**β-Galactosidase Langmuir Monolayer at Air/X-gal Subphase Interface**

Shiv K. Sharma,† Shanghai Li,‡ Miodrag Micic,§,∥ Jhony Orbulescu,‡ Daniel Weissbart,† Hiromichi Nakahara,‖ Osamu Shibata,‖ and Roger M. Leblanc†,*

†Department of Chemistry, University of Miami, 1301 Memorial Drive, Coral Gables, Florida 33146, United States
‡MP Biomedicals LLC, 3 Hutton Center, Santa Ana, California 92707, United States
§Department of Engineering Design Technology, Cerritos College, 11110 Alondra Boulevard, Norwalk, California 9265, United States
∥MP Biomedicals SAS, Parc d’innovation-Rue Geiler de Kaysersberg, Illkirch-Graffenstaden 67402, France
‖Department of Biophysical Chemistry, Nagasaki International University, Huis Ten Bosch, Sasebo, Nagasaki 859-3298, Japan

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**ABSTRACT:** This article investigates the surface chemistry properties of the β-galactosidase monolayer at the air-subphase interface at the vicinity of its substrate, X-gal. We have demonstrated that the β-galactosidase in the monolayer form remained active and performed hydrolysis of the X-gal in the subphase. We investigated the β-galactosidase Langmuir monolayer in absence and presence of X-gal in the subphase of varying concentration of X-gal with the sodium chloride solution. It was found that the limiting molecular area as well as the collapse surface pressure kept on decreasing with the increasing concentration of X-gal. In accordance to the data obtained from the isotherm it was also found that β-galactosidase forms a stable monolayer that does not aggregate at the air-subphase interface. The stability of the monolayer at the air-subphase interface was studied by using compression–decompression cycles with and without X-gal at varying concentration and different surface pressures. The infrared reflection–absorption spectroscopy (IRRAS) and Brewster angle microscopy (BAM) of β-galactosidase Langmuir monolayer was also investigated for pure and mixed β-galactosidase at the air-subphase.

**INTRODUCTION**

In this article, our aim was to study the surface properties of β-galactosidase, as a monolayer at the air-subphase interface and to identify whether it retains its hydrolytic activity in the monolayer form. The reasons for undertaking this work are 3-fold: to understand the surface chemistry behavior of this important enzyme in a model systems representing vaguely cell membrane environments,† to prove the activity of the deposited enzyme in a form of the monolayer for a possible future glycomics sample preparation microfluidics device development,‡ and to prove existence of catalytic activity and stability of monolayer for possible future biosensing applications. β-galactosidase belongs to the class of exoglycosidase enzymes§ which plays important roles to hydrolyze the β-glycosidic bond formed between a galactose and other organic residue in glycans.∥ As such, it is one of the most important metabolic enzymes. Besides fundamental importance in key biochemical pathways in vivo, β-galactosidases have a lot of tangible applications in the lab as a key life science tool in applications, such as blue-white screening, in an ELISA assays∥ based on color generation by action on X-gal or similar substrate, electrophoresis, and more recently as a key enzyme for glycans sample preparation and glycans digestion and analysis. The β-galactosidase can hydrolyze several of the β-glycosidic glycan analogs, like 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) which produces colored, luminescent, or fluorescent compounds, and as such is commonly the enzyme used in vitro assays and in biosensing applications.‡ X-gal is an artificial organic compound that consists of galactose linked to a substituted indole and is highly used in molecular biology.∥ The most commonly used substrate for β-galactosidase is X-gal, resulting in the cleavage of glycosidic linkage to galactose and indigo type dye revealing a strong indigo blue color. The β-galactosidase–X-gal reporting system is commonly used in gene expression, as a marker in gene cloning, plasmid construction, structural stabilization of fusion proteins, process monitoring, and purifications.∥∥∥∥∥∥ Nonethelss, the amphiphilic natures of vesicles and cell membrane that exist in vivo and interaction mechanism around the amphiphilic natures is not clear. This article studied the interaction of β-galactosidase and X-gal at the air-subphase interface, which can
be extensively used to mimic the amphiphilic membranes structure in vivo.

