Chemistry and Physics of Lipids 155 (2008) 24–30

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Differences in surface behaviour of galactolipoids originating from different kind of wheat tissue cultivated *in vitro*

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ARTICLE INFO

Article history:
Received 28 February 2008
Received in revised form 12 June 2008
Accepted 20 June 2008
Available online 2 July 2008

Keywords:
Langmuir monolayer
Biomembrane
Galactolipid
In vitro culture

ABSTRACT

The aim of presented researches was to investigate the physicochemical properties of Langmuir monolayer of galactolipids extracted from two different kinds of plastids: immature embryos and inflorescences. Differences between the physicochemical properties of the plastid membranes may help to explain different physiological processes, such as plant regeneration. Surface pressure (π) vs. molecular area (A) isotherms of the monogalactosyldiacylglycerol (MGDG)/digalactosyldiacylglycerol (DGDG) monolayers of various molar ratios were measured at 15 °C. Galactolipids were extracted from two different types of tissue: inflorescences and embryos. Based on the analysis of the π–A isotherms, the properties of monolayers, such as collapse pressure (π_{coll}), limiting area (A_{lim}), compressibility modulus (C_s^{-1}), excess free energy of mixing (ΔG^{EXC}) and free energy of mixing (ΔG_{MIX}), were calculated. The results show that pure MGDG and DGDG and their mixtures form liquid-expanded monolayers, independently on the kind of tissue. Galactolipids originating from inflorescences produce more compressible films at the air/water interface, with larger limiting area per molecule and lower stability against the collapse process. MGDG and DGDG are miscible and form non-ideal mixed monolayers at the air/water interface. Negative values of ΔG^{EXC} were calculated for the mixture of galactolipids originating from inflorescences, with the content of MGDG, $x_{\mathrm{MGDG}} > 0.6$. In the case of embryos, the negative values of ΔG^{EXC} were found for $x_{\mathrm{MGDG}} \approx 0.5$. Therefore, the attractive interactions between MGDG and DGDG exist in the mixtures of these compositions. As it is shown by negative values of ΔG_{MIX}, mixed monolayers are more stable compared with unmixed ones.

1. Introduction

The generation of whole plants from selected tissues is a requirement for genetic engineering-based improvement (Delporte et al., 2001). As one of the most important food crop species, wheat has been extensively investigated with respect to plant regeneration from *in vitro* culture. Regeneration of wheat plants has been achieved mainly from explant sources such as immature embryos (Maës et al., 1996) and immature inflorescences (Sharma et al., 1995). The highest rates of callus induction and plant regeneration of wheat have been obtained from the culture of immature embryos (Varshney et al., 1996). This effect was observed during practical preparation of *in vitro* cultures but mechanism of this preference in immature embryos regeneration relatively to other was not studied in detail. Many factors can affect wheat regeneration, such as explant source, donor plant growth conditions, culture medium and genotype, among which genotype is often the dominant one. In tissue culture of cereals including wheat, 2,4-dichlorophenoxyacetic acid (2,4-D) is often used for callus induction (Zheng and Konzak, 1999; Li et al., 2003). Tissue cultures of winter wheat initiated from young inflorescences and immature embryos possess the potential for regeneration of whole plants. Both types of callus used in this work were produced on Murashige and Skoog's medium with 2 mg/l 2,4-D. Centres in which the peripheral cells cease dividing, elongate and could be easily separated. Such callus had a smooth surface and characteristic chlorophyllous areas. As it was mentioned earlier, immature embryos are known to be the best explant for efficient regeneration from callus culture. However, it is difficult to obtain immature embryos throughout the year (Özgen et al., 1998). Donor plants must be grown year-round for a continuous supply, requiring greenhouse space and demanding extra labour and expense.

