Chitosan as a Lipid Binder: A Langmuir Monolayer Study of Chitosan−Lipid Interactions

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Owing to its distinct chemico−biological properties, chitosan, a cationic biopolymer, offers a great potential in multifarious bioapplications. One such application is as a dietary antilipidemic supplement to be used to reduce obesity/overweight and to lower cholesterol. The lipid-binding efficiency of chitosan, however, remains debatable. Accordingly, in this study we investigated the interactions of chitosan with selected lipids, cholesterol and fatty acids, the latter including saturated (stearic acid) and unsaturated (oleic, linoleic, α-linolenic) acids. The experiments were performed with the Langmuir monolayer technique, in which surface pressure−area isotherms were recorded for the lipid monolayers spread on the acetate buffer pH 4.0 subphase in the absence and presence of chitosan. We found that the presence of chitosan in the subphase strongly influenced the shape and location of the isotherms, proving that there existed attractions between chitosan and lipid molecules. The attractions were revealed by changes of the molecular organization of the monolayers. The common feature of these changes was that all the monolayers studied underwent expansion, in each case reaching saturation with increasing chitosan concentration. In agreement with the lipid molecular structures, the highest expansions were observed for the most unsaturated fatty acids, linoleic and α-linolenic, the lowest for stearic acid, with oleic acid and cholesterol being the intermediate cases. By contrast, the main distinguishing feature of these changes was that, although none of the monolayers studied changed its state when completely saturated with chitosan, compared to the parent ones the compactness of the monolayers was modified. The solid monolayers of stearic acid and cholesterol were loosened, whereas those of all the unsaturated acids, liquid in nature, were tightened. On the basis of these results we tentatively propose a mechanism of the chitosan action that includes both electrostatic and hydrophobic lipid−chitosan interactions as well as hydrogen bonding between them.

Introduction

Typical of developed countries, the overindulgent consumption of fat-rich food concomitant with a decrease in energy expenditure by physical activity has led to a number of prevalent health disorders, overweight/obesity and hypercholesterolemia counted among the most serious ones. For the former, the survey data suggest that their prevalence is increasing globally at an alarming rate.[1−4] In the United States alone, now approximately one-third of the adult population is classified as obese and another third as overweight.[1,2,4] In addition to the impairment of the quality of life, overweight and obesity have been linked as causative factors with serious adverse health conditions that include cardiovascular diseases, type 2 diabetes, musculo−skeletal problems, and cancer.[2,4−6] The other disorder, hypercholesterolemia on the other hand, by leading to atherosclerosis is believed to be a major risk factor for coronary heart diseases, which now have been reckoned as one of the chief causes of death among adults in the Western world.[6−9]

In view of the above data, nonprescription lipid-lowering agents that could help reduce body weight and lower cholesterol, effective, safe, and inexpensive, are being increasingly sought. As a result, irrespective of their varying and not always fully substantiated effectiveness, a multitude of over-the-counter lipid-lowering dietary supplements are being marketed.[1,2] Among them is chitosan, available in the form of capsules and tablets, advertised to be able to both lower cholesterol and produce rapid weight loss.[5,7,10−12]

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Chitosan as a Lipid Binder. Chitosan is a polyaminosaccharide derived from chitin.[13,14] Chitin, one of the most plentiful renewable organic resources in nature, found mainly in the exoskeleton of crustaceans, such as shrimp, lobster, crabs, or krills, chemically is a linear polymer composed of *N*-acetylglucosamine units. Chitosan is obtained by *N*-deacetylation of chitin to varying degrees and is consequently a copolymer of *N*-acetylglucosamine and glucosamine. Chitin and chitosan can be chemically considered to be analogues of cellulose in which hydroxyls at carbon-2 have been replaced by acetamido and amino groups, respectively (Figure 1). Chitosan possesses distinct chemical and biological properties attributable to the presence of multiple amino groups in its molecules. First and foremost the groups make chitosan a cationic polyelectrolyte ($pK_a \approx 6.5$), one of the few found in nature. Due to this basicity chitosan is soluble in aqueous acidic media and when dissolved possesses high positive charge on $-NH_3^+$ groups, thereby being able to adhere to or aggregate with negatively charged molecules. This can be exploited in a variety of processes, medical treatments included, and these are possible due to excellent biocompatibility and physiological inertness of chitosan.

