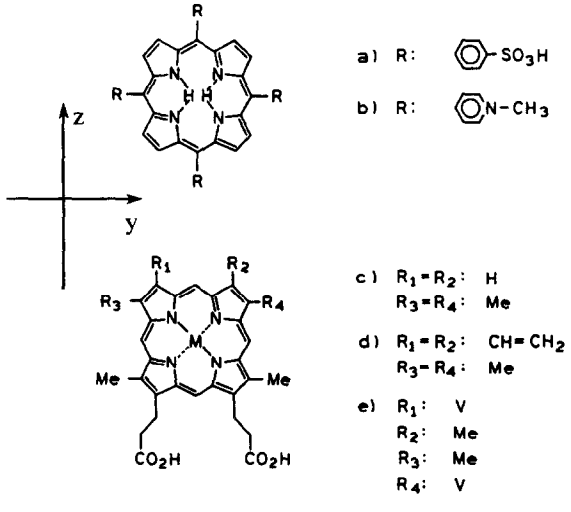

Fig. 2. Structures of different porphyrins.

Mg–protoporphyrin IX were already reported by us [39,40]. Their absorption anisotropy spectra are shown in a comparative fashion in Fig. 3a,b.

Molecules of $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin and $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-porphyrin) tetra-*p*-tosylate salt have an apparent D_{4h} [40] symmetry. As predicted by theoretical consideration [40], they show wavelength independent linear dichroism with absorption anisotropy close to $K = 0.15$.

When the molecular symmetry is lowered to the C_{2V} symmetry group in type III porphyrins, a strong wavelength dependent linear dichroism is observed. The linear dichroisms for iron-free and iron derivative of deutero- and proto-porphyrin III have a similar wavelength dependent character. It should be noticed that in the presence of metal, the whole absorption anisotropy spectrum is downward shifted to lower values.

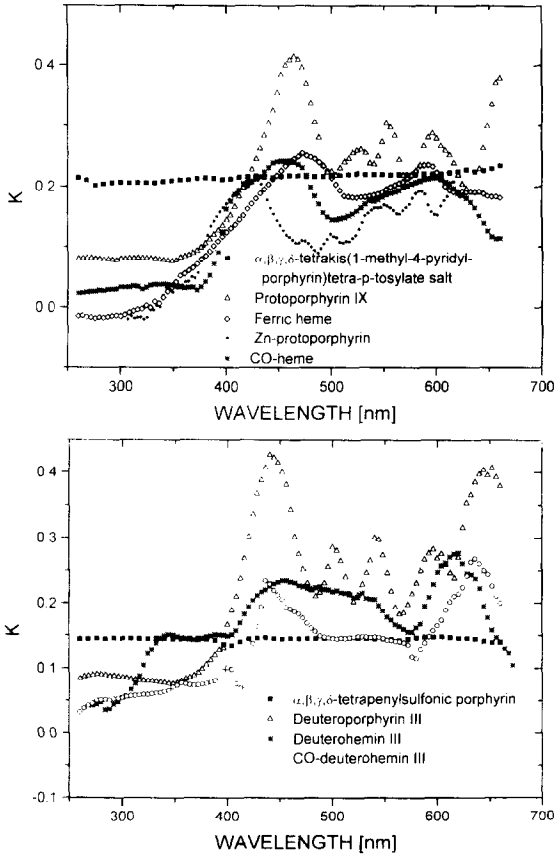

Fig. 3. Absorption anisotropy measured in stretched PVA films for different symmetry porphyrins.

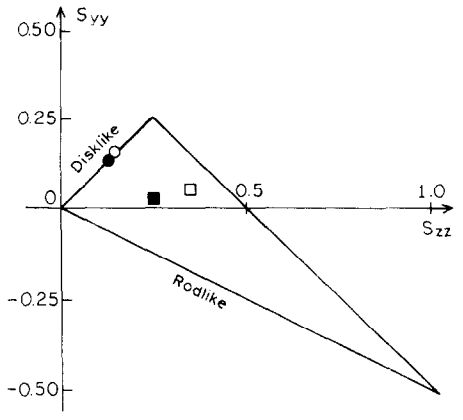

Fig. 4. Orientation triangle showing the positions determined by the Saupe parameters for $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin (●), $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-21*H*,23*H*-porphyrin) tetra-*p*-tosylate salt (○), deuterohemin III (■) and deuteroprotoporphyrin III (□)

Non-symmetrical protoporphyrin IX, Zn– and Mg–protoporphyrin IX and CO-heme show wavelength dependent linear dichroisms similar to those of the iron deutero- and proto-porphyrin III derivatives (C_{2V}). Also, in this case, the presence of the metal in the porphyrin center does not modify the wavelength dependent character of the linear dichroism producing only a down-shift of the whole absorption anisotropy spectrum.

