Screening Hypochromism in Molecular Aggregates and Biopolymers

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Abstract. An improved model of the screening effect is suggested. Mutual «shielding» of chromophores from light due to competition for the incident photon can take place in molecular aggregates and macromolecules. From a common point of view, it could be interpreted as in «interaction» of the absorption dipole moment transitions. Screening leads to a decrease in the extinction coefficient. The largest decrease is observed in the maximum of the absorption band. This is demonstrated with chromophores of adenine, tyrosine, tryptophan, retinol, porphyrin and anthracene. The model enables prediction of hypochromic spectra or evaluation of the quantity of chromophores in an aggregate or macromolecule.

Key words: Aggregate, chloroplast, DNA, erythrocyte, hypochromism, light absorption, oligonucleotide

Introduction

Hypochromism (the decrease in the extinction coefficient in the absence of strong molecular interactions) is observed in a wide diversity of systems: in dye aggregates, microcrystals, polymers, proteins, nucleic acids, chloroplasts, etc. [1–4]. Experimentally, the hypochromism value (h) at a given wavelength can be found by the equation:

$$h = 100\% (\varepsilon - \varepsilon^{\wedge})/\varepsilon, \quad (1)$$

where ε is the extinction coefficient for a solution of single chromophores, and $\varepsilon^{\wedge}$ is the average extinction coefficient to account per one chromophore of an aggregate or macromolecule. Various theoretical explanations of the hypochromic effect can be found in the literature [3–17].

The accepted theory considers hypochromism as a result of weak dipole-dipole interactions modified by the light wave [1, 5]. This theory was criticized in [6, 7] and modified and new models [6–11] were suggested. One of them interpreted hypochromism as a result of changes in the complex refractive index [8, 9, 18]. Refractive index consist of two components: the natural component and imaginary one. The imaginary part relates to absorption [18]. The models demonstrate the importance not only of the refractive index but also of the local light field [13] and of the dispersion of the effective field of the light wave [18].

Most of these models, excepting [18], operate with oscillator forces and do not permit description of the form of the hypochromic spectrum. Hypochromism was not adequately described in the framework of the mentioned approaches. Researchers have had to use the qualitative expressions and to enter correction coefficients to reconcile the theory and experiment, see [19] for example.

Other models viewed hypochromism in the context of light scattering [12, 13], for example in the frameworks of Mie theory [12]. However, their physical sense is not entirely clear, because the imaginary part of the complex refractive index is used. The Mie theory has a phenomenological character. Moreover, the amplitude of the electrical field of light wave was identified with intensity of light flux [13], which is not correct. These models are a rough approximation, because when expanding interactions in series, only the first members are considered.

And finally, other models considered hypochromism from absorption statistics for nonhomogeneous media [4, 14–16]. Some of them assume ‘sifting’ of light fluxes between particles (the ‘sieve effect’) [4, 15, 16] or emphasized multiple light scattering by particles with special distributional absorbance statistics [14]. These models operate with absorbance instead of an extinction coefficient. They are suitable for suspensions of optically dense particles of size greater than the wavelength. They are hardly suitable for small molecular aggregates or single macromolecules.

Previously, I proposed a «screening» model of hypochromism [17]. The hypochromic cross section for identical dipoles was calculated from probabilities of absorption in a stack. The screening model states that a mutual «shielding» of chromophores from light can arise in a molecular stack. Screening is not «geometrical shielding»; it is electro-optical shielding due to «competition» between chromophores for the photon inside a stack. This leads to a reduction in the extinction coefficient. From a common point of view, it could be interpreted as an «interaction» of the absorption dipol moment transitions.

The improved screening model is described below, and its application to a number of biologically important chromophores in aggregates and macromolecules is considered. The model operates directly with a molecular parameter – the extinction coefficient. The model allows to calculate the hypochromic absorption spectra from the extinction coefficients of stack-like chromophore structures directly, without using the imaginary part of complex refractive index.