One such application of chitosan is as a dietary antilipidemic supplement where, owing to limited hydrolysis by human digestive enzymes, chitosan passes along the digestive system up to the large intestine practically intact, acting effectively like a dietary fiber. It has been proposed that the antihyperlipidemic action of chitosan consists of the following.[10−12] In the acidic environment of the stomach (pH ≈ 2) chitosan swells and forms a positively charged gel. The gel attracts negatively charged molecules of fats, fatty acids (oleic, linoleic, palmitic, stearic,

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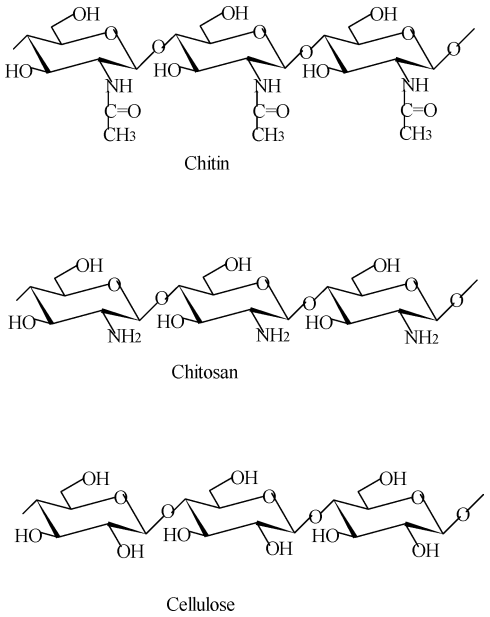

Figure 1. Structure of chitin, chitosan, and cellulose.

and linolenic) and bile acids (cholic, deoxycholic, and lithocholic), forming ionic complexes. In addition, chitosan can also interfere with emulsification of neutral lipids (cholesterol and other sterols) by binding them through hydrophobic interactions, thus forming hydrophobic complexes. Passing further along the digestive system, in the small intestine where pH is 7−8, the chitosan−lipid ionic and hydrophobic complexes solidify and as aggregates are subsequently excreted in the feces. Thus, it is by binding to chitosan that the lipid absorption from the small intestine to the bloodstream is thought to be prevented. Importantly, in cholesterol-lowering, the action of chitosan can also be viewed as that of a bile acid sequestering agent that reduces cholesterol by interrupting enterohepatic circulation.[9]

The antihyperlipidemic potential of chitosan has been studied both in vivo and in vitro.[5,6,10,11] In vivo studies included trials carried out both on animals and humans and consisted of a variety of determinations, mainly body weight, serum lipid levels, and lipid concentrations in feces. Remarkably, the data reported are conflicting. Although animal trials mostly showed reducing effects of chitosan on body weight and cholesterol levels,[7,8,11,15−20] human trials failed to show these effects.[3−6,10,21−25] Understandably, to gain a better insight into these effects (or their lack) in vitro studies of chitosan−lipid interactions are crucial. Such studies reported consisted mainly of precipitation of chitosan−lipid complexes and showed a considerable uptake of bile acids[26−28] and triglycerides[28,29] by chitosan. The mechanisms of chitosan action, however, are debatable, proving that broader research is needed to fully validate chitosan as a lipid binder. An interesting approach to look at chitosan−lipid interactions is by using a Langmuir monolayer technique.

Langmuir Monolayer Technique. The Langmuir monolayer technique makes use of the formation of one-molecule thick insoluble layers of compounds spread on an aqueous subphase.[30] Classic compounds used to prepare Langmuir monolayers are water-insoluble amphiphilic compounds that possess a hydrophilic headgroup and a hydrophobic tail. To prepare a monolayer, the compound is deposited onto the subphase as solution in a volatile solvent. A Langmuir monolayer can be compressed or expanded by modifying its area with a moving barrier in a Langmuir film balance. By measuring the surface pressure of the air−water interface during the compression, the surface pressure (π)−area (A) isotherms are obtained, in which bidimensional phases can be detected, each separated by a phase transition. The 2D phases in increasing pressure order are (1) bidimensional gas, (2) expanded liquid, (3) liquid, (4) condensed liquid, and (5) solid. Once the solid phase has been reached and the pressure is further increased collapse occurs, i.e., the monolayer breaks and aggregates or multilayers are formed. Importantly, the shape and location of the isotherms are indicative of the interactions both between molecules in the monolayer and between molecules of the monolayer and of the subphase. Accordingly, in addition to widely performed classic studies of the interactions between sterols, phospholipids, and fatty acids, whose monolayers can be regarded as model cellular membranes, the technique is also frequently applied to studies of the interactions of substances dissolved in the aqueous subphase, e.g., ions, drugs, enzymes, with the components of the monolayers formed at the interface.

In view of its great experimental potential, as yet unexploited in the field of chitosan applications,[31,32] in this work we used the Langmuir monolayer technique to investigate the interactions between chitosan and lipids. The lipids studied were cholesterol and fatty acids, the latter including stearic, oleic, linoleic, and α-linolenic, i.e., acids of the same length but differing in the degree of saturation. Chitosan was dissolved in the aqueous subphase on which the monolayers of the lipids were formed, and their $\pi-A$ isotherms were recorded. Making use of the variations of the limiting molecular area with chitosan concentration in the subphase and of the compression modulus with surface pressure (derived from the $\pi-A$ isotherms), we herein provide information on chitosan effects on a molecular organization of the lipid films at the interface and the role of saturation degree of fatty acids on the interaction with chitosan.

