Diffuse Reflectance Fourier Transform Infrared Spectroscopy of Phospholipid Adsorption onto Silicic Acid

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ABSTRACT: Diffuse reflectance Fourier transform infrared spectroscopy was used to study the mode of adsorption of phosphatidylcholine (PC) in hexane onto silicic acid (SA). PC adsorption was mainly through the charged phosphate group with minimal binding through the ester carbonyl. When the SA surface with adsorbed PC is washed with hexane, containing a small concentration of isopropanol, the desorbed PC is recovered without structural change, i.e., there is no evidence of PC hydrolysis in the adsorption process. Adsorbent moisture probably promotes PC adsorption due to the increased availability of surface water hydroxyl groups for interaction with the PC phosphate groups. Isopropanol promoted PC binding by destabilizing PC reverse miscelles in solution, thus promoting its adsorption.
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KEY WORDS: Adsorption, Fourier transform infrared spectroscopy, isopropanol, phosphatidylcholine, phospholipid, water.

Phospholipids (PL) are crude vegetable oil components that are commercially removed to produce a refined oil of acceptable quality. This is done industrially by precipitating most of the PL with 1–3% water and then removing them by centrifugation. Residual PL in the oil are then removed by adsorption on synthetic silica gel in the presence of bleaching clays. Silica has greater affinity than clays for PL, which reduces competition between PL and oil pigments for clay adsorption sites.

Adsorption studies with simple model systems of silicic acid (SA) and crude soy oil diluted with hexane, i.e., miscella, have been useful in studying lipid/adsorbent interactions. These studies have involved measuring the concentrations of oil components before and after adsorption and plotting the amount adsorbed per gram vs. residual concentration after adsorption. Such work has shown that the binding of PL (1), carotenoids (2), and triglyceride (TG) (2) from a miscella is according to a Freundlich isotherm and is by competitive adsorption.

One percent isopropanol (IPA) in the miscella and water deactivation of SA promotes adsorption of PL (1), which was proposed to occur by removal of TG from the adsorption sites. PL were subsequently desorbed by a hexane/IPA/water solvent system (6:8:1). Bound TG can also be desorbed by washing with polar solvents, and the adsorbent can be reused (1). Binding of PL was enhanced by using SA in a column, rather than in a batch system (3). The mode of PL adsorption from a soy oil/hexane miscella by rice hull silica (4) was similar to that reported for synthetic SA (1).

Although much has been learned as to how to control PL adsorption, there is limited understanding of the interaction between the SA surface and PL. However, diffuse reflectance Fourier transform infrared spectroscopy (FTIR) (5,6) is a tool that has proved useful in showing that adsorption of TG to SA is by hydrogen bonding through an ester carbonyl group, which is unaffected when TG and IPA are present in equimolar amounts (7). Nevertheless, IPA in a hexane wash, containing no TG, is able to displace previously bound TG (7). Additional FTIR studies also showed that oleic acid (OA) was hydrogen-bonded to silanol sites through its carboxyl carbonyl group. IPA probably reduced OA adsorption to SA by hydrogen bonding with OA in solution as well as by competitive adsorption to silica (8). In these studies (7,8), FTIR spectra were highly reproducible, even if experiments were repeated months later.

The objective of this study was to use FTIR to examine the nature of PL binding to SA from hexane, and the mechanism by which IPA desorbs PL. The effect of IPA and water on the adsorption was also studied.

MATERIALS AND METHODS

Lipids, solvents, and adsorbents. Phosphatidylcholine (PC) (Sigma Chemical Co., St. Louis, MO), hexane high-performance liquid chromatography (HPLC), IPA (HPLC-grade)

and SA (Bio-Sil A., 100–200 mesh; Bio-rad Laboratories, Richmond, CA) were used.