Langmuir film technique is considered as an effective way to understand how surface proteins interact with their membrane and subphase environment by studying their air-subphase behavior.20,21 In this technique, one molecule-thick monolayer of proteins is produced which ultimately guides to study its transformation on the air–water interface from the gaseous phase to condensed phase via liquid condensed phase. Basic information like protein packing and conformation studies can be obtained from measurement of the surface pressure, average molecular area and surface potential, however, detailed information about secondary structures of enzyme can be obtained by employing infrared reflection–absorption spectroscopy (IRRAS) and Brewster angle microscopic (BAM) studies gives real image of the monolayer at different surface pressures.17,18 In Langmuir monolayer, amphiphilic molecules are spread at the air–water interface. In doing so, movable barriers are employed for compression in order to control molecular packing.19 The study of Langmuir film is basically used to measure the surface pressure as a function of surface area available to surfactant molecules at constant temperature which is so-called as surface pressure–area (π–A) isotherm. This measurement gives information about the existence of different phase transitions and the stability of the film of enzymes in the absence and presence of the substrate. The isotherm behavior of the Langmuir film is usually estimated by the physical and chemical properties of amphiphilic molecules and composition of the subphase (such as salt concentration).20,21

In this work, we will explore surface chemistry properties and activity of β-galactosidase monolayer on hydrolysis of X-gal subphase. We exploit the Langmuir monolayer film technique to assimilate basic surface chemistry properties and to scrutinize the behavior of β-galactosidase at the air-subphase interface at different surface pressures that concur to different states, ranging from the gaseous phase to the liquid compressed phase. In this study we investigated the β-galactosidase Langmuir monolayer in absence and presence of varying concentration of X-gal and the sodium chloride subphase. β-galactosidase was chosen to study the Langmuir monolayer because it is an important enzyme in our metabolism,22 useful for the structural investigation of carbohydrate, determination of lactose (food-stuffs analysis) and as an enzyme label for enzyme immuno-assay.23 Furthermore, X-gal was chosen as an ingredient in the subphase because an active enzyme can be detected by using X-gal which gives the intense blue color product after cleavage by the enzyme and is easy to quantify.

■ EXPERIMENTAL SECTION

Chemicals. β-Galactosidase (EC 3.2.1.23) was obtained from MP Biomedicals (Solon, OH) having the molecular weight of 540 000 Da that was determined using matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometer. Specific activity of the enzyme as provided by company was 700 U/mg by analysis with O-nitrophenyl-β-D-galactopyranoside (ONPG). X-gal (5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside) and sodium chloride (99.5% pure) were also obtained from MP Biomedicals. Water utilized in these experiments was used from Modulab 2020 Water Purification System (Continental Water System Corp., San Antonio, TX) with the resistivity of 18 MΩ cm, surface tension of 71.6 mN/m, and pH of 6.0 at 20.0 ± 0.5 °C.

**Equipment.** Entire isotherm measurements were carried out in a clean room (class 1000) where temperature (20.0 ± 0.5 °C) and humidity (50% ± 1%) were maintained constant. A Kibron μ-trough (Kibron Inc., Helsinki, Finland) having area of (5.9 cm × 21.1 cm) was used in the study of surface pressure–area (π–A) isotherms, surface potential-area isotherms, stability, and compression–decompression cycles. Kelvin probe that consists of a capacitor-like system was utilized to measure surface potential. The vibrating plate was adjusted at 1 mm (approx.) above the surface of Langmuir monolayer and a gold-plated trough acted as a counter electrode. The Wilhelmy method was deployed to assess surface pressure with a 0.51 mm diameter alloy wire probe having sensitivity of ±0.01 mN/m.