Among others, the visible effect of callus culture regeneration is plastid differentiation into chloroplasts. The plastid is the site for a number of biosynthetic functions essential to plants, including photosynthesis, nitrogen assimilation and the synthesis of amino acids, as well as the synthesis of starch, fatty acids and numerous secondary products. These diverse biochemical

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doi:10.1016/j.chemphyslip.2008.06.004

functions rely on the controlled exchange of metabolites between the plastid and the surrounding cytosol (Fischer and Weber, 2002). Increase in interest in plastids connected with their function in cells led to investigation of their structure and composition. Plastids are double-membrane organelles. Monogalactosydiacylglyceride (MGDG) and digalactosyldiacylglyceride (DGDG) are the major lipids of plastids. Independently on plant tissues, they contain about 50 mol% of MGDG together with smaller amount of DGDG and both plastid galactolipids are highly polyunsaturated (Yamamoto, 2006). The galactolipids MGDG and DGDG carry one or two galactose moieties, respectively, linked to diacylglycerol (Kelly and Dőrmann, 2004). Owing to its small head group, MGDG has a cone-like geometry, with galactose at the tip and the two fatty acyl chains oriented towards the base of the cone. Therefore, the most stable phase that MGDG adopts in aqueous *in vitro* mixtures is the hexagonal-II (HII) phase, with the polar head groups facing towards the centre of micellar or tube-like structures. For this reason, MGDG belongs to the so-called non-bilayer lipids in mixtures with water. In contrast to MGDG, DGDG, which contains two galactose moieties in its head group, has a more cylindrical shape and forms bilayers, i.e., lamellar liquid-crystalline structures (Lα) in mixtures with water. As in all organisms, the ratio of non-bilayer-forming to bilayer-forming lipids is crucial for protein folding and insertion as well as for intracellular protein trafficking (Lee, 2000; Dörmann and Benning, 2002; Goss et al., 2007). In model artificial membranes, the ability of mixtures of mono- and digalactosyldiacylglycerol to form paracrystalline arrays, subsequently identified as bicontinuous cubic phases, is well established (Sen et al., 1982; Lindblom and Rilfors, 1989). However, NMR studies suggest that such structures are stable only over limited ranges of temperature and hydration (Brentel et al., 1985), which indicates the possibility of modification of plastid membranes by different external and internal factors. Thus, physicochemical properties of monolayers and bilayers built from lipids extracted from biological cells can be the source of information for investigation of the mechanism of different physiological processes. Our earlier results (Filek et al., 2005) proved that MGDG and DGDG form monolayers on water surface. Similar observations were reported by Bottier et al. (2007). The authors focused on the physical properties of mixtures of hydrated MGDG and DGDG found in wheat flour. They noticed that both galactolipids form monolayers exhibiting a structure of homogeneous liquid-expanded phase in agreement with the high degree of double bonds in the acyl chains that limits molecular packing. Kooijman et al. (1998) studied the influence of puroindoline-a (PIN-a) injected into the subphase on the MGDG monolayer. They found that injection of PIN-a caused an increase of galactolipid monolayer surface pressure. The data were interpreted as incorporation of PIN-a molecules into the MGDG film. In our last study, we examined the influence of auxin, the plant hormone important in wheat plant regeneration process, on surface properties of galactolipids isolated from plastids (Filek et al., 2005). The aim of the present experiments is to investigate the physicochemical properties of monolayers built from galactolipids extracted from plastids of immature embryos and inflorescences. These parameters could be helpful in studies of differences between regeneration effectivity of these explants.