Experimental Section

Materials. Chitosan (chitin of Antarctic krill shells) was obtained from the Sea Fisheries Institute in Gdynia, Poland. A fraction of grade 0.43−0.75 mm was used whose weight-average molecular weight was 3.3×10^5 and deacetylation degree was of the order of 70%.[33] A stock chitosan solution of the concentration 10 mg/mL in 0.8% (v/v) acetic acid was prepared in the following way.[34] A volume of 1 mL of 80% (v/v) acetic acid was added to the suspension of 1 g of chitosan in 100 mL of water heated to 60 °C. The suspension was mixed with a magnetic stirrer to allow the complete dissolution of chitosan. The solution was then stirred for 2 h at 60 °C and allowed to stand for 20 h at room temperature. Chitosan solutions of concentrations between 0.025 and 0.300 mg/mL to be used as subphase solutions in the Langmuir balance measurements were prepared by dilution of the stock solution with 0.6 M acetate buffer pH 4.0. Double-distilled water was used throughout. The lipids studied, stearic, oleic, linoleic, and α-linolenic acids (purity ≥99%), were purchased from Aldrich, and cholesterol (purity ≥99%) from Sigma and were used as received. The spreading solutions of the lipids were prepared by dissolving each compound in freshly distilled chloroform (POCh, Poland).

Langmuir Monolayer Measurements. A Langmuir balance (NIMA, model type 301, U.K.) furnished with a Teflon trough of total area 300 cm^2, placed on an antivibration table, was used to determine the $\pi-A$ isotherms of the lipids. For that the spreading solutions of the lipids were deposited with use of a Hamilton microsyringe precise to ±2.0 μL onto the aqueous subphase in the trough, this being either acetate buffer pH 4.0 or the same buffer containing chitosan of different concentrations in the range between 0.025 and 0.300 mg/mL. The number of deposited molecules was always 2.3×10^{16}. Upon spreading, the solutions were left for 20 min to allow the solvent to evaporate, and subsequently the $\pi-A$ isotherms were measured. The films were compressed at a rate of 20 cm^2/min. The surface pressure of the films was measured with a Wilhelmy plate made of filter paper (ashless

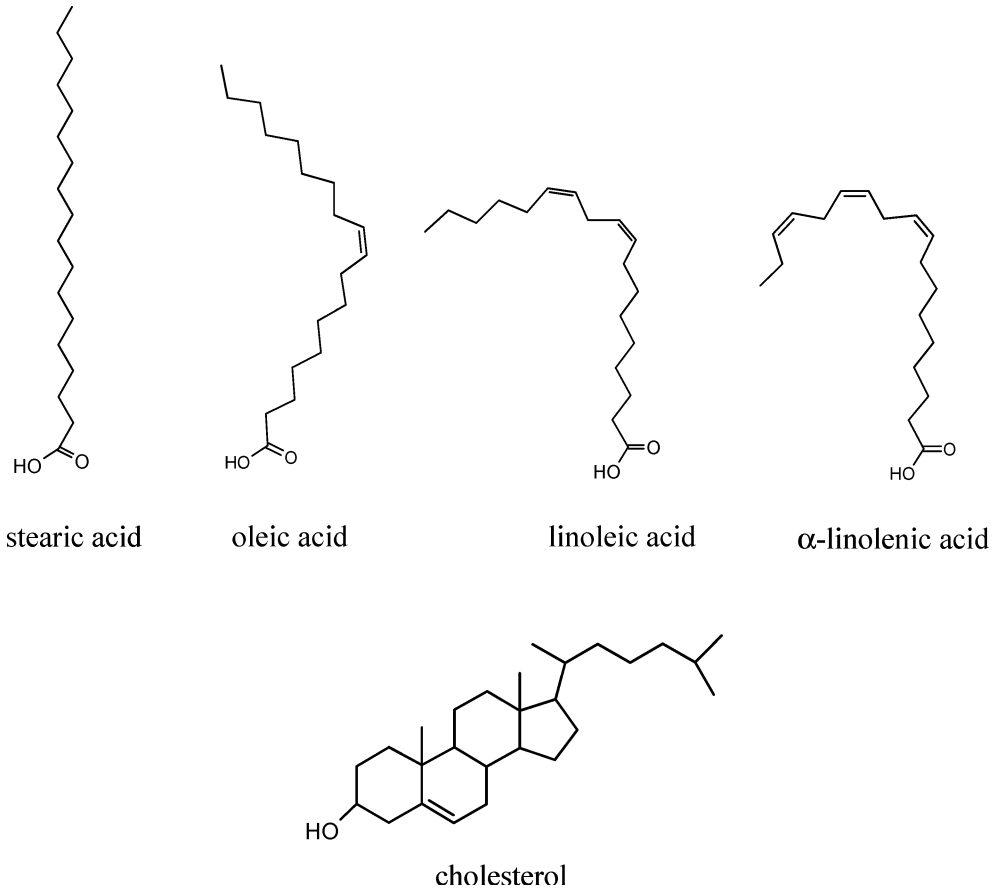

Figure 2. Structural formulas of the investigated lipids.

