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Monolayer study of plastoquinones, α-tocopherol quinone, their hydroquinone forms and their interaction with monogalactosyldiacylglycerol. Charge-transfer complexes in a mixed monolayer

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The surface pressure-area isotherms of pure plastoquinone-9 (PQ-9), plastoquinone-3 (PQ-3), α-tocopherol quinone (α-TQ), their reduced (hydroquinone) forms and mixtures of these molecules with monogalactosyldiacylglycerol (MGDG) have been studied by a monolayer technique. The collapse pressures of all hydroquinones (QH_2) were higher than those of the corresponding quinones (Q), the difference being highest between PQ-9 and PQH_2-9. The limiting molecular areas of hydroquinones were higher than those of the corresponding quinones except for α-TQH_2. All Q-QH_2 mixtures showed miscibility throughout the whole range of the components' ratios. There was no deviation from the additivity rule observed for any of the Q-QH_2 mixture, as well as for the mixtures of MGDG with PQ-3, PQH_2-9, α-TQ and α-TQH_2. On the other hand, PQ-9/MGDG and PQH_2-3/MGDG mixtures showed positive and negative deviations, respectively. All the isotherms of Q-MGDG and QH_2-MGDG mixtures showed a kink point above the collapse pressure of the Q or QH_2 examined, indicating that with the increase in surface pressure, Q or QH_2 were gradually squeezed out from the monolayer. The percent content of Q and QH_2 in the monolayer as a function of surface pressure was also calculated. The hydroquinones were more difficult to remove from monolayers than the corresponding quinones, and among the investigated quinones, PQ-9 was most easily and α-TQ most difficultly squeezed out. The surface pressure-area isotherms of the three-component mixtures of PQ-9/PQH_2-9/MGDG showed a shift to lower molecular areas in comparison with the corresponding two-component mixtures, especially at higher surface pressures. This indicates that the presence of PQ-9 lowered the PQH_2-9 content in the monolayer, especially at higher pressures, which was explained by charge-transfer complex formation upon interaction of PQ-9 with PQH_2-9. The comparison of surface potential-area isotherms of PQ-9/PQH_2-9/MGDG mixtures with those of the corresponding binary mixtures also suggest charge-transfer interaction between PQ-9 and PQH_2-9. The orientation and localization of the investigated quinones and quinols in the thylakoid membrane and significance of charge-transfer interactions in functioning of PQ-9 has been discussed.

Introduction

Prenylquinones are known to be important components of electron transport chains in chloroplasts, mitochondria and bacteria. The most essential for plant photosynthesis, plastoquinone-9 (PQ-9) was found to occur in thylakoid membranes as bound to proteins [1-3] and freely diffusing in membranes as a PQ-pool. PQ-9 of the pool functions as a proton pump across the membrane [4] and an hydrogen carrier between Photosystem II and cytochrome b_6-f complex, occurring in an equilibrium of oxidized (PQ-9) and reduced form, plastohydroquinone (PQH_2-9). Both forms show high lateral and transversal mobility in thylakoid membranes, moving mainly within the fluid interior of the membrane [5]. Plastoquinone-3 (PQ-3), a homologue of PQ-9, was so far found only in spinach chloroplasts [6]. α-Tocopherol quinone (α-TQ) is always found together with PQ-9 but in much smaller amounts [7,8]. Its function and localization in membranes remains so far unknown, but it is supposed to be not protein-bound and probably it takes part in electron and proton transport [9]. As is PQ-9, it is accompanied by its

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reduced form (α-TQH$_2$) [7]. Another prenylquinone occurring in thylakoids membranes is vitamin K$_1$ (phylloquinone). It plays the role of a protein-bound electron acceptor in Photosystem I [10].

The orientation and localization of prenylquinone molecules occurring in lipid parts of natural membranes is not sufficiently examined. For PQ-9 and ubiquinone (in mitochondria), relatively deep localization within the hydrophobic part of the membrane, close to the midplane region, is suggested [5,11,12]. On the other hand, the possibility of penetration, at least of the more polar parts of these molecules, into the regions closer to the membrane surface is also taken into account, as experiments on PQ-9 and ubiquinone-mediated hydrogen transport across liposome membranes indicate [13]. Different solubility of PQ-9 and PQH$_2$-9 in organic solvents suggests [14] that there should be a difference in localization of these molecules within the thylakoid membrane; PQH$_2$-9 being mainly near the membrane surface, whereas PQ-9 more or less equally distributed across the membrane with the preference to its hydrophobic interior. It was also found that the presence of PQ-9 enhanced the solubility of PQH$_2$-9 in hydrophobic solvents, which is partially connected with the formation of charge-transfer complexes by these molecules [14].