SA/solvent interaction. Control experiments were conducted to examine the nature of the adsorbent and the adsorption of solvents in a lipid-free system. The FTIR spectrum of SA was observed with a Nicolet Model 205 FTIR instrument (Nicolet, Madison, WI) with a diffuse reflectance unit 0030–002 (Barnes Analytical, Stamford, CT) having a resolution of 4 cm^{-1}. Spectra were obtained by co-adding 100 interferograms before transformation to spectra. These conditions were used throughout, unless stated otherwise. This was done to obtain a baseline spectrum of the adsorbent, which was subtracted from the subsequent spectra after lipid adsorption. The effect of exposing the adsorbent to hexane was examined by adding 0.5 g of silica to 100 mL hexane in a sealed vessel and mixing for 15 min (2). The SA was recovered and dried under air flow in a hood for 15 min prior to FTIR analysis as described above. The IR spectra showed that no hexane remained on the SA after 15 min drying. The spectra of the clean SA and of IPA on SA are given elsewhere (8).

PC spectra. The FTIR spectrum of PC was obtained by direct transmission of the IR beam through a film of PC (0.12M) in hexane between salt windows.

SA spectra. The FTIR spectrum of SA (4% moisture) was observed. This produced a baseline spectrum of the adsorbent that, in some studies, was subtracted from spectra after lipid adsorption.

Adsorption from hexane. Half a gram of SA (4% moisture) was added to 5 mL of 0.12M PC in hexane and stirred in a sealed vessel for 15 min at 20°C. An FTIR spectrum of the adsorbent and a transmission IR spectrum of the solution after adsorption were then obtained.

Desorption by 0.5M IPA in hexane. After adsorption, the adsorbent was washed in a sealed vessel with 25 mL of 0.5M IPA in hexane. An FTIR spectrum of SA and a transmission spectrum of the IPA solution were obtained.

Effect of adsorbent moisture content. SA was prepared with 1.0, 4.0, and 14% moisture. One percent moisture content was obtained by drying SA at 105°C for 1 h. The unmodified moisture content was 4.0%. SA with 14% moisture was prepared by adding 0.5 g SA with 0.05 g deionized water and shaking in a closed vessel for 2 h.

For each treatment, 0.5 g of SA was stirred with 100 mL of 0.5mM PC in hexane for 15 min in a sealed vessel. The SA was recovered and dried under an air flow in a hood prior to FTIR analysis.

Effect of IPA in the miscella. Adsorption studies were repeated with 100 mL 0.05M IPA and 0.5mM PC in hexane. The SA (4% moisture) was observed after adsorption.

The data were interpreted by examining the literature values of IR adsorption (9–11).

RESULTS AND DISCUSSION

PC spectra. Figure 1 shows the FTIR spectrum of PC (0.12M) in hexane as seen on a salt window in its unbound
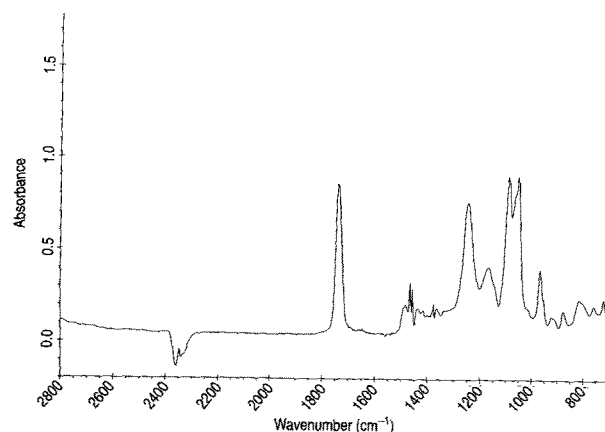

FIG. 1. The Fourier transform infrared spectrum of phosphatidylcholine 0.12M in hexane on a salt window with the hexane peaks subtracted.

form. The hexane peaks have been subtracted, as seen by the absence of C-H deformation bands for hexane at 1464 cm^{-1} and 1380 cm^{-1}. Significant stretches are the carbonyl at 1742 cm^{-1}, P=O at 1248 cm^{-1}, C-O-C (ester) at 1168 cm^{-1}, P-O-C (ester) at 1092 cm^{-1} and 1055 cm^{-1}, and P-O-C at 970 cm^{-1} (11). A C-H deformation band for PC is found at 1500 cm^{-1}.

