Biochimica et Biophysica Acta, 689 (1982) 45–54
Elsevier Biomedical Press

BBA 71219

EXCESS FREE ENERGIES OF INTERACTION OF CHLOROPHYLL *a* WITH MONOGALACTOSYLDIACYLGLYCEROL AND PHYTOL

A MIXED MONOLAYER STUDY

PIERRE TANCRÈDE *, GAÉTAN MUNGER and ROGER M. LEBLANC

Centre de Recherche en Photobiophysique, Université du Québec à Trois-Rivières, Trois-Rivières (Québec), G9A 5H7 (Canada)

(Received February 15th, 1982)

Key words: Surface pressure isotherm; Chlorophyll a; Phytol; Monogalactosyldiacylglycerol; Free energy calculation; Mixed monolayer

The surface pressure isotherms of chlorophyll *a*, monogalactosyldiacylglycerol and phytol at the air-water interface were studied on a Langmuir trough at 20.0±0.5°C. The subphase was a phosphate buffer, 10^{-3} M at pH 8.0. The extrapolated limiting areas per molecule are 115, 82 and 38 Å^2/molecule, respectively. The isotherms of eight mixtures of chlorophyll *a* with monogalactosyldiacylglycerol and eight mixtures of chlorophyll *a* with phytol, covering in both cases the whole range of molar fractions have also been measured. The results for the mixed monolayers were analysed in terms of the additivity rule. They show that a small negative deviation with respect to ideality is observed upon mixing chlorophyll *a* with monogalactosyldiacylglycerol. However, chlorophyll *a* forms an ideal two-dimensional solution when mixed with phytol. The excess free energies of mixing of chlorophyll *a* with monogalactosyldiacylglycerol as a function of concentration were calculated from the surface pressure isotherms at 10, 15, 20 and 25 mN·m^{-1}. The values are negative, reflecting the interactions prevailing between these components in the monolayers. For the four surface pressures studied, the excess free energies are symmetrical with respect to the mole fraction. The values for an equimolar mixture range from −300 to −540 J·mol^{-1} at 10 and 25 mN·m^{-1}, respectively. A comparison between the thermodynamics of mixing of chlorophyll *a* with monogalactosyldiacylglycerol and phytol suggests that the polar head of monogalactosyldiacylglycerol together with the polar groups of chlorophyll *a* are probably involved in the interaction. However, this does not completely rule out the possibility that structural effects due to a different packing of chlorophyll *a* with monogalactosyldiacylglycerol and phytol may also be involved. Furthermore it is shown that the small interactions between these constituents are not inconsistent with the specific coupling existing between the apoprotein of the chlorophyll *a*-protein complexes and chlorophyll *a*.

Introduction

Chlorophyll *a* (Chl *a*) represents the most essential pigment of green plants photosynthesis. Its importance in the various primary events of the photosynthetic process, from the capture of light (Chl *a* antenna) to the subsequent act of energy transfer and the light-energy conversion in the specialized photosynthetic centers is now well established [1]. In vivo, it appears that all the Chl *a* molecules are coupled to membrane-bound polypeptides [2]. These complexes are embedded within a lipid bilayer which also includes all the accessory pigments (e.g. carotenoids), the quinones and the specialized proteins responsible for the electron transport between the two photosystems [3,4].

---

* To whom correspondence should be sent.

Abbreviations: Chl *a*, chlorophyll *a*; MGDG, monogalactosyldiacylglycerol.

Galactolipids (monogalactosyldiacylglycerol and digalactosyldiacylglycerol) form the major class of lipids of the thylakoid membranes, representing about 60 to 70 mol% of the total lipids present in these functional units [5].

Considering the importance of Chl *a* in photosynthesis it is mandatory to have a good knowledge of the way this pigment can interact with its environment [6]. Our approach initially is to use the monolayer technique to obtain accurate data on the interactions prevailing between Chl *a* and its lipid environment and then to proceed with more biologically relevant systems by studying how these interactions are modified when proteins are dissolved in the subphase and come into contact with the Chl *a* molecules dispersed in a mixed monolayer at the interface. This paper therefore presents a monolayer study of the interactions existing between Chl *a* and monogalactosyldiacylglycerol (MGDG) and between Chl *a* and phytol. Phytol is the esterifying alcohol of Chl *a*. MGDG was chosen as a model component of the class of lipids found in the thylakoid membranes. It represents about 70–75% of the total amount of galactolipids present in this kind of membranes. The surface pressure isotherms of eight mixtures of Chl *a* with MGDG, covering the whole range of molar fractions, have been carefully measured at the air-water interface. The excess free energies of interaction have been calculated from the surface pressure isotherms. In contrast with the results found in literature [7,8], small negative free energies of mixing are measured for this system, indicating a small but definite interaction between these constituents. On the other hand, the surface pressure isotherms of eight mixtures of Chl *a* with phytol show ideal behaviour over the whole concentration range as emphasized by the fact that the miscibility rule is obeyed for all the molar fractions studied. A comparison between the thermodynamics of interaction of these two systems is presented and conclusions are drawn with respect to the type of interaction prevailing between Chl *a* and MGDG.

