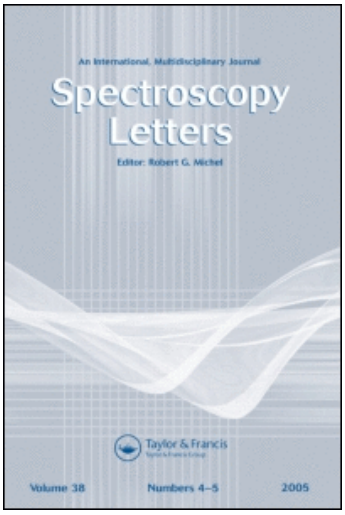

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ON THE LOCATION OF THE ELECTRONIC TRANSITIONS OF CHLOROPHYLL "A" AND PROTOCHLOROPHYLL "A" DEPENDING ON THE DEGREE OF SOLVATE STATE

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ABSTRACT

Anisotropy of optical transitions of monosolvate and disolvate species of chlorophyll "a" (Chl) and protochlorophyll "a" (PChl) have been investigated using fluorescence polarization (FP), circular dichroism (CD) and magnetic circular dichroism (MCD). The Q_x(o-o) transition of monosolvate species is found at 621-630 nm, while the Q_x(o-o) transition of Chl disolvate species at 639-642 nm. Therefore, previously accepted assignment of the Chl Q_x(o-o) transition is not correct. The longest wavelength transition of PChl is assigned to Q_x(o-o).

The most pronounced spectral change in conversion from monosolvate to disolvate species is an intensity

increase in the Chl Q_x(o-o) transition and intensity decrease in the PChl Q_y(o-o) transition.

Correlation of the transition orientations and signs of the corresponding absorption bands in the CD and MCD spectra of pigments has been discovered.

The data obtained create the experimental basis for the further theoretical calculations of the electronic structure of Chl and its derivatives.

INTRODUCTION

Spectroscopic studies of porphyrins, chlorophylls and their derivatives show that electronic absorption spectra of these molecules are due to four electronic transitions[I,2]. Two of them belong to the Soret band and two others lie in the visible region and correspond to the excitation of the first and second singlet excited states of the molecules. Only the last two transitions have been discussed in this paper. In accordance with the well known designation we denote these transitions as Q_x and Q_y. Where X and Y are the axes which unite the nitrogen atoms of the II and IV, and I and III pyrrole rings of the pigments, respectivly. The available spectral data[3,4,5] indicate that energy of the Q_x and Q_y transitions is determined by the coordination state of the Mg atom in the molecule. Usually, the Mg atom has the coordination number 5

and is solvated by one molecule of the solvent[5]. Chl, dissolved in diethyl ether, is used as an example of monosolvate species of the pigment. The staircase of the bands growing in intensity before the Q_y(o-o) transition is typical for the absorption spectra of monosolvate species of the pigment. From the MCD spectra the Q_x(o-o) transition is usually assigned to the absorption band at 575 nm and the Q_y(o-o) transition at 662 nm.

In a highly polar solvents Mg has the coordination number 6 and is solvated by two molecules of the solvent. Chl solution in pyridine or alcohol glass (mixture of diethyl ether, petroleum ether and isopropanol in the ratio of 5 : 5 : 2, respectively, at 77°K) is a typical example of disolvate species of the pigment. It has been shown by using the FP method that in alcohol glass (77°K) the Q_x(o-o) transition occurs at 639 nm[3,4]. The comparison of the available data for monosolvate and disolvate species of Chl suggests that the Q_x(o-o) transition is highly sensitive to the solvatation state of the molecule and by I700 cm^{-I} shifts.

However, these great shifts are usually observed in essential chemical modifications of the molecules. So it is unlike that such a weak perturbation as solvatation will induce such a great change in the elect-

ronic band position. For the reliable interpretation of the electronic transitions of Chl molecules in various states of solvatation systematical studies of the transition anisotropy using the FP, CD and MCD methods are necessary. The above problems are discussed in this paper. The similar investigations for PChl, porphyrin predecessor of Chl, are of a certain interest. It is known that Q_x and Q_y transitions of porphyrins are quasi-forbidden. In conversion from porphyrin to chlorin (chlorophyll) only one transition is allowed which is shifted to a long wavelength region and intensified[2]. At the same time the second electronic transition is almost unchangabled both in energy and intensity[I,2]. These common regularities may serve as the basis for the interpretation of electronic transition in the PChl molecules.