Fig. 4 shows the position of various porphyrins inside the orientation triangle as computed from the respective linear dichroism. Both $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin and $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-porphyrin) tetra-*p*-tosylate salt are located on the edge for disklike molecules. The shape of C_{2V} molecules of deuteroprotoporphyrin III derivatives shifts their location toward the rodlike edge of the triangle. Notably, the molecular orientations of deuteroprotoporphyrin III and iron–deuteroprotoporphyrin III are significantly different. This difference is too large to be explained only on the basis of molecular shape.

4.3. Emission anisotropy

For fluorescence anisotropy measurements, we used isotropic PVA films with lower protein concen-

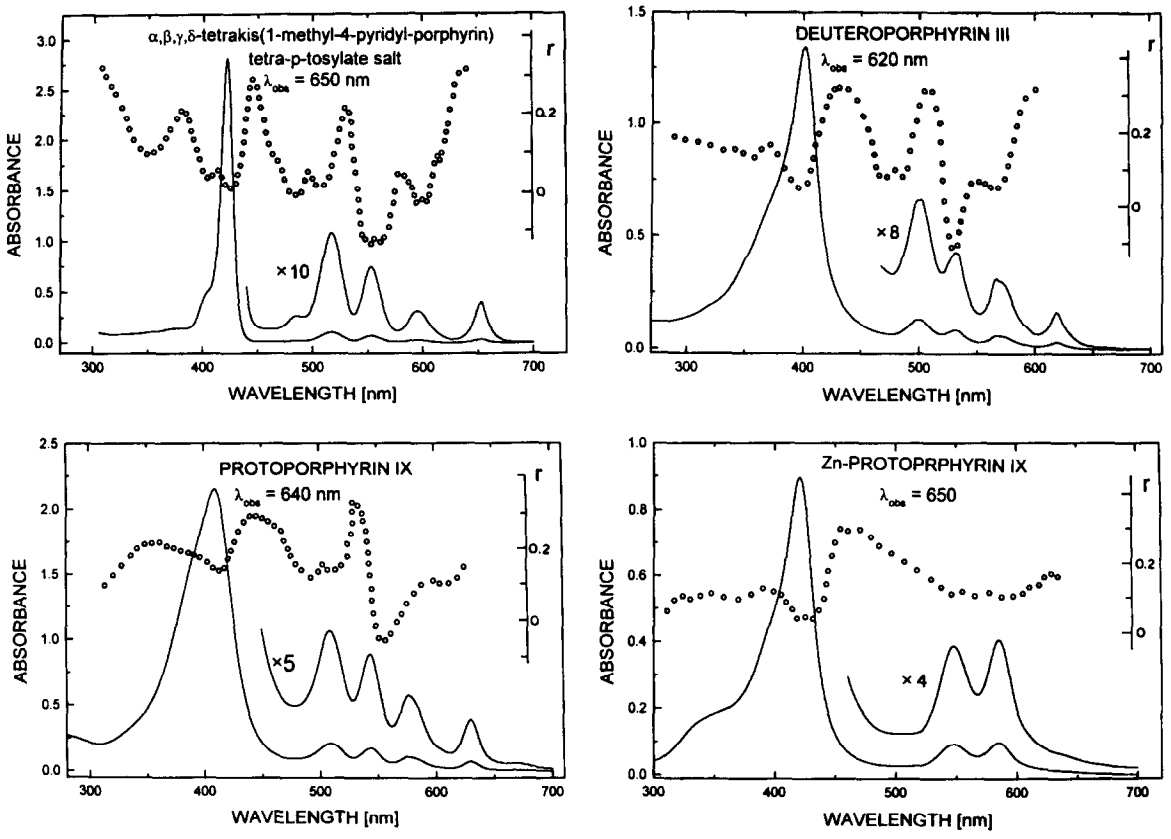

Fig. 5. Absorption spectra and excitation fluorescence anisotropy, r, of: $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-21H,23H-porphyrin) tetra-p-tosylate salt; of deuteroprotoporphyrin III, protoporphyrin IX and Zn-heme.

tration so as to eliminate the excitation energy migration [52–54]. Also the PVA matrix is known to be a rigid medium with very low reorientational depolarization effects at room temperature [52]. For comparison, we also measured the emission anisotropy of different porphyrins in viscose solvent (Kodak oil at 15°C) using the regular right angle square geometry. It is important to note that there was a very good correspondence between the excitation emission anisotropy measured in viscous solvents and in the PVA films. Also, the absorption spectra in solvents and polymer films were very similar.

