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Pigment Containing Lipid Vesicles

III. Role of Chlorophyll *a* as Sensor for Aggregational States of Lecithin

D. Walz

Department of Pharmacology, Biozentrum, University of Basel, Basel, Switzerland

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Summary. It was shown previously (Walz, 1976) that chlorophyll *a* incorporated into the membrane of lecithin vesicles is a probe which detects the aggregational state of the lipids. This phenomenon is interpreted in terms of a solvatochromism, i.e., the effect of various solvents on the absorption spectrum of the solute. The sensor characteristics can be expressed by a set of solvatochromic coefficients, which are pertinent to the electronic transitions occuring in chlorophyll *a* on excitation with light, and by means of the absorption bands associated with these transitions. An unambiguous resolution of spectra into absorption bands is not yet practicable, but at least part of the bands can be approximated by Gaussian components which then allows us to estimate the solvatochromic coefficients. From these data and based on the currently available theoretical and experimental information about solvatochromism, it is concluded that the chromophore, i.e., the porphyrin ring of chlorophyll *a*, is located adjacent to the glycerol-ester moieties of the lecithin molecules in the membrane, and that the sensor ability relies on different orientations of the chromophore for lecithin in different states of aggregation.

In a previous paper (Walz, 1976) it was reported that the absorption spectrum of chlorophyll *a* incorporated into the membrane of lecithin vesicles is slightly altered upon the addition of valinomycin to the vesicle suspension. The phenomenon was interpreted as a change in the aggregational state of the lipid molecules with chlorophyll *a* being a sensor for different lecithin aggregations. The notation "aggregational state" shall be retained here, although it is reminiscent of similar but not comparable notions such as crystalline or liquid crystalline state, and should be understood in the following sense: in a certain domain around a valinomycin molecule, this antibiotic exerts an influence on the lecithins probably due to hydrophobic interaction which reduces the motional freedom of the whole phospholipid molecules. The lipids in this domain then constitute a microphase whose state of aggregation differs from that outside the

domain. The situation may be best visualized in terms of the clusters described by Lee, Birdsall, Metcalfe, Toon and Warren (1974). Valinomycin would then nucleate the formation of clusters and tremendously increase their lifetime, thus yielding a constant population of clusters numbering in the amount of valinomycin molecules present in the membranes.

The effect of valinomycin on the surrounding lecithins appears to be very similar to (if not identical with) the boundary lipid formation observed by Jost, Griffith, Capaldi and Vanderkooi (1973) in vesicles prepared from isolated mitochondrial cytochrome oxidase and phospholipids. Using a spin labelled fatty acid, these authors found the label to record, besides the usual fluid lipid bilayer configuration, regions with very low fluidity whose proportion in the membrane depends on the lipid/protein ratio. These findings were interpreted to indicate layers of boundary lipids which are thought to be bound to the hydrophobic part of the protein in order to explain the immobilization of these lipids. The label was assumed to exchange between regions of different fluidity, and so most probably do the lipids, too. The concept of boundary lipids around proteins thus converges to that of a protein-induced cluster formation (as outlined above for the depsipeptide valinomycin) which conforms with the picture sketched by Hauser, Finer and Chapman (1970) in order to rationalize the data they obtained with the polypeptide alamethicin in lipid vesicles.

Spin-label probes are known to reflect the microviscosity of the surrounding medium; however, it is not selfevident why the absorption spectrum of chlorophyll *a* contained in a membrane should be susceptible to variations in the fluidity of the bilayer. The role of this pigment as a sensor for aggregational states of lecithin thus deserves a detailed analysis. It will be carried out in terms of a solvatochromism, i.e., the influence of various solvents on the spectral parameters of the solute, since solvatochromic effects are well established for chlorophyll *a* (*see*, e.g., Seely & Jensen, 1965) and different aggregational states of lecithin can be considered as different "solvents". This analysis, however, is not performed for the sake of a further elucidation of the spectroscopic features of chlorophyll *a*, but it shall provide insight into the molecular basis of the pigment's ability to act as a sensor. In this context it should be emphasized that a sensor is only as valuable as how much is known about the molecular events which render it a probe, a fact which is probably not duly assessed by all workers in the field of membrane biology, especially when using fluorescent probes.

Theoretical Considerations

Solvatochromism of chlorophyll *a* arises for different reasons. Solvation, aggregation of the pigment molecules as well as dispersive and electrostatic interactions between chromophore and surrounding medium, can contribute to the phenomenon in variable degrees depending on conditions.

Solvation of chlorophyll *a* occurs in solvents with nucleophilic groups. One or two solvent molecules are coordinated to the Mg atom in the porphyrin ring thus yielding solvated species whose absorption spectra are in part determined by the chemical nature of the complexed solvent molecules (Seely, 1965; Cotton, Trifunac, Ballschmiter & Katz, 1974). By far the best complexing agent was found to be water (Katz & Norris, 1973), and to obtain pure solvates with organic solvents usually requires rigorously dry conditions (Cotton *et al.*, 1974). Thus, in view of the aqueous medium in which the vesicles are prepared and suspended together with the penetration of water into the membrane, the hydrated chlorophyll *a* species has to be expected (*see also* Lee, 1975). The nucleophilic components of lecithin, viz., the phosphate group and the carbonyls in the ester groups, can in principle coordinate to the Mg atom, provided that the strong hydration of the phosphate group (Hauser, 1975) and the sterical conditions (predominant orientation and restricted mobility of the lipids in the membrane, *see* Appendix C), do allow a complexation. Even then it seems rather unlikely that these nucleophiles can successively compete with water, for hydration is strongly favored due to hydrogen bonding in addition to the Mg—OH_2 coordination (Katz & Norris, 1973); yet mixed solvates with water and lecithin, similar to the species which Seely (1965) found to be preferentially formed in binary solvents, can occur. These features of the system are hard to reconcile with a possible change in solvation due to the two different aggregational states of lecithin. Except for the incorporation of valinomycin, which does not form a complex with chlorophyll *a* (*see* Walz, 1976), there is no evidence for a change in the molecular composition of the membrane which would be a prerequisite for a change in solvation (*cf.* Seely, 1965). This possibility is therefore disregarded in the following considerations (but *see* last section in Discussion).

Aggregation of chlorophyll *a* occurs in apolar solvents without nucleophilic groups. Dimers and oligomers are formed (depending on the concentration of the pigment and the polarity of the solvent) by coordination of the keto group in the cyclopentanone ring of one chlorophyll *a* to the Mg atom of another (Katz & Norris, 1973; Cotton *et al.*, 1974). Similar

effects were also observed for chlorophyll *a* in detergent micelles (Lehoczki, 1975) and in unsonicated lipid bilayers (Lee, 1975). In the latter system as well as in sonicated vesicles (Colbow, 1973), a marked increase in aggregation was found at the transition from the liquid crystalline to the crystalline state of the lipids. One could expect, therefore, that the valinomycin-induced "phase transition" for lecithin in vesicle membranes could similarly promote an aggregation of chlorophyll *a*. However, the absorbance differences measured (*see* Fig. 3) do not clearly show the additional absorption bands typical for an aggregation (Cotten *et al.*, 1974). Moreover, aggregation does not affect the location of the fluorescence band but strongly decreases its intensity due to quenching of the fluorescent monomers by the nonfluorescent aggregates (Colbow, 1973; Lee, 1975). In contrast to this, we found the fluorescence band of chlorophyll *a* to be redshifted and its intensity to be increased upon addition of valinomycin to the vesicle suspension, thus definitely eliminating aggregation as a possible source of solvatochromism.

The absorption spectrum of a pigment in solution (solvated or not) is influenced by *dispersion interaction* between solvent and solute. In addition *electrostatic interaction* of the pigment with the surrounding solvent molecules creates a reaction field to which the pigment is subjected (Liptay, 1969). Both phenomena give rise to changes in relative positions of the energy levels in the pigment molecule, and affect the transition moments (Liptay, 1965, 1966). As outlined by Labhart (1967), rotational and vibrational sublevels need not be distinguished when dealing with a solvatochromism measured by the usual spectroscopic devices. The considerations can then be restricted to electronic states and the resulting effects are the same throughout the absorption band of a given electronic transition. In terms of the extinction coefficient, ε_t, of the absorption band associated with the t-th transition, solvatochromism causes a change of this coefficient by an increment $\Delta\varepsilon_t$ which can be written as (*cf.* Reich & Schmidt, 1972)

$$\Delta\varepsilon_t = \sum_{d=0}^{2} a_{d,t}\,\bar{\nu}\,\frac{\partial^d(\varepsilon_t/\bar{\nu})}{(\partial\bar{\nu})^d} \tag{1}$$

where $\bar{\nu}=1/\lambda$ denotes the wavenumber related to the wavelength, λ. The solvatochromic coefficients $a_{d,t}$ comprise the effects of dispersive and electrostatic interactions on the transition moments ($d=0$) and the energy levels in the pigment molecule ($d=1, 2$). Let the change of the span between the energy levels of the two electronic states associated with the

t-th transition be $hc \cdot \Delta\bar{\nu}_t$ (h = Planck constant, c = velocity of light) then

$$a_{d,t} = (-\Delta\bar{\nu}_t)^d / d \quad \text{for } d = 1, 2 \tag{2}$$

(*cf.* Reich & Schmidt, 1972). The coefficients $a_{0,t}$ cause a change in intensity of the bands, the coefficients $a_{1,t}$ create a band-shift with respect to wavenumber and the coefficients $a_{2,t}$ give rise to a band-broadening.

The overall extinction coefficient of chlorophyll *a*, ε'_c, is the sum of the extinction coefficients of the absorption bands:

$$\varepsilon_c = \sum_t \varepsilon_t . \tag{3}$$

It relates the absorbance, $A(\lambda)$, at a given wavelength λ to the concentration of chlorophyll *a*, C_c, and the optical pathlength, l (Beer-Lambert's law):

$$A(\lambda) = \varepsilon_c(\lambda)\, C_c\, l . \tag{4}$$

The extinction coefficient ε_c applies to chlorophyll *a* embedded in lecithin in the actual state of aggregation found in vesicle membranes. Chlorophyll *a* surrounded by lecithin in the valinomycin-induced state has a different extinction coefficient, ε'_c, which is again the sum of the extinction coefficients for individual bands, ε'_t [compare Eq. (3)]. Each ε'_t corresponds to a particular ε_t, since both are associated with the same electronic transition, but differs from ε_t due to solvatochromism by $\Delta\varepsilon_t$:

$$\varepsilon'_t = \varepsilon_t + \Delta\varepsilon_t . \tag{5}$$

Hence in view of Eqs. (1) and (3)

$$\varepsilon'_c = \sum_t \varepsilon'_t = \varepsilon_c + \sum_t \sum_{d=0}^{2} a_{d,t}\, \bar{\nu}\, \frac{\partial^d (\varepsilon_t / \bar{\nu})}{(\partial \bar{\nu})^d} . \tag{6}$$

Let c'_c be the concentration of chlorophyll *a* in an environment changed by valinomycin and C_c denote the total chlorophyll *a* concentration. The difference in absorbance between the cuvettes with and without valinomycin is then given by [*cf.* Eq. (4)]

$$\Delta A(\lambda) = [\varepsilon'_c(\lambda) - \varepsilon_c(\lambda)]\, c'_c\, l . \tag{7}$$

It has been shown previously that the molar ratio of chlorophyll *a* in the valinomycin-induced environment to total chlorophyll *a*, n'_c / N_c, is pro-

portional to r_l', i.e., the molar ratio of valinomycin dissolved in the membrane to lecithin [*see* Eq. (28) in Walz, 1976]. Hence we can write for the corresponding concentration ratio

$$c_c'/C_c = k r_l'. \tag{8}$$

Substituting from Eqs. (6) and (8) into Eq. (7) yields

$$\Delta A(r_l', \lambda) = k r_l' \sum_t \sum_{d=0}^{2} a_{d,t} \left[\bar{\nu} \frac{\partial^d (\varepsilon_t/\bar{\nu})}{(\partial \bar{\nu})^d} \right]_\lambda C_c l, \tag{9}$$

which, when compared to Eqs. (23) and (25) in the previous paper (Walz, 1976), shows that

$$F_2(\lambda) = \sum_t \sum_{d=0}^{2} a_{d,t} \left[\bar{\nu} \frac{\partial^d (\varepsilon_t/\bar{\nu})}{(\partial \bar{\nu})^d} \right]_\lambda = \overline{\Delta E}(\lambda) \varepsilon_c(668)/k. \tag{10}$$

This equation correlates the sensor characteristics of chlorophyll *a* to the absorption bands pertinent to the electronic transitions in this molecule (as expressed in terms of the extinction coefficients, ε_t) and to the corresponding solvatochromic coefficients, $a_{d,t}$.

