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Adsorption and enzyme activity of sucrose phosphorylase on lipid Langmuir and Langmuir–Blodgett films

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ABSTRACT

The production of bioelectronic devices, including biosensors, can be conducted using enzymes immobilized in ultrathin solid films, for which preserving the enzymatic catalytic activity is crucial for optimal performance. In this sense, nanostructured films that allow for control over molecular architectures are of interest. In this paper, we investigate the adsorption of sucrose phosphorylase onto Langmuir monolayers of the phospholipid dimyristoylphosphatidic acid, which caused the surface pressure isotherms to expand. With polarization-modulated infrared reflection absorption spectroscopy (PM-IRRAS), the amide bands from the enzyme could be identified, with the C–N and C=O dipole moments lying parallel to the air–water interface. Structuring of the enzyme into an α-helix was noted, and this structure was preserved when the mixed enzyme-phospholipid monolayer was transferred in the form of a Langmuir–Blodgett (LB) film. The latter was demonstrated with measurements of the catalytic activity of sucrose phosphorylase, which presented the highest enzyme activity for multilayer LB film. The approach presented in this study not only allows for optimized catalytic activity toward sucrose but also permits to explain why certain film architectures exhibit superior performance.

1. Introduction

Enzyme immobilization in Langmuir–Blodgett (LB) films is a current strategy for obtaining nanostructured films whose molecular architectures can be controlled at the molecular level. For this purpose, it is necessary to form stable films at the air–water interface to be subsequently transferred to solid supports. In this sense, not only the formation of films of pure enzymes at the air–water interface [1,2], but also the use of protector molecules to avoid the denaturation of the enzyme when adsorbed at the interface have been reported [3–6]. Phospholipids are molecules with this protector property because of their amphiphilic nature [3], which may provide an adequate orientation of the polypeptide moiety, enhancing the accessibility of the catalytic substrate. Thus, mixed enzyme-phospholipid films are suitable for obtaining well-ordered nanostructured systems, which can also be employed as biosensors.

Sucrose phosphorylase (SF) is an intracellular enzyme found in microorganisms responsible for catalyzing the chemical conversion of sucrose to D-fructose and α-D-glucose-1-phosphate by means of a double displacement mechanism [7]. This enzyme can therefore be used in sucrose sensing, but studies on this topic remain scarce [8,9]. Also, reports on this enzyme in nanostructured films, such as Langmuir monolayers, LB films, or layer-by-layer structures were not found in the literature.

In this sense, in this paper, the adsorption of SF at the air–water interface is investigated with emphasis on its interaction with the phospholipid dimyristoylphosphatidic acid (DMPA) and its transfer onto solid supports using the LB technique in order to consider further the ability of such structures to recognize sucrose.

2. Experimental

The water used in all experiments was purified using a Milli-Q® system (resistivity of 18.2 Ω cm^{-1}, pH ~6.0). Dimyristoylphosphatidic acid (DMPA) and sucrose phosphorylase (SF) from *Leuconostoc mesenteroides* were obtained from Sigma-Aldrich. DMPA solutions were obtained dissolving the lipid in chloroform (Synth) to a concentration of 0.5 mg/mL. SF solutions with a concentration of 0.55 mg/mL were obtained by dissolving the enzyme in an aqueous buffer solution of K_2HPO_4 (Sigma-Aldrich) and KH_2PO_4 (Sigma-Aldrich) with a salt concentration of 0.01 mol/L and pH ~7.0.

For the preparation of monolayers at the air–water interface, a Langmuir trough (KSV Instruments, model: Mini) was initially filled with the phosphate buffer solution. A DMPA solution was then spread on the buffer–air interface to obtain an area per molecule of ~100–120 $Å^2$. After the evaporation of chloroform for 20 min, the monolayers were compressed with two movable barriers at a rate of

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5 Å^2/molecule/s. The surface pressure values were monitored using a filter paper Wilhelmy plate that intercepted the interface in the center of the trough. Compression was stopped either when the monolayer collapsed or when the minimum area allowed by the equipment was attained.

For the preparation of mixed enzyme-lipid monolayers, after spreading of the DMPA solution and evaporation of the solvent, pre-determined aliquots of the enzyme solution were carefully injected below the interface into the buffer. After allowing the surface pressure to stabilize, the interface was compressed. The surface pressure was monitored in function of the molecular area.

Polarization-modulation infrared reflection absorption spectroscopy (PM-IRRAS) measurements were performed using a KSV PMI 550 instrument (KSV Instrument Ltd, Helsinki, Finland) for the Langmuir monolayers. With an incidence angle of 80°, the incoming light was continuously modulated between *p*- and *s*-polarizations at a frequency of 1500 cm^{-1}, allowing for the simultaneous measurement of the spectra for the two polarizations. The PM-IRRAS signal is obtained from the reflectivities of both the *p* and *s* fractions of the light. The difference between the signal spectra provides surface-specific information, and the sum provides the reference spectrum. The ratio between the difference and the sum gives the PM-IRRAS signal, which has effects of water vapor and carbon dioxide reduced, providing information on vibrational groups present only at the air–water interface. For such measurements, the monolayers were compressed until the desired surface pressure was achieved, and PM-IRRAS spectra were recorded for a minimum of 600 scans.