The isotropic absorption spectra together with the excitation fluorescence anisotropy spectra of four porphyrin derivatives (α, β,γ,δ-tetraphenylsulfonic porphyrin, deuteroporphyrin III, protoporphyrin IX and Zn–protoporphyrin IX) are shown in Fig. 5. The observation wavelengths for each porphyrin derivative are indicated in the figures.

The fluorescence anisotropy of all porphyrins derivatives are strongly wavelength dependent with maximum values higher than 0.3. This is a high value of steady-state anisotropy in view of the fact that depolarization due to the reorientational wobbling of the molecules are not fully eliminated.

5. Discussion

The linear dichroism of fully symmetric $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin and $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-porphyrin) tetra-p-tosylate salt are wavelength independent. This

is possible only in one of the following cases: (1) each transition moment of the molecule is generated by transitions equally distributed around the molecular center to form a so called 'circular absorber'; (2) each electronic transition originates from two energetically equivalent and orthogonal transitions; (3) an apparent molecular symmetry (like D_{4h}) produces uniform molecular orientation of the two main molecular symmetry axis.

Polarized absorption (linear dichroism) is a second rank process [55] which depends on the molecular orientation function (orientation parameters). For this reason, linear dichroism measurements are unable to distinguish between the uniform distribution of molecular planes within the oriented polymer (as expected for symmetrical disk-like molecules) and the uniform distribution of transition moments within the molecular plane ('circular absorber'). The experimental response is identical [40] and may easily lead to misinterpretations.

The wavelength independent linear dichroism of D_{4h} symmetry porphyrins have a relatively high values of absorption anisotropy, $K > 0.15$, which indicates an effective orientation of the stretched PVA system and a good orientation of the molecular plane of the porphyrins within the polymer chains. However, from only this experimental data, it is difficult to decide which of the three possibilities mentioned above is responsible for the wavelength independent linear dichroism.

On the other hand, fluorescence anisotropy due to photoselection by the excitation light is a fourth rank process [55], and can easily verify whether a planar degeneration of the electronic transitions exists as proposed by the 'circular absorber' model. The wavelength dependent emission anisotropy reported in Fig. 5 rules out the 'circular' character of the electronic transitions. The wavelength independent linear dichroism of symmetrical (D_{4h}) compounds shown in Fig. 3 and the strongly wavelength dependent fluorescence anisotropy (shown in Fig. 5) clearly prove that the observed flat linear dichroism spectra are a consequence of the molecular shape, which results in equivalent orientation of two main symmetry axis in the polymer matrix. As expected, absorption spectra follow the symmetry rule and the electronic transitions are the composition of two orthogonal transitions which lay in the molecular plane. These results are fully consistent with earlier studies of porphyrins in rare gas matrices, which resolved at low temperature the orthogonal polarizations of the allowed electronic transitions [11].

The lower (C_{2V}) symmetry deuteroprotoporphyrin III, with a preferential orientation along the α,γ-meso-axis, shows a strongly wavelength dependent linear dichroism spectrum (Fig. 3). Deuteroprotoporphyrin III is a C_{2V} symmetry molecule and its linear dichroism should be interpreted in terms of two orthogonal transition moments oriented along the main symmetry axis. The preferential orientation along the stretching direction of the longer axis of the molecular symmetry testified to the energetic separation of two orthogonal polarizations of the transition moments of the porphyrin ring. In fact, the fluorescence excitation anisotropy spectrum of protoporphyrin III (Fig. 5) is, as expected, strongly wavelength dependent with values between $r = -0.1$ and $r = 0.3$. We want to stress that the addition of iron in the center of C_{2V} symmetry porphyrins (as in proto- and deutero-hemin III [40]) affects the position and shape of the absorption bands (particularly in the visible region) conserving a general pattern of the porphyrin absorption spectra. The interesting and significant effect of iron substitution is a downward shift of the absorption anisotropy values at all wavelength.