One of the methods which seems to be useful for study of prenylquinone properties is the monolayer technique. It was already successfully applied to the investigation of mixtures of chlorophyll with plastoquinones and α-TQ [15] and mixed monolayers of ubiquinones with phospholipids [16]. Monolayers of prenylquinones transferred onto solid support (Langmuir-Blodgett films) made possible the determination of the orientation of quinone rings in such a system (unpublished data).

Since it was found that PQ-9 and α-TQ may form charge-transfer complexes in the solid state and in solutions [17], as well as in fatty acids [18], it was interesting to examine if the formation of such complexes could be detected also in a monolayer state. Apart from quinone (Q) and hydroquinone (QH$_2$) mixtures we also studied properties of these compounds in a mixture with monogalactosyldiacylglycerol (MGDG), the most abundant lipid of thylakoid membranes. Such a system, due to the presence of MGDG, would simulate to some extent native conditions.

There were already some observations reported for chlorophyll/prenylquinone mixtures [15], which suggest the possibility of charge-transfer interactions in a monolayer state.

Materials and Methods

PQ-9 and PQ-3 were supplied as a gift by Hoffman-La Roche (Basle, Switzerland) and α-TQ was obtained from Eastman Kodak. MGDG was from Lipid Products (Nutfield, Surrey, UK). All three quinones were purified by TLC as described in Ref. 15. The hydroquinones were prepared from the corresponding quinones by dissolving α-TQ and PQ-3 in methanol or PQ-9 in ethanol (at concentrations about 5 mg/ml) and adding gradually NaBH$_4$ until the solution became colourless. To prevent the formation of semiquinone forms, the pH of the mixture was maintained slightly acidic by adding droplets of diluted HCl. Then three volumes of hexane (bubbled with argon to avoid oxidation of the hydroquinone) and water (with a small amount of Na$_2$S$_2$O$_4$ to absorb oxygen) were added and the mixture was shaken for a while. After separation of phases, the upper hexane phase (containing hydroquinone) was collected and evaporated to dryness in a stream of argon. The extraction was repeated several times. All the organic solvents were distilled before use and when used as a solvent for hydroquinones, they were additionally bubbled with argon to remove dissolved air oxygen. Water used for the monolayer work was filtered and deionized in a nanopure Barnstead column (Fisher, Canada) and then bidistilled in a quartz distiller (Heraeus Quartzschmelze, Germany). The solutions of quinones and MGDG were prepared by weighing the required amount of dried sample (under vacuum) on a Cahn electrobalance (Model 2000, Ventron Instruments) and adding appropriate volumes of benzene. The solutions were prepared in glass vials equipped with Teflon mininert valves (Pierce) to avoid evaporation of the solvent. In the case of hydroquinones it was very difficult to remove traces of the solvent even by long storage under vacuum, which would introduce an error in determination of their weight. For this reason the concentrations of hydroquinones were determined spectrophotometrically, using earlier calculated molar absorption coefficients (ε) in absolute ethanol. They were determined together with ε for quinone forms by preparing a quinone solution of known concentration and then after reduction with NaBH$_4$, the spectrum of hydroquinone, of the same molar concentration as of quinone, was recorded.

The subphase for all the monolayer experiments was phosphate buffer (10^{-3} M, pH = 7.0). The alkaline pH is not recommended because it facilitates hydroquinone degradation and the oxidation is rapid. During deposition and compression of all monolayers containing hydroquinones, high purity nitrogen was fluxed over the monolayer surface to prevent their oxidation. The compression rate was 8 Å^2/molecule per min and temperature was 20 ± 2°C. The surface pressure–area isotherms were automatically recorded by an Apple II computer interfaced to the experimental setup. Surface potentials were also recorded while compressing the monolayer. They were monitored using an ionized

^{241}Am electrode (Nuclear Radiation Development, New York) at 2–3 mm above the interface and by a platinum reference electrode dipped in the subphase.

All the surface pressure–area isotherms presented in this work represent the average of at least three measurements. The difference between the individual isotherms was not more than 1 Å^2/molecule.

Results and Discussion

The molar absorption coefficients

The measured molar absorption coefficients (M^{-1} cm^{-1}) of the investigated compounds in absolute ethanol at their absorption maxima in the ultraviolet range were for:

PQ-9, $\varepsilon_{255} = 17940$

PQ-3, $\varepsilon_{255} = 16260$

α-TQ, $\varepsilon_{268} = 18830$

PQH$_2$-9, $\varepsilon_{290} = 3390$

PQH$_2$-3, $\varepsilon_{290} = 3480$

α-TQH$_2$, $\varepsilon_{287} = 3110$

Monolayers of individual compounds

The surface pressure–area isotherms for the individual compounds investigated in this study are shown in Fig. 1. Their collapse pressures, extrapolated limiting areas (to zero pressure), and surface potentials changes during compression are given in Table I. Among the three prenylquinones investigated, PQ-9 as the most hydrophobic (nine isoprenoid units) forms monolayers with the lowest collapse pressure. α-TQ shows the highest collapse pressure as the most polar molecule among the investigated quinones (four isoprenoid units and hydroxyl group at the isoprene unit closest to the headgroup) and it occupies also the largest molecular area. The hydroquinones with their rings more polar than those of the corresponding Q forms show higher collapse pressures, as expected. The difference between Q and QH$_2$ collapse pressures increased with the rise of hydrophobicity of Q, being highest for PQ-9. In the case of PQ-3 and PQ-9, the molecular areas of their QH$_2$ forms exceed those of Q forms, which is connected with the larger space occupied by a QH$_2$ ring than that of Q. This observation, however, does not apply for α-TQH$_2$, whose molecular area is even less than that of α-TQ. Probably this phenomenon is connected with the presence of a hydroxyl group in both α-TQ and α-TQH$_2$. The side chain of those compounds, which occupies larger space than an α-TQ or even an α-TQH$_2$ ring, determines the molecular area of the whole molecule, therefore the redox state of the ring does not influence the area of the whole molecule. The slightly lower molecular area of α-TQH$_2$ than that of α-TQ is probably connected with more compact side chain conformation of the hydroquinone form induced by interaction of its ring and side chain hydroxyl groups. This explanation is supported by the low limiting area of α-tocopherol (53 Å^2/molecule [19]), which is the cyclic (dehydro) form of α-TQH$_2$ depleted of its OH side chain group. The surface potentials (Table I) indicate that for all hydroquinones, their potentials are lower than for the corresponding Q forms.

TABLE I

The collapse pressures, extrapolated limiting areas (to zero pressure) and surface potential changes during compression (being a straight line from a to b) for the pure compounds used in the monolayer study

Compound	Π (mN/m)	*A* (Å^2/molecule)	*E* (mV) *a* – *b*
PQ-9	1.2	76	269 ± 6–278 ± 10
PQH$_2$-9	13.3	83	152 ± 1–193 ± 2
PQ-3	8.4	58	198 ± 8–268 ± 9
PQH$_2$-3	16.0	67	148 ± 2–202 ± 4
α-TQ	26.6	79	221 ± 9–291 ± 12
α-TQH$_2$	31.0	73	55 ± 4–144 ± 1
MGDG	44.0	82	259 ± 3–340 ± 5

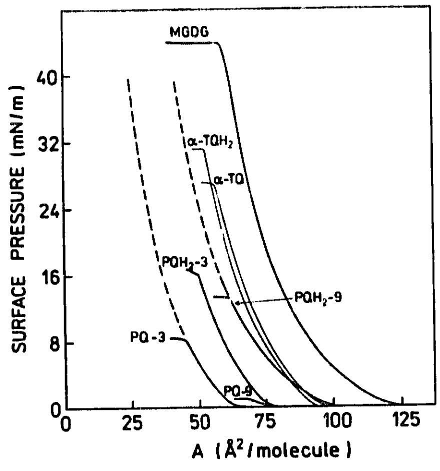

Fig. 1. Surface pressure–area isotherms of pure compounds (———) used in this work and the extrapolated isotherms (— — —) for PQ-3 and PQH$_2$-9.

Mixed monolayers of Q and QH$_2$

All three mixtures of the Q-QH$_2$ type showed the presence of only one collapse pressure, whose value changed proportionally throughout the whole range of Q/QH$_2$ ratios examined, suggesting that these systems are miscible at all ratios of components. The PQ-3/PQH$_2$-3 and α-TQ/α-TQH$_2$ mixtures showed a similar collapse pressure variation with a change in component proportions (isotherms not shown) as PQ-

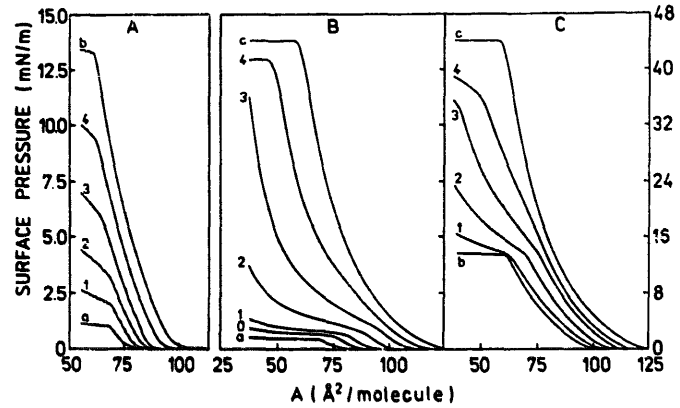

Fig. 2. Surface pressure-area isotherms of PQ-9/PQH$_2$-9 (A), PQ-9/MGDG (B) and PQH$_2$-9/MGDG (C) mixtures. Curves a, b, and c are for pure PQ-9, PQH$_2$-9 and MGDG, respectively. In curves 1–4 (A) the molar fraction of PQH$_2$-9 is 0.2, 0.4, 0.6 and 0.8, respectively, and in curves 0–4 (B, C) the molar fraction of MGDG is 0.1, 0.2, 0.4, 0.6 and 0.8, respectively.