Theory

The probability of absorption. The probability of absorption of a photon by a molecule can be presented as [1, 2]

$$P = \delta/X, \quad (2)$$

where X is the area of a molecule, δ is the absorption cross section (at a given wavelength), i.e. ‘the area that is nonpermeable for the photon’ [2]. δ does not exceed X [2]. $\delta(\text{\AA}^2)$ can be converted to molar-extinction units (ϵ) [2]; for example:

Figure 1. The dependence of the orientation factor q for linear oscillator on the spherical angles between the transition dipole and electric vector of the light wave. The marked area corresponds to the one half of chromophores that contribute to $\sim 80\%$ of the absorption of the solution.

$1\text{\AA}^2 = (6 \times 10^7 \text{ cm}^3)/(6 \times 10^{23} \text{ cm}) = 6 \times 10^4 \text{ litre mol}^{-1} \text{ cm}^{-1} = 60\,000 \text{ M}^{-1} \text{ cm}^{-1}$. At the transition from δ to the molecular extinction coefficient (E) the multiplier 2.3 arises due to replacement of natural logarithm with the decimal one [1, 2]. Instead of the indefinite parameter X , the more exact one should be used, namely, 'section of valence electrons' (S), i.e. the area of a density of the electrons responsible for optical transition. And finally, to account for the dependence of chromophore-absorbing ability on orientation [20–22] it is necessary to enter the orientation factor (q). The dependence of q on the angles between light flow and chromophore orientations in solution was described in [20]. Effective absorption of a photon occurs, when fluctuations of the electrical components of the light wave (they are perpendicular to the direction of the luminous flux) coincide with the vector of electronic transition in the chromophore plane. Value of δ of linear dipole at such concurrence is three times more than at random orientation [2, 20–22], which can be expressed by $q = 3$ (oriented) and $q = 1$ (random), respectively [20]. If one plot q as a function of spherical angles (Figure 1), it appears that the molecules with $q \sim 2$ are responsible for $\sim 80\%$ of absorption of the solution while the molecules with $q \sim 0.5$ are responsible for $\sim 20\%$ of it.

With regard to what has been said above, it is possible to write:

$$P = 2.3Eq/S, \quad (3)$$

where S can be determined with quantum-chemical methods [23] or found from results of low-temperature X-ray analysis [24]. Typical values of areas of separate bonds involving carbon, hydrogen, oxygen, and nitrogen or of electronic pairs of oxygen and nitrogen are $0.32\text{--}0.91 \text{\AA}^2$ [23]. For double and conjugate bonds these values are somewhat greater, about $0.9\text{--}1.5 \text{\AA}^2$ per one double bond. The S value

Table I. The extinction coefficients, absorption section, areas of electronic density and absorption probabilities of biologically important chromophores in solution

Substance	λ (nm)	ε (M ⁻¹ cm ⁻¹)	E (Å ²)	δ (Å ²)	S (Å ²)	P
Adenine	260	15 000	0.25	0.575	7.1	0.081
Tyrosine	275	1 270	0.021	0.048	3.1	0.016
Tryptophan	280	5 600	0.093	0.214	4.3	0.05
Retinol	498	40 000	0.7	1.61	5.4	0.3
Porphyrin	408	120 000	2.0	4.6	14.5	0.317
Chlorophyll	430	120 000	2.0	4.6	17.0	0.271
Anthracene	253	200 000	3.3	7.6	8.0	0.95

of a molecule is obtained by summation of all elementary sections of electronic densities that participate in absorption of a photon by the chromophore.

We have calculated the probabilities of absorption for a number of biologically important chromophores (Table I) using Equation 3. As maps of optical electron density have not been constructed yet for many heteroaromatic molecules, we calculate S using the previously published maps of separate bonds and fragments [23, 24]. Since S is a more precise parameter than X , the obtained probabilities are more exact (for example, compare the calculations for anthracene in [25] and in Table I).