It is important to note that deuteroporphyrin with C_{2V} symmetry is a planar system and linear dichroism analysis allows a precise calculation of the orientation parameters S_{ii}. Fig. 4 shows the orientation triangle with the positions of deuteroporphyrin III and iron–deuteroporphyrin III, obtained using the spectral step reduction method. The drastic difference in the values of the orientation parameters for deuteroporphyrin III and its iron derivatives is too large to be explained only by modifications of the orientation behavior of the porphyrin structure produced by the central iron substitution.

Very similar behavior was observed for non-symmetrical protoporphyrin IX and its metal derivatives Fe, Zn and Mg. Non-symmetrical protoporphyrin IX shows wavelength dependent linear dichroism with very large limiting values of absorption anisotropy, similar to those of deuteroporphyrin III (Fig. 3a,b). This indicates a very good orientation of the protoporphyrin IX molecule in the PVA matrices with

preferential orientation axes similar to that of deuteroprotoporphyrin III. Also, in this case, the absorption anisotropies for iron and the other metal derivatives are downward shifted along the entire absorption spectra with respect to the iron-free porphyrins. This seems to be a general phenomenon associated with the presence of the metal-atom in the porphyrin center.

5.1. Circular absorber of polarized light

Over 20 years ago, Eaton and Hochstrasser [37,38] proposed that the central iron in the heme structure induces a degeneration of the electronic transitions in the porphyrin plane producing a so-called 'circular absorber' of polarized light. This unusual iron effect would significantly alter the linear dichroism measurements. Electronic transitions produced by circular degeneration should result in a constant wavelength independent absorption anisotropy [40] with values similar to or higher than those observed for $\alpha,\beta,\gamma,\delta$-tetraphenylsulfonic porphyrin and $\alpha,\beta,\gamma,\delta$-tetrakis(1-methyl-4-pyridyl-porphyrin) tetra-*p*-tosylate salt. This effect of metal substitution on the electronic transitions is ruled out by the strong wavelength dependent linear dichroism reported in Fig. 3 for both symmetrical (C_{2V} symmetry) and non-symmetrical metallo-porphyrins.

In comparison to the metal free porphyrins, the metal derivatives exhibit consistently lower values of absorption anisotropy along the entire absorption range. The question may be asked whether a partial degeneration could be responsible for the parallel downward shift of all absorption anisotropy values. Simple considerations show that such a lowering is expected for transitions polarized along the longer molecular axis with high values of absorption anisotropy. Instead, for transitions polarized orthogonal to this axis and characterized by a low value of absorption anisotropy, circular degeneration would produce an increase of the values of absorption anisotropy. This, as expected [40], would result in a 'flattening' of the absorption anisotropy spectra. As all values are lowered, the circular degeneration cannot explain such a behavior and we conclude that the degeneration proposed by the 'circular absorber' hypothesis does not contribute in any significant way to the absorption anisotropy of these compounds.

5.2. Out-of-plane polarized transitions induced by central metal substitutions

A number of high-symmetry (D_{4h}, D_{2h}) molecules have been investigated in uniaxially oriented systems in the hope to observe significant out-of-plane polarized transitions on the background of intense $\pi\pi^*$ transitions. In almost all cases, the out-of-plane polarized transitions such as $n\pi^*$ are so weak relative to the in-plane polarized absorption that they do not affect the observed linear dichroism. Initial work with free-base tetrahenylporphyrin in stretched polyethylene [55–57] suggests the presence of a measurable out-of-plane component in the blue-side of the Soret band. However, more recent studies in polypropylene and in liquid crystal [55,58] do not show any significant lowering of linear dichroism in this spectral region. One of the most convincing examples which confirms the existence of out-of-plane polarized absorption of significant intensity in planar molecules is dibenzo[a,j]chrysene [59]. In this case (D_{2h} symmetry group), distortion from planarity increases the intensity of the forbidden out-of-plane polarized transitions to the level of nominal $\pi\pi^*$ transitions. Studies of linear dichroism in single crystals of hemoproteins also revealed a significant absorption component orthogonal to the heme plane. These out-of-plane polarized transitions with high intensities were, in this case, assigned to the porphyrin–iron charge transfer transitions [31,32].