EXPERIMENTAL SECTION

Chl "a" was obtained from dry nettle powder with a subsequent chromatographing on powdered sugar[6]. Chl "a'", diastereoisomer of Chl "a", was obtained by boilling Chl "a" in toluene with a subsequent rechromatographing the samples on powedered sugar by the mixture of the petroleum ether and propanol (0,5%).

It should be noticed that infrared and electronic absorption spectra and quantum yields of fluorescence of Chl "a" and "a'" are essentially identical[6].

They differ only in the FP[3] and CD spectra[7]. But these differences, however, are not of principle and consist in the differences of transition intencities.

PChl "a" was obtained from the cover of seeds of Ecballium elaterium which contains a high concentration of PChl. There are no typical admixtures such as 4vinylprotochlorophyll and protopheophytin (PPheo) which are usually contained in the traditional source of PChl,i.e. in the cover of seeds of pumpkin[8]. An additional purification of the PChl was obtained by multiple rechromatographing the samples on the powdered sugar using initially the petroleum ether (40-70°C) and toluene (30%) mixtures, and then the petroleum ether and propanol (0,5%) mixtures.

Spectrally pure mesoporphyrin was kindly presented by A.M.Shulga.

The absorption spectra were recorded by a "SF-10" spectrophotometer. The fluorescence spectra were recorded using a "Fica-55" spectrofluorometer. The CD and MCD spectra were recorded using a "Jasco-20" spectropolarimeter. The magnetic field was directed axially and in the sense of propagation of the light. The field strength was 8000 gauss.

The FP spectra were recorded with the instrument disigned on the base of two diffraction monochromators.

The spectra width at the excitation and record of the fluorescence was I-3 nm. For the FP the error was 5-I0%.

RESULTS

I. Chlorophyll "a" and "a ".

The studies of transitions anisotropy of Chl "a" and "a " gave similar results. It will be noted that the Chl "a " Q_x transitions are more expressive in the CD and FP spectra. Therefore, in further discussion only the data on Chl "a " will be used. The absorption, fluorescence and FP spectra of Chl "a " at 77°K are shown in Fig.IA. Independence of the fluorescence spectra shape on the excitation wavelength confirms the fact that there are only one kind of fluorescent molecules in the solution, which are assigned as earlier [3-5] to disolvate species of Chl.

The negative values of the degree of FP (Fig.IA) of the band at 639 nm indicate that the transition dipole moment is perpendicular to the transition dipole moments of the Q_y(o-o) and Q_y(o-ı) bands. This result may be accounted for by two ways: I. The band at 639 nm belongs to incompletely symmetrical vibrational satellite of the Q_y(o-o) transitions; 2. The band at 639 nm is the Q_x(o-ɔ) transition.

The FP data testify that the first of the suppositions is hardly probable. In fact in the fluorescence