The screening model. In dilute solutions of identical molecules the probability of absorption of a quantum for each chromophore is the same: $P = P' = P'' \dots$ (although the intensity of a light flux in solution decreases in accordance with the exponential law). In aggregates and macromolecules, the probability of absorption of a quantum by each chromophore must depend on the probabilities of absorption by the other ones due to shielding and/or interactions. When interchromophore distance is comparable with chromophore size (much less than the wavelength), one light wave covers many chromophores. This creates the conditions for «competition» for the given photon. From a common point of view, it could be interpreted as an «interaction» of the absorption dipol moment transitions.

As is well known from chance theory, the final probability for two accidental events is equal to $P + P' - PP'$. Therefore, for identical chromophores the probability of absorption by the second chromophore will be expressed as

$$P' = (1 - P)P, \quad (4)$$

where P is the probability of absorption by the first chromophore. For instance, if $P = 0.9$, then $P' = 0.09$, so the probability of absorption by the second chromophore in a stack-like pair will be 10 times less than by the first one or then by a single chromophore. For the third chromophore we have

$$P'' = (1 - P - P')P = (1 - P)^2 P. \quad (5)$$

For the k -th chromophore

$$P^* = (1 - P)^{k-1} P, \quad (6)$$

where k is the quantity of chromophores located at distances much less than the wavelength and competing for the photon in a given aggregate or macromolecule. The probability of absorption by a macromolecule or aggregate, consisting of k identical chromophores, will be equal to

$$P^\wedge = P + P' + P'' \dots + P^* = 1 - (1 - P)^k. \quad (7)$$

The average probability of absorption of the photon by one chromophore of the aggregate or macromolecule is $P^\wedge/k$. On the other hand, it is equal to $2.3E^\wedge q/S$ (see Equation 3). Therefore, for equally oriented identical chromophores in a solution of stacks it is possible to write down:

$$P^\wedge/k = 2.3E^\wedge q/S = [1 - (1 - P)^k]/k. \quad (8)$$

Thus, we have

$$E^\wedge = [1 - (1 - 2.3Eq/S)^k]S/2.3qk. \quad (9)$$

This expression permits prediction of the hypochromic extinction coefficients in a solution of stack-like aggregates or macromolecules if the values of E , S , q and k are known. As E changes with wavelength, Equation 9 enables description of the form of the hypochromic band.

In the screening model, extinction of stacks in solution is compared with extinction of single chromophores in solution. Stacks in a solution are randomly oriented relative to the light flux as well as single molecules. In a solution of aggregates or macromolecules, as well as in a solution of separate chromophores, $\sim 80\%$ of the absorbed light should belong to half of the chromophores with $q \sim 2$, and $\sim 20\%$ of it should be absorbed by the other half with $q \sim 0.5$. Practically only the first molecules participate in screening. The orientations of dipole transitions into each of the others in a stack is most responsible for the hypochromic effect. The heaviest hypochromism will be reached if concurrence of directions of dipole transition are competitive; these directions change under variations of wavelength [1, 22]. The largest participation in absorption and hypochromism are due to stacks in which chromophore planes are perpendicular to the light flux (a dipole transition usually places in the chromophoric plane). Therefore, for hypochromism of solutions are stack-like aggregates with identical directions of dipole transition or of solutions of rigid regular polymers, $q = 2$ can be considered a good approximation. Here, q becomes the intra-stack parameter.

In a random polymer or chaotic aggregate, chromophores compete for the photon much less effectively ('residual' hypochromism), because they have essentially

different directions of dipole transitions. In this case, the number of chromophores competing for the photon (k) is considerably less than the total number of chromophores in the aggregate or macromolecule (K). For approximate estimation of h in large aggregates and long chaotic chains, it can be assumed that $k = K$ with $q = 1$. For short random chains and small aggregates $k < K$ always; therefore, if we use $k = K$, we must select q from real orientations, because q can vary from 1 to 0.