9/PQH$_2$-9 (Fig. 2A). The interactions of the components in the mixed monolayer can be revealed by comparison (at a given pressure) the experimental molecular areas (from Fig. 2A) with the mean molecular areas calculated using the additivity rule,

$$A_{1,2} = x_1 A_1 + x_2 A_2$$

where $A_{1,2}$ 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 presents the experimentally determined mean molecular areas ($A_{1,2}$) compared with calculated results assuming the additivity rule for all three Q/QH$_2$ mixtures at different ratios of the components and at different pressures. As can be seen, there is no apparent deviation from the additivity rule for any of the mixed monolayers studied. The slight deviations of some points might be caused by experimental errors.

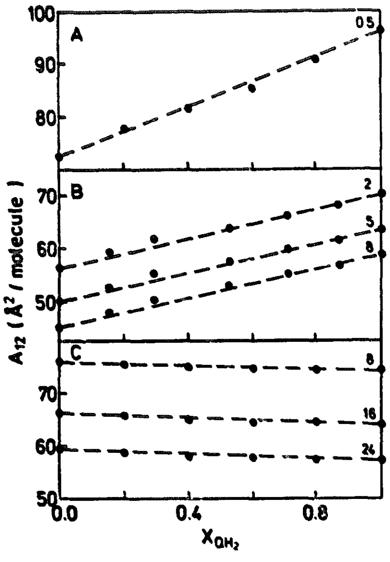

Fig. 3. Mean molecular area ($A_{1,2}$) of the mixed PQ-9/PQH$_2$-9 (A), PQ-3/PQH$_2$-3 (B) and α-TQ/α-TQH$_2$ (C) monolayers as a function of the mole fraction of the hydroquinones at the surface pressure (in mN/m) indicated; dashed lines are calculated results assuming the additivity rule; solid circles are the experimental values.

Bearing in mind that PQ-9 and α-TQ form charge-transfer complexes with their reduced forms in different organic solvents, one could expect a negative deviation from the additivity rule, originating from conformation(s) of the complex with alternately arranged substituents, for which the molecular area occupied by such a complex is lower than the sum of the individual areas. The absence of such contraction in Q-QH$_2$ mixed monolayers can be explained by the fact that the monolayer/water interface where the rings of Q and QH$_2$ reside is highly polar, and at high polarity of the medium such complexes undergo dissociation (especially the alternate conformations) into their components [17].

Mixed monolayers of Q-MGDG and QH$_2$-MGDG type

The isotherms for PQ-9/MGDG and PQH$_2$-9/MGDG are shown in Figs. 2B and 2C, respectively. In all cases (also for PQ(H$_2$)-3/MGDG and α-TQ(H$_2$)/MGDG mixtures, curves not shown), the ideal miscibility is not observed throughout the whole range of ratios examined. For all mixtures a kink point in the isotherms occurs above the collapse pressure value of Q or QH$_2$. The lower Q or QH$_2$ molar fraction in the mixture, the higher is the pressure at which the kink point is observed and it becomes also less evident (Figs. 2B and 2C). These observations indicate that the mixtures examined show miscibility only up to the collapse pressure of the Q or QH$_2$ used, but above this pressure the component with the lower collapse pressure is gradually squeezed out from the monolayer and forms a separate phase overlying the MGDG monolayer.

There was no deviation from the additivity rule (at pressures of complete miscibility) in the case of α-TQ/MGDG, PQ-3/MGDG, PQH$_2$-9/MGDG and α-TQH$_2$/MGDG mixtures as shown in Figs. 4B, 4C, 5A and 5C, respectively. On the other hand, there was apparent positive deviation for the PQH$_2$-9/MGDG mixture (Fig. 4A) and a negative deviation for that of PQH$_2$-3/MGDG (Fig. 5B) at all three surface pressures indicated. The positive deviation can be explained by the difference in the side chain lengths of both components (the PQ-9 molecule is two times longer than MGDG). The part of the PQ-9 molecule which protrudes from the monolayer can freely rotate over MGDG molecules. This rotation also induces partial rotation of the chain parts of PQ-9 that are in contact with MGDG chains. The space occupied by