Most likely, metal-atoms located in the porphyrin center cannot produce significant changes of the polarizations or the relative energy of the electronic vibrations which are dictated by the overall porphyrin structure. Instead, their presence may induce a charge separation and may significantly increase the intensity of out-of-plane polarized absorption components. It should be stressed that for well-oriented molecular planes even small contribution of out-of-plane polarization to the overall linear dichroism may significantly downward shift the observed absorption anisotropies.

The relative intensity of out-of-plane polarized transitions for metal derivatives of C_{2V} symmetric deuteroprotoporphyrin III can be derived from a simple formalism described by Matsuoka and Norden [60] or the spectral step reduction method [55,61]. According to this method, for C_{2V} symmetry as in

deuteroprotoporphyrin III, the Soupe orientation parameters are $S_{yy} = K_{max} = 0.45$ and $S_{zz} = K_{min} = 0.06$ and the A_z and A_y polarized absorption spectra are given by:

$$A_z(\lambda) = \frac{A(\lambda) \cdot 3K_{max} - 3K(\lambda)}{K_{max} - K_{max}}$$

$$A_y(\lambda) = \frac{A(\lambda) \cdot 3K_{min} - 3K(\lambda)}{K_{min} - K_{max}}$$

where K_{max} and K_{min} are maximum and minimum values of absorption anisotropy, $K(\lambda)$ and $A(\lambda)$ are the wavelength dependent absorption anisotropy and absorbance, respectively. Fig. 6 shows the polarized absorption spectra A_y and A_z calculated for iron-free deuteroporphyrin III.

Iron–deuteroporphyrin III generally shows lower values of absorption anisotropy. Linear dichroism analyses, on the assumption that all allowed electronic transition lies in the molecular plane, yield significantly different orientation parameters than deuteroporphyrin III. This different location in the orientation triangle (Fig. 3) is too large to be solely produced by a single metal atom in the center of a much larger porphyrin ring. Such a discrepancy suggests the presence of out-of-plane transition moment components induced by the central metal-atom.

From direct linear dichroism measurements, we can calculate the apparent orientation parameters S_{ii} and decompose the absorption spectrum into the A_y and A_z polarized components as shown in Fig. 7a for iron–deuteroporphyrin III. As mentioned above, the orientation parameters S_{ii} for iron–deuteroporphyrin III are very different from those of deuteroporphyrin III. Assuming that both free porphyrin and metal-substituted porphyrin have a similar orientation in the stretched polymer, we may estimate the effect produced by the central iron substitution. To estimate the contribution of the orthogonal polarization, we can use the orientation parameters of deuteroporphyrine III to decompose the absorption spectrum of its iron derivative so as to obtain new A_y and A_z polarized components. This new decomposition, presented in Fig. 7b, reveals a significantly stronger A_y (orthogonal) component. Subtracting from the A_y polarized absorption in Fig. 7b, the A_y polarized component in panel 7a, we can compute the change (ΔA_y) in y polarization produced by iron substitution. This is shown in Fig. 7c. This is the out-of-plane polarization component in-

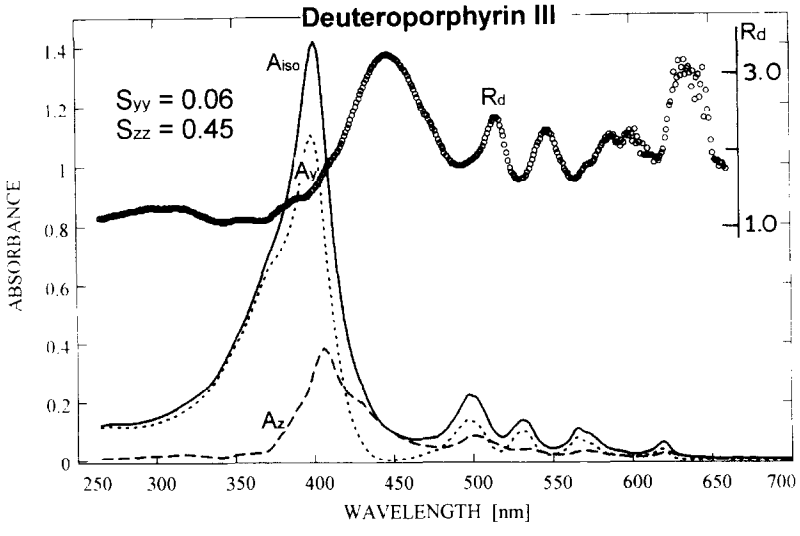

Fig. 6. Isotropic (solid line) and resolved polarized spectra in z and y direction respectively: (A_z) dashed line and (A_y) dotted line.