Results

In order to test Eq. (10) against the experimental data reported in the previous paper (Walz, 1976), the absorption bands pertinent to the different electronic transitions in chlorophyll *a* should be known, but there is no way to unambiguously resolve a spectrum into absorption bands. On the other hand, the spectrum of chlorophyll *a* in the red region is generally understood to be dominated by the band of one electronic transition whose vibrational structure yields the prominent peaks around 665 nm and 620 nm and minor contributions to the spectrum below 600 nm. The bands of other electronic transitions, which are obviously shifted to the blue, can contribute in some cases to the 620 nm peak but usually determine the spectrum below about 600 nm. These features are deduced from the mirror symmetrical characteristics of absorption and emission spectrum, from fluorescence polarization measurements, as well as from interpretations of solvatochromism (Goedheer, 1966). Hence, as a first approximation, the chlorophyll *a* spectrum in the red region above 600 nm can

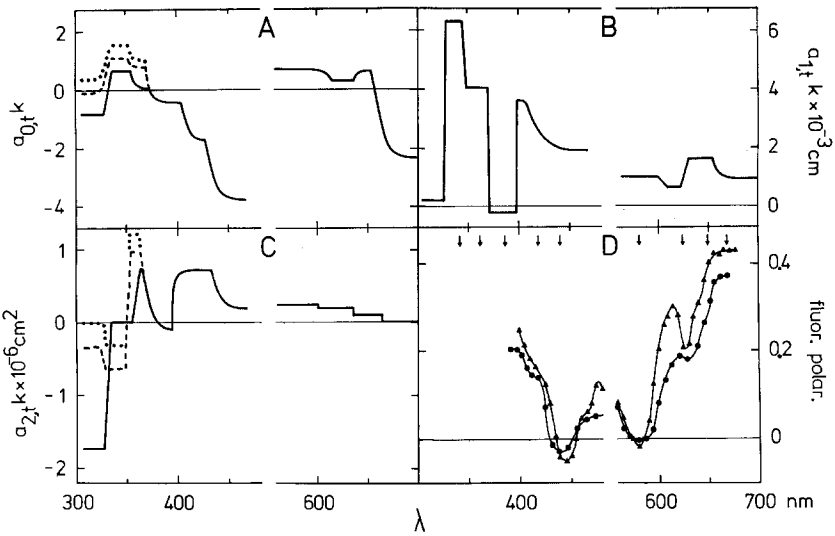

Fig. 1. (*A*)–(*C*). Solvatochromic coefficients $a_{d,i}$, estimated by means of the spectrum instead of the absorption bands as described in Appendix A.1, and their dependence on the wavelength λ for $d=0$(*A*), $d=1$(*B*), and $d=2$(*C*). The factor k is pertinent to the valinomycin-lecithin interaction and thus independent of λ. In (*A*) and (*C*), three curves are shown in the wavelength range below 370 nm corresponding to vesicles with different chlorophyll *a* content (*see* Appendix A.1): $q=0.015$ (——), $q=0.01$ (------) and $q<0.005$ (.....); $q=$molar ratio of chlorophyll *a* to lecithin. (*D*). Fluorescence polarization spectrum of chlorophyll *a* in castor oil, replotted from the data reported by Bär *et al.* (1961, ●) and by Gouterman and Stryer (1962, ▲). The arrows on the top indicate the locations (i.e., λ_i) of the Gaussian components found in the chlorophyll *a* spectrum (*see* Fig. 2)

be considered to represent essentially the band of the first electronic transition (*see also* Seely & Jensen, 1965) which should allow us to estimate the solvatochromic coefficients $a_{1,d}$ $(d=0,1,2)$ according to Eq. (10). The pertinent numerical evaluation is described in Appendix A.1, and the results, which for comparison were calculated for the whole experimentally covered wavelength range, are shown in Fig. 1 *A–C*. These data clearly indicate that the above mentioned approximation is not valid for the present spectra and that the situation in the red region is similar to that in the Soret region where the spectrum is known to be composed of several bands with comparable intensities pertinent to different electronic transitions. In particular, there seems to be an additional electronic transition whose 0–0 vibrational peak should be located around 650 nm. Such a peak indeed appears in the spectrum of chlorophyll *a* recorded at low temperature where all bands are shifted slightly to the red and sharpened (Freed & Sancier, 1951). Similarly, emission spectra of highly purified chlorophyll *a* at low temperature show a peak

at 700 nm corresponding to the 650 nm peak (Brody & Broyde, 1963). Moreover, the contributions of this band and that of the next electronic transition to the 620 nm peak seem to be much larger than mentioned above. These conclusions are supported by the fluorescence polarization spectrum (Fig. 1 *D*) for chlorophyll *a* in castor oil. Finally, the fluorescence spectrum of chlorophyll *a* in lecithin vesicles was measured and compared with the absorption spectrum. The mirror symmetrical features of these two spectra again suggest an additional band starting around 650 nm and indicate that the band of the first electronic transition contributes only about 30 % to the 620 nm peak.

Another approach to the assessment of the absorption bands in a spectrum makes use of the assumption that the contour of a band may be approximated by a sum of several Gaussian curves, $\varepsilon_{t,v}(\bar{\nu})$, where the index v is related to a successive numbering of the vibrational peaks occurring in the absorption band:

$$\varepsilon_t = \sum_v \varepsilon_{t,v}(\bar{\nu}) = \sum_v \varepsilon^*_{t,v} \exp\left\{-\frac{1}{2}\left(\frac{\bar{\nu}-\bar{\nu}_{t,v}}{\sigma_{t,v}}\right)^2\right\}. \tag{11}$$

The maximal values of the curves, $\varepsilon^*_{t,v}$, are located at $\bar{\nu}_{t,v}$ while their widths are determined by $\sigma_{t,v}$. Therefore it seems reasonable to deconvolute the spectrum into a set of Gaussian components, $\gamma_i(\bar{\nu})$:

$$\varepsilon_c = \sum_i \gamma_i(\bar{\nu}) = \sum_i \gamma^*_i \exp\left\{-\frac{1}{2}\left(\frac{\bar{\nu}-\bar{\nu}_i}{\sigma_i}\right)^2\right\} \tag{12}$$

where γ^*_i denotes the maximal value, $\bar{\nu}_i$ the location of the maximum and σ_i the width of the i-th component. When substituting from Eq. (11) into Eq. (3) and comparing this with Eq. (12), it can be inferred that, depending on the relative positions and intensities of the absorption bands pertinent to the different electronic transitions, some of the Gaussian components obtained from the deconvolution of the spectrum are very likely to coincide with particular curves of the sets defined in Eq. (11) while others will be not resolvable sums of several strongly overlapping curves. This procedure thus allows for at least a partial resolution of the spectrum with respect to absorption bands which will prove to be sufficient for the present analysis.

The deconvolution of the normalized spectra of chlorophyll *a*, which was carried out as described in Appendix A.2, revealed 11 Gaussian components (Fig. 2). The spectrum between 490 and 550 nm was not

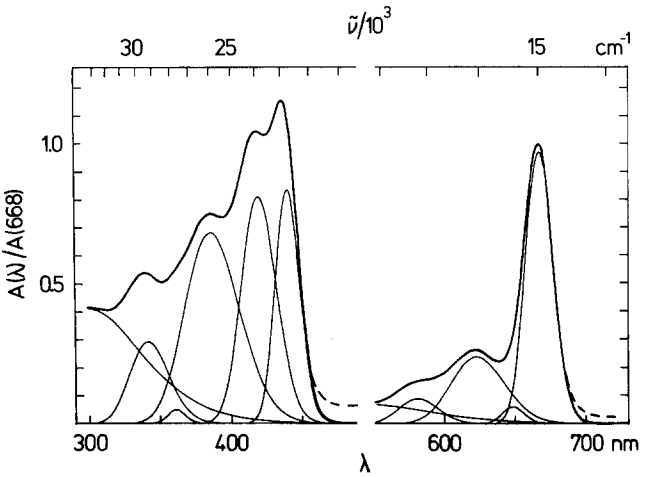

Fig. 2. Normalized absorption spectrum of chlorophyll *a* deconvoluted into Gaussian components. Same spectrum as in Fig. 1 of the previous paper (Walz, 1976) where the details are shown in the legend. The heavy line indicates the spectrum calculated as the sum of the components (thin lines); it coincides with the measured spectrum (broken line) except for those wavelength ranges where components were neglected

deconvoluted since no valinomycin-induced absorbance changes were observed in this range (Walz, 1976). It should be mentioned then that the component which appears at the lower wavelength end of the red region comprises the contributions of all components located in the omitted range of the spectrum in the red region. Similarly, the component with a maximum around 300 nm comprises the contributions of the components located below this wavelength and, in addition, is distorted to a certain extent by light scattered by the vesicles which interfered with the absorbance measurements (*see* Walz, 1976). The small components to be found on the upper wavelength end of the Soret and the red region were neglected. Table 1 summarizes the data obtained for the parameters of the Gaussian components from all spectra. The variations observed, especially in the Soret region of the spectra for chlorophyll *a* in vesicles from different preparations as well as on aging of vesicles from the same preparation (Walz, 1976), recurred in the form of similar variations in the parameters for the components.

The Gaussian components can be considered as substitutes for the unknown absorption bands, ε_i; Eq. (10) then has to be rewritten in terms of $\gamma_i(\bar{\nu})$ and a set of 33 "pseudosolvatochromic" coefficients should be determined. In view of the large number of coefficients it seems doubtful whether this determination could be carried out without ambiguity.