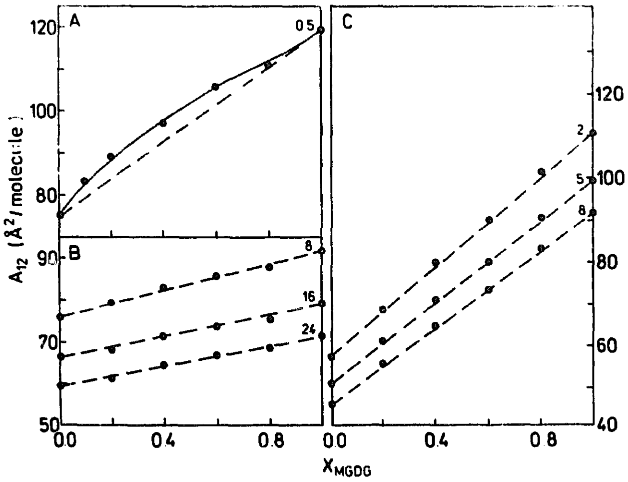

Fig. 4. Mean molecular area ($A_{1,2}$) of the mixed PQ-9/MGDG (A), α-TQ/MGDG (B) and PQ-3/MGDG (C) monolayers as a function of the mole fraction of MGDG at the surface pressures indicated; dashed lines are calculated results assuming the additivity rule; solid circles are the experimental values.

thermal motions of PQ-9 chains increases the molecular area of the whole molecule as compared to the PQ-9 molecule in a pure monolayer. The highest deviation should occur at approximately equal amounts of PQ-9 chains and acyl chains of MGDG, i.e., at molar ratios of PQ-9 to MGDG equal 2, which is consistent with the experimental data obtained (Fig. 4A). A similar phenomenon was found for mixtures of long chain alcohols with different chain lengths [20] and mixed monolayers of ubiquinones with phospholipids [16]. The lack of the analogical effect for PQH_2-9/MGDG mixture (Fig. 5A) may be caused by the association of hydroxyl groups of the PQH_2-9 ring with carbonyl groups of ester bonds in MGDG (hydrogen bridges), which partially can inhibit the rotation of PQH_2-9 molecules.

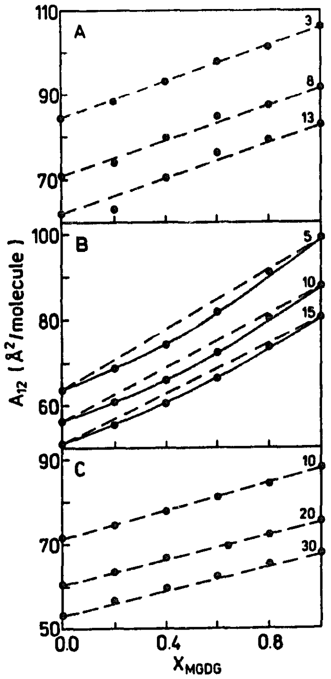

Fig. 5. Mean molecular area ($A_{1,2}$) of the mixed PQH_2-9/MGDG (A), PQH_2-3/MGDG (B) and α-TQH_2/MGDG (C) monolayers as a function of the mole fraction of MGDG at the surface pressures indicated; dashed lines are calculated results assuming the additivity rule; solid circles are the experimental values.

On the other hand, the contraction of PQH_2-3/MGDG monolayers (Fig. 5B) can be connected with hiding of PQH_2-3 molecules under MGDG chains (cavity effect), the latter being about 6 carbon atoms longer than PQH_2-3 molecules. Moreover, MGDG chains at their ends are curved (kinked) because of the presence of *cis* double bonds, which causes the occurrence of empty spaces under the fatty acyl residues of MGDG. However, such a contraction does not occur for PQ-3 mixed with MGDG (Fig. 4C) probably because PQ-3 is not hydrogen bonded to MGDG, therefore it shows higher mobility than PQH_2-3 and this could compensate the hiding effect. Moreover, these two components occupy slightly different positions in the monolayers; PQH_2-3 should be located closer to the subphase than PQ-3 molecules. Absence of such hiding effects for mixed monolayers of MGDG with α-TQ(H_2) (Figs. 4B and 5C) may result from longer and larger α-TQ(H_2) molecules than those of PQH_2-3. The side chains of α-TQ(H_2) must be also more mobile because of their full saturation.

The additivity rule enables us to examine interactions of components in a mixed monolayer only below the collapse pressure of the component with the lower collapse pressure. As previously mentioned, in the Q(H_2)/MGDG mixtures, Q(H_2) was gradually expelled from the MGDG monolayer while increasing surface pressure. It is of interest to determine the proportion of the component that was squeezed out and that remaining in the monolayer at a given surface pressure. To perform such calculations we should know the molecular areas of Q(H_2) molecules at pressures higher than their own collapse pressures. We can calculate approximately these values by extrapolation of Q(H_2) isotherms to higher surface pressures, assuming that they change on increase of external surface pressure in the same manner below and above the collapse pressures. Bearing in mind these assumptions we can approximate Q(H_2) curves by an exponential function:

$$y = A \cdot e^{B \cdot x}$$

and calculate the molecular areas of the investigated compounds above their collapse pressures as shown, for example, for PQ-3 and PQH_2-9 in Fig. 1. As calculated for MGDG the whole isotherm is well approximated by the exponential function. The highest error in extrapolation may occur for PQ-9 because of its low collapse pressure. For the calculations of the percent content (mol%) of $Q(H_2)$ present in the monolayer at a given pressure, we used the following formula:

$$\%\text{cont.} = \frac{b - c \cdot d}{(1-d) \cdot a} \times 100\%$$

where a, b, and c are the molecular areas of $Q(H_2)$, the mixture and MGDG, respectively; d is the molar fraction of MGDG. On the grounds of such calculations one can plot the percent content of $Q(H_2)$ in the monolayer as a function of surface pressure for a given mixture (Fig. 6). For the simplicity of calculations it was assumed that there is no deviations from the additivity rule for all mixtures. As can be noticed from Fig. 6 a gradual removal of Q and QH_2 occurs above collapse pressures of the investigated compounds. The quinones, as possessing less polar groups, are squeezed out more easily from the mixed monolayer than the corresponding hydroquinones, and then the lower the $Q(H_2)$ content, the more difficult are they removed from the monolayer. Comparing Figs. 6A, B and C it is apparent that α-$TQ(H_2)$, as the most polar, undergo ejection at highest pressures, and for $PQ(H_2)$-9 as the least polar, this occurs at the lowest pressures. It is worth mentioning that the difference in percent content between PQ-9 and PQH_2-9 is the highest of all Q-QH_2 systems investigated.

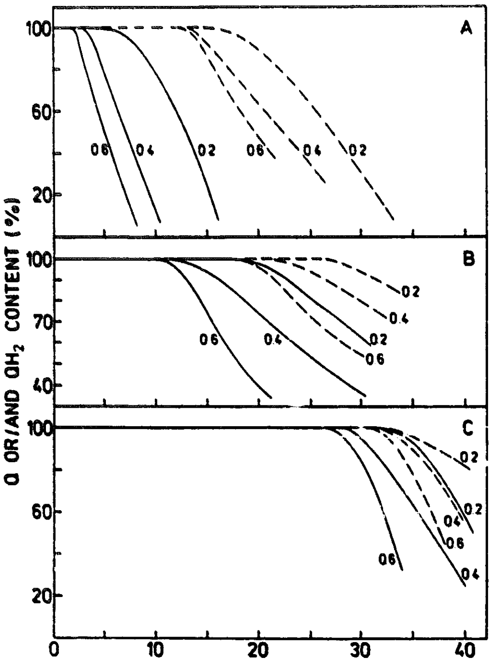

Fig. 6. The percent content of quinone (———) and hydroquinone (— — —) in the monolayer for $PQ(H_2)$-9/MGDG (A), $PQ(H_2)$-3/MGDG (B) and α-$TQ(H_2)$/MGDG (C) mixtures, as a function of surface pressure. The numbers stand for the molar fraction of $Q(H_2)$.

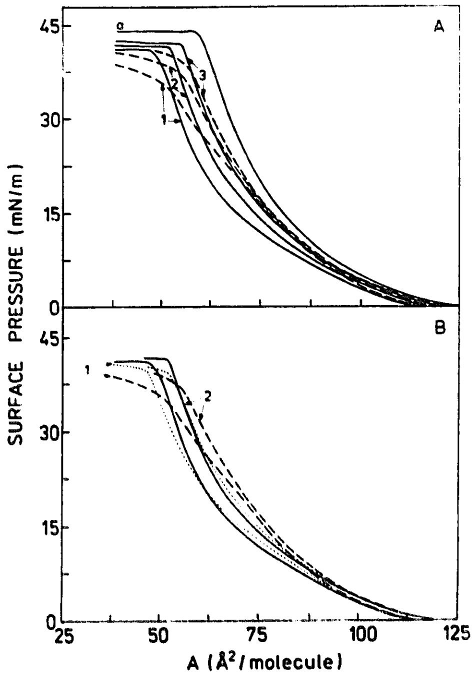

Fig. 7. Surface pressure–area isotherms of PQ-9/MGDG (———), PQH_2-9/MGDG (— — —) and PQ-9/PQH_2-9/MGDG (······) mixtures. In curves 1–3 the molar fraction of MGDG is 0.8, 0.89 and 0.94, respectively. For the three-component mixture, Q/QH_2 molar ratio is 1 : 1.