Material and Methods

Chl *a* was extracted from spinach leaves and purified according to the method described by Iriyama et al. [10,11] and Omata et al. [12]. The pigment was crystallized from isooctane/water (100:0.5, v/v) in the last step of the purification procedure and a single spot was observed by TLC on silica gel (5 × 20 cm plates, 250 μm layer; Analtech, distributed by Mandel Scientific, Montréal, Canada) using *n*-pentane/*tert*-butanol (9:1, v/v) as developing solvent. MGDG from spinach leaves was a commercial sample purchased from Serdary (London, Canada). The product was shown by TLC to be contaminated with coloured pigments (carotenoids and chlorophylls). Degradation products were also observed as streaking and a few more spots were revealed in addition to MGDG and the coloured pigments. However, an adequate purification of the compound, specially developed for monolayers studies, could be achieved by using preparative TLC as described in details by Tancrède et al. [13]. The purified sample was analyzed by TLC using chloroform/methanol/water (90:25:2, v/v) as developing solvent. The lipid showed only two narrowly separated spots, characteristic of the aliphatic chains of MGDG ($R_F = 0.72$ and 0.69). It was stored in solution in benzene under nitrogen at −20°C in the dark and was always used within 10 days after purification as degradation was apparent by TLC when kept for a longer time period. Phytol was bought from Sigma (Saint-Louis, MO). It was distilled three times under a 1.5 torr pressure (200 Pa) at 145 to 150°C.

Benzene (Fisher Scientific Co., Montréal, Québec) was used as the spreading solvent throughout this study. It was distilled prior to utilization on a Vigreux column 30 cm high [13] because it was found to give a significant response on the monolayer trough when a blank experiment was performed. The phosphate salts (Na_2HPO_4 and NaH_2PO_4, 99.5 mol%) used to prepare the buffer were obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ) and used without further purification. The buffer was prepared from quartz-distilled water (Hereaus, Model BI 18, Amersil Inc., Sayreville, NJ) with a specific resistivity better than $17 \cdot 10^6\ \Omega \cdot cm$ and a surface tension larger than $70\ mN \cdot m^{-1}$.

The surface pressure isotherms were automatically recorded on a float type torsion balance. A description of the system is presented in Ref. 14.

The isotherms were measured at the air-water interface, identical results being obtained when air is substituted for nitrogen. Surface potentials were also recorded while compressing the monolayer. They were monitored using an ionizing ^{241}Am electrode (Nuclear Radiation Development, New York) at 2–3 mm above the interface and by a platinum reference dipping in the subphase. They were mainly used to identify more precisely the collapse pressures of the isotherms, a sudden change in slope of ΔV vs. the molecular area being apparent at these pressures. Otherwise, their general variation with the surface pressure show the same general pattern as the surface pressure isotherms. The isotherms were measured on a phosphate buffer subphase (10^{-3} M, pH = 8.0). The temperature of the subphase as well as that of the stainless-steel glove-box enclosing the monolayer trough were kept at 20.0 ± 0.5°C for all the systems studied. When Chl *a* was used, the measurements were done under a dim green light. The benzene solutions of the pure components or the mixtures were deposited dropwise from a microsyringe (Pierce Chemical Co., Rockford, IL) at the air-water interface. About 50 to 100 μl of the solutions were needed. The monolayer was compressed at the speed of 1.9 $\mathrm{cm \cdot min^{-1}}$ (rate of surface area reduction: 0.44 $\mathrm{cm^2 \cdot s^{-1}}$). The molar weights of Chl *a*, phytol and MGDG are 894, 297 and 775 $\mathrm{g \cdot mol^{-1}}$, respectively.

The solutions of Chl *a*, MGDG and phytol used for the measurements of the surface pressure isotherms were prepared according to Ref. 15. The solutions were prepared in glass vials previously cleaned using a solution of No-Chromix (Godax Laboratories, New-York) and then soaked in a sodium hydroxide solution (0.01 M) for about two hours. The vials were closed with a Teflon mininert valve (Pierce Chemical Co., Rockford, IL). This device allows the withdrawal of sample fractions with minimal losses due to evaporation. The total volume of the batch solutions of the pure products was 4.00 ml. About 1.0 mg of Chl *a*, 1.5 mg of MGDG and 2.0 mg of phytol were weighed on a Cahn electrobalance (Model RG 2000, Ventron Instrument Corp., CA) to achieve the desired concentrations. Mixtures of a given composition were then prepared from these batch solutions of the pure components by transferring into a vial using a precise gas-tight syringe (Pierce Chemical Co., Rockford, IL), the amount of the components required to obtain the molar fraction desired. The total volume of the solution when the mixtures were prepared was 0.60–1.00 ml.

Results and Discussion

Surface pressure isotherms of the pure components

The surface pressure-area isotherms of the pure components measured at 20.0 ± 0.5°C are shown in Fig. 1. Curve a represents the isotherm measured for phytol, curve b for MGDG and curve c for Chl *a*.