Table 1. Parameters for the Gaussian components in chlorophyll *a* spectra

Component no (*i*)	λ_i (nm)	G_i^*	$\Delta\lambda_i$ (nm)
1	667.5–668.4	0.968–0.969	22.6
2	648.8–694.3	0.061–0.066	18.5–19.4
3	623.4–624.1	0.234–0.237	43.3–44.4
4	581.7–584.0	0.081–0.088	32.0–33.0
5	539.8–541.0	0.070–0.085	103–114
6	439.7–440.4	0.752–0.954	19.2–21.6
7	419.0–419.6	0.676–0.899	24.9–32.3
8	385.4–387.6	0.666–0.755	37.7–50.6
9	360.9–362.8	0.033–0.074	10.2–15.5
10	340.7–343.0	0.308–0.425	32.0–36.6
11	298.9–304.0	0.369–0.454	47.4–75.5

The numbering of components is in the order of increasing wavenumber. Components number 1 to 5 constitute the red region and components number 6 to 11 the Soret region of the spectrum. λ_i denotes the location of the maximum, $G_i^* = \gamma_i^*/\varepsilon_c(668)$ is the maximal value and $\Delta\lambda_i$ is defined as the wavelength span between the two half-maximal values on the curve: $\Delta\lambda_i = 2\sigma_i(-2\ln 0.5)^{1/2}/(1/\lambda_i^2 + 2\sigma_i^2 \ln 0.5)$. The data indicate the ranges found for the parameters by the deconvolution of all spectra measured with vesicles from different preparations whose molar ratio of chlorophyll *a* to lecithin was larger than 1:100.

However, the degree of freedom inherent in this procedure is considerably reduced when going back to the individual difference spectra and taking their r_l' dependence into account. Rewriting Eq. (9) for $\gamma_i(\bar{\nu})$ yields

$$\Delta A(r_l', \lambda) = \sum_i \sum_{d=0}^{2} f_{d,i}(r_l') \left[\bar{\nu} \frac{\partial^d(\gamma_i/\bar{\nu})}{(\partial\bar{\nu})^d} \right]_\lambda \tag{13}$$

with

$$f_{d,i}(r_l') = p_{d,i} r_l' . \tag{14}$$

The pseudosolvatochromic coefficients are denoted by $p_{d,i}$, and

$$p_{d,i} \simeq a_{d,t} k \quad \text{for } \gamma_i(\bar{\nu}) \simeq \varepsilon_{t,v}(\bar{\nu}), \tag{15}$$

i.e., for a component which coincides with a Gaussian curve pertinent to the *t*-th absorption band [*see* Eq. (11)]. Sets of $f_{d,i}$ values can now be determined for each difference spectrum separately [Eq. (13)], and only those parameters $f_{d,i}$ are meaningful whose values are proportional to r_l' [Eq. (14)]. Based on these two equations, an algorithm was designed (*see* Appendix A.3) and the values for $p_{d,i}$ thus obtained are listed in Table 2.

Table 2. Pseudosolvatochromic coefficients $p_{d,i}$ and assignment of Gaussian components to electronic transitions

Gaussian component no (i)	Assigned to electronic transition no (t)	$p_{0,i}$	$p_{1,i}$ ($10^3\,cm^{-1}$)	$p_{2,i}$ ($10^5\,cm^{-2}$)
1	1	−3.3 (0.5)	1.1 (0.1)	0
2	2	38 (7)	4.1 (0.7)	6.2 (1.0)
3	–	0	0	0.5 (0.3)
4	–	0	0.2 (0.1)	0
6	$3+t_1$	−5.8 (0.7)	2.4 (0.4)	0
7	$4+t_1$	0	2.4 (0.4)	5.6 (1.5)
8	–	0	0	3.3 (1.5)
9	–	0	0.9 (0.5)	0
10	$5+t_2$	0	0.2 (0.1) 1.0 (0.3)	20 (3)

The numbering of Gaussian components is the same as in Table 1; for their assingment to electonic transitions, *see* text. The values for the coefficients $p_{d,i}$ were calculated as described in Appendix A.3; standard deviations are given in parentheses. The coefficients for components number 5 and 11 were all found to be zero. Two values are listed for $p_{1,10}$ as explained in the text.

Fig. 3 shows, by way of example, how a difference spectrum calculated by means of Eqs. (13) and (14) and with the data in Table 2 agrees with the experimentally determined absorbance changes. In the *red region*, the difference spectra can be essentially ascribed to a solvatochromic effect on the Gaussian components number 1 and 2 (Fig. 4). The values for $f_{d,i}$ plotted *vs.* r_l' in Fig. 4 indicate a proportionality between the two parameters in every case, and the fit between experimental points and calculated curve in the wavelength range above 620 nm is satisfactory (*see* Fig. 3). Hence it is legitimate to assign these two components to those Gaussian curves [*cf.* Eq. (11)] which constitute the zero vibrational peaks in the absorption bands of the first two electronic transitions (*see* Table 2). The first component is associated with the main peak of the spectrum in the red region while the second component does not give rise to a discernable peak (except at low temperature), but such an electronic transition was already inferred from the data shown in Fig. 1. The absorbance differences in the red region below 620 nm are small and the parameters $p_{d,i}$ for components numbers 3 to 5, which were difficult to estimate, are rather insignificant (*see* Table 2). These components then represent sums of Gaussian curves pertinent to different absorption bands and therefore cannot be assigned to a particular electronic transition.

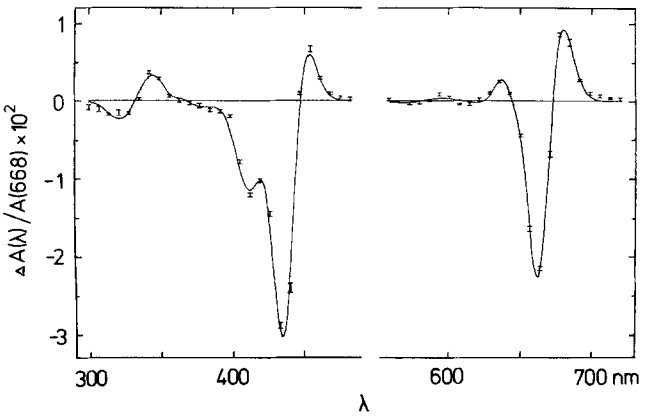

Fig. 3. Example of a difference spectrum calculated by means of the coefficients $p_{d,i}$ and the Gaussian conponents of the spectrum. The measured data are marked by a bar representing ± standard deviation. Same difference spectrum as in Fig. 2 of the previous paper (Walz, 1976) where the experimental conditions are given

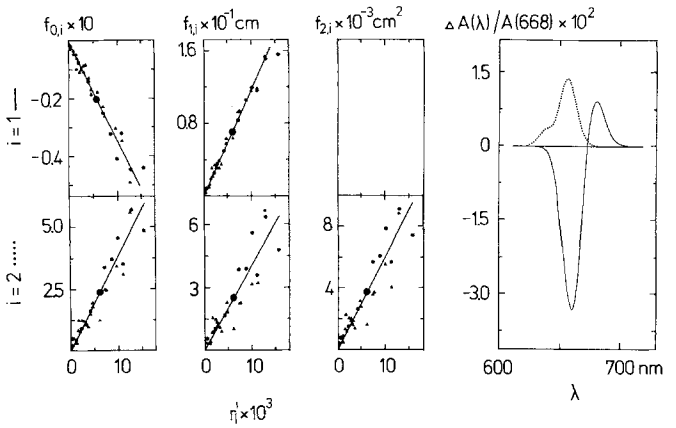

Fig. 4. Plot of the parameters $f_{d,i}$ *vs.* r_l' (left) and contribution to the difference spectra (right) for Gaussian components numbers 1 and 2 which essentially determine the absorbance differences in the red region of the chlorophyll *a* spectrum. An empty box on the left indicates that the values for this parameter $f_{d,i}$ were found not to be proportional to r_l' [*cf.* Eq. (14)] and that the corresponding $p_{d,i}$ was therefore set equal to zero. The curves on the right are pertinent to the difference spectrum shown in Fig. 3, and the corresponding $f_{d,i}$ values are marked by heavy dots on the left. Vesicles with $q=0.0154$ (●), vesicles with $q=0.0113$ and 0.0103 (▲); q = molar ratio of chlorophyll *a* to lecithin

A similar situation is found in the *Soret region.* Here a solvatochromic effect on the Gaussian components number 6, 7 and 10 predominantly determines the difference spectra (Fig. 5). The values for $f_{1,10}(r_l')$ shown in Fig. 5 are grouped around two straight lines through the origin, which is due to the fact that light scattered by the vesicles disturbed the absorbance

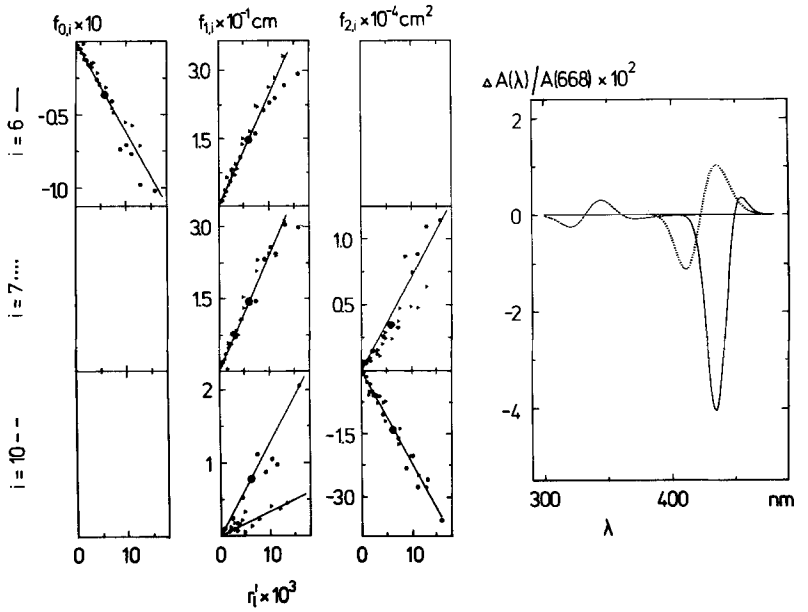

Fig. 5. Plot of the parameters $f_{d,i}$ *vs.* r_i' (left) and contribution to the difference spectra (right) for Gaussian components numbers 6, 7 and 10 which essentially determine the absorbance differences in the Soret region of the chlorophyll *a* spectrum. Other conditions as in Fig. 4

measurements below about 390 nm (see Walz, 1976; *compare* also Fig. 1), and this effect recurred in one of the parameters of component number 10. What has been mentioned above for components numbers 3 to 5 applies as well to components numbers 8, 9 and 11. Hence it is again legitimate to assign components numbers 6, 7 and 10 to the electronic transitions numbers $3+t_1$, $4+t_1$ and $5+t_2$, respectively (*see* Table 2). The symbols t_1 and t_2 shall indicate that additional electronic transitions exist, giving rise to absorption bands which either appear in the omitted spectral range between 490 and 550 nm or totally overlap with other bands and therefore do not essentially determine one of the Gaussian components.

Discussion

The analysis carried out, though based on the phenomenologically determined Gaussian components instead of the theoretically required absorption bands, has shown that the measured absorbance differences conform to a solvatochromism of chlorophyll *a*. The same conclusion could have been drawn from the data shown in Fig. 1 *A–C;* however, the

values for the solvatochromic coefficients presented there are not reliable. The overlap of several absorption bands in both regions of the spectrum leads to wrong estimates for the coefficients since, e.g., a substantial over-estimation of the values pertinent to one band is easily compensated by correspondingly wrong values for the coefficients of another overlapping band and vice versa. The values for the pseudosolvatochromic coefficients, $p_{d,i}$, obtained with the Gaussian components are not falsified by this effect, but may be somewhat over- or under-estimated due to the imperfect representation of part of an absorption band by a Gaussian component. The assignment of these components to electronic transitions appears reasonable when confronted with the fluorescence polarization spectrum (*cf.* Fig. 1 *D*) and is at least not contradicted by the conclusions which Gouterman and Stryer (1962) have drawn from this spectrum. Hence, the coefficients $p_{d,i}$ are considered as useful approximations of the solvatochromic coefficients $a_{d,t}$, which justifies their interpretation.