Adsorption from hexane. Figure 2 shows the diffuse reflectance FTIR spectrum of the PC bound from 5 mL of 0.12M PC in hexane by 0.5 g SA of 4% moisture. The spectrum of SA has been subtracted. The SA spectrum was identical to that reported previously (7,8). The PC methylene stretch of the bound lipid shows weak asymmetric and symmetric C-H stretching vibration of CH$_2$ at 2929 and 2857 cm^{-1}. The C-H stretch for a R-C=C(H)R' group appears weakly at 3009 cm^{-1}. The CH$_2$ and CH$_3$ deformation bands appear around 1500–1460 cm^{-1}. The positive and negative peaks in the 1100–1400 cm^{-1} are due to changes in the Si-O-
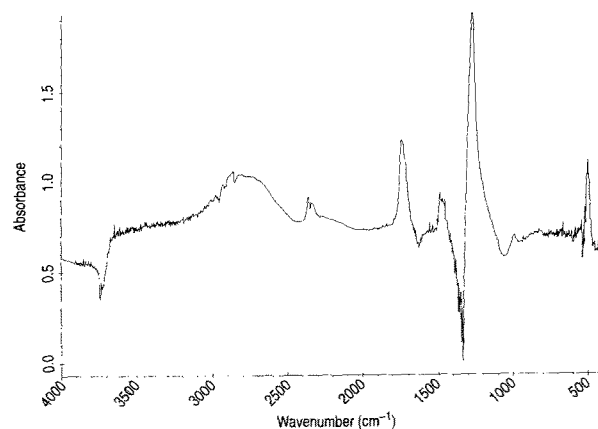

FIG. 2. The diffuse reflectance Fourier transform infrared spectrum of 0.5 g silicic acid (SA) with adsorbed phosphatidylcholine, which was bound by incubating with 0.12M solution in 5 mL hexane for 15 min. The SA spectrum has been subtracted.

Si stretching and Si-OH deformation bands changing with adsorption.

The broadening of the carbonyl stretch (1742 cm^{-1}) relative to the free form (Fig. 1) suggests that the carbonyl groups of the PC are interacting somewhat with the SA. Because the PC carbonyl peak is at the exact wavenumber as it is in the free form (Fig. 1), it appears that the carbonyl plays, at most, only a small role in PC binding to SA. Usually, strongly adsorbed functional groups are shifted to a lower wavenumber, relative to the unbound form. This is shown by TG and OA carbonyl groups complexing with SA (7,8).

The reason that no distinct phosphate bands are evident is that the interaction of highly polar charged PC phosphates with the adsorbent is so strong that the corresponding bands (970–1400 cm^{-1}) are greatly broadened. This makes the peaks difficult to identify against the shifting SA background.

Figure 3 shows the transmission IR spectrum of 0.12M PC in hexane as seen on salt windows after adsorption by 0.5 g of SA. The band frequencies and relative intensities are the same as those in Figure 1 before exposure to the SA. This similarity indicates that there are no detectable catalytic reactions that produce a change in the PC during the adsorption process.

Desorption by 0.5M IPA in hexane. Figure 4 is the diffuse reflectance FTIR spectrum of SA that was washed with 25 mL of 0.5M IPA in hexane after PC adsorption. The intensity of the carbonyl stretch at 1742 cm^{-1} shows that washing with IPA in hexane has little effect on the adsorbed PC. This is in sharp contrast to the behavior of adsorbed OA and TG, which in our previous work were shown to readily desorb upon washing with IPA in hexane (7,8).

