Colloids and Surfaces B: Biointerfaces 135 (2015) 639–645

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

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

Algal polysaccharides as matrices for the immobilization of urease in lipid ultrathin films studied with tensiometry and vibrational spectroscopy: Physical–chemical properties and implications in the enzyme activity

Audrey Kalinouski de Brito, Cristina S.F. Nordi, Luciano Caseli*

Instituto de Ciências Ambientais, Químicas e Farmacêuticas, Universidade Federal de São Paulo, UNIFESP, Diadema, SP, Brazil

ARTICLE INFO

Article history:
Received 30 April 2015
Received in revised form 19 August 2015
Accepted 20 August 2015
Available online 22 August 2015

Keywords:
Exopolysaccharides
Langmuir
Langmuir–Blodgett
Urease
Enzyme activity
Air–water interface
Monolayers

ABSTRACT

Currently, many biological substances extracted from algae have received special attention because of their intrinsic characteristics, which can be applied to different areas of biotechnology. Therefore, in the current study, exopolysaccharides (EPS) from the microalgae *Cryptomonas tetrapirenoidosa* were employed as an aqueous subphase of a monolayer formed by the lipid dioctadecyldimethylammonium bromide (DODAB). The primary objective of this approach was to evaluate whether EPS could serve as a matrix for the immobilization of the enzyme urease to produce biosensors for urea. After DODAB was spread on the EPS solutions, urease was injected into the aqueous subphase, and the surface was submitted to compression using lateral barriers. The monolayers were subsequently characterized by surface pressure-area isotherms and polarization modulation infrared reflection-absorption spectroscopy (PM-IRRAS). The results indicated that EPS enhanced the adsorption of the enzyme on the lipid monolayer. The mixed films were later transferred to solid supports using the Langmuir–Blodgett (LB) technique and were characterized by transfer ratio, PM-IRRAS, quartz crystal microbalance, and atomic force microscopy. The immobilization of the enzyme on solid supports was fundamental for providing an ideal geometrical accommodation of urease because the interaction of EPS with urease in solution causes a decrease of the relative activity of urease. Therefore, these LB films are promising for the fabrication of future urea biosensors, the architecture of which can be manipulated and enhanced at the molecular level.

1. Introduction

Microalgae are present in several aquatic environments around the world. Due to their intrinsic characteristics, it is possible to use their excreted organic matter for biotechnological purposes. These organisms have been employed in the food industry as stabilizers and thickeners [1] and in the pharmaceutical industry for the production of molding materials and anti-coagulants [2,3].

These applications are possible due to the advantages offered by this type of organism compared with other materials, such as ease of cultivation and availability of isolated clones, which can enable the production of a homogeneous material appropriate to use to obtain cells isolated from other biological cultures [4,5].

Moreover, the ability of excreted systems obtained from *Cryptomonas tetrapirenoidosa* to interact with lipids forming a monolayer at the air–water interface has been recently reported [6]. The excreted system is composed of exopolysaccharides (EPS), and because they are negatively charged, they are successfully adsorbed on positively charged lipids. Sequential studies on this issue were performed by transferring the mixed EPS-lipid monolayer from the air–water interface to solid supports using the Langmuir–Blodgett (LB) technique [7]. This transfer enabled the investigation of the mixed film as a system able to recognize ions, which was inspired by the fact that it is reported that EPS can remove certain metallic ions from aqueous environments [8].

Another interesting property of EPS that can be explored is their eventual ability to serve as a matrix for the immobilization of enzymes. Particularly, it has been reported that polysaccharides serve as a facilitator for enzymes not only in terms of a matrix for their immobilization but also to better conserve their biological activity [9]. Therefore, EPS are interesting compounds for use in for the conservation of the activity of biomolecules and for the construction of matrices for the immobilization of enzymes. They are

* Corresponding author.
E-mail addresses: lcaseli@unifesp.br, lcaseli@gmail.com (L. Caseli).

http://dx.doi.org/10.1016/j.colsurfb.2015.08.033
0927-7765/

also interesting because they offer the possibility of using a natural biological surface.

Among the enzymes able to be part of a biosensor, urease is of interest because quantification of urea is important in different areas, such as in medicine, where the detection of urea in blood serum may indicate certain disorders, such as renal diseases [10–12]. The detection of urea is also of interest for environmental purposes because urea is considered a pollutant in some aquatic ecosystems [13]. Urea is important in agriculture and in the food industry because it is present in nitrogen-release fertilizers [14] and is considered a contaminant in dairy products [15].

However, the successful construction of a biosensor requires several steps in which the choice of the biological component, as well as its characterization and adjustment, is important. A key step for the construction of a biosensor is the immobilization of the biological recognition element on a suitable substrate to maintain its biological activity. Therefore, the construction of a urea biosensor with the enzyme accommodated in nanostructured systems would require a detailed study on methods to immobilize the biomacromolecule. In this regard, urease has been immobilized in lipid LB films [16] and in mixed carbon-nanotube/lipid LB systems [17]. The use of LB films for biosensing has been employed in the last decades, exploiting the high molecular control provided by this technique. Enzymes confined in bio-inspired and bio-based nanostructured systems present benefits related to enzymes immobilized in disorganized matrices whose efficiency as a sensor is reduced [18,19]. Furthermore, the use of Langmuir and Langmuir–Blodgett films of lipids has been reported as an excellent strategy to conserve the structure of biomolecules [20–25].