The screening model is limited by the condition that chromophores are together in a small space, considerably less the wavelength. If the size of a chromophore aggregate or a macromolecule is more than the wavelength, the length of the elementary absorbing site will not be more than the wavelength, and k will not change with length any more.

The main attributes of screening hypochromism are the following: 1) changing the hypochromism value along the absorption spectrum as a function of the initial extinction coefficient; 2) the most hypochromic value is in the maximum of the absorption band; 3) strong dependence on chromophore orientations; 4) absence of an appearance of new bands or 'transfer' of intensities into other bands.

In systems where hypochromism is caused by molecular interactions, it is also possible to use the final equation, formally suggesting that the interactions result in the same reduction of the absorption probability. However, the physical sense of the equation is changed.

Materials and Methods

The following chemically pure compounds were used: adenine, adenosine, adenosine monophosphate, L-tyrosine, D,L-tryptophan, ribonuclease T1, bovine phospholipase A2, etc. Adenosine and oligoadenines were dissolved in buffer solution containing 100 mM lithium chloride, 10 mM sodium cacodylate with pH 7.0. Rat erythrocytes were allocated by the standard method. Erythrocytes were suspended in 150 mM NaCl and kept on ice; hemolysate was obtained by hemolysis of erythrocytes in distilled water.

The absorption spectra are measured in Specord UV-visible and Perkin-Elmer-Coleman spectrophotometers in quartz cuvettes by various optical pathlength, from 0.1 to 5 cm. If appreciable scattering by a suspension was observed (for tyrosine aggregates and erythrocytes), the cuvettes were placed in the special part of the Specord, near to the detector. In this case the effect of light scattering by particles was reduced to a minimum due to collection of the scattered light by the detector; the residual scattering was subtracted from the total absorbance using the scattering absorbance curve extrapolated from the region remote from the absorption band. All measurements were performed at 20 °C, except for oligoadenines and adenine that were measured at 6 °C or 10 °C.

Figure 2. The extinction spectra of adenosine (1), penta-adenosine phosphate (2) and the calculated spectrum with $k = 5$ and $q = 2$ (3). Aqueous solution contains 100 mM lithium chloride, 10 mM sodium cacodylate, pH 7.0; temperature is 6 °C.

Results and Discussion

NUCLEOTIDES AND OLIGONUCLEOTIDES

The most investigated and relatively simple hypochromic structures are oligonucleotides [26, 27]. It was established that oligoadenine chromophores of the chain in aqueous solutions at temperatures less than 10 °C are arranged nearly a stack.

For adenosine-monophosphate, adenosine or adenine in aqueous solution, ϵ is evaluated of 14 200 – 16 800 $\text{M}^{-1} \text{cm}^{-1}$ at 259 nm [26–28, 30]. We accept on the average that $\epsilon = 15\,000 \text{ M}^{-1} \text{cm}^{-1}$; it corresponds to $E = 0.25 \text{ Å}^2$. The projection of valence electron density of the adenine chromophore is about 7.1 Å^2 ($\sim 0.9 \text{ Å}^2 \times 4$ for projection of four double bonds and $\sim 0.7 \text{ Å}^2 \times 5$ for electronic pair of five nitrogens). Therefore, the probability of photon absorption by the adenine chromophore in solution is 0.081 (at chaotic orientation, $q = 1$). Such is sufficient to observe some shielding for oligomers.