Lower Limit for the Factor k

The solvatochromic coefficients $a_{d,t}$ differ from the coefficients $p_{d,i}$ by the factor k [*see* Eq. (15)] which is pertinent to the valinomycin-lipid interaction. The upper limit $k<36$ was found previously (Walz, 1976), and a lower limit can now be estimated by means of the coefficients $p_{0,i}$ and the so-called integral absorption of a band.

Since unpolarized measuring light was used, the chlorophyll *a* molecules were randomly oriented with respect to the electric vectors of the light. The extinction coefficient, ε_t, and the absolute value of the transition moment, $|\boldsymbol{\mu}_{ge,t}|$, for the t-th transition are then correlated by (Liptay, 1966; Reich & Schmidt, 1972)

$$\int_{g\to e} \frac{\varepsilon_t}{\bar{\nu}}\, d\bar{\nu} = \frac{2\pi^2 N_L}{3nhc\varepsilon_0 \ln 10} |\boldsymbol{\mu}_{ge,t}|^2 . \tag{16}$$

The integration has to be carried out over the whole band which is associated with the transition from the ground state g to the excited state e thus yielding the integral absorption of this band. N_L, h and c denote Loschmidt's number, the Planck constant and the velocity of light, respectively; n is the refractive index of the surrounding medium. The constant $\varepsilon_0 = 8.85 \cdot 10^{-12}$ As/Vm has to be introduced when using the

Giorgi system. Substituting from Eq. (1) into Eq. (5) yields

$$\varepsilon_t' = \varepsilon_t(1 + a_{0,t}). \tag{17}$$

The contributions of the coefficients $a_{1,t}$ and $a_{2,t}$ are several orders of magnitude smaller than that of $a_{0,t}$ and can be neglected. From Eq. (16) and a similar equation written for ε_t' and $|\boldsymbol{\mu}_{ge,t}'|$, one obtains on substituting from Eq. (17)

$$|\boldsymbol{\mu}_{ge,t}'|^2/|\boldsymbol{\mu}_{ge,t}|^2 = 1 + a_{0,t}. \tag{18}$$

The quantity on the left hand side of this equation is always positive which means $a_{0,t} > -1$. It then immediately follows from Eq. (15) and the values for $p_{0,i}$ listed in Table 2 that $k > 6$.

Solvatochromism Due to Dispersive and Electrostatic Interactions

The transition moment, $\boldsymbol{\mu}_{ge,t}$, of the t-th electronic transition in a pigment molecule, which is influenced by dispersive and electrostatic interactions with the surrounding medium, can be expressed by (Liptay, 1966, 1969)

$$\boldsymbol{\mu}_{ge,t} = \boldsymbol{\mu}_{ge,t}^0 + \varepsilon_0 \boldsymbol{\alpha}_{ge,t} \boldsymbol{F} + \frac{2}{b^3} \frac{n^2 - 1}{2n^2 + 1} G(n^2, S) \boldsymbol{W}_{ge,t} \tag{19}$$

where $\boldsymbol{\mu}_{ge,t}^0$ is the permanent transition moment inherent in the pigment molecule. The second term in this equation, i.e., the vectorial product of the so-called transition polarizability tensor, $\boldsymbol{\alpha}_{ge,t}$, with the electric field $\boldsymbol{F}$, arises from the electrostatic interaction. $\boldsymbol{F}$ is the reaction field which itself can be represented by a rather complex relation comprising permanent and induced dipole moments of the pigment molecule as well as the dielectric constant and the refractive index of the solvent (Liptay, 1965). The third term in Eq. (19) is due to dispersion interaction. The polarizability of the surrounding medium is expressed by its refractive index, n, while the vector $\boldsymbol{W}_{ge,t}$ is essentially determined by molecular parameters of the pigment molecule. The so-called radius of interaction, b, is an empirical parameter and G denotes a geometrical factor depending on S, a parameter which comprises the characteristic dimensions of the cavity in which the pigment molecule is located (Liptay, 1965).

The location of the absorption band pertinent to the t-th electronic transition is expressed by the wavenumber, $\bar{\nu}_t$, which can be written as

(Liptay, 1965, 1969; Reich & Schmidt, 1972)

$$\bar{\nu}_t = \bar{\nu}_t^0 + \delta_{v,t} - (\boldsymbol{\mu}_{e,t} - \boldsymbol{\mu}_g)\,\boldsymbol{F}/\mathrm{hc} - \varepsilon_0(\boldsymbol{\alpha}_{e,t}\,\boldsymbol{F} - \boldsymbol{\alpha}_g\,\boldsymbol{F})\,\boldsymbol{F}/\mathrm{hc} - \frac{2}{b^3}\,\frac{n^2-1}{2n^2+1}\,G(n^2, S)\,W_t \tag{20}$$

where $\delta_{v,t}$ accounts for transitions between different vibrational sublevels (rotational sublevels are not considered) and $\bar{\nu}_t^0$ indicates the location of the peak arising from the $0-0$ transition for a pigment molecule in the gaseous phase. Electrostatic interaction contributes via the third and fourth term in Eq. (20), i.e., the scalar products of the reaction field, $\boldsymbol{F}$, with the differences of the permanent dipole moments $(\boldsymbol{\mu}_{e,t} - \boldsymbol{\mu}_g)$ and the induced dipole moments (vectorial products of the polarizability tensors $\boldsymbol{\alpha}_{e,t}$ and $\boldsymbol{\alpha}_g$ with $\boldsymbol{F}$) of the pigment molecule in the excited state reached by the t-th transition and in the ground state, respectively. Dispersion interaction adds a term identical with that in Eq. (19) except for the vector $\boldsymbol{W}_{ge,t}$ being replaced by the scalar W_t (Liptay, 1965).

The relations in Eqs. (19) and (20) together with the expression for the reaction field were tested against the experimental data obtained with several pigments (examples are given by Liptay, 1965, 1966; *see* also Liptay, Schlosser, Dumbacher & Hünig, 1968) and were found to be very suitable for the interpretation of the observed phenomena. Many data about the effect of solvatochromism on the main red peak and the first peak in the Soret region of the spectrum of chlorophyll *a* have been published by Seely in 1965, i.e., before Liptay has worked out his detailed theory, and Seely's evaluation of these data had, therefore, to rely on the less profound theoretical background available at that time. Appendix B then presents a re-evaluation of Seely's data using Liptay's theory and the results can serve for the interpretation of the coefficients $a_{d,t}$.

Interpretation of the Solvatochromic Coefficients

In view of the dominating role of dispersion interaction for the solvatochromism of chlorophyll *a* (see Appendix B) one would expect that the absorbance differences result from a change in refractive index of the surrounding medium due to the incorporation of valinomycin and as a consequence of the altered lipid aggregation. The refractive index, n', of the domains in the membrane which are composed of one valinomycin molecule and k lecithin molecules in the altered state of aggregation can

be expressed by means of the molar refractions [*cf.* Eq. (A 16)]

$$\frac{n'^2-1}{n'^2+2} \equiv Q'_R(m) = \rho'_l \frac{mR_v + (1-m)R'_l}{m(\rho'_l/\rho_v - 1)+1} \tag{21}$$

where ρ'_l and ρ_v are the molar densities of lecithin in the altered state of aggregation and valinomycin in the membrane, respectively, while

$$m = 1/(1+k) \tag{22}$$

denotes the mole fraction of valinomycin. The molar refraction of valinomycin, R_v, can be calculated from the increments of atoms and groups or from the increments of binding electrons and electron octets (see Bauer & Fajans, 1949) and $R_v = 0.280\,\mathrm{cm}^3/\mathrm{mmole}$ was thus obtained. The molar refraction, R'_l, and by this the refractive index, n'_l [*cf.* Eq. (A 14)] of lecithin in the valinomycin-induced state of aggregation can then be calculated by means of Eq. (21) and the following relation for $Q'_R(m)$ which is derived from Eq. (A 11)

$$Q'_R = \frac{\bar{\nu}_t^0 - \bar{\nu}_t - B_t\, Q_P/(Q_P+1)}{A_t - \bar{\nu}_t^0 + \bar{\nu}_t + B_t\, Q_P/(Q_P+1)} \tag{23}$$

where

$$Q_P(m) \equiv \frac{D-1}{D+2} = \rho'_l \frac{mP_v + (1-m)P_l}{m(\rho'_l/\rho_v - 1)+1}. \tag{24}$$

P_v and P_l are the molar polarizations related to the dielectric constants, D_v and D_l [*cf.* Eq. (A 15)] of valinomycin and lecithin, respectively, while D denotes the dielectric constant in the above-mentioned domains. From Eqs. (2), (15) and (22) it follows

$$\Delta\bar{\nu}_t = \bar{\nu}_t - \bar{\nu}_t(m=0) = -p_{1,i}\, m/(1-m) \tag{25}$$

where the relation between the indices i and t is given by the assignment of Gaussian components to electronic transitions (*see* Table 2). For lecithin membranes without valinomycin, $k=\infty$, i.e., $m=0$, and Eqs. (21) to (24), and (A 14) allow then to estimate the refractive index, n_l, of lecithin in the unaltered state of aggregation while $\bar{\nu}_t(m=0)$ indicates the location of the absorption bands for chlorophyll *a* in this lipid environment.