The results obtained for phytol (curve a) are the average of six different measurements done on two different batches of the pure component in benzene. The maximum deviation between the extremes was 3 Å^2/molecule. The collapse pressure and limited extrapolated areas are 27.2 $\mathrm{mN \cdot m^{-1}}$ and 38 Å^2/molecule, respectively. The extrapo-

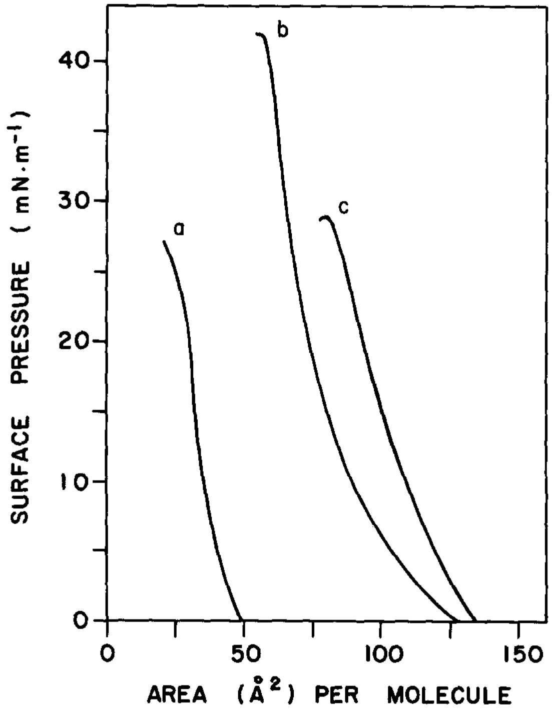

Fig. 1. Surface pressure-area isotherms of the pure components at the air-water interface at 20.0±0.5°C. Curves a, b and c are the isotherms for phytol, MGDG and Chl *a*, respectively. The subphase was a phosphate buffer 10^{-3} M at pH 8.0.

lated limiting area found here for phytol is almost identical with the one given by Hirsch and Brody [16], 41 Å^2/molecule, all the conditions (buffer subphase and temperature) being the same. However, $CHCl_3$ was used as the spreading solvent in the latter work. In general, our surface pressure isotherm for this compound is shifted towards lower molecular areas when compared to the one given in Ref. 16. The maximum of deviation between the two sets of results is less than 5 Å^2/molecule at $\Pi = 20$ mN · m^{-1}, the highest surface pressure given by these authors. However, our results for phytol differ considerably from those presented by Liljenberg and Selstam [8,9], which have obtained molecular areas much larger than the ones presented in Fig. 1. For example, at $\Pi = 15$ mN · m^{-1}, they found for phytol a molecular area of about 86 Å^2/molecule as compared to 36.5 Å^2/molecule in Ref. 16 and 32 Å^2/molecule in the present work. The large molecular areas reported in Refs. 8 and 9 are difficult to reconcile with the chemical nature of phytol and the molecular areas usually found with this class of compounds.

The surface pressure isotherm obtained for the purified sample of MGDG (curve b) represents the average of ten different measurements done on five independent purifications of the commercial product, the difference between the extremes being about 4 Å^2/molecule. These results are similar to our previously published isotherms for this lipid [13], the maximum difference found being less than 2 Å^2/molecule over the whole range of molecular areas. The extrapolated limiting area (82 Å^2/molecule) and collapse pressure (42 mN · m^{-1}) are identical with those already reported [13]. A detailed comparison between our results for the surface pressure isotherm of MGDG and those found in the literature has been presented in Ref. 13.

Curve c of Fig. 1 presents the results obtained for Chl *a*. The surface pressure isotherm is the average of ten different measurements done on five independent extractions of the pigment from spinach leaves. The maximum difference between the extremes in this case was 6 Å^2/molecule. The extrapolated limiting area and collapse pressure are 115 Å^2/molecule and 29 mN · m^{-1}, respectively. The results obtained here are almost identical with those given by Hirsch and Brody [16]. However, our surface pressure isotherm is slightly shifted towards higher molecular areas when compared to the ones published by Trosper et al. [7] and Bellamy et al. [17]. For example, at 15 mN · m^{-1}, the values for the molecular area of Chl *a* is 93 and 89 Å^2/molecule for Refs. 7 and 17, respectively, as compared to 100 Å^2/molecule for the present work. Liljenberg and Selstam [8,9] have also reported data for the surface pressure isotherm of Chl *a* but their values are much higher than those found in the present work (152.5 Å^2/molecule at $\Pi = 15$ mN · m^{-1}). It is difficult, from the results published in the literature, to establish the reasons for the small difference observed in the surface isotherm of Chl *a* among the various authors. This difference may be related to the various techniques used to extract and purify the pigment, although in most cases extreme care is taken to avoid contamination from surface active components in the various steps of the purification procedure. However this explanation remains speculative and further experimental work would be required to solve this problem.

Chlorophyll a and monogalactosyldiacylglycerol mixtures

The surface pressure-area isotherms of eight mixtures of Chl *a* and MGDG, covering the whole range of molar fractions have been measured at the air-water interface. Fig. 2A presents the surface pressure-area isotherms of three of these mixtures, curves 1 to 3, the molar fraction of Chl *a* being 0.220, 0.500 and 0.800, respectively. Curves a and b are the surface pressure isotherms obtained with MGDG and Chl *a*, respectively. Fig. 2B presents the surface potential isotherms for the same systems. The other systems studied correspond to a molar fraction of Chl *a* of 0.100, 0.400, 0.600, 0.700 and 0.900. However, due to the overlapping of some of the surface pressure isotherms, these isotherms are not presented in Fig. 2. The surface pressure isotherms for all the systems are the average of three different measurements. The maximum deviation observed for all the mixtures was 3 Å^2/molecule. Furthermore, they result from different purification batches of Chl *a* and MGDG over the range of mole fractions. Some systems ($x_{\mathrm{Chl}\,a} = 0.500$, 0.600 and 0.700) were reproduced

