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Short communication

Langmuir monolayers of fractions of cork suberin extract

A. Barros-Timmons[a,*], M.H. Lopes[a], C. Pascoal Neto[a], A. Dhanabalan[b], O.N. Oliveira Jr.[b]

[a] *CICECO and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal*
[b] *Instituto de Física de São Carlos, U.S.P., CP 369-13560-970 São Carlos, SP, Brazil*

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ABSTRACT

The wide variability in composition and molecular weight of natural polymers has hampered understanding of their physicochemical properties and ultimately their use in noble applications, especially in the cases where surface properties need to be probed at the molecular level. A useful approach to analyse data from surface monolayers of complex mixtures is to try distinguishing the effects from the distinct fractions in such mixtures. The cork suberin extract investigated here is known to contain aliphatic monomers with terminal carboxylic acid and methyl ester groups as well as long esterified aliphatic chains dispersed in a polymeric aliphatic matrix. The role of such terminal groups was studied and the results showed that depending on the nature of the terminal groups the monolayers present distinct isotherms due to the different interactions with the water subphase. Fractionation strategies based on different solubilities of the cork suberin components in chloroform were also employed to probe their effect on the monolayer characteristics. From the two sets of experiments it is clear that the presence of monomers with terminal carboxylic acids in the suberin extract affects considerably the monolayer-forming ability. This approach may be used as a complementary, relatively simple route to assess suberin genetic engineering strategies towards resistance to environmental stress.

1. Introduction

There has been a search in recent years for more noble applications of natural polymers and macromolecules motivated by the increasing need to develop environmentally-friendly processes and products, which is illustrated by extensive research into cellulose, chitosan, lignins, suberins and humic substances, to name just a few [1–3]. A variety of properties can be exploited in such materials, including biodegradability and biocompatibility, but an obvious difficulty, particularly for those having complex structures, lies in the need to establish structure–function relationships, particularly at the molecular level. In this context, the Langmuir monolayer technique, which is normally used to study monolayers at the air–water interface of synthetic amphiphilic polymers and fabricate Langmuir–Blodgett (LB) films for a variety of applications [4,5], has also been used to study complex natural polymers such as lignin and cellulose [6–9]. Langmuir films, however, have not been used in studies of suberin extracts – to the best of our knowledge. Suberin is the main component of cork, the outer bark of *Quercus suber* L. tree, contributing to about 40% of its dry weight [10]. Although the structure of suberin is not yet fully understood, it is now established that it is a biopolyester composed mainly by aliphatic chains and minor amounts of aromatic structures such as ferulic acid [11–14]. A structural model proposed by Lopes et al. for cork suberin and its organisation in cork cell wall is shown in Fig. 1 [11]. More recently, Graça and Santos proposed a model in which long alkyl chains would be anchored to polyaromatics [15]. *In situ* suberin is a macromolecular network insoluble in all solvents. It can however be decrosslinked by chemical processes *via* cleavage of esters bonds [10,11,16,17]. The most common method involves alkaline alcoholysis.

Previous work carried out in our laboratory indicated that cork suberin extracts consist of a complex mixture containing *ca.* 30% of monomeric compounds such as aliphatic ω-hydroxy acids, diacids and fatty acids, with chain lengths of C16–C26 (see Table 1), and a remaining fraction of higher molecular mass structures (>1000 g mol^{-1}) [11,16]. Moreover, microscopic studies using polarised light showed that a substantial portion of cork suberin extract has a microcrystalline character which confers its viscous behaviour that is both plastic and tixotropic at room temperature [18]. This behaviour is thought to be due to the interactions between the molecules in the liquid phase and the microcrystals suspended in it [18]. These features may find application as property-enhancing additives in the optical quality of coating surfaces or as rheology modifiers [19]. Other applications for suberin or knowledge derived from suberin depolymerisation studies may include the use in adhesives, printing inks [20], building blocks for polymers with novel architectures [21], improvement of resistance

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* Corresponding author. Tel.: +351 234370708; fax: +351 234370084.
E-mail address: anabarros@ua.pt (A. Barros-Timmons).

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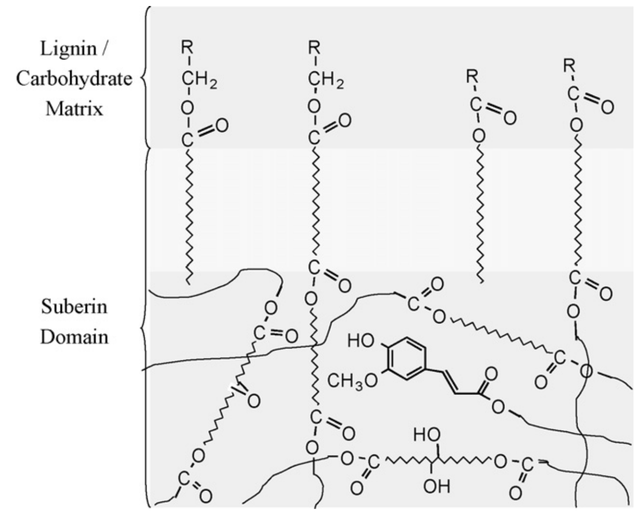

Fig. 1. Proposed model for the structure of cork suberin [11].
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of crop plants [22,23] and as anti-ageing, smoothing product for the human skin [24].