An FTIR spectrum of the wash solution after desorption was observed on a salt window to see any modification of PC removal during the adsorption/desorption process (Fig. 5). It shows a low-intensity carbonyl stretch at 1742 cm^{-1} and stretches at P=O at 1248 cm^{-1}, P-O-C at 1092 cm^{-1}, and 1055

FIG. 4. The diffuse reflectance Fourier transform infrared spectrum of silicic acid (SA) binding phosphatidylcholine (PC) after incubation with 0.12M PC in hexane and subsequent desorption with 0.5M isopropanol in hexane. The SA spectrum has been subtracted.

cm^{-1}, C-O-C at 1168 cm^{-1}, and P-O-C at 970 cm^{-1}, in the same relative intensity to each other as the original PC bands shown in Figure 1. This suggests no change in the PC molecule during the adsorption/desorption process for the small amount of PC desorbed in the washing process.

Effect of adsorbent moisture content. Figure 6 shows the FTIR spectra of PC bound to SA of differing moisture content. The SA spectrum has been subtracted. The PC carbonyl band is present in all spectra. However, the phosphate peaks are not evident due to their being greatly broadened by interaction with surface OH groups of the SA, as discussed with respect to Figure 2. The importance of these spectra is that the carbonyl peak height becomes greater with increasing moisture content. The increasing carbonyl peak height indicates greater PC adsorption as the water content of the SA increases. The previous spectra have indicated a strong interaction between the phosphate group and the surface. These
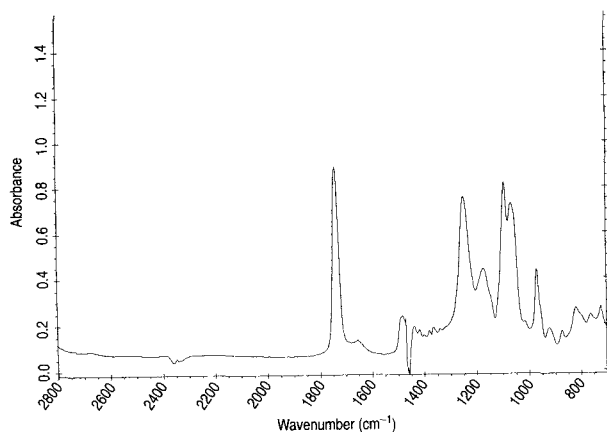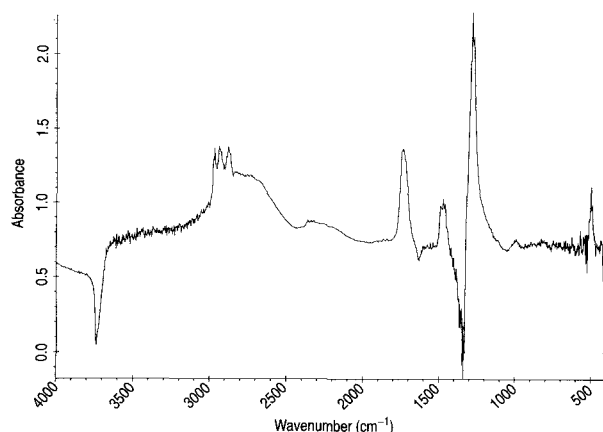

FIG. 3. The Fourier transform infrared spectrum of 0.12M phosphatidylcholine in hexane on salt windows after incubating with 0.5 g silicic acid for 15 min. The hexane peaks have been subtracted.
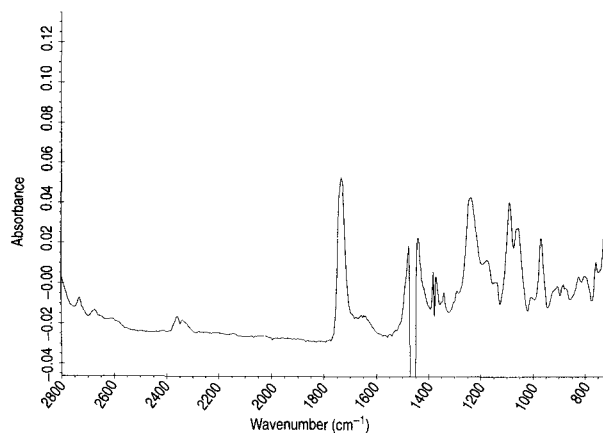