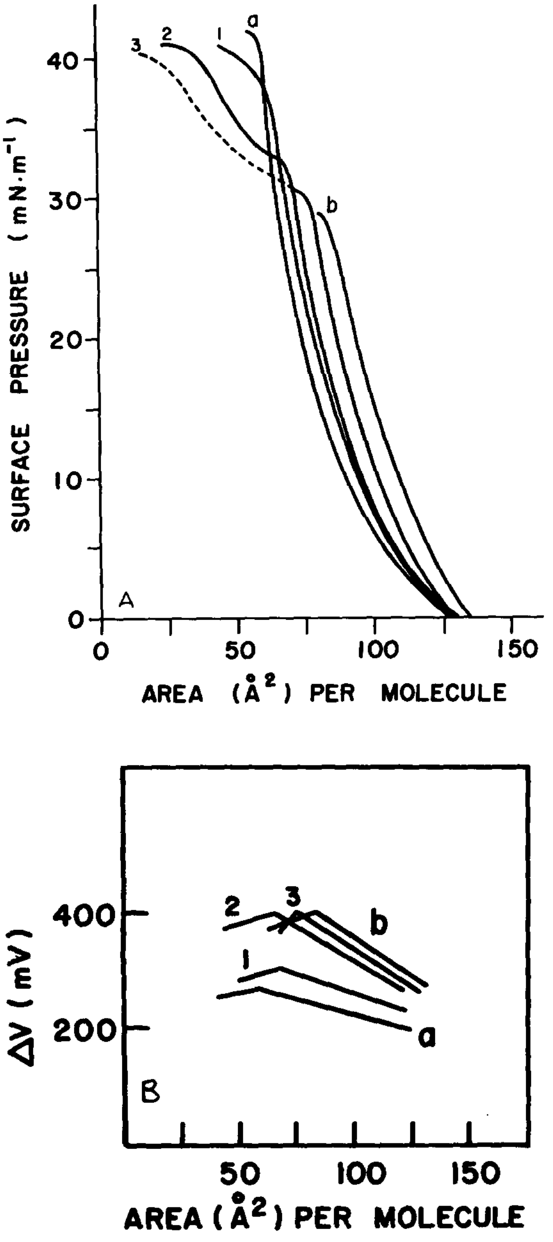

Fig. 2. (A) Surface pressure-area isotherms of some of the Chl *a*-MGDG mixtures. The experimental conditions are as described in the legend of Fig. 1. Curves a and b are for MGDG and Chl *a*. Curves 1–3, the molar fraction of Chl *a* is 0.220, 0.500 and 0.800, respectively. (B) The surface potential isotherms of the same mixtures and the pure components.

with different batches of the pure components to check the internal consistency of the results. The surface pressure isotherms of all the mixtures studied were also fitted to the following expression

$$A = \sum_{i=0}^{n} a_i \Pi^i \qquad (1)$$

where A is the molecular area (Å^2/molecule) and Π is the surface pressure (mN · m^{-1}). This expression allows a mathematical expression of the isotherms obtained. Therefore, the isotherms of the mixtures in Fig. 2 that were not drawn due to overlapping problems can be reproduced precisely using Eqn. 1. Furthermore, this representation is also useful because the best fit parameters can be used to calculate the excess free energies of mixing for the various systems, presented below. Table I presents the least-squares fitting parameters, a_i, obtained from the experimental results of all the surface pressure isotherms, together with the standard deviation, S.D., calculated for each mixture. A third order polynomial was found satisfactory to reproduce the data within 0.5 Å^2/molecule.

The results for the mixed monolayers show that an almost constant collapse pressure is observed at a surface pressure of about 41 mN · m^{-1} for the mole fractions of Chl *a* ranging from 0.100 to 0.500. Furthermore, an apparent collapse pressure is observed at 34, 33 and 30 mN · m^{-1} for $x_{\mathrm{Chl}\,a} =$ 0.400, 0.500 and 0.600, respectively. Both the true and apparent collapse pressures could be located precisely as a change of slope in the surface potentials recorded for the isotherms. Curve 2, in Fig. 2A, shows both the collapse pressure at 41 mN · m^{-1} and the apparent collapse pressure at 33 mN · m^{-1} for an equimolar mixture of Chl *a* and MGDG. For the mole fractions of Chl *a* larger than 0.500, an apparent collapse pressure was also obtained at a surface pressure of about 30 mN · m^{-1} as exemplified by curve 3 in Fig. 2A. Furthermore, this curve has been drawn as a dotted line because for molar fractions of Chl *a* larger than 0.500, the monolayer was rather unstable at surface pressures larger than 30 mN · m^{-1} and the reproducibility for this portion of the isotherm was rather poor. The existence of these apparent collapse pressures is associated with the rejection, partial or total, of one of the components from the monolayer [15,18]. An analysis of the results in terms of the surface phase rule [19] shows that

TABLE I

SURFACE PRESSURE ISOTHERMS DATA FOR THE CHLOROPHYLL *a*-MONOGALACTOSYLDIACYLGLYCEROL MIXTURES

System [a]	a_0 [b] (Å^2/molec.)	a_1 [b] ((Å^2/molec.)/(mN·m^{-1}))	a_2 [b] ((Å^2/molec.)/(mN·m^{-1})^2)	a_3 ($\times 10^3$) [b] ((Å^2/molec.)/(mN·m^{-1})^3)	S.D. [c] (Å^2/molec.)
0.100	117.09	−4.40	0.138	−1.67	0.156
0.220	125.37	−4.76	0.165	−2.28	0.228
0.400	115.12	−4.19	0.167	−2.80	0.134
0.500	123.39	−4.18	0.164	−2.96	0.449
0.500	126.83	−4.86	0.200	−3.38	0.262
0.600	117.33	−4.53	0.219	−4.42	0.297
0.600	122.77	−3.74	0.124	−1.78	0.085
0.700	129.76	−3.64	0.112	−1.66	0.065
0.700	124.44	−3.51	0.112	−1.64	0.058
0.800	124.29	−3.07	0.084	−1.13	0.037
0.900	127.95	−3.28	0.103	−1.63	0.062

[a] The numbers indicated to identify the mixtures refer to the mole fraction of Chl *a*.