Whatman Chr1) connected to an electrobalance, to an accuracy of ±0.1 mN/m. The measurements were performed at 20 ± 0.1 °C.

Data Analysis. The principal characteristics of Langmuir monolayers from which data on the molecular organization of the monolayers and molecular interactions therein can be drawn include the course, shape, and the location of the $\pi-A$ isotherms. First, the limiting molecular area (A_{lim}) that corresponds to the surface area occupied by one molecule in a highly compressed monolayer is estimated, and this is done by extrapolation of the steep linear part of the $\pi-A$ isotherms to zero surface pressure. Second, the collapse pressure (π_{coll}), that is, a pressure at which a monolayer breaks, is determined from the point where an isotherm halts to rise upon monolayer compression. Third, to characterize the state of the monolayers and the phase transitions, the compression modulus (C_S^{-1}) is calculated. The compression modulus, which is the reciprocal of the monolayer compressibility, is defined as[35]

$$C_S^{-1} = -A\frac{d\pi}{dA} \quad (1)$$

The states of monolayers are classified on the basis of the maximal values of C_S^{-1} in the plots of C_S^{-1} versus π in the following way:[35] $C_{S,max}^{-1} = 12.5-50$ mN/m, liquid-expanded; $C_{S,max}^{-1} = 50-100$ mN/m, liquid; $C_{S,max}^{-1} = 100-250$ mN/m, liquid-condensed; $C_{S,max}^{-1} > 250$ mN/m, solid. The minima in the plots of C_S^{-1} versus π on the other hand, correspond to the phase transitions. In this work the compression moduli were obtained by numerical calculation of the first derivative from the isotherm data points with the OriginPro-7 program.

In this study the uncertainty of A_{lim} determinations was ±0.5 Å^2/lipid molecule, that of π_{coll} was ±0.1 mN/m, and that of C_S^{-1} was ±5 mN/m.

Results and Discussion

Fatty Acids. The fatty acids under investigation included stearic, oleic, linoleic, and α-linolenic acids. Their structural formulas are presented in Figure 2. The acids have the hydrocarbon chains of the same length but unsaturated to different degrees. Saturated fatty acids are known to form straight chains resembling rods. Unsaturated fatty acids, by contrast, have one or more double bonds which in most naturally occurring acids are in the cis conformation. This conformation makes the chain bend, and accordingly, the more double bonds the chain has in the cis configuration, the more curved the chain in its molecule becomes. For example, oleic acid, with one double bond, has a kink in the hydrocarbon chain and its molecule has a shape of a boomerang, whereas linoleic and α-linolenic acid with two and three double bonds, respectively, are similar to each other but have an increasingly pronounced bend in their hydrocarbon chains. The molecules of these acids favor a hooked shape. As a result of the specific molecular geometry, in restricted environments, such as in the monolayers studied in this work, unsaturated fatty acids, unlike saturated ones, have a limited ability to be tightly packed. These geometrical effects on the behavior of the Langmuir monolayers of fatty acids spread on the buffer and on the chitosan-containing buffer subphases were examined and are discussed below.

Fatty Acids Monolayers on the Buffer Subphase. The $\pi-A$ isotherms recorded for the fatty acids spread on the buffer subphase are shown in Figure 3, and the corresponding compression moduli C_S^{-1} are plotted against surface pressure π in the inset. The numerical characteristics of the isotherms are listed in Table 1.

As presented in Figure 3, the monolayer of stearic acid exhibited the characteristic isotherm with a change of slope at $\pi \approx 25$ mN/m and area of 18 Å^2/acid molecule, corresponding to the phase transition from the liquid-condensed to the solid state. This phase transition is also seen as a minimum at $\pi \approx$

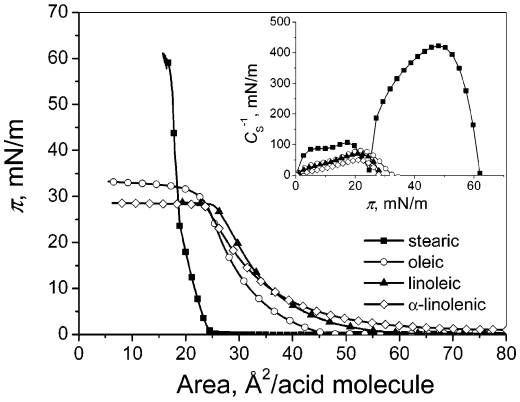

Figure 3. Surface pressure−area ($\pi-A$) isotherms of the fatty acids monolayers on the buffer subphase (inset: variation of the compression modulus C_S^{-1} with surface pressure π).