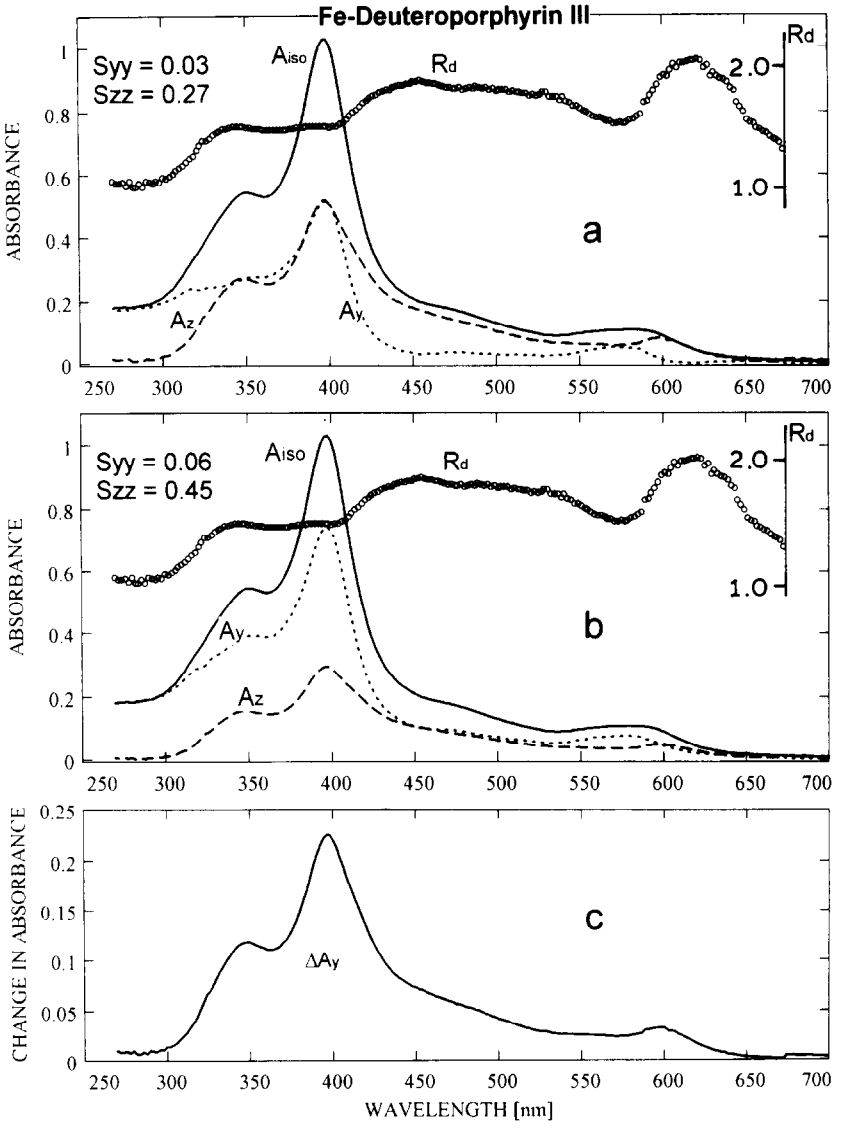

Fig. 7. Isotropic (solid line) and resolved polarized spectra in z and y direction respectively ferric deuteroporphyrin III; (A_z) dashed line and (A_y) dotted line. (a) resolved polarized generated with the orientation parameters obtained from spectral analysis of iron-deuteroprotoporphyrin III; (b) resolved polarized generated with the orientation parameters obtained for deuteroprotoporphyrin III; (c) difference in the orthogonal y polarized absorption components.

duced by the metal-atom in the center of the porphyrin. It should be stressed that the ΔA_y spectrum, so computed, represents a maximum possible contribution of the orthogonal transitions induced by the iron.

