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Interactions of ibuprofen with hybrid lipid bilayers probed by complementary surface-enhanced vibrational spectroscopies

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Abstract

The incorporation of small molecules into lipid bilayers is a process of biological importance and clinical relevance that can change the material properties of cell membranes and cause deleterious side effects for certain drugs. Here we report the direct observation, using surface enhanced Raman and IR spectroscopies (SERS, SEIRA), of the insertion of ibuprofen molecules into hybrid lipid bilayers. The alkanethiol-phospholipid hybrid bilayers were formed onto gold nanoshells by self-assembly, where the underlying nanoshell substrates provided the necessary enhancements for SERS and SEIRA. The spectroscopic data reveal specific interactions between ibuprofen and phospholipid moieties and indicate that the overall hydrophobicity of ibuprofen plays an important role in its intercalation in these membrane mimics.

Introduction

The interactions of amphiphilic molecules, such as nonsteroidal anti-inflammatory drug (NSAIDs), with cellular membranes are biologically important. The clinical use of NSAIDs for their analgesic, antipyretic and anti-inflammatory properties is extremely widespread; however, these compounds cause serious side effects such as gastrointestinal bleeding and peptic ulcer disease.¹ It has been postulated that these side effects occur due to the interaction of amphiphiles with phospholipid layers covering the gastrointestinal mucosa.^{2,3} In support of this hypothesis, salicylate has been shown to interact with and change the physicochemical properties of lipid membranes in a way that enhances the formation of membrane pores.⁴ Ibuprofen, one of the most heavily prescribed NSAIDs⁵, is known to partition into synthetic and biological membranes and cause changes in the permeability, fluidity, mechanical, and structural properties of the membrane.^{3,6} It has been shown using optical-trapping confocal Raman microscopy that ibuprofen causes a high level of disorganization in lipid phosphatidylcholine (PC) acyl chains in a dose-dependent manner.⁷

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Supporting Information Available:

Detailed experimental procedures and the unenhanced Raman spectrum for a 500 mM aqueous solution of sodium ibuprofenate are provided. This information is available free of charge via the Internet at <http://pubs.acs.org>.

Recently there has been growing interest in probing biological membrane function by coupling the properties of nanostructured materials with lipid membranes or membrane mimics.⁸⁻¹¹ Hybrid lipid bilayers provide a very important materials system for this approach. They typically consist of an alkanethiol self-assembled monolayer (SAM)¹², bound to a noble metal substrate, with an associated outer layer of phosphatidylcholine (PC) lipids.¹³ Phospholipids with phosphocholine headgroups are known to exist in high abundance within the plasma and intracellular membranes of eukaryotic cells.¹⁴ In a hybrid lipid bilayer, the phospholipid layer assembles onto the SAM with its hydrophobic tail groups directly adjacent to the alkane chains of the SAM, forming a structural analog to the natural lipid bilayer structures found in biological systems.¹⁵ Hybrid bilayers are a good composite system for investigation because they mimic the composition of biological membranes¹⁶ with the additional advantages that they are robust, stable, and can be dried and rehydrated.¹⁷ Additionally, they can be fabricated to cover large surface areas, are easy to form through self-assembly techniques, and have been characterized by a variety of analytical methods.¹⁸

In this work, hybrid bilayers are assembled onto Au nanoshells, whose plasmon-derived properties allow them to serve as strongly enhancing substrates for both surface enhanced vibrational spectroscopies,¹⁹ surface enhanced Raman scattering (SERS) and surface enhanced infrared absorption spectroscopy (SEIRA). Au nanoshells are spherical nanoparticles consisting of a dielectric (silica) core and a thin metallic (gold) shell.²⁰ Nanoshells have the unique property that their plasmon resonance can be tuned across a broad region of the optical spectrum by varying the relative dimensions of the core and shell layers of the nanoparticle.^{21,22} Surface enhanced spectroscopies require large local electromagnetic fields at the substrate surface, which are an important characteristic of plasmon-resonant nanoparticles. With nanoshells, the large local electromagnetic field can be resonantly engineered in a frequency range of choice by controlling the internal nanoshell geometry,²³ and by forming small aggregates or arrays.^{24,25} For SERS, the single particle plasmon resonance of the substrate is tuned near the frequency of the excitation laser, while for SEIRA, the local fields in the interparticle junctions must be intense across the entire infrared wavelength range over which the molecules will be probed. SERS and SEIRA enhancements observed with nanoshells as the plasmonic substrate are both strong and highly reproducible. By utilizing geometries with closely adjacent nanoshells, both Raman and infrared spectroscopies have been shown to be enhanced simultaneously on the same nanoshell-based substrate.^{25,26}