Table 1. Characteristics of the Langmuir Monolayers of the Fatty Acids on the Buffer Subphase

fatty acids	A_{lim}, Å²/acid molecule	π_{coll}, mN/m	$C_{S,max}^{-1}$, mN/m
stearic	20.0	62	420
oleic	34.5	32	82
linoleic	40.5	29	68
α-linolenic	40.0	28	49

25 mN/m in the plot of C_S^{-1} versus π (inset to Figure 3). The limiting molecular area A_{lim} of stearic acid yielded 20.0 Å²/acid molecule. Under the compression conditions used in this work, the film collapsed at surface pressure $\pi_{coll} \approx 62$ mN/m and area 17 Å²/acid molecule. Similar numerical characteristics of the monolayers of stearic acid on aqueous subphases were reported elsewhere.[32,36] Importantly, a steep shape of the isotherm of stearic acid as compared to the other acids in this study proves that this acid forms the most condensed monolayer. In contrast, the unsaturated fatty acids, oleic, linoleic, and α-linolenic, formed liquid-type monolayers (Figure 3), which collapsed in a less sharp manner at surface pressures lower than that of stearic acid (Table 1). A much less condensed character of these monolayers is also demonstrated both by the lower values of the compression moduli C_S^{-1} (inset to Figure 3) with their maximal values $C_{S,max}^{-1}$ also lower than that of stearic acid (Table 1) and by the limiting molecular areas A_{lim} that were found to be much higher than that of stearic acid (Table 1). The foregoing effects can be attributed to the molecular geometry of the acids discussed earlier. Namely, owing to its slim geometry stearic acid molecules occupy the smallest area, whereas linoleic and α-linolenic having the bulkiest molecules, the largest area in the monomolecular films. Consequently, for stearic acid the films are the most and for linoleic and α-linolenic the least condensed.

Fatty Acids Monolayers on the Buffer Subphase Containing Dissolved Chitosan. The $\pi-A$ isotherms measured for the fatty acids spread on the buffer subphase containing chitosan at different concentrations between 0.025 and 0.300 mg/mL are shown in Figure 4. The parameters derived from the isotherms, i.e., A_{lim} and $C_{S,max}^{-1}$, are plotted as a function of chitosan concentration in Figure 5, parts a and b, respectively.

As observed in Figure 4, following an increase in chitosan concentration in the subphase, the isotherms of all the fatty acids studied were shifted toward higher molecular areas, meaning that the monolayers underwent expansion. Most importantly, this expansion indicates that there exist strong attractive interactions between the fatty acids and chitosan. One way to measure the magnitude of this expansion is with the limiting molecular area A_{lim}. Figure 5a shows that the A_{lim} increases with an increase in chitosan concentration to reach saturation at concentrations of 0.050, 0.075, 0.100, and 0.100 mg chitosan/mL for stearic, oleic, linoleic, and α-linolenic acid, respectively. This means that, depending on the type of acid, at certain characteristic chitosan concentrations, no more chitosan molecules could interact with the acids molecules to further expand their monolayers. Apparently, the bulkier is the fatty acid molecule (more double bonds), the higher concentration of chitosan is required to achieve this saturation. Likewise, the magnitude of this expansion is dependent on the type of acid. If calculated as a difference between A_{lim} of the monolayer for two chitosan concentrations, at the saturation and zero (Figure 5a), ΔA_{lim} amounts to 1.8, 5.6, 9.7, and 9.2 Å²/acid molecule, for stearic, oleic, linoleic, and α-linolenic acid, respectively.

In addition to the expansion of the isotherms, the interactions of the fatty acids with chitosan were also manifested in other properties of the isotherms, again depending whether of saturated or unsaturated acids (Figure 4). The properties include the slope and the collapse pressure π_{coll} of the isotherms. On increasing chitosan concentration, stearic acid showed the reduction of the slope of its isotherms and the collapse pressures π_{coll} slightly decreased. By contrast, the unsaturated acids shared a common pattern of behavior, and unlike stearic acid, both the slopes and π_{coll} of their isotherms increased.

The reduction of the slope of the stearic acid isotherms, accompanied with the progressive vanishing of the phase transition, proves that the presence of increasing chitosan changed the monolayer of this acid to more fluid. This progressive change is also reflected in the variation of the compression modulus $C_{S,max}^{-1}$ with chitosan concentration shown in Figure 5b, in which up to a chitosan concentration of 0.1 mg/mL, the $C_{S,max}^{-1}$ underwent reduction to reach a stable value of about 285 mN/m, which correspondingly means that the monolayer is still within the range of the solid state though more fluid and more flexible than in the parent state. This in turn proves that due to the interactions with chitosan, stearic acid molecules changed their orientation from perpendicular with respect to the surface to tilt. Apparently, this less solid state of the monolayer is also responsible for an easier collapse of the monolayer as evidenced by the π_{coll} lowered by ca. 6 mN/m.