The absorption spectra of adenosine and 3'-5'-desoxyribopenta-adenosine phosphate (*pApApApApA*) and the calculated spectrum, designed from the adenosine spectrum using Equation 9 with the assumption that $k = 5$ and $q = 2$ (stack), are presented in Figure 2. The k value was taken = 5, because *pApApApApA* consists of 5 adenine chromophores. The ϵ values of adenosine at each wavelength were used for calculation by Equation 9. The experimental and calculated spectra of *pApApApApA* are similar. The shift of the experimental spectrum to the short-wave region is only 2 nm or 340 cm^{-1} , which corresponds to only 4 kJ mol^{-1} . The area between the adenosine spectrum and the calculated *pApApApApA* spectrum

Figure 3. The calculated dependencies of oligoadenine extinction on the number of adenine chromophores at $q = 1$ (1), at $q = 2$ (2) and at $q(k) = 2 \times 0.95^{k-1}$ (3). Experimental points for oligoadenines at $\sim 10^\circ\text{C}$ from [27] (●) and [26] (+) and own data (○) are shown.

is ~ 30 times larger than the area of the 'red' experimental *hyperchromic* band of *pApApApApA* at 282 nm. This means that the interactions are weak and there is no considerable 'transfer' of intensity from one band to the other. The 'red' band is the result of increasing and shifting of the $n - \pi^*$ transition of adenine in polyadenines [28], probably due to intrachain dimer formation (our unpublished data).

The calculated dependencies of the extinction coefficient on the quantity of chromophores in an oligoadenine chain at $q = 1$, $q = 2$ and $q(k) = 2 \times 0.95^{k-1}$ (q decreasing from 2 to 1 by 5% with each k increasing by 1) are shown in Figure 3.

The experimental dependencies for oligoadenines from [26, 27] and our data are added. The experimental points at small k magnitudes lie close to the calculated curve with $q = 2$; the divergence at $k > 6$ is caused by the known ability of long oligoadenines to break and fold [26, 27, 29], i.e. by the disruption of the stack arrangement. With lengthening of the oligoadenine chain, the experimental dependence is bent to the curve with $q = 1$. The best agreement of the experimental points with theory is obtained when q varies from 2 to 1 with k .

In the aqueous solution an increase in concentration of adenine, adenosine or AMP from several μM to several mM is accompanied by gradual reduction in ε [30] because of the occurrence of dimers, and with further concentration increasing (between 5 and 50 mM) a sharp fall in ε is observed, caused by the occurrence of large aggregates. These changes are not connected with an increase in light-scattering [30]. Having transformed the initial and hypochromic molar ε of adenosine from [30] into the molecular E ($15\,800\text{ M}^{-1}\text{ cm}^{-1} = 0.263\text{ \AA}^2$; for dimers $\varepsilon^\wedge = 14\,500 - 15\,000\text{ M}^{-1}\text{ cm}^{-1} = 0.241 - 0.25\text{ \AA}^2$, $\varepsilon^\wedge$ of aggregates $\sim 12\,000\text{ M}^{-1}\text{ cm}^{-1} = 0.199\text{ \AA}^2$) and substituted them into Equation 9 with $S = 7.1\text{ \AA}^2$ and $q = 1$, it is possible to find k . The substitution shows that at small concentrations (0.05 – 0.1 mM) k is equal to 2–3, and at large concentrations, $k \sim 8$. Thus, the equation indicates dimerization of adenine derivatives and their subsequent association correctly.

DNA

The energies of inter-chromophore interactions between nucleotides and excitonic interactions in DNA are small [28, 29], and could hardly induce a significant hypochromic effect.

Assuming adenine, guanine, thymine and cytosine are present in approximately identical proportions, we find the average E at 260 nm to be $\sim 8\,350\text{ M}^{-1}\text{ cm}^{-1} = 0.14\text{ \AA}^2$. The average S is $6.5\text{ \AA}^2$. The length of the rectilinear site of DNA in the ionic solution is evaluated of 200–300 $\text{\AA}$ [29], and the pitch of the helix is evaluated at 34.6 $\text{\AA}$. Only two nucleotides per the 34.6 $\text{\AA}$ pitch that share the same orientation (one of the 5' strand and one on the 3' strand). It means that at the mentioned site length there are approximately 14 nucleotides with strictly identical orientation relative to each other and to the incidence photon. Thus, we obtain that $k \sim 14$. The majority of nucleotides are turned by others angles, and participate in the competition for the photon only a little (at the fixed wavelength there is a prevailing direction of transition moment adequate for absorption, but other directions are not active [1, 22]). When substituting the mentioned E , S and k values into Equation 9 with $q = 2$ for the stack chain, we obtain $E^\wedge = 0.0775\text{ \AA}^2$. With Equation 1 we find that $h = 44.6\%$. The last value is in good agreement with known [1, 3] $\sim 40 - 45\%$ hypochromism of DNA in solutions. In crystalline DNA, stronger hypochromism should be expected. A crystal has long rectilinear sites the size of which can appreciably exceed the wavelength. The effective site length