[b] Constants for Eqn. 1 to reproduce the experimental molecular area from the surface pressures.

[c] Standard deviation.

below the apparent collapse pressure, the two components would be completely miscible while rejection, or partial rejection, of one of the components would begin when the surface pressure is further increased. A kink would appear in the surface pressure isotherm at that point [20]. In the case under study here, the more easily collapsible component, Chl *a*, would be squeezed out of the monolayer. The effect is particularly clear for molar fractions in the range 0.220–0.500. For the higher molar fractions, the apparent collapse pressure becomes very close to the true collapse pressure of Chl *a*. However, the observed variation in the apparent collapse pressure together with a thermodynamic analysis of the results, presented below do provide evidence for miscibility below 28 mN · m^{-1} over the whole concentration range.

A better understanding of the interactions between Chl *a* and MGDG is provided by a comparison of the molecular areas, calculated assuming ideality of the mixing through the additivity rule, and the experimental molecular areas given in Fig. 2 and Table I. Fig. 3 shows the experimental mean molecular areas for the mixtures plotted as a function of the mole fraction of Chl *a* at four different surface pressures, 10, 15, 20 and 25 mN · m^{-1}. The dashed lines were calculated assuming the additivity rule,

$$A_{12} = x_1 A_1 + x_2 A_2 \qquad (2)$$

where A_{12} is the average molecular area at a given surface pressure in the two-component film, x_1 and x_2 are the mole fractions of the components in the mixed monolayer, and A_1 and A_2 are the molecular areas of the pure components at the same surface pressure. Fig. 3 shows that negative deviations from ideality are observed for the four surface pressures studied, the maximum of the effect being located around $x_{\mathrm{Chl}\,a} = 0.500$. For example, at 20 mN · m^{-1}, the difference between the experimental and calculated molecular areas is found to be 4 Å^2/molecule. These negative deviations provide evidence for a small interaction between Chl *a* and MGDG, particularly for molar fractions of Chl *a* in the range 0.2–0.8. Trosper et al. [7,21] reported that Chl *a* and MGDG formed an ideal mixture. However, their investigation only covered molar fractions of Chl *a* in the range 0.05–0.155 for which the mean molecular area of the mixed monolayer is indeed, within experimental error, identical to the ideal value calculated from Eqn. 2. Liljenberg et al. [8,9] have also reported that MGDG and Chl *a* form miscible

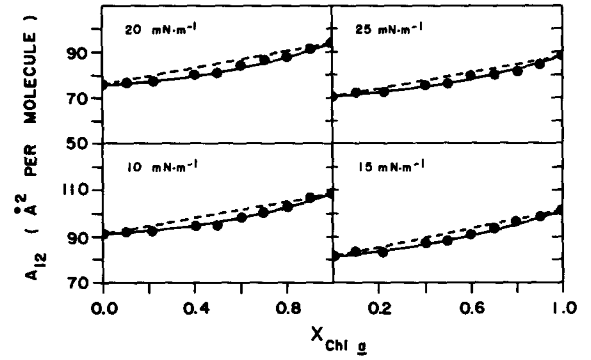

Fig. 3. The mean molecular area (A_{12}) of the mixed Chl *a*-MGDG monolayers as a function of the mole fraction of Chl *a* at the different surface pressures indicated. Dashed curves, calculated results assuming the additivity rule. Solid circles, the experimental values.

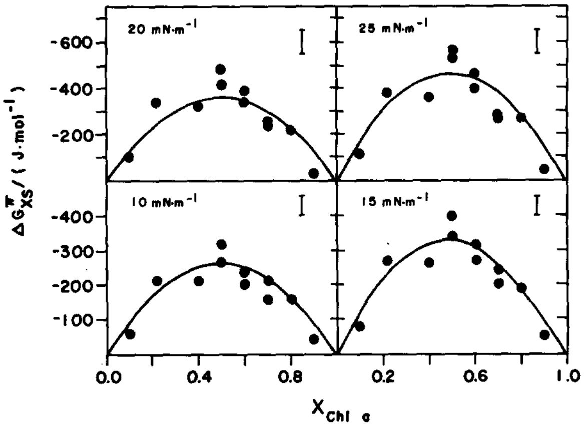

Fig. 4. The excess free energies of mixing, ΔG^{Π}_{xs}, as a function of the mole fraction of Chl *a* for Chl *a* + MGDG at the different surface pressures indicated. The bars at the upper right represent the maximum experimental error on the excess free energies.

monolayers for MGDG/Chl *a* ratios of 6:1 and 3:1. However, the unusually large molecular areas they obtain for Chl *a* may cast some doubts on the validity of their results when the mixtures are considered.