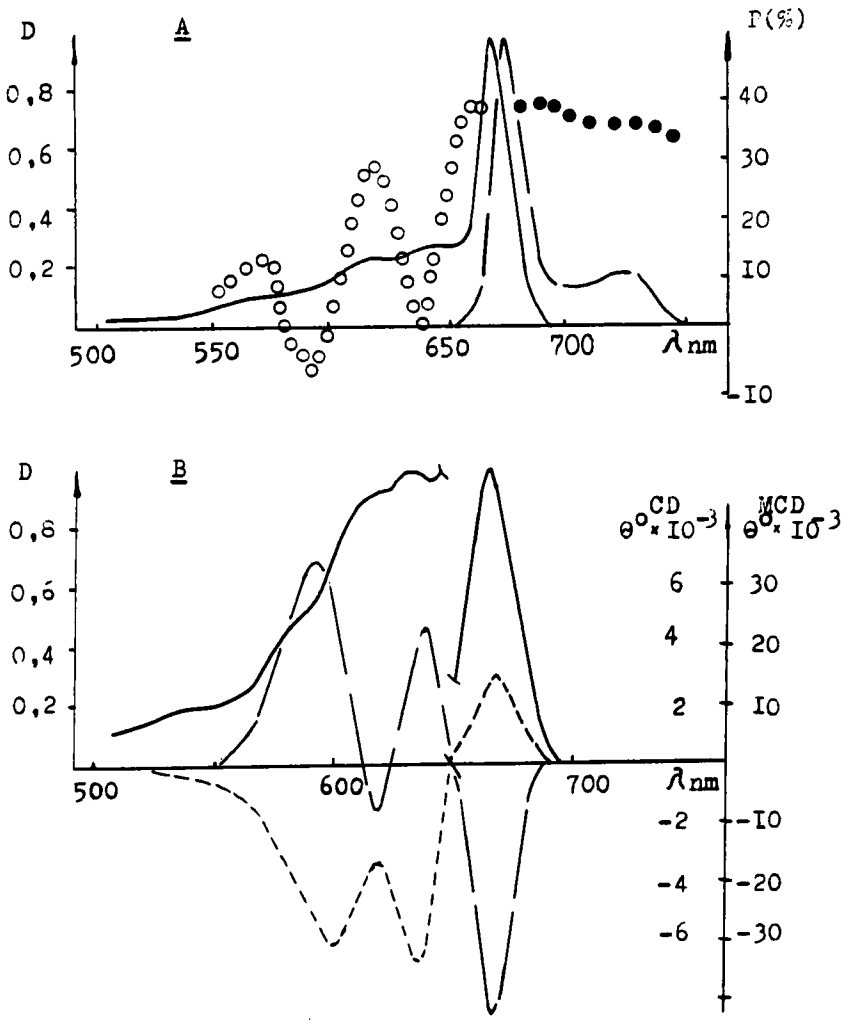

Fig.I

A. Absorption (————) and fluorescence (— ——) spectra, excitation spectrum of FP (○○○○○○) and FP along the emission spectrum (●●●●●●) of Chl "a'" in alcohol glass at 77°K.

B. Absorption (————), CD(— ——) and MCD (- - - - - -) spectra of Chl "a'" in pyridine at room temperature.

spectra there is no mirror imaging absorption band that corresponds to the absorption band at 639 nm. The rest of the region of the fluorescence spectra is the mirror symmetrical to the absorption band. Moreover, the FP spectra, measured in the region of fluorescence band (λ_{ex} = 660 nm) do not contain the gap that corresponds to the negative polarized band at 639 nm in the excitation spectra of FP.

These data permit to conclude that the band at 639 nm belongs to the Q_x(o-o) transition.

The analogous information may be obtained from the CD and MCD data (Fig.IB) of Chl "a'" disolvate. In Fig.IB it is obvious that the signs of the MCD spectrum of pyridine solutions of Chl coincide with the signs of the FP spectrum of Chl in alcohol glass. The first (the most wavelength) band in MCD spectrum of Chl is positive. This result is the particular case of the common rule, that consists in that the sign of the first band in MCD spectra is always positive. This rule has no exceptions for all investigated MCD spectra of both chlorophylls and porphyrins. The report in[7] on the negative sign of the long wavelength band of some porphyrins and in particular mesoporphyrin is not confirmed by our experiments.

So in accordance with the MCD data the Q_x(o-o) transition of pyridine solution of Chl is located at

639 nm. The CD method as distinct from the MCD apparently "feels" absolute orientation of the transitions in respect of the molecule skeleton. A detailed analysis of our results and data available[1,9,10] on chlorophylls and their derivatives allows to suppose that the Q_x transitions always have the positive CD sign and the Q_y transitions have the negative CD sign. The assigning of the transitions of PChl and PPheo are the exceptions to this rule. However, below the experimental data will be given which allow to propose the new assignments of the Q_x(o-o) and Q_y(o-o) transitions of PChl, confirming the community of the rule about the CD signs of the X and Y transitions.