Due to the complementary nature of these vibrational spectroscopies, the combination of both SERS and SEIRA provides a uniquely information-rich method for probing molecular systems at the nanoscale. Specifically, ibuprofen's aromatic ring structure is known to have strong Raman-active modes²⁷ whereas the polar headgroups of the lipid layer have strong IR-active vibrational modes. By utilizing both SERS and SEIRA, we can spectroscopically monitor both Raman and IR active functional groups of both the intercalant molecules and the hybrid bilayer. These two combined spectroscopies could be potentially used to chemically differentiate between similar analogs of an analyte molecule with high specificity.

Two different effects are thought to contribute to the total enhancement in both SERS and SEIRA: electromagnetic and chemical effects. The electromagnetic contribution consists of the enhancement associated with the local surface plasmon excitation²⁸ while the chemical contribution is due to the electronic coupling interactions between the adsorbate molecules and the substrate²⁹. These interactions lead to the observed changes in frequency of Stokes modes in SERS when compared to unenhanced Raman spectroscopy.³⁰ It is interesting to note that in addition to their relevance as a biological mimic, hybrid bilayers and their intercalant species also provide a simple approach for isolating electromagnetic from chemical effects in SERS^{31–33}, since the bilayer-intercalated molecules are adjacent to, but not directly interacting with, the substrate surface.

A schematic diagram of the nanoparticle-hybrid bilayer complex is shown in Figure 1. The sample geometries consist of (A) ibuprofenate adsorbed onto bare nanoshells for SERS and (B) ibuprofen intercalated in hybrid bilayers for SERS (where the single particle plasmons are utilized), and (C) ibuprofen intercalated in hybrid bilayers on nanoshell aggregates for SEIRA. (Ibuprofenate adsorbed onto nanoshell aggregates for SEIRA is not shown for simplicity).

Materials and Methods

Au nanoshells of core radius 60 nm with shell thickness 20 nm and core radius 190 nm with shell thickness 35 nm were fabricated for SERS and SEIRA measurements respectively, according to previously described protocols.²⁰ The core and shell dimensions of the nanoshells used for SERS were adjusted so that the plasmon resonance provided a near-field enhancement maximum near 785 nm in water, to provide enhancement at the 785 nm pump laser wavelength. The dimensions of the nanoshells used for SEIRA measurements were adjusted to provide a plasmon resonance absorbance maximum in air in the mid-infrared (mid-IR) region: the full SEIRA bandwidth originates from both plasmon-resonant and lightning rod effects.²⁶ The nanoshell dimensions were then confirmed by scanning electron microscopy (SEM).

The formation of a SAM on Au nanoshells requires the nanoshells to be dispersed in an ethanolic solution of dodecanethiol. The aqueous solutions of $[r_1, r_2] = [60, 80]$ nm and $[r_1, r_2] = [190, 125]$ nm nanoshells were centrifuged at 350 RCF for 30 minutes and 90 RCF for 20 minutes, respectively. The particles were then resuspended in absolute ethanol (200 proof, AAPER Alcohol and Chemical Co.).

Alkanethiol monolayers were first prepared on the nanoshells by separately making a solution of 10 mM 1-dodecanethiol (Aldrich 98%) in absolute ethanol. This was diluted to 30 μ M solutions with the Au nanoshells in ethanol, and allowed to incubate overnight in the dark for covalent attachment. 30 μ M solutions were chosen based on the nanoshell surface area and concentration, and based on the size of the dodecanethiol molecule³⁴ to provide monolayer coverage in ten times excess. After incubation, the nanoshells had settled and the supernatant was removed, so that the nanoshells were redispersed in fresh ethanol and any unreacted thiol was removed from solution. The dodecanethiol functionalized nanoshells were then allowed to dry completely.