In contrast to stearic acid, the isotherms of the unsaturated fatty acids, upon the addition of chitosan to the subphase, became more condensed, this being revealed by progressively steeper isotherms (Figure 4). This finding is in keeping with the variations of the compression modulus. In Figure 5b we see that unlike for stearic acid, the $C_{S,max}^{-1}$ values for the unsaturated acids grew with an increase in chitosan concentration to reach plateaus above 0.1 mg chitosan/mL of ca. 106, 96, and 70 mN/m for oleic, linoleic, and α-linolenic acid, respectively. Remarkably, when compared with the parent ones (Table 1), these values demonstrate that the monolayers did not change their liquid state, however, became more rigid. Apparently responsible for this rise in rigidity are some specific acids−chitosan interactions altering the monolayer structure to more ordered. As a result, the collapse pressure of these monolayers on chitosan grew to be higher than that of the initial monolayers by 16 mN/m on average.

Cholesterol. The molecular structure of cholesterol is presented in Figure 2. Like stearic acid, cholesterol belongs to typical amphiphilic compounds whose film-forming properties at the air−water interface are exploited in Langmuir monolayer

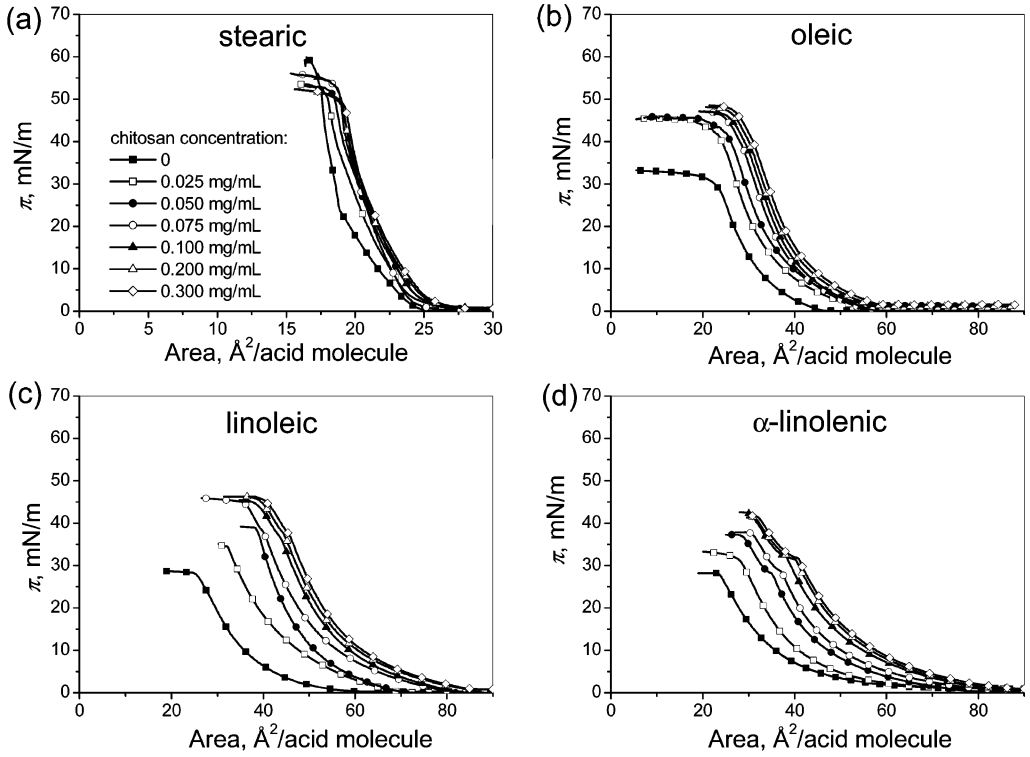

Figure 4. Surface pressure−area ($\pi-A$) isotherms of the fatty acids monolayers on the buffer subphase containing dissolved chitosan.