Figure 4. The dependence of extinction of tyrosine on its concentration in distilled water (pH 7.0; ionic strength is ~ 0).

where nucleotides compete for the photon will probably be about the wavelength, 2600 Å. Only 75 nucleotides of identical orientations lie into this length. Substituting $k = 75$ into Equation 9, we find that $E^{\wedge} = 0.0188 \text{ Å}^2$. With Equation 1 we find that $h = 86.6\%$.

TYROSINE

For tyrosine in aqueous solution, $\varepsilon = 1270 \text{ M}^{-1}$ (at 275 nm) that corresponds to $E = 0.021 \text{ Å}^2$. S of tyrosine is about 3.1 Å^2 . This means that $P = 0.016$ (at $q = 1$). This value is too small to cause shielding in dimers. However, in large aggregates shielding must take place.

Indeed, dimerization and small association of the tyrosine molecules caused only insignificant changes in absorption (Figure 4). At concentrations more than 2 mM the aggregates are formed, that is accompanied by a sharp fall in absorption. At 40 mM, all tyrosine molecules were in the aggregated form; free tyrosine was practically absent. The aggregates formation was not accompanied by any spectral shift, 'transfer' of band or an appearance of a new band (not shown); both tyrosine bands, at 275 and 222 nm, were hypochromized, though to various degrees.

In large aggregates the length of an elementary shielding site is limited by wavelength. Assuming the average distance between chromophore centres in the tyrosine aggregates to be $\sim 10 \text{ Å}$ (in view of the size of the amino acid), we can conclude that no less than 270 molecules can be stack-like arranged along one axis within the distance 2700 Å (wavelength). The calculation agrees with the experimental value (Table II). Here the order of k is estimated and is not its exact value. If k is several times smaller, the calculated spectrum will differ only a little, because at large k hypochromism changes not as sharply as at average and small k . For example, if $k = 135$, we obtain an h value that differs only by a factor of 1.3 from h with $k = 270$.

Table II. Extinction coefficients and hypochromism in chromophore aggregates and macromolecular structures

Aggregate or structure	k	q	$E^\wedge (\text{\AA}^2)$	$\epsilon^\wedge (\text{M}^{-1} \text{cm}^{-1})$	$\epsilon^\# (\text{M}^{-1} \text{cm}^{-1})$	$h(\%)$	$h^\#(\%)$
<i>pApApApApA</i>	5	2	0.1811	10 860	11 100	27.6	26
DNA in solution	14	2	0.0775	4 650	—	44.6	$\sim 40^*$
DNA in crystal	75	2	0.0188	—	—	86.6	—
Tyrosine in aggregates	270	1	0.0068	406	530	75.7	58
Tryptophan in aggregates	30	1	0.0488	2 940	2 800	47.5	~ 50
Phospholipase A2	8	> 1	0.211	12 660	—	~ 11	$< 35^*$
Ribonuclease T1	10	—	0.236	14 160	—	~ 15	$< 49^*$
Hemoglobin in erythrocytes	20	1	0.432	25 920	36 000	78.4	~ 70
Chlorophyll in <i>Chlorella</i>	7	1	0.94	56 400	57 600	53.0	52^*
Rhodopsin in rods	10	1	0.228	13 680	—	67.4	—
Anthracene in aggregates	10	1	0.3478	20 870	20 700	89.5	90

Footnotes: $\epsilon^\#$ and $h^\#$ are the experimental hypochromic values, $E^\wedge$ and h are the calculated values. * The experimental hypochromic values for DNA, *Chlorella*, and phospholipase with ribonuclease are borrowed from the literature [1, 3], [4], and [29], respectively.