For in solution measurements, an initial stock solution of ibuprofen sodium salt was prepared according to Du et al.³⁵ by dissolving ibuprofen (Sigma, USA) in sodium hydroxide (1N, Fisher Scientific) and then adjusting the pH with hydrochloric acid (1N, Fisher Scientific). Since the pKa of ibuprofen is about 4.6,³⁶ the molecule is predominantly in the ionized form under basic conditions. For the unenhanced powder measurements, the ibuprofen sodium salt utilized was purchased from Sigma.

Lipid solutions of either dimyristoylphosphatidylcholine (DMPC) or 1,2-Dimyristoyl-D54-sn-Glycerol-3-Phosphocholine-1,1,2,2-D4-N,N,N-trimethyl-D9 (DMPC-D54) (Avanti Polar Lipids, Inc., Alabaster, AL) were prepared by solubilizing the lipids in isopropyl alcohol (Fisher Scientific) (50 μ L per 2 μ mol of lipid).¹⁵ The DMPC-D54 lipids and ibuprofen stock solution were added to alkanethiol functionalized nanoshells to give a final concentration of 100 μ M DMPC lipids and final concentrations of 0.01 mM, 0.1 mM, 1 mM, 10 mM, and 100 mM of ibuprofen respectively. The solutions were placed in an ultrasonicator bath (VWR Model 150D) for 30 minutes at 30 °C, above the main phase transition temperature of the DMPC lipid ($T_m = 24$ °C)³⁵ and DMPC-D54 lipid ($T_m = 18.7$ °C)³⁷ where the lipid is in the liquid crystalline phase and the hydrocarbon chains are more compressible and fluid.³⁸ All measurements were then performed at room temperature around 22 °C.

The hybrid bilayer phase behavior was characterized using the fluorescent probe Laurdan, following the techniques of Parassis et al.^{39,40}, Bagatolli et al.⁴¹⁻⁴³ and Zhou et al.⁴⁴. For these measurements, an appropriate amount of hybrid bilayers formed with either DMPC-D54 or DMPC were mixed with a methanolic solution of Laurdan (Sigma Aldrich) to achieve a lipid/probe ratio of 300:1. The emission spectra between 400 nm and 600 nm were obtained at a fixed excitation chosen between 320 nm and 390 nm.

SERS substrates consisted of fused quartz (Technical Glass Products, Inc., Painesville Twp., OH), while SEIRA substrates consisted of silicon (Sumco Oregon Corp., Salem, OR). Both substrates were coated with poly(4-vinylpyridine) (PVP). Substrates of this composition combine the advantages of colloidal suspensions and the stability of solid substrates.⁴⁵ Cut fused quartz or silicon substrates were first treated with a piranha solution (a concentrated solution of sulfuric acid and hydrogen peroxide) for two hours followed by rinsing with ethanol and drying with nitrogen. Films of poly(4-vinylpyridine) (Aldrich Chemical Company) were deposited by immersing in dilute (0.1%) solutions in absolute ethanol (AAPER Alcohol, Shelbyville, KY) for two hours followed by rinsing with ethanol, drying with nitrogen and were then allowed to cure overnight.⁴⁶ The hybrid bilayer ibuprofen solutions were drop dried on the functionalized fused quartz or silicon substrates and then examined with either an in Via Raman microscope (Renishaw) with a 63 $\times$ water immersion objective after rehydrating the sample at the appropriate pH, or a normal incidence transmission FTIR system (Thermo Nicolet) in air using a liquid nitrogen cooled MCT detector (4 cm^{-1} resolution, 256 scans). All IR and SEIRA spectra were background corrected using the Omni software package. Water purified by a Milli-Q water system was used throughout the experiments. The transmission electron microscopy (TEM) images presented in Figure 2 were obtained with a JEM 2010 Cryo-TEM. Excitation generalized polarization spectra were obtained using a JOBIN YVON UV-vis Fluorolog.

Results and Discussion

Representative TEM images for both a bare nanoshell and a hybrid bilayer functionalized nanoshell are shown in Figure 2A and 2B, respectively. Additional evidence for the formation of hybrid bilayers on nanoshells is demonstrated by the optical image (Figure 2C), which shows nanoparticle solubility at various stages of hybrid bilayer formation. Unfunctionalized nanoshells easily disperse in aqueous solvent (Figure 2C, i), however, nanoshells functionalized with an alkanethiol are hydrophobic and preferentially disperse in an organic carbon disulfide phase, rather than a water phase (Figure 2C, ii). Once a lipid layer self-assembles atop of the alkanethiol, the nanoshells are readily dispersed back into water (Figure 2C, iii).