In accordance with the signs of CD bands (Fig.1B) the Q_x(o-o) transition of Chl corresponds to the band at 639 nm and its vibrational satellite to the band at 595 nm. The energy difference between Q_x(o-o) and Q_x(o-1) is about 1200 cm^{-1}.

Therefore, it is evident that each of the above methods gives the same results.

In order to establish the location of the Q_x(o-o) transition in the absorption spectra of monosolvate species of Chl the search for the proper system of solvents which provides retaining the spectral properties of the monosolvated species of the pigments at 77°K and is good for FP measurements has been carried out. The mixture of

castor oil and diethyl ether (I:I) satisfies these requirements. The shape of fluorescence spectra of the pigments dissolved in the mixture of castor oil and diethyl ether does not depend on the excitation wavelength. This is an evidence that there is only one kind of fluorescent molecules in the mixture. For measurements of the CD and MCD spectra of Chl monosolvate the ether solutions of the pigment were used. The results are presented in Fig.2. In order to achieve better intensity of the CD spectra the optical density in the orange-yellow region of the absorption spectra was about one.

The excitation spectra of FP (Fig.2A) indicate that there is a transition in the region of 630 nm and its orientation is essentially perpendicular to the orientation of the Q_y transitions. It will be observed that the FP along the fluorescence spectra remains nearly constant. Thus, the transition at 630 nm is the Q_x(o-o) transition of monosolvate species of Chl. It is almost forbidden and covered by vibrational satellite of the Q_y(o-o) transition.

The MCD and CD data (Fig.2B) confirm the conclusion that the Q_x(o-o) transition is located at 62I on the long wavelength slope of the Q_y(o-I) transition. The band at 578 nm differed from the Q_x(o-o) transition by about I200 cm^{-I} can be attributed to the Q_x(o-I)