**Langmuir Monolayer Preparation.** The β-galactosidase solution was prepared in pure water (pH 6.0) at the concentration of 3.7 × 10^{-7} M NaCl (0.1M) solution and varying concentration of X-gal were used to prepare the subphase. X-gal was dissolved in minimal amount of dimethylformamide (DMF) and further diluted by using pure water. The β-galactosidase was spread uniformly over the air-X-gal/NaCl interface by using a 100 μL syringe (Hamilton Co., Reno, Nevada). The spreading volume of the enzyme solution was 45 μL for the surface chemistry and spectroscopic measurements. After spreading the solution, Langmuir monolayer was allowed to attain the equilibrium state approximately for 20 min. Then, monolayer was compressed with the rate of 3990 Å².molecule⁻¹.min⁻¹.

**Infrared Reflection–Absorption Spectroscopy (IRRAS).** Bruker Equinox55 FTIR instrument (Billerica, MA) equipped with the XA-511 accessory for the air–water interface was used at the air-subphase interface to obtain infrared spectra. Kibron μ-trough S (Helsinki, Finland) with dimensions of 5.9 cm × 21.1 cm was employed for the experiment. The measurements were carried out by the use of p-polarized light and a mercury–cadmium-telluride (MCT) liquid-nitrogen-cooled detector. Each spectrum was acquired by the coaddition of 1200 scans at a resolution of 8 cm⁻¹.

**Brewster Angle Microscopy (BAM).** The BAM measurements of Langmuir films at air-subphase interface were performed by the use of KSV Optrel BAM 300 from KSV Instrumentals Ltd., Finland, linked to a film balance system (KSV Minitrough, KSV Instrumentals Ltd., Finland). A standard laser of a 20 mW He–Ne emitting p-polarized light of 632.8 nm wavelength and a 10× objective was employed. The angle of incidence was set at 50° at 298.2 K. Triply distilled water having surface tension 71.9 mN/m and resistivity 18 MΩ cm was used in preparing the subphase with NaCl (from Nacalai Tesque; Kyoto, Japan) roasted at 900 K for 24 h to eliminate surface-active organic impurities) and X-gal. A high grade charge-coupled device (CCD) camera (EHDkamPro02, EHD Imaging GmbH, Germany) was used to record the reflected beam and the BAM images were digitally saved.

**RESULTS AND DISCUSSION**

**Surface Pressure and Surface Potential-Area Isotherms.** The Langmuir monolayer behavior and interfacial properties of β-galactosidase on the surface of X-gal/NaCl was investigated by spreading 45 μL of the enzyme having concentration of 3.7× 10^{-7} M on the 9.78 × 10^{-3} M X-gal and 0.1 M NaCl subphase solution. These conditions reproducibly displayed the proper formation of two-dimensional enzyme Langmuir monolayer. The basic reason for spreading the enzyme on the subphase that contains an active
substrate is to investigate and compare the surface phenomenon that would make useful in industrial and medical applications by using the surface property of the enzyme. An initial zero in surface pressure starting at 120 000 Å²/molecule for a spreading amount of $3.7 \times 10^{-7}$ M of enzyme is ideal correlating the gaseous phase (Figure 1). The enzyme molecules continue to condense until 45 000 Å²/molecule when the sharp increase in surface pressure can be observed which is referred as liquid expanded phase. When the maximum compression is reached the phase reached at around 25 000 Å²/molecule. The liquid condensed film of the enzyme monolayer is observed in the range of 35 000 to 23 000 Å²/molecule.

Basically surface pressure quantifies the interactions between molecules in close contact (van der Waals interaction) and surface potential evaluates the potential difference or dipole moment difference above and below the Langmuir monolayer film in the same experiment, which is the interaction of molecules at longer distances regarded as dipole–dipole interaction. The surface potential-area isotherms exhibit the molecular interactions that occur before and during phase change of the monolayer as seen during the compression.24

As per expectation, when compression of the monolayer is started the surface potential-area isotherm exhibits increase in the surface potential. This is better explained by the charges that are present on β-galactosidase. When the monolayer begins to move from a liquid expanded phase to liquid condensed phase there appears the kink on the surface potential curve at about 95 000 Å²/molecule and change in surface potential is observed.