A phase-sensitive fluorescent probe, Laurdan, was utilized to examine possible hybrid bilayer gel-phase characteristics at both pH 3 and 10. Excitation generalized polarization is defined as

$$GP_{\text{ex}} = \left(\frac{I_{444} - I_{484}}{I_{444} + I_{484}} \right) \quad \text{Eq. 1}$$

where I_{444} and I_{484} are fluorescence intensities at 444 and 484 nm, respectively. The excitation generalized polarization spectra (GP_{ex}) can be utilized to indicate phase behavior below, near, and above the main phase transition temperatures.⁴³ The GP_{ex} spectra for hybrid bilayers with both deuterated DMPC-D54 and nondeuterated DMPC lipids are presented in Figure 3. Both deuterated and nondeuterated systems were probed because it has been experimentally observed that deuterating the lipid can lead to an approximate 4–5 degree decrease in the main phase transition temperature⁴⁷. For hybrid bilayers formed using nondeuterated DMPC, the GP_{ex} spectra show no appreciable tilt either downward or upward, indicating that they are not affected by the excitation wavelength. This evidence suggests that hybrid bilayers formed with nondeuterated DMPC are below the main phase transition temperature and have gel phase characteristics under the employed experimental conditions at both pH 3 and 10. For the deuterated hybrid bilayers, however, a slight downward tilt of the GP_{ex} spectra is observed for both pH values. Since GP_{ex} spectra show a downward tilt above the main phase transition temperature, it is reasonable to conclude that under these experimental conditions the deuterated hybrid bilayers reside in a liquid phase. These conclusions are in accord with the established result that the T_m for a deuterated lipid is lower than its nondeuterated analog.

Figure 4 shows the unenhanced Raman and IR spectra of sodium ibuprofenate along with the SERS and SEIRA spectra of ibuprofenate on bare nanoshells. The enhanced Raman and infrared (IR) spectra of sodium ibuprofenate differ from their respective unenhanced spectra, revealing significant chemical interaction between the molecule and the Au nanoshell surface (Fig. 4). The major peaks in the SERS spectrum (Figure 4A, iii) are attributable to the sodium ibuprofenate molecule and indicate its interaction with the surface. The intense low wavenumber peak at 249 cm^{-1} has been assigned to the adsorption of ibuprofenate onto the Au nanoshell surface via the COO^- moiety, and arises from the CO_2 -Au vibration.⁴⁸ Several peaks appear to be shifted in the SERS spectrum with respect to the unenhanced

Raman spectrum. For example, the in-plane ring deformation⁴⁹ at $\sim 637\text{ cm}^{-1}$ in the unenhanced spectrum shifts to $\sim 663\text{ cm}^{-1}$ in the SERS spectrum. The peaks at 1381 and 1579 cm^{-1} in the SERS spectrum can be attributed to the symmetric and asymmetric stretching vibrations of the COO^- group.⁵⁰ The presence of the peak at 1703 cm^{-1} in the SERS spectrum on bare nanoshells is assigned to the C=O stretch. The appearance of these three peaks allows us to infer that not all of the carboxylate groups of ibuprofen are in an ionized state. It is reasonable that both protonated and deprotonated forms of the molecule may be present, since the aqueous nanoshell solution ($\text{pH} \approx 5.2$) to which the ibuprofenate was added was near the pK_a of ibuprofen. Additionally, the $\sim 1185\text{ cm}^{-1}$ and $\sim 1610\text{ cm}^{-1}$ peaks are relatively intense in the unenhanced Raman but not in the SERS spectrum, which may be due to conformational variability of the ibuprofenate as it is adsorbed onto the Au nanoshell surface.