The interaction observed between Chl *a* and MGDG can be analysed more quantitatively. The surface pressure isotherms presented in Fig. 2 for the pure components and the mixtures can be used to evaluate the excess free energy of interaction, ΔG^{Π}_{xs}, for the components at a given surface pressure. If we assume that the two components in the mixed monolayer are miscible and behave ideally in the limit of zero pressure, then

$$\Delta G^{\Pi}_{xs} = \int_0^{\Pi} (A_{12} - x_1 A_1 - x_2 A_2)\, \mathrm{d}\Pi \qquad (3)$$

where A_1, A_2 and A_{12} are, respectively, the areas per molecule at pressure Π for the pure components 1 and 2 and for the mixture, x_1 and x_2 being the mole fractions of the components in the monolayer. The surface pressure isotherms of the pure components and the mixtures have been fitted with Eqn. 1 up to 28 mN · m^{-1}, in order to maximize the precision of the curve fitting in this portion of the isotherms. The excess free energies of mixing are presented in Fig. 4 as a function of the mole fraction of Chl *a* at 4 different surface pressures, 10, 15, 20 and 25 mN · m^{-1}. The excess free energies of mixing are negative for the four surface pressures studied. The maximum of the deviation is found around $x_{\mathrm{Chl}\,a} = 0.500$. The small negative excess free energies of interaction are also consistent with the slight contraction of the mixed monolayer observed in Fig. 3 and discussed above. They also bring further weight to the assumption made above that Chl *a* and MGDG are miscible components at the air-water interface.

It is difficult, from the results presented here, to assess the exact nature of the weak interactions prevailing between MGDG and Chl *a*. The final state of interaction of the components in the mixed monolayer results from the breaking of hydrophobic interactions between the aliphatic chains of the pure components and the breaking of polar interactions between the polar groups of the component molecules among themselves and the water molecules of the subphase to establish similar kinds of interactions in the mixed monolayer. It is not possible from the free energy data themselves to determine to which extent the hydrophobic and/or the polar interactions are involved in the slight contraction of the mixed Chl *a*-MGDG monolayer observed here. The surface potentials, being sensitive to changes involved in the dipole moments of the molecules, could have provided evidence that the polar interactions were involved in this case. This did not turn out to be the case. For example, the experimental surface potentials obtained for an equimolar mixture of Chl *a* and MGDG are 290, 310 and 330 mV at 10, 15 and 20 mN · m^{-1}, respectively. These figures are identical, to within experimental error, to those calculated from the pure components assuming a simple additivity rule, 305, 330, and 346 mV for these three surface pressures. On the other hand, it is possible by a comparison of the thermodynamics of various systems to obtain indications on the preponderance of one type of interaction over the other one. In this aspect, a study of the molecular interactions between Chl *a* and phytol has been undertaken. Phytol is a long chain alcohol, the esterifying hydrocarbon found in Chl *a*. The polar head of this molecule is much smaller than the galactosyl group of MGDG and it would therefore have a much more aliphatic character. The comparison between the thermodynamics of Chl *a* with MGDG and with phytol could therefore provide information about the kind of interactions (polar or hydrophobic) prevailing between Chl *a* and MGDG.

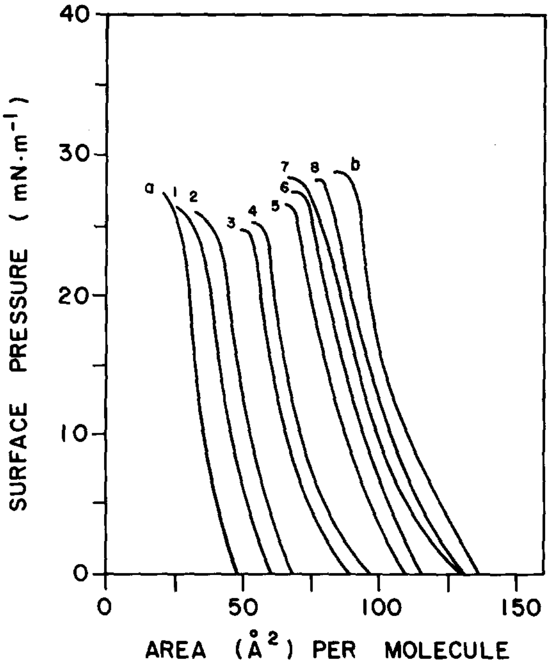

Fig. 5. Surface pressure-area isotherms of the Chl *a*-phytol mixtures. The experimental conditions are described in the legend of Fig. 1. Curves a and b are for phytol and Chl *a*, respectively. Curves 1–8, the mole fraction of Chl *a* is 0.100, 0.200, 0.400, 0.500, 0.700, 0.800, 0.838 and 0.900, respectively.

Chlorophyll a and phytol mixtures

The surface pressure isotherms at the air-water interface for Chl *a* mixed with phytol are presented in Fig. 5. Curves a and b are the surface pressure isotherms for the pure components, phytol and Chl *a*, respectively. Curves 1 to 8 are the surface pressure isotherms for eight mixtures of these components, the molar fraction of Chl *a* being 0.100, 0.200, 0.400, 0.500, 0.700, 0.800, 0.838 and 0.900, respectively. The surface pressure isotherms for all the systems are the average of three different measurements. As for the mixtures studied in the previous section, the isotherms of Fig. 5 result from independent determinations done on three different batch solutions of the pure components in benzene. The maximum deviation between the extremes in this case was 3 Å^2/molecule. The isotherms were also fitted to Eqn. 1 and the results of the best fit parameters are presented in Table II.