Understanding the surface properties of suberin extract may therefore be rewarding not only in terms of furthering knowledge of its physicochemical properties but also of allowing new applications. Due to the amphiphilic nature of the suberin extract the Langmuir monolayer technique appears to be promising to obtain further insight into its surface properties at the molecular level, which has prompted us to report the monolayer results to be presented here. We build upon our experience with lignins, in which we found essential to identify the contributions from the various material components to the properties of the final film properties [8]. Accordingly, we have manipulated the suberin extract with chemical treatments and fractionation methods to obtain components that were rich in carboxylic acids or methyl esters, of different molecular weights, and investigated the film properties of the suberan, a high molecular weight non-hydrolysable aliphatic fraction of suberin [25]. The main purpose of the present work is therefore to identify which components of suberin are responsible for its surfactant properties.

2. Experimental

The methods used for cork suberin extraction were already described elsewhere [11]. Basically it involves the depolymerisation of suberin with a dry methanolic solution of sodium methoxide followed by acidification and extraction of the depolymerisation products with chloroform. This extract is hereafter designated as “suberin extract”. The identification and quantification of suberin monomers was carried out by gas chromatography in tandem with mass spectrometry (GC–MS) of the methyl ester trimethylsilyl ether derivatives, as described in detail elsewhere [11]. Gel permeation chromatography (GPC) was performed with a Waters Instrument connected to a Model 440 Absorbance Detector at 254 nm. A PLGel (5 μ) pre-column was followed by three PLGel columns series: 10^3 Å (5 μ); 500 Å (10 μ); 100 Å (10 μm). Tetrahydrofuran (THF) was used as eluent with a flow rate of 1 ml min^{-1} at 25 °C. Polystyrene (68,000, 28,000, 12,500, 7600, 3700, 1800, 1050, 580, 160) and ethyl benzene (106) were used as standards. Ultrapure water supplied by a RO60 Millipore filter connected to a Milli-Q system was used to prepare the subphase solution with $CdCl_2$, 4×10^{-4} M, whose pH was maintained at 6.0 by addition of a sodium bicarbonate solution (5×10^{-5} M). The resistivity of the ultrapure water was 18.2 MΩ cm, while its surface tension was 73.2 mN m^{-1}. Monolayer studies were carried out in a Nima-611D trough (with dimensions of 25 cm vs 20 cm) placed in a class – 10 000 clean room at 22 °C and relative humidity of 60%. Suberin extracts and its fractions were dissolved in a mixture of THF (Merck, 88%) and chloroform (Merck, HPLC grade) (4:96 by volume). The typical concentration used was about 0.4 mg/ml. The solutions were spread at the water surface and upon evaporation of the solvent (during 5 min) the monolayers were compressed at 30 cm^2/min. The surface pressure was measured with a Wilhelmy plate. In the surface pressure (π_c) vs area isotherms, the mean molecular area axis is based on the weight average molecular masses (M_w) determined by GPC. In Fig. 4 the area of the mixed monolayer was calculated using the M_w of sample **5** and the M_w of stearic acid (octadecanoic acid) which were both determined by GPC for the purpose of comparison. These components were used in a ratio of 50:50.

Table 1
Average monomeric composition of suberin extract obtained by alkaline methanolysis of cork (as for sample **1**). Determined by GC–MS as % area of the methyl ester trimethylsilyl ether derivative peaks [11].

Monomers of the suberin extract	%
Alkanoic acids (C16–C24)	2.6
α,ω-Alkanedioic acids (C16–C24)	17.3
ω-Hydroxyalkanoic acids (C16–C24)	34.2
Mid-chain substituted ω-hydroxyalkanoic acids (C16–C20)	29.6
Mid-chain substituted alkanedioic acids (C18)	10.3
1-Alkanols (C16–C26)	4.7
Ferulic acid	1.3
Yield of GC–MS analysis (w/w)	29

3. Results and discussion

The rationale behind the choice of experiments with Langmuir monolayers of suberin was based on the finding that for complex natural polymers important information is obtained from the effects of different functional groups comprising the polymer [8]. We have therefore manipulated the suberin extract to be able to identify these effects, as will be explained below.

The suberin extract, referred to as sample **1**, was obtained from alkaline methanolysis of a cork sample previously treated with organic solvents to remove the very low molecular mass compounds, as described elsewhere [11]. The alkaline methanolysis of cork should yield a mixture of aliphatic monomers containing terminal free carboxylic acids and terminal methyl esters since the methanolysis conditions were not completely anhydrous. In addition, it was shown previously that under the conditions used the depolymerisation of suberin was not completed and some of the suberin long aliphatic chains in the extract consist of oligomers linked *via* ester bonds [26]. The GC–MS analysis showed that 90% of sample **1** correspond to alkanedioic acids and hydroxyalkanoic acids (see Table 1).

In order to probe the effect of the terminal methyl ester and carboxylic acid groups at the air–water interface, a portion of the suberin extract was hydrolysed using H_2SO_4/methanol to yield sample **2**, and another portion was methylated using diazomethane to produce sample **3**. These samples were characterised by FTIR spectroscopy. The FTIR spectrum of sample **1** showed a strong broad band with a shoulder, between 1730 and 1708 cm^{-1} (δ C=O, $COOCH_3$ and COOH), while the spectrum of sample **2** showed a small band at 1730 cm^{-1} and a sharp intense band at 1708 cm^{-1} (δ COOH) which shifted to 1511 cm^{-1} (δ COO^-) upon treatment with $N(CH_2CH_3)_3$. This confirms that the latter band was due to carbonyl groups of carboxylic acids. Finally, the FTIR spectrum of sample