Although ibuprofen is a commonly prescribed analgesic, there have been few spectroscopic studies of this molecule in the literature,^{49–51} and none reported for sodium ibuprofenate. Also, ibuprofen exhibits a strong IR spectral dependence on its degree of solvation and local environment.^{52–56} The SEIRA spectrum (Fig. 4B, ii) shows several shifted and enhanced modes of ibuprofenate compared to its unenhanced spectrum. The various in-plane ring modes assigned by Jubert et al.⁴⁹ and Gordijo et al.⁵⁰ (at 847 , 1090 , 1365 , 1466 , and 1510 cm^{-1}) seen in the SEIRA spectra are shifted from their corresponding peak positions in the normal IR spectra. The significantly different spectra obtained for the unenhanced and enhanced Raman and IR cases indicate that both chemical interactions and electromagnetic effects are contributing to both SERS and SEIRA in these studies.

Hybrid bilayer-coated nanoshell substrates using a deuterated lipid (DMPC-D54) were exposed to a range of concentrations of ibuprofen at two different pH values to study its intercalation into the hybrid bilayers using SERS (Fig. 5A,B). The pH values three and ten were chosen so that ibuprofen remains predominantly in either the protonated or deprotonated form. Figure 5A,B provides spectral evidence for the deuterated lipid where there are additional peaks that occur due to C–D stretches in the 2000 cm^{-1} to 2200 cm^{-1} region. These peaks reveal that the lipid component of the hybrid bilayer is present near the nanoshell surface and allow for spectral segregation of different aspects of the hybrid bilayer system under study. By increasing the ibuprofen concentration at both pH values (Fig. 5A,B (ii–vii)), several ring modes of the molecule at 803 , 1185 , 1205 and 1610 cm^{-1} in the SERS spectra were observed to increase in intensity, confirming its presence in the lipid bilayer.^{49,57} Figure 5C shows the gradual increase of ibuprofen partitioning into the bilayers for both pH values obtained by monitoring the normalized SERS intensity of the strongest mode at 1610 cm^{-1} . The increase in intensity as a function of ibuprofen loading concentration displays an isotherm-like response. Interestingly, at low pH when the ibuprofen molecule is predominately protonated, the signal from ibuprofen is stronger, allowing for better detection than at high pH. The stronger ibuprofen signal intensity at low pH may result from a change in the hydrophobicity of the molecule. In the protonated form, ibuprofen is anticipated to be more hydrophobic in nature than in the dissociated form. Due to the hydrophobic effect, a decrease in pH may allow for more ibuprofen molecules to intercalate into the hybrid bilayer, increasing their local concentration and in turn, allowing for the ibuprofen molecules to get closer to the hydrophobic acyl chains.

Ibuprofen itself is a known surfactant and has a critical micelle concentration (CMC), above which it can exist in an aggregate, rather than monomeric form. The interactions between ibuprofen and lipids and its toxicity depend on the aggregation pattern of ibuprofen. High concentrations above the CMC for ibuprofen can damage the integrity of lipid bilayers. While electrochemical studies, such as cyclic voltammetry and impedance spectroscopy, can act as a direct way to examine membrane permeability and integrity,⁵⁸ similar studies to characterize pore formation for this system are difficult. The system presented here is unique in that it is the first demonstration of hybrid bilayers on a nanoparticle surface, but the small size of the nanoparticles and the lack of an electrically conductive continuous film of nanoparticles hinder their use as electrode probe, as finite interparticle separations are imperative for large spectroscopic enhancements. However, the observed gradual decrease in the C–D stretch intensity as a function of increasing ibuprofen concentration (shown in the SERS spectra in Figure 5A,B) may provide an indirect indication that hybrid bilayer is being disrupted by the presence of ibuprofen.

Al-Saidan et al. has reported a CMC value of 0.83 mM for ibuprofen solutions prepared in aqueous 0.2 M disodium hydrogen phosphate.⁵⁹ The 0.1 mM concentration detected at both pH 3 and 10 in the SERS measurements (Figure 5) is below the CMC of ibuprofen, and therefore, provides physiologically relevant information on the interaction of ibuprofen and hybrid lipid bilayers in a liquid-crystalline phase, characteristic to that of biologically functional cell membranes⁶⁰.