The results from Fig. 5 show that the collapse pressure varies slightly with composition, decreasing gradually from about 26.5 mN · m^{-1} for $x_{\mathrm{Chl}\,a} = 0.100$ to 24.6 mN · m^{-1} for $x_{\mathrm{Chl}\,a} = 0.400$. It then increases slowly from 25.4 mN · m^{-1} for $x_{\mathrm{Chl}\,a} = 0.500$ to $x_{\mathrm{Chl}\,a} = 0.900$. This variation of the collapse pressure with composition may provide miscibility for the components in the mixed monolayer [19]. However, the magnitude of the variation of the collapse pressures for the various mixtures is small, reflecting the small difference between the collapse pressures of Chl *a* and phytol.

An analysis of the surface pressure isotherms of the mixtures in terms of the additivity rule through Eqn. 2 has also been undertaken. The results are presented in Fig. 6, which shows the mean molecular areas of the various mixtures taken from Fig. 5 plotted as a function of the mole fraction of Chl *a* at four different surface pressures, 10, 15, 20 and 25 mN · m^{-1}. The dashed lines in Fig. 6 were calculated assuming the additivity rule. The results show that for the four surface pressures studied the mixtures show an ideal behaviour over the whole range of concentration. These findings, obtained here for Chl *a* mixed with phytol over the concentration range do not bring support to any contraction of the monolayer [16] upon mixing of

TABLE II

SURFACE PRESSURE ISOTHERMS DATA FOR THE CHLOROPHYLL *a*-PHYTOL MIXTURES

System [a]	a_0 [b] (Å^2/molec.)	a_1 [b] ((Å^2/molec.)/ (mN·m^{-1}))	a_2 [b] ((Å^2/molec.)/ (mN·m^{-1})^2)	a_3 ($\times 10^3$) [b] ((Å^2/molec.)/ (mN·m^{-1})^3)	S.D. [c] (Å^2/molec.)
0.100	59.58	−2.28	0.123	−3.15	0.192
0.200	68.60	−2.40	0.102	−2.06	0.065
0.400	87.23	−3.35	0.151	−2.98	0.209
0.500	96.71	−2.66	0.046	−1.01	0.320
0.700	108.30	−2.79	0.084	−1.35	0.021
0.800	115.23	−2.77	0.072	−9.88	0.047
0.838	123.65	−3.32	0.110	−1.90	0.116
0.900	129.85	−3.35	0.099	−1.56	0.089

[a] The numbers indicated refer to the mole fraction of Chl *a*.

[b] Constants for Eqn. 1 to reproduce the experimental molecular area from the surface pressures.

[c] Standard deviation.

these constituents. The reasons for this discrepancy are difficult to understand. Hirsch and Brody [16] have used $CHCl_3$ as the spreading solvent. It is well known, particularly with phospholipids, that $CHCl_3$ may drag some of the surface active material in the subphase when deposited at the air-water interface, thereby yielding to lower molecular areas. However, this effect does not seem to be observed here, the molecular areas of Chl *a* and phytol given in Ref. 16 being almost identical with the ones presented here.

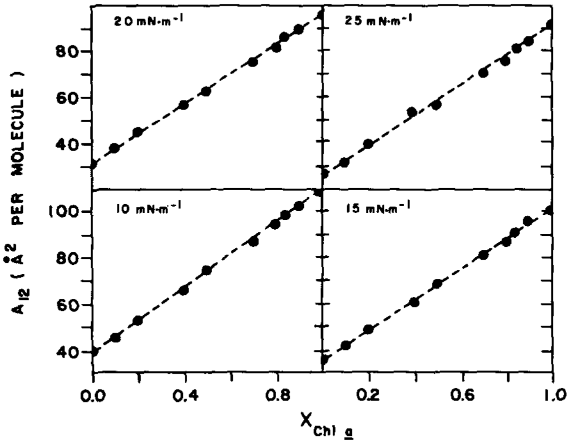

Fig. 6. The mean molecular areas (A_{12}) of the mixed Chl *a*-phytol monolayers as a function of the mole fraction of Chl *a* at the different surface pressures indicated. Dashed curves, calculated results assuming the additivity rule. Solid circles, the experimental values.

The comparison between the behaviour of Chl *a* when mixed with MGDG and phytol brings insights into the nature of the interactions prevailing between Chl *a* and MGDG. Phytol is a much less polar molecule than MGDG. Actually, this molecule is essentially hydrophobic in nature, being essentially a long chain saturated hydrocarbon with a small polar head. In a sense, it can therefore be considered as a model component of the aliphatic chains present in MGDG. The fact that negative excess free energies of interactions are observed when Chl *a* is mixed with MGDG together with the fact that Chl *a* forms an ideal two-dimensional solution ($\Delta G_{xs}^{\Pi} = 0$) with phytol both suggest the interaction to occur among the polar head of MGDG and the polar groups present in Chl *a*. However this conclusion may not be completely warranted, considering the fact that the fatty acid moieties of MGDG are unsaturated (α-linolenic acid is the major component). It is possible that, owing to structural effects [23], the Chl *a* molecules are packed differently when interspersed with MGDG rather than with phytol.