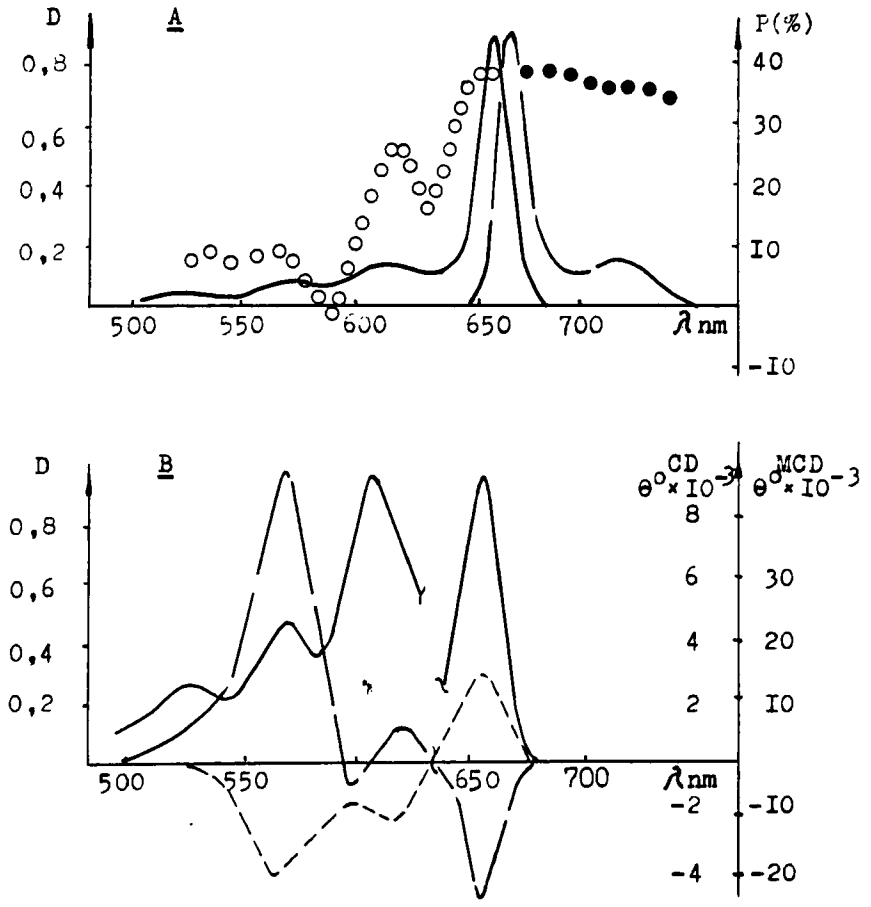

Fig. 2

A. Absorption (———) and fluorescence (— ——) spectra, excitation spectrum of FP (○○○○○○) and FP along the emission spectrum (●●●●●●) of Chl "a' " in the mixture of castor oil and diethyl ether (I:I) at 77°K.

B. Absorption (———), CD(— —) and MCD(------) spectra of Chl "a' " in diethyl ether at room temperature.

transition in accordance with the CD and MCD data. But it is difficult to neglect that the band at 578 nm belong to the third electronic transition, which is oriented in the X direction. Therefore, the results obtained bear out the fact that the previous interpretation of the Q_x(o-o) transition[9,10] is not correct. The similar conclusions are valid for some chlorophyll derivatives.

The comparison of the absorption spectra of Chl dissolved in ether and in pyridine shows that the most pronounced spectral change in conversion from monosolvate to disolvate species is the rise of intensity of the Q_x(o-o) transition.

The strong red shift of the spectrum apparently connect with the differences of the refractive indices of ether and pyridine. Really, in solvents where the value of the refractive index is high (castor oil), the most wavelength maximum of the monosolvate Chl shifts to the 672 nm and coincides with the location of the absorption maximum of disolvate species of Chl in pyridine.

It is interesting that the Q_x(o-o) shifts more stronger than Q_y(o-o), and their vibrational satellites shifts exactly as the corresponding electronic transitions.

2. Protochlorophyll "a"

The studies of PChl in various solvents show that PChl has two characteristic types of the absorption spectra. In the absorption spectrum of PChl in ether one can observe two band systems I,3,5 and 2,4,6 (Fig.3) These bands are shifted relative to each other by about 500 cm^{-I}. By analogy with the Chl we assign such type of the spectrum to monosolvate species of PChl. While PChl is dissolved in pyridine, alcohol glass or in the mixture of pyridine and alcohol (77°K) the absorption spectra consist of only I,3,5 bands (Fig.3). It is evident that this type of spectrum is due to the disolvate species of PChl. Disappearing the bands 2,4,6 is likely to connect with the strengthening the quasi-prohibition of the second electronic transition and vibrational satellites of the transition are the main spectroscopic properties of the disolvated PChl.

The data on the properties of the electronic transitions and their vibrational satellites for monosolvate PChl and Chl species in diethyl ether and disolvate species in pyridine or alcohol glass obtained by the FP, CD and MCD methods are given in the Table I. These data show that the transition I of monosolvated PChl has the same energy as the Q_x(o-o) transition of monosolvated Chl while both pigments dissolved in diethyl ether. Transformation from monosolvate to disolvate species of