Unlike the SERS spectra of ibuprofenate on bare nanoshells (Figure 4A (iii)), the SERS spectra of intercalated ibuprofen exhibit similar peak positions to the unenhanced Raman spectrum of ibuprofen (Figure 5A,B (i)). For example, the 1610 cm^{-1} mode of ibuprofen, which was not a strong spectral feature in the SERS spectrum of ibuprofenate on nanoshells (Fig. 4A (iii)) is clearly visible in the SERS spectrum of the intercalated ibuprofenate (Fig. 5 (ii–vii)). Conversely, the spectral features in the $1165\text{--}1230\text{ cm}^{-1}$ region are intense in the unenhanced Raman (Fig. 5A,B (i)) and intercalated SERS spectra (Fig. 5A,B (ii–vii)), but not in the SERS spectrum of ibuprofenate on bare nanoshells (Fig. 4A (iii)). The lack of significant spectral shifts of the ibuprofenate modes in the case of hybrid bilayer intercalation indicates negligible chemical interactions between the ibuprofenate and the nanoshell surface. However, the molecule is nonetheless in close proximity to the nanoshell surface, since strong SERS enhancement is observed.

To test whether the Raman scattering cross section of isolated ibuprofen can account solely for the observed signal strength under these experimental conditions (1.18 mW, 60 seconds integration, 100 mM, see supporting information Figure S1, C) the Raman spectra were acquired in solution. The Raman spectrum of 100 mM ibuprofen without nanoshells shows negligible signal, indicating the presence of significant SERS enhancements when nanoshells are utilized as substrates (Fig. 5A,B (ii–vii)).

SEIRA provides a means to spectrally observe the effects of ibuprofen intercalation on the DMPC lipid portion (chemical structure shown in Fig. 6A) of the hybrid bilayer structure. Figure 6B (i) shows the normal incidence transmission SEIRA spectrum of hybrid bilayers on Au nanoshells. The IR absorption peaks of the hybrid bilayer are identifiable at various

frequencies and are listed in Table 1. The methyl symmetric and asymmetric bending modes at 1375 cm^{-1} and 1462 cm^{-1} , characterizing the aliphatic part of the hybrid bilayer, are all easily observable. The zwitterionic, polar headgroup of the hybrid bilayer also presents characteristic peaks (see Table 1). The symmetric and asymmetric PO_2^- stretches, the symmetric and asymmetric $\text{N}(\text{CH}_3)_3^+$ stretches, and the phosphate skeletal vibration (CO-P-O-C stretch) are also all clearly observable in the SEIRA spectrum. The asymmetric CO-O-C stretch and the ester carbonyls (primary and secondary) of the lipid appear as strong features.^{57, 61–63}

Figure 6B (ii, iii) shows the normal incidence transmission SEIRA spectra of ibuprofen intercalated into hybrid bilayers at ibuprofen loading concentrations of 200 mM and 500 mM, respectively. In these two spectra one can observe significant differences between the SEIRA spectra of hybrid bilayers (Fig. 6B (i)) and ibuprofen intercalated bilayers (Fig. 6B (ii, iii)). The SEIRA spectra of intercalated hybrid bilayers have molecular peaks from the hybrid bilayer with additional distinct peaks attributable to the ibuprofen intercalant (Table 2). Various ring modes of ibuprofen are clearly seen at 847, 1361, 1400, and 1510 cm^{-1} . These modes grow in intensity as the concentration of the ibuprofen loading solution is increased. An asymmetric CO_2^- stretch is observable at 1582 cm^{-1} and a carbonyl peak around $1730\text{--}1740\text{ cm}^{-1}$. The presence of the asymmetric carboxylate peak can be solely attributed to the deprotonated form of ibuprofen, since the lipid itself has a ketone rather than a carboxylate moiety. The carbonyl stretching peak, however, can be attributed to both the protonated form of ibuprofen and the lipid structure. Therefore, the presence of a protonated form of ibuprofen in the hybrid bilayers cannot be ruled out in addition to a deprotonated form interacting with the lipid. It is expected that the presence of ibuprofen would cause changes in the packing and ordering of DMPC molecules. In fact, it causes several peaks of the hybrid bilayer system to shift when compared to the case of nonintercalated bilayers. The presence of ibuprofen is observed to primarily affect the peaks arising from the acyl linkage (CO-O) and the polar phosphocholine headgroup of the hybrid bilayer. Symmetric and asymmetric CO-C stretches have undergone a shift in the peak position from 1067 to 1057 cm^{-1} and from 1175 to 1167 cm^{-1} , respectively. The ester carbonyl group develops a more prominent double peak structure in the intercalated system, and the symmetric phosphate stretching mode band also undergoes a slight but readily observable broadening. These spectral changes may arise from alterations in the local chemical environment of the lipid molecules when ibuprofen is present. For example, ibuprofen may change the conformational freedom of the lipid headgroups and/or disrupt the spatial packing of the lipids. Since the observed spectral peak shifts appear primarily for the functional groups in the polar headgroup and the backbone portion of the lipid, these results indicate that the interaction of predominantly ionized ibuprofen in hybrid bilayers takes place primarily at the zwitterionic polar headgroup and acyl chain region of the lipid.