TYROSINE AND TRYPTOPHAN IN PROTEINS

Hypochromism of tyrosine residues was observed for phospholipase A2 and ribonuclease T1 [31]. The calculated approximate data on hypochromism for seven tyrosines and one tryptophan of bovine phospholipase A2 and for nine tyrosines and one tryptophan of RNase T1 are given in Table II.

ERYTHROCYTES

For one hemoglobin sub-unit in the Soret band the ϵ value = $120\,000 \text{ M}^{-1} \text{cm}^{-1}$, which corresponds to $E \sim 2 \text{ \AA}^2$. The S of heme is $\sim 20 \text{ \AA}^2$. Therefore, the P value is about 0.23 (at $q = 1$).

In erythrocytes, the Soret band is strongly hypochromised (Figure 5 and Table II) and in comparison with that of the hemolysate solution where the absorption is determined basically by free hemoglobin. For big particles ($1\text{--}10 \mu\text{m}$), light-scattering in the red and UV regions is not too different; the small component of erythrocyte light-scattering in UV was subtracted due to the extrapolation of absorption from 700 nm. The h value varies along the UV-visible spectrum and achieves its

Figure 5. Absorption spectra of the erythrocyte suspension (1), its hemolysate (2) and spectrum (3) calculated with $k = 7$ and $q = 1$ from hemolysate spectrum.

maximum value at a maximum of the Soret band. The low-absorbing bands at 530–590 nm are almost not hypochromised. Hypochromism at a maximum of the Soret band varied from 40 to 70% depending on the conditions of the experiment. There was no ‘transfer of bands’ and new bands.

Because the erythrocyte sizes are large, the size of an elementary site where hemes compete for the photon is limited by the wavelength. It is approximately 4 000 Å for the Soret band. An erythrocyte is filled with a 34% hemoglobin solution corresponding to a concentration of 0.005 M. At such a concentration the centre-to-centre intermolecular distances are about 100 Å. Thus, about 40 protein globules could be stacked over 4 000 Å, i.e. $k < 40$. As it is not known whether hemoglobin within the erythrocyte is distributed on a lattice or in regular intervals smaller than the wavelength, the exact k value cannot be established. The best agreement of the theory with the experiment is obtained with k from 7 to 20. If the spectrum of fresh hemolysate is calculated by Equation 9 with $k = 7$, $S = 20 \text{ Å}^2$ and $q = 1$, the resulting hypochromic spectrum differs insignificantly from the erythrocyte spectrum (Figure 5). These data confirm that some part of hemoglobin hypochromism in the suspension may be caused by shielding from light in each single erythrocyte, but not only by the sieve effect.

Strong hypochromism of the Soret band of the heme-octapeptide aggregates was observed in [32] (h depends on the wavelength; $h \sim 50\%$ at maximum). Substitution of the initial absorption spectrum of heme-octapeptide into Equation 9 results in spectra (not shown) similar to the hypochromic ones presented in [32]. Thus, hypochromism of the heme-octapeptide aggregates can be explained by shielding.

VISUAL RODS

For 10 rhodopsin molecules arranged in a chain with $q = 1$, we have $h = 67.4\%$ (Table II). In a stack with $q = 2$, we have $h = 83\%$. In outside segments of visual rods a sharp distinction between the absorption spectrum of isolated rhodopsin and

the action spectrum of rod vision was observed [33]. This was explained (without calculations) by self-shielding [33].

CHLOROPLASTS

For chlorophyll (Table II), according to Equation 9, appreciable hypochromism ($h = 27\%$) can be obtained on stack-like dimers ($k = 2, q = 2$) in the Soret band.