FIG. 5. The infrared spectrum of 0.5M isopropanol (IPA) in hexane solution, on a salt window, after using it to wash silicic acid, which was previously exposed to 0.12M phosphatidylcholine in hexane. The peaks of the unused 0.5M IPA in hexane solution have been subtracted.
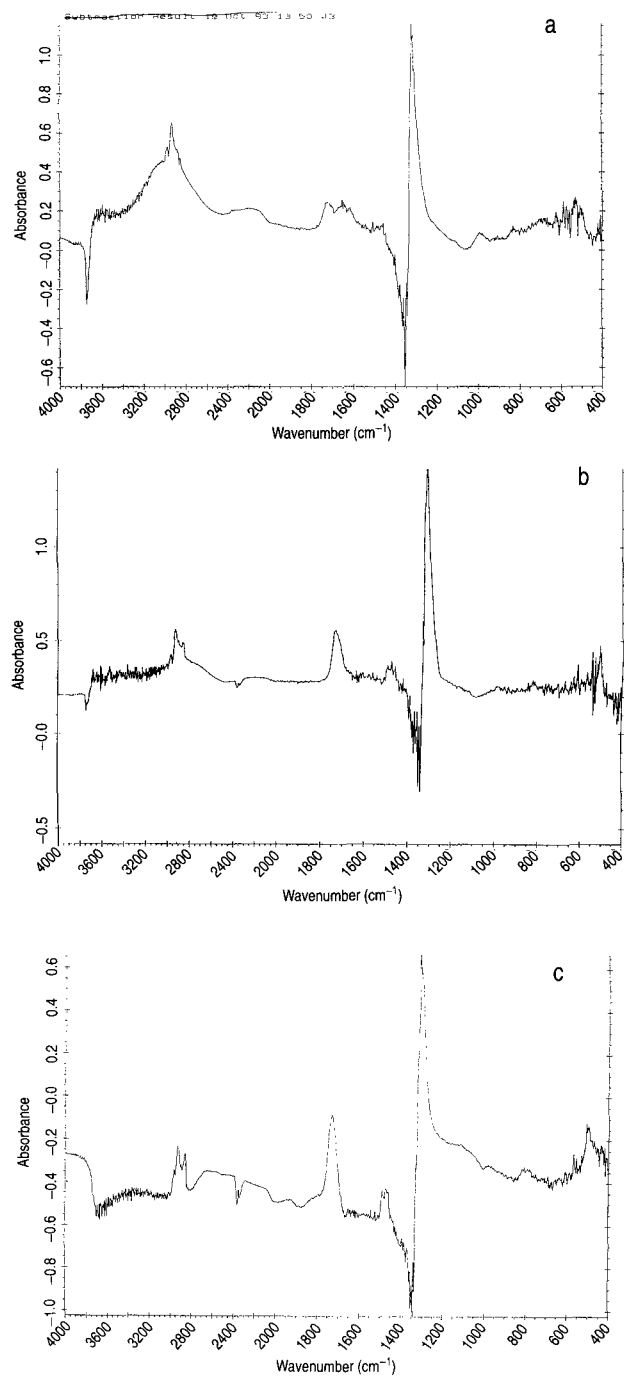
FIG. 7. The diffuse Fourier transform infrared spectrum of silicic acid recovered from 0.5 mM phosphatidylcholine in 0.05M isopropanol (IPA)/hexane with the spectrum of the adsorption without IPA subtracted.