The other small bumps seen in the surface potential curve at very low surface pressure are due to β-galactosidase molecules rapidly moving on the subphase surface under the vibrating electrode. The maximum voltage of 120 mV relates to the compact packing structure of the enzyme molecules. At this value, due to short distance among molecule some of the dipole–dipole interactions get canceled out which ultimately results in a steady value in surface potential.

**Concentration Effect of X-gal in the Aqueous Subphase.** The experiments were conducted by using NaCl aqueous solution and NaCl/X-gal aqueous solutions. Figure 2 shows the π–A isotherms for 0.1 M NaCl and mixed 0.1 M NaCl—(2.45, 4.89, 7.34, and 9.78) × $10^{-3}$ M of X-gal. As barrier goes on compressing the enzyme monolayer, the surface pressure starts to rise and at lift-off area due to repulsion of the particles that start to order and hence interact as so-called two-dimensional liquid. For NaCl (0.1 M) subphase the limiting molecular area was 79 300 Å²/molecule and adding definite amount of X-gal in the preparation of the subphase subsequently lowered the limiting molecular area as shown in Table 1. The limiting molecular area of Langmuir monolayer depicts the minimum cross-sectional area per molecule. This value can alter with subphase conditions. For example: the conformation of an enzyme gets affected due to the changes in pH there by destabilizing certain vital components throughout the molecule. Similarly, the collapse surface pressure for the NaCl (0.1 M) subphase was 43 mN/m and starts getting lowered when X-gal is added to the subphase solution (see Table 1).

So, we can conclude that when NaCl (0.1M) is only used as a subphase then the limiting molecular area as well as collapse surface pressure is higher but when X-gal is added then the limiting molecular area as well as the collapse surface pressure also goes on decreasing. This fact can be best described as the increased interaction among the X-gal and β-galactosidase due to concentration effects. This trend goes on when the concentration of X-gal is increased. The notable decrease in the limiting molecular area in case of NaCl (0.1 M)-X-gal

![Figure 1. Surface pressure and surface potential versus area/molecule when the subphase was NaCl (0.1M) and X-gal (9.78× $10^{-3}$ M).](image1)

![Figure 2. Surface pressure versus mean molecular area isotherms for 3.7 × $10^{-7}$ M β-galactosidase spread on 0.1 M NaCl and (2.45, 4.89, 7.34, and 9.78) × $10^{-3}$ M of X-gal.](image2)

### Table 1. Data Showing the Limiting Molecular Area and Collapse Surface Pressure with Different Subphase Used

<table>
<thead>
<tr>
<th>subphase</th>
<th>limiting molecular area (Å²/molecule)</th>
<th>collapse surface pressure (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M NaCl (only)</td>
<td>79 300</td>
<td>43</td>
</tr>
<tr>
<td>0.1 M NaCl + 1 mg/mL X-gal</td>
<td>58 200</td>
<td>38</td>
</tr>
<tr>
<td>0.1 M NaCl + 2 mg/mL X-gal</td>
<td>52 200</td>
<td>33</td>
</tr>
<tr>
<td>0.1 M NaCl + 3 mg/mL X-gal</td>
<td>44 050</td>
<td>28</td>
</tr>
<tr>
<td>0.1 M NaCl + 4 mg/mL X-gal</td>
<td>41 000</td>
<td>17</td>
</tr>
</tbody>
</table>

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aqueous subphase is interpreted as the larger interaction between β-galactosidase and X-gal.