The surface pressure–area isotherms of $PQ(H_2)$-9/MGDG mixtures at the molar fractions of $PQ(H_2)$ of 0.2, 0.11 and 0.06 show (Fig. 7A) that for both PQ forms a change (although for PQ-9 very slight) in the collapse pressure with the composition change of a mixture can be observed. For the same molar fraction of MGDG the collapse pressure of PQH_2-9/MGDG mixture is in all three cases lower than that of PQ-9/MGDG mixtures, despite the fact that collapse pressure for pure PQH_2-9 is higher than that of pure PQ-9, which indicates the poor miscibility of PQ-9 even at its low content in MGDG monolayer. Similar to PQH_2-9/MGDG mixture, also for $PQ(H_2)$-3/MGDG and α-$TQ(H_2)$ mixtures at low $Q(H_2)$ molar fractions (0.2, 0.11 and 0.06) there could be observed an apparent change in collapse pressures with the change of composition of these mixtures, indicating a miscibility of the mixture components (data not shown). As found for higher $PQ(H_2)$-9 molar fractions, also for their lower contents (Fig. 8), it holds true that the lower the $Q(H_2)$ molar fraction in the mixture, the more difficult it is to remove from MGDG monolayer. At the same content of Q and QH_2, the latter, as a more polar molecule, is more difficult to expel. The same rules also applied to $PQ(H_2)$-3/MGDG and α-$TQ(H_2)$/MGDG mixtures at low (0.2, 0.11 and 0.06) $Q(H_2)$ molar fractions (data not shown).

Charge-transfer interactions in mixed monolayers of Q-QH_2-MGDG type

In Fig. 7B there are compared isotherms of three-component mixtures of PQ-9/PQH_2-9/MGDG with isotherms of PQ(H_2)-9/MGDG binary mixtures with the same molar fraction of MGDG. If there was no interaction between PQ-9 and PQH_2-9 in the monolayer, the three-component isotherm should be the average of the two corresponding Q(H_2)-MGDG isotherms. As can be seen in Fig. 7B, this is not the case. In both three-component mixtures the curve is shifted to lower molecular areas compared to the expected average values. This effect is especially pronounced at higher pressures ($>$ 15 mN/m) and for the mixture of higher PQ-9 and PQH_2-9 molar fractions. This negative deviation can be quantitatively distinguished in Fig. 8. In the case of PQ(H_2)-9/MGDG binary mixtures, because of the relatively high difference in collapse pressures between PQ-9 and PQH_2-9, at some pressures (e.g., 18 mN/m) and the same Q(H_2) molar fractions (0.2), nearly all PQH_2-9 molecules would reside in the monolayer whereas nearly all PQ-9 molecules would be expelled (Fig. 8A). If we have a three-component mixture of PQ-9/PQH_2-9 (1 : 1) with MGDG of total Q and QH_2 molar fraction equal 0.2, the theoretical curve (q in Fig. 8A) should be the average of the corresponding individual curves (0.2 molar fractions) for PQ-9 and PQH_2-9. However, at the higher pressures there are much less molecules left (mainly PQH_2-9) in the monolayer than expected. For example at 22 mN/m theoretically 40% of all molecules should still be in the monolayer (as PQH_2), however nearly complete ejection of these molecules from the monolayer is observed. The presence of Q lowers the content of QH_2 in the monolayer probably by charge-transfer interaction, which is especially active in hydrophobic solvents [17]. There was observed an increased solubility of PQH_2-9 and α-TQH_2 in hexane upon the presence of corresponding quinones [14]. A similar effect in monolayers can explain the drawing in of PQH_2-9 by PQ-9 molecules into the hydrophobic region (acyl side chains of MGDG) of the monolayer, which is caused by charge-transfer interaction. The same behavior is also observed at lower PQ(H_2)-9 concentrations (Fig. 8B), but the deviation from the theoretical curve (q) is already observed at lower surface pressures. For PQ-3/PQH_2-3/MGDG and α-TQ/α-TQH_2/MGDG mixtures such an effect was relatively very low and occurred only at high pressures ($>$ 30 mN/m) (data not shown).

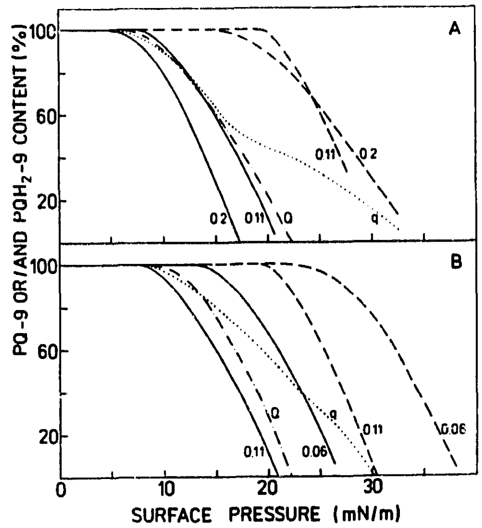

Fig. 8. The percent content in the monolayer of PQ-9 (———), PQH_2-9 (— — —) and PQ-9 plus PQH_2-9 (·–·–·) for their mixtures with MGDG as a function of surface pressure. The numbers stand for the molar fraction of PQ(H_2)-9 in the mixture. In the three-component mixture (Q) the molar fractions of PQ-9, PQH_2-9 and MGDG are 0.1, 0.1 and 0.8, respectively (A), and 0.055, 0.055 and 0.89, respectively (B). q (······) stands for the theoretical shape of the curve Q.