A good estimation for the intensities of out-of-plane polarized transitions in protoporphyrin IX derivatives can be obtained with the assumption that the Soupe orientation parameters for these compounds are not much different from those of deuteroprotoporphyrin III. In fact, the limiting values of absorption anisotropies of protoporphyrin IX are

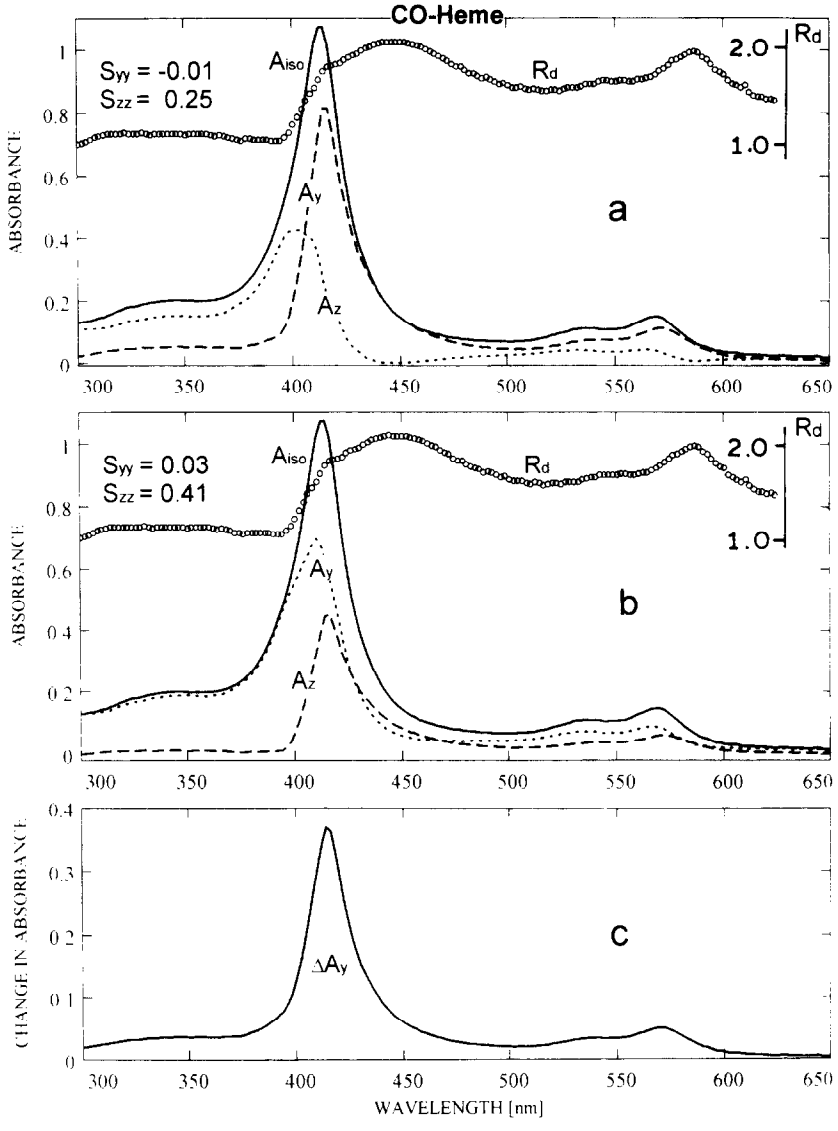

Fig. 8. Isotropic (solid line) and resolved polarized spectra in z and y direction respectively for CO- heme: (A_y) dashed line and (A_y) dotted line. (a) resolved polarized generated with the orientation parameters obtained from spectral analysis of CO-heme; (b) resolved polarized generated with the orientation parameters obtained for protoporphyrin IX; (c) difference in the orthogonal y polarized absorption components.

similar to those of deuteroprotoporphyrin III with estimated orientation parameters $S_{yy} = 0.05$ and $S_{zz} = 0.43$. Fig. 8 shows the resolved polarized spectra of the CO derivative of iron–protoporphyrin IX (CO-heme). Also in this case, the additional component ΔA_y (Fig. 8) is significant and should be assigned to the out-of-plane polarization component induced by the metal in the center of the heme. Contribution of the out-of-plane components are significantly larger in non-symmetrical protoporphyrin IX than in the C_{2v} symmetry deuteroporphyrin III.