**Compression–Decompression Cycles.** The compression–decompression reveals the extent of loss of analyte to desorption from the interface into the subphase or/and change in the conformation of the enzyme known as hysteresis. The active compression–decompression Langmuir monolayer films at the air/subphase interface gives minute data on molecular dimensions, phase transitions of the film, and its packing properties. Moreover, assessing the compression–decompression cycle gives information relating the stability of the film, attribute thermodynamics, and kinetics. As β-galactosidase has more negative charges than positive charges on the surface and due to this fact β-galactosidase molecules should ward off each other when monolayer is compressed. Our hypothesis was confirmed using the compression and decompression cycles of β-galactosidase. Figure 3 (column A) reveals that the β-galactosidase monolayer was compressed 3 fold. The surface pressure for compression and decompression was chosen as 5, 15, and 30 mN/m. For the successive 3 compression/decompression cycles that followed, a hysteresis behavior of the isotherm was observed. These cycles reveal that when compressed to 5 mN/m there is small hysteresis due to the fact that stable monolayer has not been formed yet. It has been found that only 6% of the initial isotherm has been reduced in comparison of the first and last cycle, whereas 15

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**Figure 3.** Compression–decompression of Langmuir isotherm of β-galactosidase. Column A: absence of X-gal in the subphase maintained at the surface pressure of 5, 15, and 30 mN/m. Column B: presence of X-gal (4 mg/mL, 9.78 × 10⁻³ M) in the subphase maintained at surface pressure of 5, 10, and 15 mN/m.
and 30 mN/m only exhibit hysteresis difference of 4.0 and 3.0%, respectively.

These results show that β-galactosidase molecules reorganize at the interface while the salt subphase renders the partial insoluble enzyme molecules. Beside the solubility of enzyme in the subphase, other possible explanations might be considered such as (i) the denaturation of enzyme (protein) (ii) the alteration in the conformation of the protein with time. The small difference in hysteresis after three compressions at different upper limit in surface pressure (5, 10, and 15 mN/m) is therefore integrated as a reorganization of the enzyme Langmuir film.

Like in absence of X-gal, three different compression–decompression cycles were examined by taking the higher concentration of X-gal, i.e., 4 mg/mL (9.78 × 10⁻³ M) in the case of presence of X-gal. The maximum surface pressure for compression and decompression was taken at 15 mN/m because there was the collapse of monolayer called collapse surface pressure at 17–18 mN/m. The surface pressure for compression and decompression was chosen as 5, 10, and 15 mN/m. In this case, there was lesser hysteresis in comparison to the absence of X-gal. It has been found that only 4.0% of the initial isotherm has been lost in comparison of the first and last cycle, whereas 10 and 15 mN/m show 3.0 and 2.5%, respectively.

This fact can be best described on the basis of increased interaction between the X-gal and β-galactosidase molecules at the interface to give a soluble galactose and 5-bromo-4-chloro-3-hydroxyindole. The latter dimerizes and oxidizes to give 5,5′-dibromo-4,4′-dichloro-indigo, an intense blue colored product.29

It has been shown that there is more hysteresis when the subphase is only 0.1 M NaCl.24 This is because the desorption of enzyme on NaCl subphase is greater than the subphase of X-gal + NaCl solution. This fact can be also be attributed to a loss of film forming molecules in the bulk which is also known as the dissolution of the monolayer.30 Hitherto we showed that Langmuir monolayer of β-galactosidase was more stable when

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**Figure 4.** Normalized p-polarized IRRAS spectra at the air-subphase interface of the β-galactosidase Langmuir monolayer compared at different surface pressures in absence and presence of X-gal in the subphase at a 45° incident angle.
spread on the X-gal+ NaCl subphase than when subphase was NaCl or pure water and the reproducibility of the surface pressure–area isotherms was good despite the difference in the concentration of X-gal. The stability measurements at constant pressure was also performed during the absence and presence of X-gal in the subphase (not shown here) at constant pressure over an extended time period (about 70 min) which verified that the monolayer was more stable in presence of X-gal in the subphase.