Grana of chloroplasts are similar to stacks. The distances between chlorophyll molecules are $\sim 10 \text{ \AA}$, the absorption splitting is small ($\sim 100 \text{ cm}^{-1}$) or negligible, and, in many cases, this splitting is not caused by quantum mechanical excitonic interactions [34]. The absorption spectrum of chloroplasts is strongly hypochromised in comparison with the spectrum of free chlorophyll. For example, $h = 52\%$ was observed at 440 nm in the *Chlorella* suspension [4]. Using $h = 52\%$ and Equations 1 and 9, we obtained that the k value is ~ 4 at $q = 2$, and ~ 7 at $q = 1$. These k values agree with the known chlorophyll content in the pigment-protein light-harvesting complexes. In turbid chloroplast suspensions and plant cells, the sieve effect should also be taken into account [4, 35, 36].

ANTHRACENE

The evaluation of probabilities of photon absorption by anthracene was presented in [25]. We shall try to calculate it more accurately, using anthracene $S = 8 \text{ \AA}^2$ ($> 1.1 \text{ \AA}^2$ for each of the seven double bounds) and the more exact ε value from [37]. The largest ε for anthracene is obtained in ethanol or hexan at $\sim 252 \text{ nm}$: $200\,000 \text{ M}^{-1} \text{ cm}^{-1}$ [37] which corresponds to $3.3 \text{ \AA}^2$. Therefore, $P = 0.95$. Such a high P value indicates that almost each photon is absorbed. The transition in the mentioned band can be described as circular oscillator, i.e. q never exceeds 1. At 375 nm $\varepsilon = 7\,500 \text{ M}^{-1} \text{ cm}^{-1}$, which corresponds to $0.125 \text{ \AA}^2$. This means that $P = 0.036$. So, in anthracene aggregates the band at 253 nm should be strongly hypochromized, but the band at 375 nm will be hypochromized only a little.

In anthracene aggregates formed in water with small quantities of anthracene ethanolic solution added, very strong hypochromism of the 253 nm band is observed (Figure 6, Table II). Hypochromism of the long-wavelength bands is small. Absorption spectra of anthracene dimers and microcrystals are presented in [38]. The anthracene spectrum in water is similar to that of the microcrystals but not of dimers. Thus, anthracene in water is aggregated, but dimers are absent.

The spectral modifications at the 375-nm band clearly show an interaction (long-wavelength shift, $\sim 1200 \text{ cm}^{-1}$). However, no noticeable shift was observed at the 252-nm band. Probably, oscillators at 252 nm and 375 nm interact independently. A small shift without hypochromism of the 375-nm band, but 50% hypochromism without of a shift in the 252-nm band were observed in the anthracene-contained polymers (where interactions are negligible) [39]. Perhaps, a shift and hypochromism have differ reasons. In Langmuir-Blodgett anthracene-contained

Figure 6. Spectra of anthracene extinction in ethanol (1), in water (2) and the calculated spectrum with $k = 10$ and $q = 1$ (3).

films [37], the both bands, 252-nm and 375-nm, are shifted, but only the 252-nm band is hypochromised. This hypochromism hardly can be explained by excitonic interactions, but the screening model helps to explain.

The length of the anthracene aggregate was less than 100 Å (the size was estimated by scattering). In the aggregate, the distances between planes are ~ 3 Å. These two values determine the number of molecules, which compete for the photon in the anthracene aggregate. We have obtained that $k < 30$. In the short-wave region, the spectrum of anthracene aggregates calculated from Equation 9 with $k = 10$ is similar to the experimental one (Figure 6). If we take $k = 30$, then h will be equal to 96% at 253 nm, which does not differ much from the experimental value and from h with $k = 10$.

Thus, known hypochromism of anthracene in Langmuir-Blodgett films [37], microcrystals [38] and polymers [39] may be caused by screening. Screening hypochromism may also be important in estimation of the efficiency of energy transfer in macromolecules [40, 41].

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