One may raise questions about the possible consequences for the in vivo system of the weak interactions found here to exist between Chl *a* and MGDG. As already mentioned in the introduction, it is well established [2] that in the thylakoid membranes, Chl *a* is tightly coupled to proteins. One may argue from our results that an interaction between Chl *a* and MGDG, if sufficiently strong, could prevent the formation of the Chl *a*-protein complexes provided that the free energy of interaction between Chl *a* and MGDG is larger in magnitude than the free energy of interaction between Chl *a* and its apoproteins. The interaction found here between Chl *a* and MGDG is small, the excess free energy being of the order of a few hundred joules only. On the other hand, the free energy values for the interaction between Chl *a* and the apoproteins are not known. However, a recent study [24] has shown that the addition of MGDG to solubilized thylakoid membranes do not break the Chl *a*-protein association, thereby suggesting that this association is much stronger than the Chl *a*-MGDG interactions. The weak interactions prevailing between Chl *a* and MGDG are therefore not inconsistent with the existence of the Chl *a*-protein complexes.

In conclusion, the work presented here has shown that small negative excess free energies of mixing are found when Chl *a* is mixed with MGDG. On the other hand, Chl *a* forms an ideal mixture when mixed with phytol over the whole concentration range. A comparison between the two sets of results together with considerations on the chemical nature of phytol and MGDG provide evidence for the role played by the interactions among the polar heads of MGDG and Chl *a* in the understanding of the thermodynamics of their mixtures. However, it is possible that structural effects, due to a different packing of the Chl *a* molecules with MGDG as compared with phytol may also be involved. Experimental work now in progress in this laboratory, particularly with pheophytin *a* and digalactosyldiacylglycerol, should provide more detailed information pertaining to these interactions. Furthermore, a study of the adsorption of proteins to mixed Chl *a*-MGDG monolayers has also been undertaken, which should yield a better understanding of the relative magnitude of the Chl *a*-protein interactions as compared to the interactions prevailing between Chl *a* and MGDG.

Acknowledgments

The Natural Sciences and Engineering Research Council of Canada and the Ministère de l'Education du Québec are gratefully acknowledged for financial support.

References

1 Sauer, K. (1979) Annu. Rev. Phys. Chem. 30, 155–178

2 Thornber, J.P., Markwell, J.P. and Reinman, S. (1979) Photochem. Photobiol. 29, 1205–1216

3 Gregory, R.P.F. (1977) Biochemistry of Photosynthesis, 2nd edn., pp. 22–45, John Wiley and Sons, New York

4 Katz, J.J., Norris, J.R., Shipman, L.L., Thurnauer, M.C. and Wasielewski, M.R. (1978) Annu. Rev. Biophys. Bioeng. 7, 393–434

5 Nishihara, M., Yokota, K. and Kito, M. (1980) Biochim. Biophys. Acta 617, 12–19

6 Katz, J.J. (1979) in Advances in the Biochemistry and Physiology of Plant Lipids (Appelqvist, L.-Å. and Liljenberg, C., eds.), pp. 37–56, Elsevier, Amsterdam

7 Trosper, T. and Sauer, K. (1968) Biochim. Biophys. Acta 162, 97–105

8 Liljenberg, C. and Selstam, E. (1980) Physiol. Plant 48, 428–434

9 Liljenberg, C. and Selstam, E. (1979) in Advances in the Biochemistry and Physiology of Plant Lipids (Appelqvist, L.-Å. and Liljenberg, C., eds.), pp. 199–204, Elsevier, Amsterdam

10 Shiraki, M., Yoshiura, M. and Iriyama, K. (1978) Chem. Lett., 103–104

11 Iriyama, K. and Yoshiura, M. (1979) J. Chromatogr. 177, 154–156

12 Omata, T. and Murata, N. (1980) Photochem. Photobiol. 31, 183–185

13 Tancrède, P., Chauvette, G. and Leblanc, R.M. (1981) J. Chromatogr. 207, 387–393

14 Dijkmans, H., Munger, G., Aghion, J. and Leblanc, R.M. (1981) Can. J. Biochem. 59, 328–331

15 Tancrède, P., Parent, L., Paquin, P. and Leblanc, R.M. (1981) J. Coll. Interface Sci. 83, 606–613

16 Hirsch, R.E. and Brody, S.S. (1979) Photochem. Photobiol. 29, 589–596

17 Bellamy, W.D., Gaines, G.L. and Tweet, A.G. (1968) J. Chem. Phys. 39, 2528–2538

18 Feher, A.I., Collins, F.D. and Healy, T.W. (1977) Aust. J. Chem. 30, 511–516

19 Gaines, G.L. (1966) Insoluble Monolayers at Liquid-Gas Interfaces, pp. 281–300, John Wiley and Sons, New York

20 Nakagaki, M. and Honda, T. (1976) Bull. Chem. Soc. Japan 49, 880–885

21 Trosper, T.L. (1967) Chlorophyll-*a* Interactions in Lipid Monolayers, Doctoral Dissertation, University of California, Berkeley

22 Cadenhead, D.A. and Kellner, B.M.J. (1974) J. Coll. Interface Sci. 49, 143–145

23 Cadenhead, D.A. and Müller-Landau, F. (1980) J. Coll. Interface Sci. 78, 269–270

24 Heinz, E. and Siefermann-Harms, D. (1981) FEBS Lett. 124, 105–111