Infrared Reflection–Absorption Spectroscopy (IRRAS). IRRAS of lipid/protein monolayer films in situ at the air/subphase interface provides unique conformational and orientational information from the film constituents. This technique is considered important in studies of lipid/protein interaction in a physiologically pertinent environment. Different types of applications, like the elucidation of lipid chain conformation and tilt as well as determination of protein secondary structure, are reviewed by using this technique.31–33

Presented in Figure 4 are the normalized p-polarized IRRAS spectra of the enzyme monolayer that is collected at various surface pressures during compression at an incident angle of 45° in absence and presence of different concentration X-gal. This angle was selected because it showed highest signal-to-noise ratio. Typically, the p-polarized light has the highest signal and therefore the best signal-to-noise ratio at about the Brewster angle. In absence of X-gal, there was no appreciable variation in band position and no disappearance of bands during compression which clearly means that there is no alteration in secondary structure of the β-galactosidase Langmuir monolayer.

Band position in the amide I region (1700–1600 cm−1) is due to C==O (70–80%) stretching vibrations of peptide bonds and it demonstrates that the β-sheet conformation dominates the secondary structure which is in accordance with the previous study (refer to the Supporting Information Figure S2). The amide II region (1600–1500 cm−1) shows the α-helical and unordered structures (random coils) content. Being in crystalline structure, the abundance of β-sheet in the IRRAS spectra is expected for this enzyme34 and in a study, it has been found that the native secondary structure of β-galactosidase comprises of 22% beta-turns, 14% parallel β-sheets, 25% antiparallel β-sheets, 34% unordered structures (random coils), and only 5% α-helices.35

We were more interested in the molecular structure and orientation at the presence of X-gal in the subphase. As seen from the figure, in the region of amide I, the most prominent bands are centered at 1674, 1676, 1679, and 1682 cm−1 for change in concentration of X-gal and this represents the shifting of the peak due to the products formed by the cleavage of X-gal in the interface by β-galactosidase as well as the change in secondary structure of the enzyme. This amide I region is directly related to the backbone conformation. The band observed at 1673 cm−1 is assigned to α-helices. The bands corresponding to α-helices are higher in intensity at amide I region as compared to the β-sheet. The likely reason for this observation might be due to the presence of carbonyl groups in α-helices that are lying parallel to the air-subphase interface in collation with the carbonyl groups present in β-sheet. The amide II region is due to the C–N stretching (20–40%) vibrations in combination with N–H bending (40–60%). The amide II bands at 1556 and 1541 cm−1 correspond to α-helices and the band at 1521 cm−1 corresponds to β-sheets. At different surface pressures we found that the band intensities remained almost constant which reveals that the amide chains are oriented parallel to the air-subphase interface. The C–H stretching peaks are observed in the range of 2800–3000 cm−1 and C–O peaks are observed around 1200 and 1300 cm−1 (not shown). The signals are very weak and, therefore, are difficult to assign. From the data, we came to the conclusion that presence of X-gal in the subphase alters the secondary structure of enzyme and the band positions are shifted with the increase in the amount of X-gal in the subphase. But, this necessarily does not mean that the overall conformation of the enzyme changes because the conformation of enzyme is based on primary, secondary, tertiary, and quaternary structure. Being acquainted with the fact that use of D2O in the subphase helps

Figure 5. Brewster angle micrographs for β-galactosidase spread on the (A) 0.1 M NaCl, (B) 0.1 M NaCl + 2.45 × 10−3 M X-gal, and (C) 0.1 M NaCl + 9.78 × 10−3 M X-gal at different surface pressures during compression. The scale bar represents 100 μm.
in the identification of the vibrations of the side chain moieties from the secondary structure of protein, here we have used X-gal and NaCl as subphase because our purpose is to investigate our hypothesis of the conformation and change in orientation of the enzyme monolayer observed at different concentration of X-gal.