Using the values for $\bar{\nu}_t^0$, A_t and B_t obtained from the evaluation of Seely's data together with the values for $p_{1,i}$ given in Table 2, values for n_l and $\Delta n_l = n'_l - n_l$, the difference in refractive index for lecithin in the valinomycin-induced and unaltered state of aggregation, were calculated as outlined above. As mentioned in the introduction, the mobility of the lipids in the domain around valinomycin is reduced which most probably

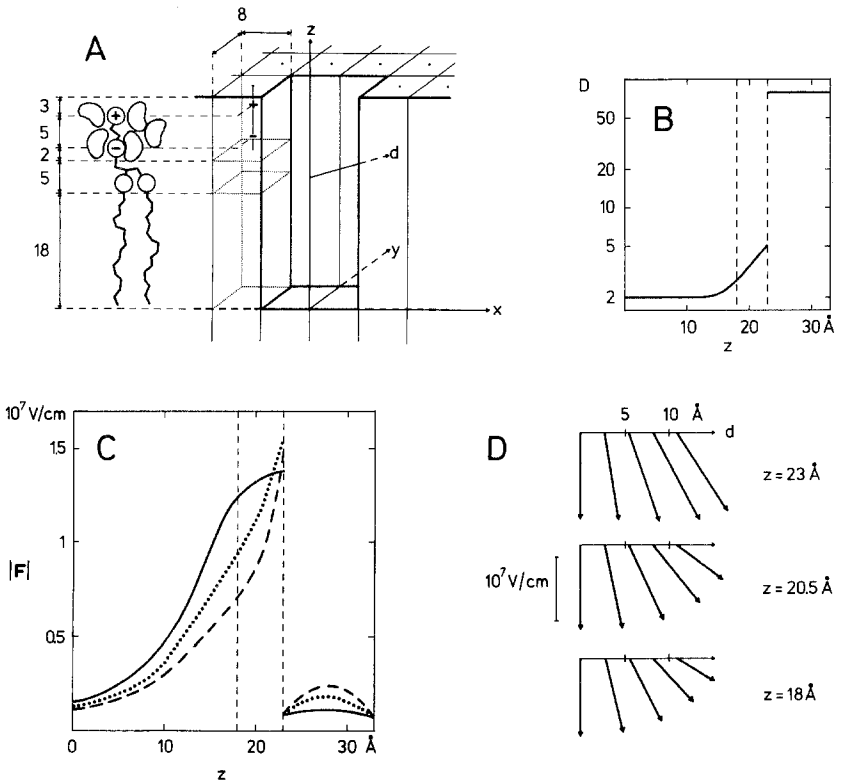

Fig. 6. Model used to estimate the electric field within a vesicle membrane, and some results obtained from this model. (*A*) *Left:* Sketch of a lecithin molecule in the membrane. Circles with + and − signs symbolize the choline and the phosphate group, respectively; empty circles indicate the ester bonds. The bean-shaped symbols represent water molecules. *Right:* Diagram of the model, drawn as a cross section through one half of the membrane. The other half below the broken line is mirror symmetrical to the plane determined by the *x*- and *y*-axis of the coordinate system, except for the gap. For further explanations *see* Appendix C. Figures indicate distances in Å-units. (*B*) Dependence of the dielectric constant, *D*, on *z*. The broken lines indicate the boundaries of the three domains, i.e., hydrocarbon, ester and aqueous layer (from left to right). (*C*) Electric field strength $|\boldsymbol{F}|$ as a function of *z* for different positions along the diagonal *d* in the gap [*see* (*A*)]. Solid line: $x=y=0$, dotted line: $x=y=6$ Å, broken line: $x=y=8$ Å. Calculated as described in Appendix C with all charges whose *x*- and *y*-coordinates are between ± 125 Å. The contribution of the remaining charges was estimated to be less than 5 %. For the vertical broken lines *see* (*B*). (*D*) Electric field $\boldsymbol{F}$ at different positions along the diagonal *d* at three values of *z* in the domain of the glycerol-ester moieties. The vectors (represented by the arrows) lie in the plane determined by the diagonal and the *z*-axis and point towards the negative direction of this axis

leads to an increased molar density of these lipids, and $\rho_l \leqq \rho_l' \leqq 1.2\,\rho_l$ seems to be reasonable in view of the change in density associated with the transition from the liquid crystalline to the crystalline state. The values $\rho_l = 1.03$ mmole/cm^3 and $\rho_v > 0.36$ mmole/cm^3 were used in the previous paper (Walz, 1976) and since $\rho_v > \rho_l$ is very unlikely in view of

Table 3. Refractive index n_l and n_l' for lecithin in the valinomycin induced and unaltered state of aggregation, respectively

Solvatochromic data from	n_l	ρ_l'/ρ_v	$\Delta n_l = n_l' - n_l$			
			$k=6$	$k=20$	$k=36$	range (%)
v.s.	1.54	1	0.420	0.093	0.049	5
		3	0.976	0.173	0.089	6
ac/hp	1.53	1	0.346	0.079	0.042	8
		3	0.820	0.155	0.081	7
et/hp	1.41	1	0.102	0.025	0.013	16
		3	0.325	0.077	0.041	10

The values were calculated by means of Eqs. (21) to (25) and (A14) using the data for $p_{1,i}$ given in Table 2. For k, the factor pertinent to the valinomycin-lecithin interaction, the lower and upper limit and an average value were chosen. Results obtained with Seely and Jensen's data for the position of the main red and the first Soret peak of chlorophyll *a* in various solvents (*see* Appendix B.1) are marked by *v.s.*, those obtained with Seely's data for the red peak position of the acetone- and ethanol-solvates in heptane (*see* Appendix B.2) are marked by ac/hp and et/hp, respectively. Mean values for Δn_l are listed and the last column indicates the range ($\pm$ % of mean) in which the actual values varied due to independent variations of the parameters ρ_l', D_l and D_v within the limits $\rho_l \leqq \rho_l' \leqq 1.2\rho_l$, $2 \leqq D_l$, $D_v \leqq 10$.

the ring structure of valinomycin on the one hand and the dense packing of lipids in the membrane on the other hand, $1 \leqq \rho_l'/\rho_v \leqq 3.4$. The dielectric constant of valinomycin and lecithin were independently varied within the reasonable limits of 2 and 10 (*cf.* Fig. 6*B*). Neglecting the contribution of electrostatic interaction, i.e., $A_t = 2W_t/b^3$ and $B_t = 0$ (*cf.* Appendix B.1), yields $n_l = 1.54$ (*see* upper part of Table 3) which compares very well with the value given in the literature (Hoff, 1974). Taking electrostatic interaction into account (*cf.* Appendix B.2) also yields reasonable values for n_l (*see* lower part of Table 3), although $n_l = 1.41$ is somewhat low (this might indicate that an alcohol-like solvate is not present in vesicle membranes). The values for Δn_l, however, are unrealistically high. Such an increase in refractive index, which cannot be explained by a denser packing of the lipids since $\rho_l' > \rho_l$ accounts for this effect, is hard to reconcile with a change in aggregation. Hence, an altered refractive index of the surrounding medium and thus a change in dispersion interaction cannot be the main reason for the absorbance differences. This conclusion is confirmed by the rather large values for the coefficients $p_{0,i}$ which express the solvatochromic effect on the transition moments [*cf.* Eqs. (18) and (19)]. As outlined in Appendix B.1, dispersion interaction has little or no effect on the transition moments in chlorophyll *a* (as in many other pig-

ments); the change in absorbance caused by the altered lipid aggregation thus predominantly arises from a different phenomenon, and the *actual* effect of dispersion interaction can yield only a minor contribution to the absorbance differences (but *see* concluding remarks).

The contribution of electrostatic interaction, which was included in the calculation of the Δn_l values given in the lower part of Table 3, is based on the concept of a reaction field as it arises in a bulk solvent with freely movable and orientable molecules (Liptay, 1965). One part of the reaction field is then due to the orientational effect exerted on the dipoles of the solvent molecules by the electric field associated with the dipole moment of the pigment molecule; the other part arises from dipole moments induced by this electric field in the solvent molecules. In contrast to this, the lecithin molecules in the vesicle membrane are already highly oriented and their electrostatic polarizability is low. The charged head groups, however, constitute layers of strong dipoles on both sides of the membrane which essentially determine the electric field within the membrane, while the comparatively low dipole moments of the few chlorophyll *a* molecules in the membrane have little effect. Seelig, Limacher and Bader (1972) as well as Griffith, Dehlinger and Van (1974) indeed observed a strong polarity profile in bilayer structures of different lipids as determined with spin label probes. They explained it by the electric field due to the oriented polar head groups of the lipids in the bilayer and supported this conclusion by model calculations. A similar model adapted to the chlorophyll *a* containing vesicle membrane is described in Appendix C, and the results obtained with this model (Fig. 6*C* and *D*) can serve for a more realistic estimation of the effects due to electrostatic interaction.

Unfortunately, Eq. (19) cannot be exploited since the relation between the solvatochromic coefficients $a_{0,t}$ and the transition moments $\boldsymbol{\mu}_{ge,t}$ involves the integral absorption of the bands [*cf.* Eqs. (16) and (17)] which is not yet available. A further exploitation of Eq. (20) requires data about the permanent dipole moments and the polarizabilities as well as the orientation of chlorophyll *a* in the membrane, but little or nothing is known about these parameters. Moreover, the electric field in the membrane is not time independent, for it fluctuates as a consequence of the continuous movement of the lipids, and strongly changes over distances comparable to the size of the chromophore (*see* Fig. 6*C*). The latter difficulty can be overcome by introducing a time and space averaged field effective at the site of the chromophore as was successfully done in similar cases, e.g., when calculating the reaction field (Liptay, 1965) or the actual

Table 4. Terms pertinent to a solvatochromism arising from electrostatic interaction

Electronic transition no (t)	$\lvert(\mu_{e,t}-\mu_g)(F'-F)\rvert$ (10^7 DV/cm)	$\lvert(\alpha_{e,t}-\alpha_g)(F'^2-F^2)\rvert$ (10^{14} Å^{3}V^2/cm^2)
1	0	125
2	1.5	100 1020
$3+t_1$	0	270
$4+t_1$	1.4	260 800
$5+t_2$	2.7	935 1070

Dipole moments ($\mu_{e,t}, \mu_g$) and polarizabilities ($\alpha_{e,t}, \alpha_g$) as well as the electric field (F', F) sensed by chlorophyll *a* are characterized by scalars as explained in the text. Values were calculated from Eqs. (27) and (28) using the pertinent data for $p_{d,i}$ according to the assignment of Gaussian components to electronic transitions (*see* Table 2) and $k=20$. Where two values are listed in the last column, the first one corresponds to the negative and the second one to the positive sign in Eq. (28), respectively.

field for a pigment in a solution exposed to an externally applied electric field (Liptay & Walz, 1971). Despite the lack of data, we can gain some information if we replace, in gross simplification, the vectors and tensors in Eq. (20) by scalar quantities which are then understood to yield rough estimates of the pertinent terms including the yet unknown respective orientations. When neglecting the minor contribution of dispersion interaction we then obtain from Eq. (20)

$$-\Delta\bar{\nu}_t=(\mu_{e,t}-\mu_g)(F'-F)/\mathrm{hc}+\varepsilon_0(\alpha_{e,t}-\alpha_g)(F'^2-F^2)/\mathrm{hc} \qquad (26)$$

where F' and F denote (in scalar notation) the electric field sensed by chlorophyll *a* for lecithin in the valinomycin-induced and in the unaltered state of aggregation, respectively. Substituting from Eqs. (26) and (2) into Eq. (15) yields, on rearranging and considering terms up to the second power of F' and F only,

$$\lvert(\mu_{e,t}-\mu_g)(F'-F)\rvert=\mathrm{hc}(2p_{2,i}/k)^{1/2} \qquad (27)$$

and

$$\lvert(\alpha_{e,t}-\alpha_g)(F'^2-F^2)\rvert=\mathrm{hc}/\varepsilon_0\,\lvert p_{1,i}/k\mp(2p_{2,i}/k)^{1/2}\rvert. \qquad (28)$$

By means of these two equations and with the data of Table 2, the estimates listed in Table 4 were calculated for the two terms in Eq. (20) which are pertinent to a solvatochromism arising from electrostatic interaction.

Several authors have performed electro-optical measurements on different chromophores and thus obtained values for permanent dipole moments and polarizabilities in ground and excited states (compiled by Labhart, 1967, and Liptay, 1969; more recent data by Liptay, Walz, Bau-

mann, Schlosser, Deckers & Detzer, 1971, and Schmidt & Reich, 1972). Comparing these data with those listed in Table 4 indicates that reasonable values for the molecular parameters of chlorophyll *a* are obtained if $|F' - F|$ is taken to be of the order of 10^7 V/cm.