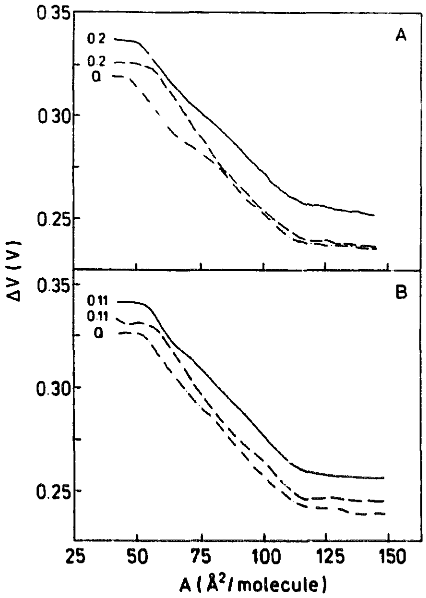

Fig. 9. The surface potential-area isotherms of PQ-9/MGDG (———), PQH_2-9/MGDG (— — —) and PQ-9/PQH_2-9/MGDG (·–·–·) mixtures. The numbers stand for the molar fraction of PQ(H_2)-9. In the tree-component mixtures (Q) the molar fractions of PQ-9, PQH_2-9 and MGDG are 0.1, 0.1 and 0.8, respectively (A) and 0.055, 0.055 and 0.89, respectively (B).

The surface potential for PQH_2-9/MGDG mixtures (Figs. 9A and B) increases more quickly than that of PQ-9/MGDG mixture below approximately the same molecular areas as the rejection of PQH_2-9 from the monolayer starts. This additional increase in surface potential of PQH_2-9/MGDG mixture is probably connected with the change of the surface potential of PQH_2-9 (which for pure compounds is lower than that of PQ-9 and MGDG), which in an apolar (hydrophobic) environment (out of the monolayer) may have higher surface potential than in a polar medium (mixed in the monolayer). Upon the additional presence of

PQ-9, surface potential of such a mixture (Q in Fig. 9) does not show any abrupt increase, which may indicate that PQH$_2$-9 on contact with PQ-9 (in the form of a charge-transfer complex) behaves as if it was in a more polar environment. This is in agreement with observations that the dipole moment of a charge-transfer complex is higher than the sum of their component dipole moments [21].

The measurements of charge-transfer interactions at still lower Q(H$_2$) than the above applied (more physiological) faced difficulties connected with the fact that the expected small differences between individual isotherms would lie within the experimental error of the method.

Although the concentrations of Q(H$_2$) used in this study are higher than those in the native systems (lipid to PQ-9 molar ratio of about 100 and for α-TQ even higher), the above presented results can give us some information about possible localization and orientation of the examined quinones and hydroquinones in native membranes, bearing in mind the limitations of an analogy between a monolayer model system and the thylakoid membrane which differ from each other, e.g., by the presence of proteins and central hydrophobic region of a bilayer in a native membrane. Nevertheless, the general conclusions obtained from the monolayer study should be useful in determination of prenylquinone(ol) properties in native membranes.

Both PQ(H$_2$)-3 and α-TQ(H$_2$) would have at native surface pressures (approx. 30–40 mN/m [22]) and natural concentrations their headgroups in contact with the surface of membranes and with their isoprenoid side chains arranged rather parallel to glycolipid acyl chains. PQH$_2$-9 would occupy a similar position, but with a tendency to penetrate deeper regions of the thylakoid membrane. Its long chain would make it difficult to arrange parallel to glycolipid molecules. On the other hand, PQ-9 would mainly reside in the hydrophobic interior with its side chain oriented rather parallel to the membrane surface, but with partial ability to penetrate the surface regions of the thylakoid membrane where its reduction by photosystem II takes place [2]. Such a PQ-9 localization is consistent with results of other experiments on its location in thylakoid membranes [1,5,12] but different localization of PQH$_2$-9 in comparison with PQ-9, as show the above presented results, was only recently suggested [14,23]. The increased penetration of PQH$_2$-9 into deeper regions of the membrane under the influence of PQ-9 would be of great importance since it explains the high diffusion rate of PQH$_2$, which is necessary to perform its function as a proton shuttle. Such efficient diffusion is especially effective in the hydrophobic part of the membrane and it would be facilitated by charge-transfer interactions between PQ-9 and PQH$_2$-9. Moreover, the efficient transversal diffusion of plastoquinol is required to its functioning as a proton pump across the thylakoid membrane.

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