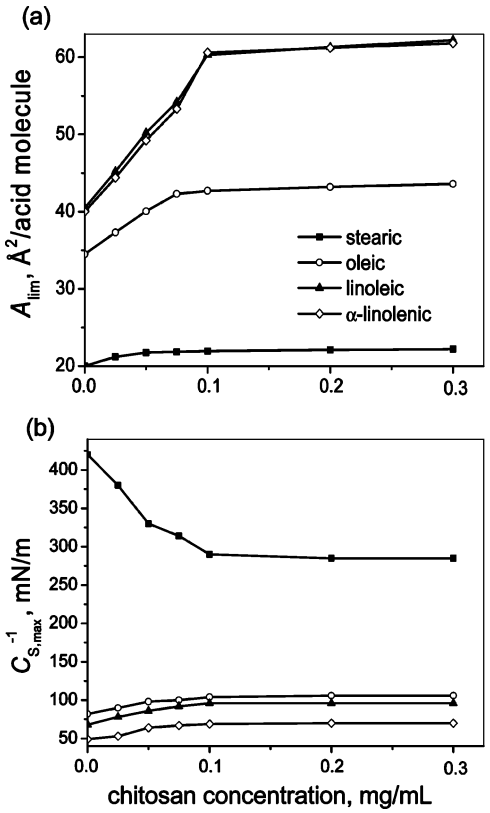

Figure 5. Properties of the fatty acids monolayers as a function of chitosan concentration in the subphase: (a) limiting molecular area A_{lim} and (b) the maximal compression modulus $C_{S,max}^{-1}$.

studies of molecular interactions in model mammalian cellular membranes.[36−39]

Cholesterol Monolayer on the Buffer Subphase. The $\pi-A$ isotherm for a cholesterol monolayer spread on the buffer subphase alone is shown in Figure 6 together with the isotherms for cholesterol monolayers on chitosan solutions in the buffer. The characteristics of the monolayer on the buffer subphase are listed in Table 2.

The $\pi-A$ isotherm for the cholesterol film on the buffer subphase possesses a steep shape typical of condensed monolayers. In the inset to Figure 6 we show the variation of C_S^{-1} with π for the film of pure cholesterol. The variation yields a very high maximal value of the compression modulus $C_{S,max}^{-1} =$ 751 mN/m attributable to a solid state of the film. The cholesterol isotherm is located at the limiting area $A_{lim} = 37.2$ Å²/cholestrol molecule, and it collapses at the surface pressure $\pi_{coll} = 45$ mN/m when an area of 35 Å²/cholestrol molecule is reached. Similar numerical data characterizing cholesterol films formed on water subphases were found by other authors.[31,32,36−39]

Cholesterol Monolayers on the Buffer Subphase Containing Dissolved Chitosan. As presented in Figure 6, similar to the action on the fatty acids, when added to the subphase, chitosan gave rise to the expansion of the cholesterol monolayer. Figure 7a shows that the expansion, if measured by the limiting area A_{lim}, saturated when chitosan concentration of 0.1 mg/mL was employed, and ΔA_{lim} amounted to 4.7 Å²/cholestrol molecule. As in the case of stearic acid, the compression modulus $C_{S,max}^{-1}$ of the cholesterol monolayers dropped with an increase in chitosan concentration (Figure 7b). The stable value it reached when the concentration exceeded 0.2 mg chitosan/mL was ca. 580 mN/m, proving that like stearic acid, the chitosan monolayer remained in the solid state, but its structure changed into less compact. This loss in compactness is a resultant of some cholesterol−chitosan binding bringing about the change of cholesterol orientation from perpendicular to tilt with respect to the surface.

Mechanisms of Lipid−Chitosan Interactions. The foregoing analysis of the effects brought about by chitosan present in the subphase to the monolayers of lipids, fatty acids, and cholesterol is supportive of significant lipid−chitosan attractive interactions. The analysis revealed that as a result of these

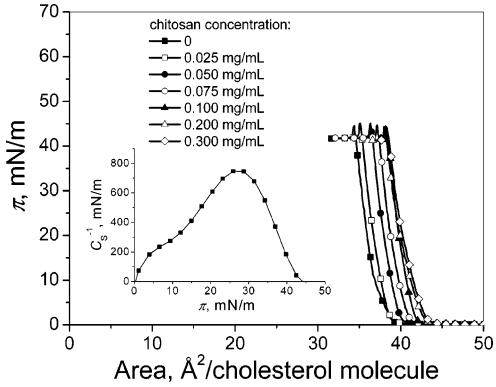

Figure 6. Surface pressure−area ($\pi-A$) isotherms of cholesterol monolayers on the buffer and on the buffer subphase containing dissolved chitosan (inset: variation of the compression modulus C_S^{-1} with surface pressure π for cholesterol monolayer on the buffer subphase).

Table 2. Characteristics of the Langmuir Monolayer of Cholesterol on the Buffer Subphase

	A_{lim}, Å²/cholestrol molecule	π_{coll}, mN/m	$C_{S,max}^{-1}$, mN/m
cholesterol	37.2	44	751

interactions chitosan is capable of changing the molecular organization of the monolayers. The common feature of these changes is that all the monolayers studied underwent expansion, in each case reaching saturation with increasing chitosan concentration. The extents of this expansion were different. The highest were observed for the most unsaturated fatty acids, linoleic and α-linolenic, the lowest for stearic acid, with oleic acid and cholesterol being the intermediate cases. By contrast, the main distinguishing feature of these changes is that, although none of the monolayers studied changed its state when completely saturated with chitosan, compared to the parent ones the compactness of the monolayers was modified. The monolayers of stearic acid and cholesterol were loosened, whereas those of all the unsaturated acids were tightened.