Molecular Basis of Chlorophyll's Sensor Ability

The rough estimate for $|F' - F|$ provides a means to localize the chromophore of chlorophyll *a* (i.e., the porphyrin ring) in the membrane. We have to look for a domain where field strengths of at least 10^7 V/cm occur. Despite the error inherent in the rough estimate for $|F' - F|$, the results of the model calculation (*see* Fig. 6*C*) then suggest but one possibility, viz., the domain of the glycerol-ester moieties, a location which is in line with those proposed by other authors (*see* below). The difference between F' and F can arise from an actual change of the field strength as well as from an altered orientation of the chromophore in the field, for the scalar notation of the field comprises both possibilities. As mentioned in the introduction, the mobility of the lipids in the valinomycin-induced state of aggregation is reduced; however, it is rather unlikely that the concomitant decrease in fluctuation of the electric field alone can account for the estimated change in F. Measurements of dichroism indicate that most absorption bands of chlorophyll *a* arise from $\pi - \pi^*$ transitions (Goedheer, 1966), which means that the transition moments – and most probably the dipole moments too – are confined essentially to the plane of the porphyrin ring (*see also* Hoff, 1974). The π-electron configuration of this ring suggests large components of the polarizability tensors within the plane of the ring and small ones perpendicular to it. Hence, the effective part of the electric field is essentially the component with respect to this plane. In view of the inhomogeneity of the electric field in the domain where the chromophore is located (Fig. 6*D*), a large change of the effective field can result from a *reorientation of the porphyrin ring due to the change in lecithin aggregation.* The red-shift of the absorption bands (positive values for the coefficients $a_{1,t}$) indicates an increase of the electric field strength, and the most plausible explanation for this would be an increased angle of tilt for the porphyrin ring of chlorophyll *a* in a lipid environment influenced by valinomycin compared to that for a chromophore in the unaltered vesicle membrane.

Several authors have discussed the location and orientation of chlorophyll *a* or *b* in black lipid bilayers (Cherry, Hsu & Chapman, 1972; Steinemann, Stark & Läuger, 1972), in unsonicated liposome membranes

(Nicholls, West & Bangham, 1974; Lee, 1975) or in oriented lipid multilayers (Hoff, 1974). Using different experimental evidences, all agree on the location of the porphyrin ring close to the water-lipid interface with an angle of some 40 to 50 degrees between the porphyrin plane and the membrane surface. No consensus has yet been reached as to which side of the interface the porphyrin ring protrudes. The present investigation does not provide information about the actual orientation of the porphyrin ring, it only demonstrates a reorientation depending on the lipid aggregation, and the forementioned tilt of the ring most probably applies to the vesicle membrane as well. [The dimensions of the gap used in the model (*see* Fig. 6*A*) and the scheme in Fig. 8 of the previous paper (Walz, 1976) should not be mistaken to imply an orientation of the ring parallel to the membrane.] The proposed location of the chromophore then suggests that the "hydrophilic edge" of the porphyrin ring, i.e., the cyclopentanone ring and the ester group, is close to or in the aqueous layer containing the polar head groups while the "hydrophobic edge" dips in the domain of the fatty acid chains (*cf.* Fig. 6*A*). This configuration yields a surface area of about 100 $Å^2$ per chlorophyll *a* molecule which, when compared to the surface pressure-area isotherm determined by Karan and Brody (1974) for chlorophyll *a* at a heptane-water interface, allows for ample variation of the chromophore's orientation. The reduced mobility of the lipid molecules interacting with valinomycin (*see* the introduction) probably leads to a more rigid structure which corresponds to an increased surface pressure and thus can explain the reorientation of the porphyrin ring.

Concluding Remarks

As emphasized in the introduction, the main concern of this paper is *not* with the solvatochromic behavior of chlorophyll *a* but to find out how the pigment can sense different aggregational states of lecithin. This question was tackled with the admittedly limited theoretical and experimental information about solvatochromism (particularly of chlorophyll *a*) currently available. Thus, the present theoretical treatment of dispersion interaction involves an averaging over a random orientation of the solvent molecules. A refined treatment adapted to the particular supramolecular structure of the vesicle membrane might reveal a larger effect of dispersion interaction than estimated; provided, however, that a change in relative orientation of the chromophore to the solvent molecules concomitant with the transition from one aggregational state of lecithin to another is taken into account. Similarly, the special molecular architecture

in the membrane may allow only for a partial coordination of one of the lipid's nucleophilic sites to the Mg atom in chlorophyll *a* to an extent which depends on the relative position of the porphyrin ring to the lipid molecules. Again, a reorientation of the porphyrin ring due to an altered lipid aggregation would then be a prerequisite for a change in solvation being a reason for the observed absorbance differences. The answer to the question raised above, though based on an approximation of absorption bands by Gaussian components, thus appears to be valid irrespective of the extent to which solvation, dispersive and electrostatic interaction in particular, contributes to the solvatochromism of the chromophore. Despite the lack of precise data about the molecular parameters which govern the light absorption process in chlorophyll *a*, it could be demonstrated that this sensor reflects certain aspects of the supramolecular structure of the membrane, and this feature can make it a valuable tool in the field of membrane biology. Obviously the information obtainable with this sensor will be improved when more about its molecular parameters will be known.

Up to now, the influence of valinomycin on lecithin and in turn of lecithin on chlorophyll *a* has been extensively discussed. It should be emphasized, however, that similar effects in reversed order have to be expected too, i.e., an influence of chlorophyll *a* on lecithin [indicated by the decrease of the average radius of the vesicles by about 10 Å upon incorporation of chlorophyll *a* (Ritt & Walz, 1976)] and an influence of lecithin on valinomycin (*see*, e.g., Grell, Funck & Eggers, 1975). The system can then be considered to exemplify a more general phenomenon, viz., a "long range" interaction between nonlipid components in a membrane, mediated and possibly modulated by the lipid phase. This should be kept in mind when dealing with problems such as lipid-protein interactions or effects of polypeptide antibiotics in biological membranes other than those related to their ionophoric properties.

It is a pleasure to thank Profs. H. Labhart, P. Läuger and F. Grün for valuable criticism and helpful discussions, and Dr. A. Steinemann, who measured the fluorescence spectra.

Appendix A

1. Estimation of the Coefficients $a_{d,i}$ by Means of the Unresolved Spectrum

In this approximation, the extinction coefficients, ε_t, of the absorption bands are replaced by the overall extinction coefficient for chlorophyll *a*,

ε_c. We then obtain from Eq. (10) by virtue of Eq. (4) and after some algebraic manipulations

$$\overline{\Delta E}(\lambda) = a_{0,t}\, k E(\lambda) + a_{1,t}\, k \left[\frac{\partial E}{\partial \bar{\nu}} - \frac{E}{\bar{\nu}}\right]_\lambda + a_{2,t}\, k \left[\frac{\partial^2 E}{(\partial \bar{\nu})^2} - \frac{2}{\bar{\nu}}\frac{\partial E}{\partial \bar{\nu}} + \frac{2E}{\bar{\nu}}\right]_\lambda \quad \text{(A 1)}$$

where

$$E(\lambda) \equiv A(\lambda)/A(668) \quad \text{(A 2)}$$

denotes the normalized spectrum of chlorophyll *a*. The indices t pertinent to the electronic transitions are, of course, optional in the context of Eq. (A 1) but shall be retained for the sake of a consistent notation. The first and in turn the second derivative of the normalized spectra were calculated by means of a series of approximations in overlapping wavelength ranges based on fourth order polynomials and using five data points spaced at 7 or 3.5 nm if the curvature of the spectrum was large. The data for the function $\overline{\Delta E}(\lambda)$ are given in the previous paper [*see* Eq. (21), Fig. 3 and Table 3 in Walz, 1976].

Eq. (A 1) was written for three successive values of λ at which the difference spectra were evaluated (*see* Walz, 1976), and values for the three coefficients $a_{d,t}\, k$ ($d = 0, 1, 2$) were calculated from this system of linear equations which are valid for the wavelength range determined by the chosen λ values. This procedure was repeated for λ values taken from the whole experimentally covered wavelength range. Since $E(\lambda)$ and $\overline{\Delta E}(\lambda)$ slightly varied for different vesicle preparations, this evaluation was carried out with the data for each preparation separately. All values for $a_{d,t}\, k$ thus obtained, when plotted versus λ, can be represented by a typical curve for each value of d (Fig. 1*A–C*), except for the wavelength range below about 370 nm. There, three curves had to be drawn corresponding to vesicles with different chlorophyll *a* content. This results from light scattered by the vesicles which interfered with the absorbance measurements (*see* Walz, 1976).

2. *Deconvolution of the Spectra into Gaussian Components*

Several methods have been described to deconvolute spectra into Gaussian components (for references *see* Katz & Norris, 1973) which were developed especially in order to cope with such complex spectra, e.g., as found for chlorophylls in chloroplasts. The present spectra are

comparatively simple, and therefore the following technique proved to be adequate.

From Eqs. (4), (12) and (A 2) together with $\bar{\nu}=1/\lambda$, $G_i=\gamma_i/\varepsilon_c(668)$, $G_i^*=\gamma_i^*/\varepsilon_c(668)$ and $\lambda_i=1/\bar{\nu}_i$ it follows

$$E(\lambda)=\sum_i G_i=\sum_i G_i^* \exp\left\{-\frac{1}{2}\left(\frac{1/\lambda-1/\lambda_i}{\sigma_i}\right)^2\right\} \tag{A 3}$$

for the normalized spectra. It can be easily verified that for a single Gaussian component G_i:

$$\frac{\partial G_i/\partial\bar{\nu}}{G_i}=\frac{1}{\sigma_i^2\,\lambda_i}-\frac{\bar{\nu}}{\sigma_i^2} \tag{A 4}$$

which means that the quantity on the left hand side plotted *vs.* $\bar{\nu}$ yields a straight line whose slope and intercept determine the parameters λ_i and σ_i. Therefore, the ratio $(\partial E/\partial\bar{\nu})/E$ [$\partial E/\partial\bar{\nu}$ calculated as described in section 1] was plotted *vs.* the wavenumber, $\bar{\nu}$, which gave a zigzag-line crossing the abscissa. For those λ-(or $\bar{\nu}$-) ranges where a component G_i predominantly contributes to the spectrum, the line was almost straight (with a negative slope) and allowed to estimate a first approximation for the parameters λ_i and σ_i according to Eq. (A 4). The corresponding parameters G_i^* could then be calculated from a system of linear equations obtained from Eq. (A 3) written for those values of λ which were closest to the estimated λ_i values. Further approximations were obtained with the same algorithm except for the following modification. When dealing with a given component, the contributions of the other components to the spectrum and its derivative as calculated with the approximate values for λ_i, σ_i and G_i^* were subtracted. This procedure straightened the corresponding plots of $(\partial E/\partial\bar{\nu})/E$ *vs.* $\bar{\nu}$ considerably and therefore allowed more reliable estimates of λ_i and σ_i for the component under consideration. This technique was numerically stable and converged after 6 to 10 approximations.