**Brewster Angle Microscopy (BAM).** The lack of hysteresis and increase in stability of isotherms in presence of X-gal in comparison to the absence of X-gal in the subphase (Figure 3) was described as the lack of aggregate formation of the enzyme. In order to confirm this finding and to explore the topography of the monolayer surfaces we decided to use BAM. This technique is very responsive to interfacial changes due to properties of phase domains in monolayers like surface density, anisotropy, interfacial roughness, and thickness.\(^{36,57}\) As Brewster angle for the air-subphase interface is estimated as 50° (for air–water interface being 53°) and under this condition the image of a pure air-subphase appears black since no light is reflected and addition of enzyme to the interface modifies the local refractive index and hence small amount of light is reflected which gets displayed within the image. Figure 5 shows the micrographs obtained during compression of the \(\beta\)-galactosidase Langmuir monolayer in the subphase in absence and presence of X-gal. Figure 5A shows the micrographs obtained at the surface pressures of 1, 5, and 15 mN/m in absence of X-gal that does not reveal any aggregate or domain formation which comprehends the basis of homogeneity of the micrographs. The BAM micrographs obtained during compression at surface pressures 1, 5, and 15 mN/m with 2.45 \(\times\) 10\(^{-3}\) M X-gal in the subphase are shown in Figure 5B.

The micrographs substantiate the conclusion drawn on the basis of Figure 3 as no aggregate or domain formation is revealed. Figure 5C shows the micrographs obtained at surface pressures of 1, 5, and 15 mN/m in presence of 9.78 \(\times\) 10\(^{-3}\) M X-gal in the subphase. The BAM micrograph obtained during 1 mN/m also does not exhibit any aggregate or domain formation. Additional compression of the monolayer to attain surface pressures 5 and 15 mN/m revealed a number of tiny needle-like structures. These tiny structures are due to the 5,5'-dibromo-4,4'-dichloro-indigo which is the dimerized and oxidized form of the 5-bromo-4-chloro-3-hydroxyindole formed due to the cleavage of X-gal which are not soluble in the subphase. The BAM images during decompression are given in Supporting Information Figures S3, S4, and S5.

**CONCLUSIONS**

We have found that the \(\beta\)-galactosidase Langmuir monolayer limiting molecular area and the monolayer collapse surface pressure kept on decreasing with the increasing amount of X-gal in the subphase. Based on the results of the BAM imaging we conclude that \(\beta\)-galactosidase forms a stable monolayer which does not aggregate at the air-subphase interface. In general, subphase stabilizes the monolayer, and in the case of the \(\beta\)-galactosidase we have demonstrated that the monolayer is more stable with less hysteresis in presence of X-gal than compared to monolayer in the absence of X-gal in the subphase. We have demonstrated using the p-polarized infrared absorption–reflection spectroscopy (IRRAS) that the enzyme secondary structure is altered due to presence of X-gal in the subphase. The BAM imaging provides visual evidence of the absence of aggregates and domains, thus indicating that the \(\beta\)-galactosidase monolayers have less hysteresis and hence are more stable due to the presence of X-gal in the subphase. The presence of the indigo hue formation in the subphase indicates that the activity of the enzyme is preserved in the monolayer form, opening a door for the future investigations of possible use of \(\beta\)-galactosidase and other glycolytic enzymes for the glycan sample preparation and biosensors applications.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jpcb.6b09020.

Infrared-reflection-absorption spectroscopy (IRRAS) and Brewster angle microscopy (BAM) images of \(\beta\)-galactosidase spread on aqueous subphase (PDF)

**AUTHOR INFORMATION**

Corresponding Author

* E-mail: rml@miami.edu; Phone +1-305-284-2194; Fax: +1-305-284-6367.

ORCID

Roger M. Leblanc: 0000-0001-8836-8042

**Notes**

The authors declare no competing financial interest.

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