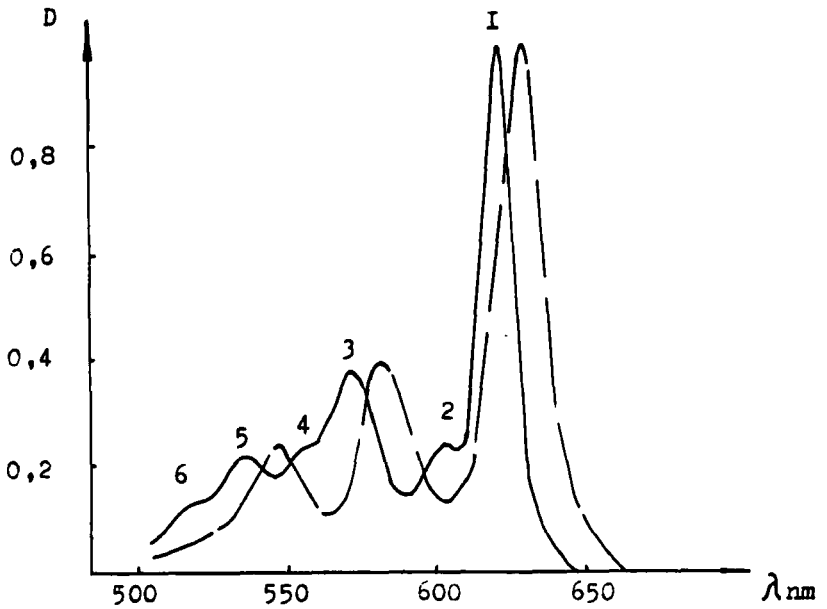

FIG.3

Absorption spectra of PChl in ether at room temperature (————) and in alcohol glass at 77°K (—— ——).

the pigments induces similar long wavelength shifts of the transition I of PChl and Q_x(o-o) of Chl.

So, it is natural to identify the first band in the absorption spectrum of PChl with the Q_x(o-o) transition and the bands 3 and 5 correspond to its vibrational satellites. The Q_y(o-o) transition is almost forbidden and located in the shorter wavelength region (band 2).

TABLE I

Optical properties of the PChl and Chl pigments

solvate state	assign-ments	abs. max (nm)	$\bar{\nu}$ cm^{-1}	$\bar{\nu}$ cm^{-1}	signs CD	signs MCD	FP
Chl							
mono	Q_y(o-o)	662	I5I05	247	-	+	+0,40
di		673	I4858		-	+	+0,37
mono	Q_y(o-1)	6II	I6366	237	-	+	+0,23
di		620	I6I29		-	+	+0,22
mono	Q_x(o-o)	624	I6077	452	+	-	+0,I5
di		640	I5625		+	-	0,00
mono	Q_x(o-1)	578	I730I	495	+	-	0,00
di		595	I6806		+	-	-0,08
PChl							
mono	Q_x(o-o)	624	I6025	452	+	+	+0,37
di		640	I5625		+	+	+0,27
mono	Q_y(o-o)	605	I6528	242	-	-	+0,I2
di		6I4	I6286		-	-	-0,0I

It is interesting to note that in the framework of the interpretation proposed the coincidence of the CD signs of the band I of PChl and the Q_x(o-o) band of Chl is not accidental,it is a consequence of the common rule for chlorophyll derivatives. In accordance with this

rule the Q_x transitions has always positive signs and the Q_y transitions has always negative signs. To base this rule further experimental and theoretical investigations are necessary.

When summarizing the PChl data it should be emphasized that the main spectroscopic effect of PChl solvatation is the strengthening of the quasi-prohibition in the Q_y transitions. Reduction of the PChl to Chl level intensifies and shifts by 1400 cm^{-1} to the long wavelength region the Q_y(o-o) transition, and the Q_x(o-o) transition does not change its location in the absorption spectra. So, although this assignment differs from the accepted one[II], it is in good agreement with experimental results obtained.

CONCLUSION

1. Analysis of the available data shows that the long wavelength transition in the MCD spectra of porphyrins, chlorophylls and their derivatives has always a positive sign.

2. From the CD data it follows that the Q_x transition of Chl and PChl derivatives has always positive signs and the Q_y transition has always negative signs in the CD spectra. Probably, this rule can serve as the criterion for the transition assignment.

3. In the Chl molecules the Q_x(o-o) transition is at long wavelength side of the Q_y(o-1) transition or

lies between the $Q_y(o\text{-}o)$ and $Q_y(o\text{-}1)$ transitions depending on the solvate state of Mg atom in the molecule. The first electronic transtion of PChl is assigned to $Q_x(o\text{-}o)$.

4. The main spectroscopic effect of disolvatation of the Chl and PChl is the change in the intensitv of one of the transitions. The intensity of the Chl $Q_x(o\text{-}o)$ transition increases and the intensity of the PChl $Q_y(o\text{-}o)$ decreases.

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