The Langmuir films were then transferred onto solid glass supports, which had previously been cleaned with KOH and ethanol, by vertically withdrawing or immersing the support across the air/film interface with an immersion speed of 5 mm/min and at a constant surface pressure of 30 mN/m. Films with 1–3 layers at each side of the substrate were deposited. Since the solid supports are hydrophilic, the first layer was transferred during the withdrawal, with transfer ratio of about 0.9–1.0 for further analysis. For analysis of the LB films and examination of co-transfer of the enzyme, fluorescence spectroscopy (Spectrophotometer model RF-5301PC, Shimadzu) was employed using an excitation wavelength of 285 nm with the glass directly placed in the fluorimeter holder.

The catalytic activity of the enzyme was measured according to a method previously described in the literature [10,11]. For this purpose, a solution containing 50 mmol/L sucrose (Synth), 50 mmol/L 2-amino-2-hydroxymethyl-propane-1,3-diol (Synth), 1 mmol/L ethylenediaminetetraacetic acid (Exôdo Científica), and 5 mmol/L magnesium sulfate (Synth) was prepared. One microgram aliquots of β-NAD (Sigma-Aldrich), phosphoglucomutase (Sigma-Aldrich), and glucose-6-phosphate dehydrogenase (Sigma-Aldrich) were added to the solution with a final volume of 1 mL, and the pH was adjusted to 7.0 using hydrochloric acid (Synth). After insertion of the enzyme or enzyme-containing LB film into the cuvette, the absorbance at 340 nm was followed with time. The determination of the enzyme activity in homogeneous medium was carried out for an enzyme solution with a concentration of 1 μg/mL.

All experiments were performed at a temperature of 25.0 ± 0.2 °C.

3. Results and discussion

To determine whether SF possesses some surface activity, the enzyme solution was spread on the top of the buffer subphase and the area of the air–water interface was reduced with mobile barriers. For that, 50 μL of 0.55 mg/mL of SF was spread on the buffer solution and 1 h was waited prior to compression. Because

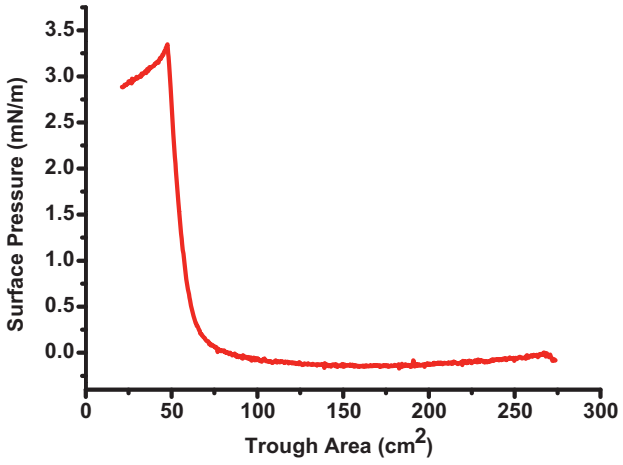

Fig. 1. Surface pressure-area isotherm for 50 μL of 0.55 mg/mL SF (total concentration in the trough: 0.100 μg/mL) spread on the buffer–air interface.

SF is soluble in water, it does not form an actual Langmuir monolayer; however, because SF is an amphiphilic compound, it can form a Gibbs monolayer, in which the enzyme molecules are partitioned between the interface and the aqueous subphase, with some surface excess of the enzyme. In fact, as shown in the surface pressure-area isotherm in Fig. 1, SF presents some surface activity and increases the surface pressure at the end of compression to values as high as 3.3 mN/m. Because SF is soluble in water, the amount of enzyme that remains at the interface cannot be determined; therefore, the *x*-axis shows the total area of the trough rather than the area per molecule, as customary for this type of isotherm.

For DMPA monolayers (Fig. 2), a typical isotherm is observed, as expected. It is shown in the literature [12,13] that DMPA monolayers at the air–water interface present a liquid-expanded phase followed by a liquid-condensed one, with a plateau at 3–10 mN/m, indicating a first-order transition (for an example, see Supplementary Data). This plateau is usually highly sensitive to the experimental conditions, such as temperature, pH and presence of ions in the subphase, primarily because of the negative charge of DMPA. For the phosphate buffer employed in the present work, the liquid-expanded to liquid-condensed transition appears to occur at about 18 mN/m, as evidenced by a kink in the DMPA isotherm

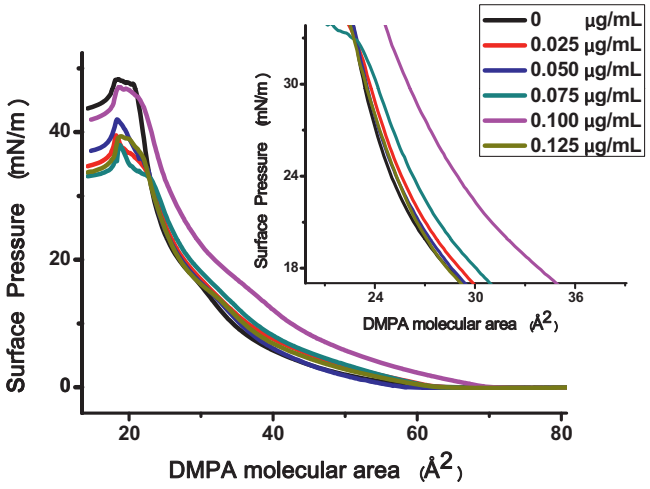

Fig. 2. Surface pressure-area isotherms for DMPA in several concentrations of SF in the buffer subphase as indicated in the inset. Furthermore, magnified isotherms in the region close to the surface pressure of 30 mN/m are also shown.