2. Materials and methods

MGDG and DGDG were isolated from winter wheat cells of calli cv. Kamila, as described previously (Filek et al., 2005). Callus cells were initiated from two types of wheat tissue, i.e., immature inflorescences and embryos. Both cultures were in the non-embryogenic stage. The percentage composition of fatty acid (mol% of total fatty acids) of MGDG and DGDG extracted from (a) inflorescences—MGDG: 35.50% 16:0 (palmitic acid), 25.00% 18:0 (stearic acid), 6.10% 18:1 (oleic acid), 24.40% 18:2 (linoleic acid), 9.00% 18:3 (linolenic acid); DGDG: 34.00% 16:0, 5.00% 16:1 (palmitoleic acid), 15.10% 18:0, 11.80% 18:1, 23.10% 18:2, 11.00% 18:3; (b) embryos—MGDG: 46.63% 16:0, 1.43% 16:1, 37.59% 18:0, 7.30% 18:1, 7.05% 18:2; DGDG: 30.53% 16:0, 9.33% 16:1, 31.62% 18:0, 14.91% 18:1, 7.32% 18:2, 6.29% 18:3. Chloroform and methanol were purchased from Sigma–Aldrich.

The surface isotherms of the galactolipids at the air–water interface, i.e., the plots of surface pressure (π) vs. the mean molecular area (A), were measured with a Langmuir trough system, KSV 1000 (KSV, Helsinki, Finland) equipped with two moving barriers and platinum Wilhelmy plate. As subphase, distilled water was used. The purity of the bare water surface was checked before each measurement by a maximum compression. On the basis of our *in vitro* experiments we have found that the optimum temperature for regeneration process of winter wheat cv. Kamila is 15 °C. Therefore, the monolayer experiments were also conducted at that temperature. The temperature of the water subphase was maintained constant (15 ± 0.05 °C) using a circulating water bath system. Galactolipids were dissolved in 4:1 (v/v) mixture of chloroform and methanol and spread onto the water surface using a Hamilton microlitre syringe. Mixed solutions containing different mole fractions of MGDG (0, 0.2, 0.4, 0.5, 0.6, 0.8 and 1) were prepared from calculated volumes of respective stock solutions. Approximately 20 min were allowed for the solvent to evaporate. The compression rate was 6 mm/min. The experimental setup was enclosed in a box for constant humidity and minimization of surface contamination. All isotherms were recorded at least twice. The measurement accuracy was ±0.01 mN/m for surface pressure and ±0.01 $Å^2$/molecule for the area.

3. Results and discussion

Fig. 1 presents π–A isotherms for pure MGDG and DGDG and mixed MGDG/DGDG monolayers extracted from inflorescences, at 15 °C. Surface pressure increases monotonically until it started to collapse at π = 35.1 and 42.0 mN/m for MGDG and DGDG, respectively. No phase transition is detectable. Compared with MGDG monolayer, the stability of pure DGDG and mixed monolayers are enhanced, as attested by their higher collapse pressures. This may be explained by the greater ability of DGDG headgroup to form hydrogen bonds with the water beneath the monolayer. The slope of the isotherm, which is proportional to the compressibility modulus of the monolayer, systematically increases with increasing DGDG fraction in mixtures. The limiting area can be obtained by extrapolating the slope of the π–A isotherm at high surface pressure to zero pressure. The point at which this line intersects the x-axis gives the hypothetical area occupied by one molecule in the condensed phase (Li et al., 2007). The limiting areas for the pure MGDG and DGDG extracted from inflorescences are 70.46 and 77.27 $Å^2$/molecule, respectively. With the increase of mole fraction of DGDG in mixed monolayers, an increase in area per molecule is observed (Fig. 2). Isotherms show lift-off (the first increase in the surface pressure) at molecular areas between 100 and 115 $Å^2$. The lift-off areas indicate the emergence of a uniform LE phase from the gas/LE coexistence.

Fig. 3 shows π–A isotherms of galactolipids originating from embryos. Isotherms have trends similar to those recorded for inflorescences. However, the collapse pressure values are higher (39.8 and 42.3 mN/m for MGDG and DGDG, respectively) indicating a greater stability against the collapse process of galactolipid monolayer. The increased stability of the monolayer can be attributed to the greater content of saturated chains present in galactolipid fraction extracted from embryos. Since galactolipids derived from embryos contain smaller amount of unsaturated fatty