In summary, based on the following observations we can conclude that ibuprofen is most likely interacting with the lipid portion of the bilayer rather than the alkanethiol layer. First, the SERS spectra (Fig. 5A (ii–vii)) indicate that with increasing ibuprofen concentration in the hybrid bilayer, the trans carbon-sulfur stretch ($\nu(\text{C-S})_{\text{T}}$) at 710 cm^{-1} remains significantly stronger than the gauche carbon-sulfur stretch ($\nu(\text{C-S})_{\text{G}}$) at 638 cm^{-1} .⁶⁴ Second, the low-wavenumber peak at 324 cm^{-1} , which corresponds to coupling between the

gold-sulfur stretch and the longitudinal acoustic modes of the alkane chain, does not shift in frequency with increasing ibuprofen concentration.⁶⁵ Ibuprofen intercalation into the alkanethiol layer would disorder the alkane chain packing, a disorder that would likely shift the longitudinal acoustic mode frequencies⁶⁶. These aforementioned observations indicate that the underlying alkanethiol layer remains ordered and largely unperturbed. Third, as discussed earlier, the SEIRA data (Fig. 6B) show significant differences in the peak positions of the lipid headgroup and acyl chain when predominately ionized ibuprofen is present in the bilayers, indicating that ibuprofenate is affecting the outer leaflet of the hybrid bilayer with a greater impact on the headgroup portion of lipid. Fourth, since the lipid headgroup is zwitterionic, when the ibuprofen is in an ionized form, it is certainly plausible for there to be electrostatic interactions between the ibuprofenate and the DMPC near the headgroup. And finally, while the SEIRA data suggests that it is the predominantly ionized state which shows an association with the headgroup and acyl region, the SERS data indicates that the hydrophobic effect dominates for intercalation of the protonated form. Together, these two experimental findings provide a more complete picture of ibuprofen interacting with and intercalating in hybrid lipid bilayers.

Conclusion

We have reported a study of ibuprofen intercalation into a hybrid bilayer structure, a membrane mimic system, using surface enhanced vibrational spectroscopies. The spectral features of ibuprofen appearing in the pH dependent SERS spectra indicate incorporation of the analyte into the bilayer. Stronger SERS signals of ibuprofen are observed at low pH, where hydrophobicity of the molecule plays a dominant role in its intercalation. Shifts in the lipid peak positions upon ibuprofen intercalation in the SEIRA spectra reveal that the headgroup portion of the lipid structure has been affected, indicating that predominantly deprotonated form interacts near the interfacial region of the hybrid bilayer. The spectroscopic results combined from SERS and SEIRA studies provide chemical insight into the nature of ibuprofen-lipid interactions and have clinical importance in understanding the effects of NSAIDs on the integrity and permeability of the gastric mucosal membrane. The plasmonic nanostructures utilized in these studies are applicable for spectroscopic investigation of other biologically relevant phenomena in membrane mimics, such as the effect of cholesterol on membrane fluidity, the role of glycolipids in membrane structure, and the binding of peripheral membrane proteins.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. Schematic illustration of (A) an ibuprofenate functionalized nanoshell and (B) a hybrid bilayer with ibuprofen intercalation on a nanoshell for SERS measurements. (C) The interparticle junction of two hybrid bilayer nanoshells with ibuprofen intercalation (dimer shown for simplicity) used for SEIRA measurements.

Figure 2.