3. Determination of the Parameters $f_{d,i}$

Eq. (13), rewritten for the normalized difference spectra, $\Delta E(r_l', \lambda)$, with a new notation, yields [*cf.* Eq. (4)]

$$\Delta E(r_l', \lambda)\equiv\Delta A(r_l', \lambda)/A(668)=\sum_{j=1}^{3n} X_j(r_l')\,S_j(\lambda) \tag{A 5}$$

where n denotes the number of Gaussian components. The parameters $X_j(r_l')$ and $S_j(\lambda)$ are defined as

$$X_j(r_l') = f_{d,i}(r_l') \quad \text{for } j = 3i + d - 2 \tag{A 6}$$

and [*cf.* Eq. (A 3)]

$$S_j(\lambda) = \left[\bar{\nu} \frac{\partial^d (G_i/\bar{\nu})}{(\partial \bar{\nu})^d} \right]_\lambda \quad \text{for } j = 3i + d - 2. \tag{A 7}$$

Multiplying Eq. (A 5) successively by S_j ($j = 1, 2, \ldots, 3n$) and summing up over all values of λ at which the absorbance differences were evaluated (denoted by $\sum_\lambda$) yields a system of $3n$ linear equations which can be written as

$$\mathscr{Y}(r_l') = \mathscr{X}(r_l') \cdot \mathscr{K} \tag{A 8}$$

where $\mathscr{Y}(r_l')$ and $\mathscr{X}(r_l')$ are column-matrices whose elements

$$Y_j(r_l') = \sum_\lambda S_j(\lambda) \cdot \Delta E(r_l', \lambda) \tag{A 9}$$

and $X_j(r_l')$ [see Eq. (A 6)], respectively, are dependent on r_l'. $\mathscr{K}$ is a $3n \times 3n$ square matrix with the elements

$$K_{jk} = \sum_\lambda S_j(\lambda) \cdot S_k(\lambda). \tag{A 10}$$

Solving Eq. (A 8) for $\mathscr{X}(r_l')$ yields the parameters $f_{d,i}$ as the elements of the column-matrix $\mathscr{Y}(r_l') \cdot \mathscr{K}^{-1}$, with $\mathscr{K}^{-1}$ denoting the inverse matrix of $\mathscr{K}$. The inversion was performed numerically with a computer program taken from the Hewlett-Packard program library which employs a modified Gauss-Jordan reduction technique using the maximum pivot strategy.

The numerical evaluation based on Eqs. (A 5) to (A 10) requires a certain level of accuracy for the $\Delta E(r_l', \lambda)$ values, a condition which was not fulfilled by all difference spectra due to experimental limitations [solubility of valinomycin in water and of chlorophyll *a* in vesicle membranes, sensitivity of the spectroscopic device (*see* Walz, 1976)]. The data obtained with vesicles whose chlorophyll *a* content with respect to lecithin was below 1:100 could not, therefore, be used. For the other 31 difference spectra, the parameters $f_{d,i}$ were determined as outlined above and with the data for the Gaussian components obtained from the corresponding spectrum. Since none of the components in the Soret region contributed

substantially to the spectrum in the red region and vice versa (*see* Fig. 2), the two regions could be treated seperately.

In order to check the $f_{d,i}$ values, difference spectra were calculated with these data [Eq. (13)] and compared with the experimentally determined absorbance differences. Moreover, the $f_{d,i}$ values were plotted *vs.* r_l' [Eq. (14)] which allowed for a selection of the meaningful parameters $f_{d,i}$. The determination of the parameters $f_{d,i}$ was then repeated but now with all those parameters found not to conform with Eq. (14) set equal to zero. The values thus obtained were again tested against their r_l'-dependence and judged by the agreement of the calculated difference spectra with the experimental data. After several repetitions of this procedure, 8 out of 15 parameters for the red region and 10 out of 18 parameters for the Soret region had been eliminated. The other parameters $f_{d,i}$ were accepted as meaningful, and the corresponding pseudosolvatochromic coefficients $p_{d,i}$ were determined as proportionality factors of the linear correlations between $f_{d,i}$ and r_l' [Eq. (14)].

Appendix B

Seely and Jensen (1965) have measured the location and the extinction coefficient for the main red peak and the first peak in the Soret region of the spectrum of chlorophyll *a* dissolved in 40 different solvents. In addition the half-width of the red peak was estimated. In a second paper dealing with solvation, Seely (1965) presents data about the location and the half-width of the main red peak for the chlorophyll *a*-solvates with acetone, ethanol and pyridine in binary mixtures of each of these polar solvents with the three apolar and nonsolvating solvents heptane, benzene and α-methylnaphthalene, respectively.

From all this information, the data for the peak positions are the most useful and will be analyzed; the other data allow but qualitative statements. As mentioned in the context of Eq. (19), the expression for the reaction field $\boldsymbol{F}$ is rather complex, but it is reduced to a practicable form if we make the reasonable assumption that the dipole moments of the pigment in the ground and excited state are more or less parallel. From Eq. (20) it then follows [compare Eq. (100) in Liptay, 1965]

$$\bar{\nu}_t = \bar{\nu}_t^0 - \frac{n^2-1}{2n^2+1} A_t - \frac{D-1}{2D+1} B_t \qquad \text{(A 11)}$$

for a 0−0 transition ($\delta_{v,t}=0$). D denotes the dielectric constant of the surrounding medium,

$$A_t=\frac{2W_t}{b^3}+\frac{(\mu_{e,t}-\mu_g)^2}{4\pi\varepsilon_0 b^3 hc} \tag{A 12}$$

and

$$B_t=\frac{(\mu_{e,t}-\mu_g)\mu_g}{2\pi\varepsilon_0 b^3 hc} \tag{A 13}$$

($\mu_{e,t}$ and μ_g refer to the absolute values of these vectors). The geometrical factor G was neglected here which is justified for it is usually compensated by the empirical parameter b. Moreover, the polarizability of the pigment molecule could not be taken into account because otherwise the experimental data would not suffice for an analysis. This can lead to an underestimation of the effects due to electrostatic interaction.

1. Data of Seely and Jensen (1965)

Solvation of chlorophyll *a* with various solvents yields new chemical species with different dipole moments. Hence, the peak position is not only changed due to the variable chemical nature of the solvates but also as a consequence of different reaction fields. Nevertheless, the authors found a fair correlation between the wavelength of the red (or the Soret peak) and $(n^2-1)/(2n^2+1)$ which indicates that dispersion interaction dominates the solvatochromism of chlorophyll *a*. Analyzing the data according to Eq. (A 11) with $B_t=0$ and $A_t=2W_t/b^3$ (including electrostatic interaction would be unreasonable in view of the variation of dipole moments with type of solvate) yields $\bar{\nu}_t^0=15501$ and 24165 cm^{-1}, $2W_t/b^3=$ 2294 and 5263 cm^{-1} for the red and the Soret peak, respectively. Thus, despite the additional effects on peak positions mentioned above and the variation of dispersion interaction with type of solvate to be expected (*see* section 2), reasonable values for $2W_t/b^3$ are obtained which compare well with the data for other pigments (*see*, e.g., Table 2 in Liptay, 1965). According to the relevance of Gaussian components to absorption bands of electronic transitions as discussed in Results and refering to Fig. 2, it seems justified to assign the data obtained for the red and the Soret peak to electronic transitions number 1 and $3+t_1$, respectively (*cf.* Table 2).

Seely and Jensen found that the product of extinction coefficient and half-width for the red peak is more or less constant for all solvents. This

product can be considered as an approximate measure for the integral absorption of the band pertinent to the first electronic transition, and the authors' finding then means that neither dispersive nor electrostatic interaction substantially affect the transition moment of this electronic transition [*cf.* Eqs. (16) and (19)]. This conclusion thus confirms Liptay's statements (Liptay, 1966) that the effect of dispersion interaction on the transition moments is usually very small, and that a change of the transition moment associated with an absorption band of medium strength (such as the red band; *cf.* the oscillator strength of about 0.2 estimated by Seely and Jensen, 1965) arises only for pigments with sufficiently large transition polarizabilities, $\alpha_{ge,t}$, and with dipole moments of medium strength [i.e., several Debye units (*see*, e.g., Liptay *et al.*, 1968) compared to about 1 *D* for chlorophyll *a*-solvates (*see* following section)]. The observed reciprocal variation of extinction coefficient and half-width then results from the band-broadening effect which concomitantly occurs with a band-shift [*see* Eqs. (1) and (2)] as already tentatively postulated by Seely and Jensen (1965, p. 1842).

2. Data of Seely (1965)

In order to analyze these data, the refractive index, n, and the dielectric constant, D, in a binary mixture should be known. These parameters can be estimated by means of the molar refraction (Bauer & Fajans, 1949)

$$R_i = \frac{1}{\rho_i} \frac{n_i^2 - 1}{n_i^2 + 2} \tag{A 14}$$

and the molar polarization (Smyth, 1949)

$$P_i = \frac{1}{\rho_i} \frac{D_i - 1}{D_i + 2} \tag{A 15}$$

where n_i, D_i and ρ_i denote the refractive index, the dielectric constant and the molar density of the i-th (pure) solvent, respectively. Both quantities are additive with respect to mole fraction under certain conditions and, when assuming ideal binary mixtures (i.e., additivity for volumes),

$$\frac{n^2 - 1}{n^2 + 2} \equiv Q_R(m) = \frac{m R_1 + (1 - m) R_2}{m/\rho_1 + (1 - m)/\rho_2} \tag{A 16}$$

where m denotes the mole fraction for solvent 1 in the binary mixture (Bauer & Fajans, 1949). An analogous equation holds true for $Q_P(m)$ with D and P_i replacing n and R_i, respectively (Smyth, 1949). Seely indicated

the composition of the binary mixtures by the volume fraction, m', and for an ideal mixture

$$m = m'\rho_1/[m'\rho_1 + (1-m')\rho_2]. \qquad \text{(A 17)}$$

By means of Eqs. (A 14) to (A 17) and with Seely's data for the refractive index and the dielectric constant of the pure solvents, n and D for the binary mixtures were calculated and Seely's data for the peak position in dependence of composition of the mixtures could then be analyzed according to Eq. (A 11).

This analysis yields $\bar{\nu}_1^0 = 15626$ and $16132\,\mathrm{cm}^{-1}$, $A_1 = 2678$ and $5491\,\mathrm{cm}^{-1}$, $B_1 = 81$ and $196\,\mathrm{cm}^{-1}$ for the acetone and ethanol solvates of chlorophyll *a*, respectively, both in the corresponding binary mixture with heptane. The results for these two solvates in binary mixtures with benzene or α-methylnaphthalene were still reasonable but less reliable because additivity of molar polarizations for mixtures of a polar and an apolar but easily polarizable solvent (such as an aromatic compound) is rather poor (*see* Smyth, 1949). Moreover, an interaction between the π-electron systems of the aromatic solvent and chlorophyll *a* can occur, an effect which Liptay's theory does not account for. This phenomenon becomes even more pronounced when the solvating molecule itself is aromatic, and the results for the pyridine solvates were accordingly unreasonable. However, since neither a benzene-like environment nor a solvate of an aromatic amine has to be expected for chlorophyll *a* in lecithin membranes, the lack of data for these two cases is irrelevant for the interpretation of the solvatochromic coefficients.

Experience has shown that the values for $\mu_{e,t}$ range from 1.5 μ_g to 3 μ_g (*see*, e.g., Table I in Liptay, 1969) while $b \simeq 5$ Å for molecules with planar configuration and π-electron systems (*see* Liptay, 1965; Liptay *et al.*, 1968). Hence, by virtue of Eqs. (A 12), (A 13) and with the values for A_1 and B_1, we find $2W_t/b^3 = 2630 \pm 30$ and $5370 \pm 75\,\mathrm{cm}^{-1}$ for the acetone- and ethanol-solvates of chlorophyll *a*, respectively, while the corresponding values for μ_g (or $\mu_{e,1} - \mu_g$) range from 0.7 to 1.4 D and 1.1 to 2.2 D, respectively.