FIG. 6. (a) The diffuse reflectance Fourier transform infrared (FTIR) spectrum of silicic acid (SA), at 1% moisture after incubation with 0.5 mM phosphatidylcholine (PC) in hexane. (b) The diffuse reflectance FTIR spectrum of SA at 4% moisture after incubation with 0.5 mM PC in hexane. (c) The diffuse reflectance FTIR spectrum of SA at 14% moisture after incubation with 0.5 mM PC in hexane.

supporting this mode of interaction is found in studies of water trapped within reverse PC miscelles, which indicate that PC hydrogen bonds to water molecules through a P-O and quaternary ammonium nitrogen groups (12). Previous work in this series (7,8) suggests that TG adsorption on SA is due to the interaction of ester carbonyl with the free OH groups of the SA surface. In the case of PL, the adsorption process is dominated by the interaction of the phosphate group with surface molecular water on SA. Thus, the phosphate interaction is stronger than that of the carbonyl.

Effect of IPA in the miscella. Figure 7 shows the diffuse FTIR spectrum obtained when the scan of SA recovered from 0.05 mM PC in hexane is subtracted from a similar spectrum obtained with 0.05M IPA. The spectrum shows a carbonyl stretch at 1742 cm^{-1}, with the C-H stretches at 2982, 2946, and 2880 cm^{-1}. Stretches at 2982 and 2880 cm^{-1} correspond to the C-H stretch typical of IPA on SA at 2982 cm^{-1} and 2884 cm^{-1}. The presence of PC causes the C-H stretch of IPA at 2884 cm^{-1} to be shifted to 2880 cm^{-1}. However, the stretch at 2982 cm^{-1} remains unchanged. Another significant peak is the C-H bending vibration at 1464 cm^{-1}. The phosphate stretch is widened between 900 and 1300 cm^{-1} in the presence of IPA, as it was in its absence. The intensity of the carbonyl band indicates enhanced binding of PC, which has also been reported in isotherm studies (1,4). These observations can be explained by IPA disrupting PC reverse micelles in hexane by attraction to the polar PC regions. Thus, the loss of micelle stability enhanced the probability of the polar groups of PC and IPA binding to adsorption sites.

To summarize, PC adsorbed to SA primarily through the phosphate group, but there is some evidence of hydrogen bonding through carbonyl groups. Nevertheless, much of the PC remained in solution. Desorption with IPA treatment recovered some PC from the adsorbent with no catalysis. In contrast to the adsorption of TG and fatty acids, carbonyl groups played a minimal role in PC adsorption. The data indicate that phosphate is the functional group holding PC to

spectra in Figure 6 indicate that this interaction is with the surface layer of molecular water because it is this layer of molecular surface water that increases to accommodate more PC as the moisture content of SA is increased. This interpretation is in agreement with reports that more PL is adsorbed on SA that has been water-deactivated (3). Further evidence
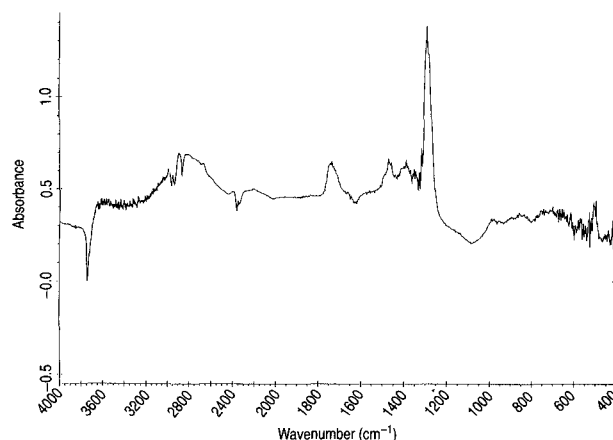
SA, although quaternary ammonium nitrogen may also play a role (12). In further contrast to the desorption of TG and fatty acids, PC desorption does not occur upon washing with IPA in hexane. This is mainly due to the strong interaction of phosphate and/or choline groups with the surface molecular water layer on SA. Adsorbent moisture probably promotes PC adsorption by providing a more reactive surface, whereas IPA promotes binding by disrupting stable PC reverse micelles in nonaqueous solvents.

ACKNOWLEDGMENTS

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