TEM images of (A) a bare Au nanoshell and (B) hybrid bilayer functionalized Au nanoshells deposited on TEM grids (as shown by the line and contrast in B). (C) Optical images of Au nanoshells (i) dispersed in water, (ii) functionalized with an alkanethiol (which preferentially disperse in an organic carbon disulfide layer on the bottom, rather than the top water layer), and (iii) functionalized with a hybrid lipid bilayer dispersed in water.

Figure 3.
The excitation generalized polarization (GP_{ex}) spectra of hybrid bilayers on nanoshells (HBL) and deuterated hybrid bilayers on nanoshells (HBL-D54) with Laurdan at pH 3 and 10.

Figure 4.

Raman spectra (A) of (i) ibuprofen sodium salt powder (50× objective, 25.5 mW laser power), (ii) sodium ibuprofenate in aqueous solution (2 M, 63× objective, 40.6 mW laser power), and (iii) surface enhanced Raman spectrum of sodium ibuprofenate (50 mM, 63× objective, 0.144 mW) adsorbed onto Au nanoshells. IR spectra (B) of (i) ibuprofen sodium salt powder and (ii) surface enhanced IR spectrum of sodium ibuprofenate (50 mM) adsorbed onto Au nanoshells (inset shows high frequency regime). (Spectra offset for clarity).

Figure 5.

Raman spectra of (i) ibuprofen in aqueous solution at (A) pH 10 and (B) pH 3 and SERS spectra of hybrid bilayer functionalized nanoshells with deuterated DMPC as a function of ibuprofen concentration: (ii) 0 mM, (iii) 0.01 mM, (iv) 0.1 mM, (v) 1 mM, (vi) 10 mM, and (vii) 100 mM at (A) pH 10 and (B) pH 3. The dashed lines indicate the peaks from clearly identifiable modes of the ibuprofen, indicating its presence in the hybrid bilayer (spectra offset for clarity). (C) Normalized SERS intensity of ibuprofen ring mode ($I_{1610-1585}/I_{1434-1388}$) as a function of ibuprofen concentration in hybrid bilayers at pH 10 (■) and pH 3 (●) with best-fit Langmuir isotherm (lines). The fitting equation and parameters used are $y = (a \cdot b \cdot x^{(1-c)}) / (1 + b \cdot x^{(1-c)})$, where at low pH $a = 1.64 \pm 0.22$, $b = 1.53 \pm 0.87$, and $c = 0.42 \pm 0.21$ and at high pH $a = 0.57 \pm 0.09$, $b = 0.42 \pm 0.12$, and $c = 0.48 \pm 0.11$.

Figure 6.

(A) Chemical structure of 1,2-Dimyristoyl-*sn*-Glycero-3-Phosphocholine (DMPC) highlighting the phosphate and acyl linkage groups. (B) SEIRA spectra for (i) hybrid bilayers (30 μM dodecanethiol with 100 μM DMPC lipids), (ii) hybrid bilayers with 200 mM, and (iii) hybrid bilayers with 500 mM ibuprofenate loading solution. The stars indicate the peaks from clearly identifiable modes of the ibuprofenate, indicating its presence in the hybrid bilayer. The lines indicate DMPC modes (spectra are offset for clarity).

Table 1IR Peak Assignments for Hybrid Bilayers on Au Nanoshells^{57,61-63}

Wavenumbers (cm ⁻¹)	Band assignment
1740, 1730	C=O stretch (primary, secondary)
1462	CH ₂ scissor, Methyl asymmetric bend
1375	Methyl symmetric bend
1236	Asymmetric PO ₂ ⁻ stretch
1175	Asymmetric CO-O-C stretch
1094	Symmetric PO ₂ ⁻ stretch
1067	CO-P-O-C stretch
966	Asymmetric N(CH ₃) ₃ ⁺ stretch
920	Symmetric N(CH ₃) ₃ ⁺ stretch
871	Methyl rock
812	CH ₂ rock-twist
714	C-S stretch, Methylene rocking-twisting

Table 2IR Peak Assignments for Ibuprofen^{49,50}

Wavenumbers (cm ⁻¹)	Band assignment
847, 1361, 1400	C4–C6 ring stretch, in plane CH ring bend, CH bend
1510, 1549	Ring vibration
1582	Asymmetric CO ₂ ⁻ stretch