Appendix C

The lecithin molecules in vesicle membranes are regularly arranged and show a predominant orientation; their mobility consists of intramolecular vibrations and rotations, of lateral diffusion within the spherical shell of the membrane and of an occasional flip-flop across the bilayer. According to Hauser (1975), the polar heads are more or less perpendicular

to the membrane surface and each is surrounded by about 20 molecules of water (*see* Fig. 6*A*, left). In the model, each lipid molecule is confined to a rectangular box (8 Å × 8 Å × 33 Å) and the polar head group is replaced by a positive and a negative charge located on the long axis of the box. The membrane is then assumed to be built up of a hydrophobic phase, consisting of a 36 Å thick hydrocarbon core with two 5 Å thick ester-like boundary domains, which is sandwiched between two 10 Å thick aqueous layers containing a regular array of positive and negative charges (*see* Fig. 6*A*, right). Four lecithins are removed on one side of the membrane thus yielding a rectangular gap (16 Å × 16 Å × 33 Å) which easily accommodates a chlorophyll *a* whatever its actual orientation in the membrane (*cf.* dimensions given by Cotton *et al.*, 1974). The dielectric properties in this gap are assumed to be the same as in the adjacent part of the membrane.

Let (x_i, y_i, z_i) and (x_j, y_j, z_j) denote sets of coordinates for the positive and negative charges, respectively, with respect to the coordinate system shown in Fig. 6*A*. The values of the x- and y-coordinates for charges with either sign are ± 4 Å, ± 12 Å, ± 20 Å, ..., but with $z_i = \pm 30$ Å and $z_j = \pm 25$ Å, except for the sets $(x_i, y_i, z_i) = (\pm 4$ Å, ± 4 Å, 30 Å) and $(x_j, y_j, z_j) = (\pm 4$ Å, ± 4 Å, 25 Å) corresponding to the removed lecithin molecules. Irrespective of the problems involved with an assignment of continuum parameters to bimolecular structures, a dielectric constant, $D(z)$, is attributed to the "model membrane" which is constant within an x, y-plane but depends on z as indicated in Fig. 6*B*. The electric field $\boldsymbol{F}$ created by the charges at a point with the coordinates (x, y, z) in the gap can then be expressed by its components with respect to the three axes of the coordinate system:

$$F_x(x, y, z) = \frac{e}{4\pi\varepsilon_0 D(z)} \left(\sum_i \frac{x - x_i}{r_i^3} - \sum_j \frac{x - x_j}{r_j^3} \right) \qquad \text{(A 18)}$$

and analogous equations for F_y and F_z. The positive direction of the field vector is defined as pointing from the positive to the negative charge; $e = 1.902 \times 10^{-19} As$ is the elementary charge. The distances r_i between the locations of the positive charges and a given point (x, y, z) are

$$r_i = [(x - x_i)^2 + (y - y_i)^2 + (z - z_i)^2]^{1/2}, \qquad \text{(A 19)}$$

and an analogous equation can be written for r_j pertinent to the negative charges.

Some results obtained with this model are shown in Fig. 6*C* and *D*; they represent an upper limit for the field, the actual values are lower because of the motion of the lipid molecules and the variation in orientation

of the polar heads. The curvature of the membrane, which is considerable in vesicles with radii of the order of 100 Å, was neglected. A similar model based on a curved membrane would yield even larger electric fields which are different for gaps oriented towards the outer or inner surface of the vesicle membrane.

Evidence has been obtained recently that the orientation of lecithin in the membrane is different from that shown in Fig. 6*A* (Dr. J. Seelig, *personal communication*). The glycerol backbone should be perpendicular to the membrane surface with one of the fatty acids being bent at a right angle and the polar head oriented more or less parallel to the membrane surface. Such an orientation would yield somewhat different data for the electric field (compare calculations of Griffith *et al.*, 1974) but does not invalidate the conclusions drawn in this paper.

References

Bär, F., Lang, H., Schnabel, E., Kuhn, H. 1961. Richtung der Übergangsmomente der Absorptionsbanden von Phthalocyaninen aus Fluoreszenzpolarisationsmessungen. *Z. Elektrochem.* **65**:346

Bauer, N., Fajans, K. 1949. Refractometry. *In:* Physical Methods of Organic Chemistry. A. Weissberger, editor. Vol. I (second ed.), p. 1141. Interscience, New York

Brody, S.S., Broyde, S.B. 1963. A low-temperature emission band from diluted solutions of pure chlorophyll *a*. *Nature (London)* **199**:1098

Cherry, R.J., Hsu, K., Chapman, D. 1972. Structure and reflection spectra of chlorophyll-lipid membranes. *Biochim. Biophys. Acta* **288**:12

Colbow, K. 1973. Chlorophyll in phospholipid vesicles. *Biochim. Biophys. Acta* **381**:4

Cotton, T.M., Trifunac, A.D., Ballschmiter, K., Katz, J.J. 1974. State of chlorophyll *a* in vitro and in vivo from electronic transition spectra, and the nature of antenna chlorophyll. *Biochim. Biophys. Acta* **368**:181

Freed, S., Sancier, K.M. 1951. Absorption spectra of chlorophylls in solutions at low temperatures—equilibria between isomers. *Science* **114**:275

Goedheer, J.C. 1966. Visible absorption and fluorescence of chlorophyll and its aggregates in solution. *In:* The Chlorophylls. L.P. Vernon and G.R. Seely, editors. p. 174. Academic Press, New York

Gouterman, M., Stryer, L. 1962. Fluorescence polarization of some porphyrins. *J. Chem. Phys.* **37**:2260

Grell, E., Funck, Th., Eggers, F. 1975. Structure and dynamic properties of ion specific antibiotics. *In:* Membranes—A Series of Advances. G. Eisenman, editor. Vol. 3, p. 1. Marcel Dekker, New York and Basel

Griffith, O.H., Dehlinger, P.J., Van, S.P. 1974. Shape of the hydrophobic barrier of phospholipid bilayers (evidence for water penetration in biological membranes). *J. Membrane Biol.* **15**:159

Hauser, H. 1975. Water phospholipid interactions. *In:* Recent Advances in Food Science. Vol. 5. Academic Press, New York and London *(in press)*

Hauser, H., Finer, E.G., Chapman, D. 1970. Nuclear magnetic resonance studies of the polypeptide alamethicin and its interaction with phospholipids. *J. Mol. Biol.* **53**:419

Hoff, A.J. 1974. The orientation of chlorophyll and bacteriochlorophyll molecules in an oriented lecithin multilayer. *Photochem. Photobiol.* **19**:51

Jost, P.C., Griffith, O.H., Capaldi, R.A., Vanderkooi, G. 1973. Evidence for boundary lipid in membranes. *Proc. Nat. Acad. Sci. USA* **70**:480

Karan, J., Brody, S.S. 1974. Chlorophyll *a* and cytochrome *c* at a heptane-water interface. *Z. Naturforsch.* **29c**:506

Katz, J.J., Norris, J.R. 1973. Chlorophyll and light energy transduction in photosynthesis. *In:* Current Topics in Bioenergetics. D.R. Sanadi and L. Packer, editors. Vol. 5, p. 41. Academic Press, New York and London

Labhart, H. 1967. Electrochromism. *Adv. Chem. Physics.* **13**:179

Lee, A.G. 1975. Segregation of chlorophyll *a* incorporated into lipid bilayers. *Biochemistry* **14**:4397

Lee, A.G., Birdsall, N.J.M., Metcalfe, J.C., Toon, P.A., Warren, G.B. 1974. Clusters in lipid bilayer and the interpretation of thermal effects in biological membranes. *Biochemistry* **13**:3699

Lehoczki, E. 1975. New chlorophyll-*b* forms in a chlorophyll-detergent photosynthetic model system. *Biochim. Biophys. Acta* **408**:223

Liptay, W. 1965. Die Lösungsmittelabhängigkeit der Wellenzahl von Elektronenbanden und die chemisch-physikalischen Grundlagen. *Z. Naturforsch* **20***a*:1441

Liptay, W. 1966. Die Lösungsmittelabhängigkeit der Intensität von Elektronenbanden. I. Theorie. *Z. Naturforsch.* **21***a*:1605

Liptay, W. 1969. Elektrochromie-Solvatochromie. *Angew. Chem.* **81**:195

Liptay, W., Schlosser, H.-J., Dumbacher, B., Hünig, S. 1968. Die Beeinflussung der optischen Absorption von Molekülen durch ein elektrisches Feld. VIII. Die Lösungsmittelabhängigkeit der Lage und Intensität von Elektronenbanden einiger Farbstoffe. *Z. Naturforsch.* **23***a*:1613

Liptay, W., Walz, G. 1971. Die Beeinflussung der optischen Absorption von Molekülen durch ein elektrisches Feld. X. Erweiterung der Theorie unter Berücksichtigung der Fluktuation der effektiven elektrischen Felder. *Z. Naturforsch.* **26a**:2007

Liptay, W., Walz, G., Baumann, W., Schlosser, H.-J., Deckers, H., Detzer, N. 1971. Die Beeinflussung der optischen Absorption von Molekülen durch ein elektrisches Feld. XI. Elektrische Polarisierbarkeiten einiger organischer Moleküle im Grundzustand und im ersten Singulett-Anregungszustand. *Z. Naturforsch.* **26a**:2020

Nicholls, P., West, J., Bangham, A.D. 1974. Chlorophyll *b* containing liposomes: Effect of thermal transitions on catalytic and spectral properties. *Biochim. Biophys. Acta* **363**:190

Reich, R., Schmidt, S. 1972. Über den Einfluß elektrischer Felder auf das Absorptionsspektrum von Farbstoffmolekülen in Lipidschichten. I. Theorie. *Z. Elektrochem.* **76**:589

Ritt, E., Walz, D. 1976. Pigment containing lipid vesicles. I. Preparation and chracterization of chlorophyll *a*-lecithin vesicles. *J. Membrane Biol.* **27**:41

Schmidt, S., Reich, R. 1972. Über den Einfluß elektrischer Felder auf das Absorptionsspektrum von Farbstoffmolekülen in Lipidschichten. II. Messung an Rhodamin B. III. Elektrochromie eines Carotinoids (Lutein). *Z. Elektrochem.* **76**:599 and 1202

Seelig, J., Limacher, H., Bader, P. 1972. Molecular architecture of liquid crystalline bilayers. *J. Am. Chem. Soc.* **94**:6364

Seely, G.R. 1965. Calculation of equilibrium constants for the solvation of chlorophyll from spectral data. *Spectrochim. Acta* **21**:1847

Seely, G.R., Jensen, R.G. 1965. Effect of solvent on the spectrum of chlorophyll. *Spectrochim. Acta* **21**:1835

Smyth, C.P. 1949. Determination of dipole moments. *In:* Physical Methods of Organic Chemistry. A. Weissberger, editor. Vol. I (second ed.), p. 611. Interscience, New York

Steinemann, A., Stark, G., Läuger, P. 1972. Orientation of the porphyrin ring in artificial chlorophyll membranes. *J. Membrane Biol.* **9**:177

Walz, D. 1976. Pigment containing lipid vesicles. II. Interaction of valinomycin with lecithin as sensed by chlorophyll *a*. *J. Membrane Biol.* **27**:55