In the present work, the enzyme urease was co-immobilized with EPS in a positively charged lipid monolayer, providing an enzyme–polysaccharide–lipid LB film. The presence of the positive lipid was necessary to construct a matrix composed of insoluble materials at the air–water interface, allowing the incorporation of polysaccharides and enzyme. The relative activity of urease was then evaluated and compared with other systems. The primary intention was to construct a device capable of detecting urea, maintaining or enhancing the catalytic activity of the immobilized enzyme using EPS.

2. Materials and methods

2.1. Obtaining EPS from C. tetrapirenoidosa

The excreted exopolysaccharide system from the culture of the microalgae *C. tetrapirenoidosa* (EPS) was prepared according to the literature [26]. The culture was submitted to centrifugation, filtration using a hollow fiber cartridge that allows the separation of the high molecular size polysaccharide, dialysis and lyophilization to obtain a pellet ready to be dissolved in purified water (Milli-Q® system from Millipore – pH 5.6; resistivity 18 MΩ cm^{-1}) and to be further used as part of the aqueous subphase of lipid Langmuir films. All of the materials from the microalgae were obtained from the same, certifiably clean and axenic inoculum from a single cell, which was obtained from the Barra Bonita Reservoir in the state of São Paulo, Brazil, thus maintaining the quality assurance and uniformity of the results. The composition of the extract was described in the literature [27] as 29.3% glucose, 24.2% mannose, 21.9% rhamnose, 7.8% xylose, 6.6% glucuronic acid, 5.6% fucose, 2.0% galactose, 1.8% galacturonic acid, and 0.8% arabinose.

2.2. Langmuir films

The Langmuir and Langmuir-Blodgett films were fabricated using a mini-trough (KSV Instruments) equipped with the following: (i) two mobile barriers capable of sweeping the aqueous surface; (ii) a filter paper intercepting the air–water interface to measure the surface tension using the Wilhelmy method; (iii) a device for the deposition of Langmuir–Blodgett (LB) films; and (iv) a polarization modulation infrared reflection–absorption spectrometer (PM-IRRAS) from KSV Instruments.

The cationic lipid dioctadecyldimethylammonium bromide (DODAB), obtained from Sigma–Aldrich with a purity above 99%, was dissolved in chloroform (Synth) to reach concentrations of approximately 0.6 mg/mL. For the experiments in which EPS were present, the polysaccharide extract was used as the subphase solution for the lipid monolayers, with a final concentration of 0.5 mg/mL and a pH of 6.2. The effects of the opposite charges between the components of EPS (negatively charged) and DODAB (positively charged) were analyzed previously [6], which showed a successful interaction between the lipids and the polysaccharides. Urease, obtained from jack beans (Sigma–Aldrich), was dissolved in ultrapure water to render a concentration of 0.5 mg/mL.

Aliquots of 25 μL of DODAB were carefully spread on the air–water interface drop-by-drop. At least 10 min was allowed for solvent evaporation. DODAB has negligible solubility in water and forms Langmuir monolayers at the air–water interface. From the experiments in which urease was present, an aqueous solution of the enzyme (0.5 mg/mL) was inserted into the aqueous subphase after the monolayer formation at the surface pressure of 0 mN/m. As a result, urease is incorporated in the monolayer by diffusion from the subphase. Films containing algal material were prepared, filling the trough with the EPS solution (0.5 mg/mL) as the aqueous subphase. Surface pressure–area (π–A) isotherms were obtained by compressing the air–water interface at a rate of 4 $Å^2$ lipid $molecule^{-1}$ min^{-1}. Monolayers were also characterized by PM-IRRAS (KSV Instruments Ltd., Helsinki, Finland) at an incidence angle of 80° to the normal, at which the intensity is maximized and the noise level is the lowest. Selected surface pressure values were chosen for each spectrum. Further explanation of this technique and the methods employed are described elsewhere [28].

The water employed in these experiments was purified with a Milli-Q® system from Millipore (pH ~6.2), and the temperature for all of the experiments was 25 ± 1 °C. All of the other reagents employed in this study were of the highest purity available.

2.3. Langmuir–Blodgett films (LB)

To produce the LB films, Langmuir monolayers were compressed until the surface pressure reached 30 mN/m. This value of surface pressure was maintained constant using mobile barriers, and then a solid support (glass, quartz or silicon), previously immersed in the aqueous subphase, was removed from it towards the air phase, passing vertically to the plane of the monolayer. The success of the transfer of the monolayer from the air–water interface to the solid support could be demonstrated by the transfer ratio (TR), which is the ratio between the area swept by the barriers to maintain the monolayer surface pressure constant and the solid support area in contact with the air–water interface during the procedure. For an ideal transfer, a TR value of approximately 1.0 is desirable. For all of the data shown here, TR was 1.0 ± 0.1, revealing the quality of the films. The characterization of the LB films was conducted with PM-IRRAS (KSV Instruments Ltd., Helsinki, Finland) at an incidence angle of 80° to the normal. Atomic force microscopy (AFM) was also employed for further characterization, and the height images were obtained in the tapping mode, employing a resonance frequency of approximately 300 kHz, a scan rate of 1.0 Hz, and scanned areas of 5.0 × 5.0 μm (Digital AFM-Nanoscope IIIA instrument). LB films were also characterized by nanogravimetry through a quartz crystal microbalance (SRS – Stanford Research Systems model QCM200). The mass of the film deposited on a surface bound by gold elec-