The spectral decomposition for the Zn derivative

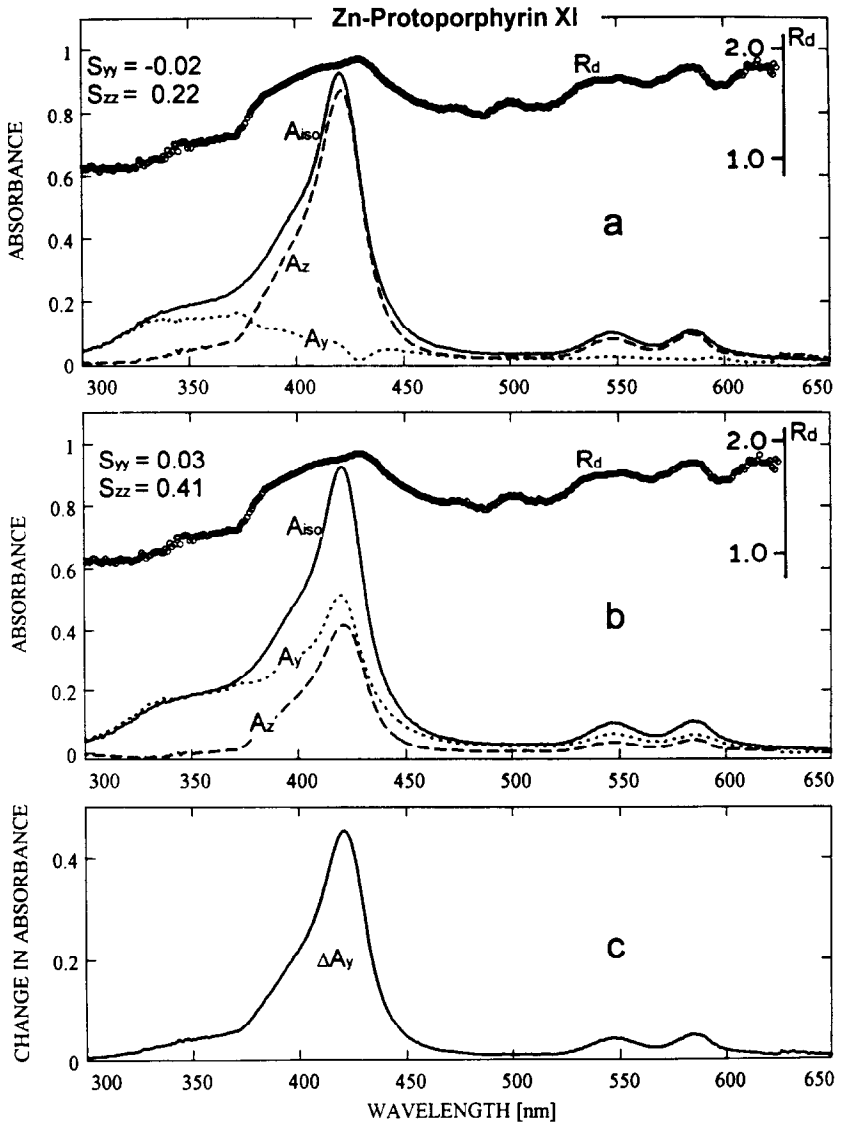

Fig. 9. Isotropic (solid line) and resolved polarized spectra in z and y direction respectively for Zn-heme; (A_z) dashed line and (A_y) dotted line. (a) resolved polarized generated with the orientation parameters obtained from spectral analysis of Zn-heme; (b) resolved polarized generated with the orientation parameters obtained for protoporphyrin IX; (c) difference in the orthogonal y polarized absorption components.

of protoporphyrin IX is presented in Fig. 9. The results are similar to those of iron-PPIX derivatives with even larger contributions of out-of-plane absorption components.

We want to stress that the contributions of out-of-plane components (ΔA_y) reported in Figs. 7–9 for metal derivatives represent maximum possible values. The out-of-plane components for some porphyrin derivatives were already observed in polyethylene films [56,57] and more precisely for the heme in the single crystals of hemoproteins [31,32]. Our results in PVA films are consistent with this observations, confirming the possibility of existence of out-of-plane components of significant intensities.

6. Conclusion

It appears that porphyrin compounds are endowed with linear transition moments with well-defined orientations in the plane of the porphyrin. The presence of metal in the center of these molecules does not produce any appreciable degeneration of the orientation of these transitions in the molecular plane. Instead, metal substitutions induce the out-of-plane absorption components of significant intensities. The linear nature of electronic transition moments must be taken into consideration for the interpretation of data of polarized spectroscopy obtained from these compounds.

Acknowledgements

This work was supported in part by PHS NIH grant POI-HL-485 17, HL-22252 and RR-08119 to the Center for Fluorescence Spectroscopy. Computer time and facilities were supported in part by the computer network of the University of Maryland at Baltimore, and College Park, MD.

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