A number of possible phenomena may be regarded as effectively contributing to these interactions: (i) the molecules of fatty acids may form electrostatic complexes with chitosan through interactions of their carboxylic groups with $-NH_3^+$ groups of chitosan, (ii) chitosan molecules may get accommodated in the monolayers, possibly through hydrophobic interactions, (iii) hydrogen bonds between hydroxylic groups of cholesterol and of chitosan may be formed, and possibly (iv) chitosan may also have an effect on the conformation of the lipids in the monolayers. Of these, as the most likely, contributions of electrostatic and hydrophobic interactions have been most extensively discussed in the literature. For instance, in the formation of complexes of chitosan with anionic surface-active compounds (SDS), it was demonstrated that the interactions were predominantly electrostatic in origin.[40] Also, when chitosan was applied for adsorption of bile acids, sodium taurocholate, sodium taurodeoxycholate, and sodium glycocholate, water-insoluble hydrophobic bile acid−chitosan salts were precipitated, mainly by electrostatic interactions.[26] However, when in the same study the salts were further used for adsorption of butter and oils, these were found to be collected by the salts to high extents, much higher than those reported for plain chitosans, interestingly, with no discrimination of their components, cholesterol and fatty acids included. It was concluded that the hydrophobic interactions prevailed in these processes.

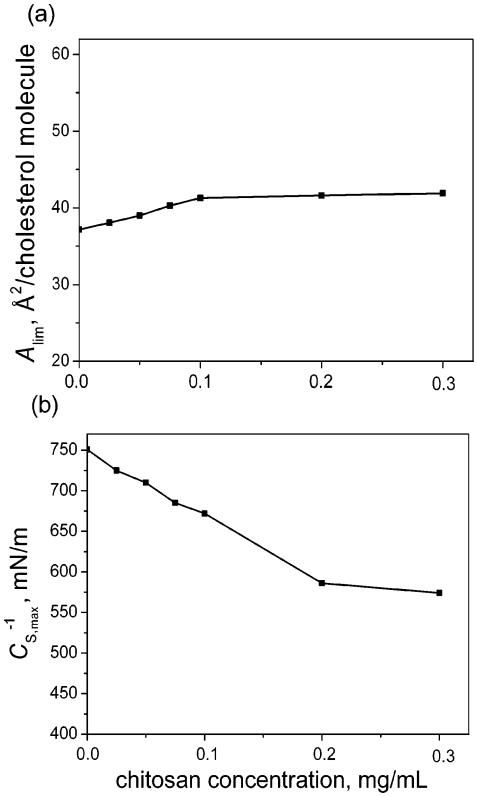

Figure 7. Properties of the cholesterol monolayers as a function of chitosan concentration in the subphase: (a) limiting molecular area A_{lim} and (b) the maximal compression modulus $C_{S,max}^{-1}$.

These findings are in agreement with other data, e.g., for *O,O′*-dipalmitoyl chitosan, a highly hydrophobic amphiphilic compound that readily adsorbed cholesterol from polar and nonpolar solvents.[41] The adsorption was found to be mainly dependent on the hydrophobic interaction. In another report it was observed that the capacity of dialkylaminoalkyl chitosans to adsorb sodium glycocholate increased with the number of carbons in the alkyl groups, indicating that the hydrophobic interaction played a major role in the sequestration of bile acids.[42] Likewise, the capacity of 6-oxychitosan for cholic acid decreased with increasing degree of oxidation, i.e., loss of cationicity.[43] A similar conclusion that cationicity is not needed to collect sterols and fatty acids from fats and oils was drawn from the experiments where chitin, chitosan, *N*-lauryl chitosan, and *N*-dimethylaminopropyl chitosan, the last two endowed with higher hydrophobicity and cationicity, respectively, were utilized.[29] Also, in a study of stearic acid and cholesterol Langmuir monolayers formed on subphases containing different kinds of chitosans, it was shown that a hydrophobically modified chitosan had a higher affinity to the lipids.[32] By contrast, in another Langmuir monolayer study of cholesterol on a chitosan solution, a suggestion was offered that the cholesterol−chitosan interaction consisted of hydrogen bond formation between hydroxyls in cholesterol and chitosan.[31] A similar finding based on an observation that the presence of multiple hydroxyl groups on bile acids enhanced their adsorption on chitosans was reported in ref 28. In the same work fat- and bile acid binding on chitosans of different physicochemical properties was studied, and a general conclusion was proposed that the hypolipidemic activity of chitosan may involve multimechanisms. Also very importantly, in a study of systems composed of chitosan and nonionic sorbitan esters, the authors concluded that for the mechanism of interaction between

chitosan and surfactants, the concentrations must be considered, as depending whether in dilute or concentrated solutions different interactions and different structures may be operative.[44]

With the results obtained in our study, a clear quantification of the effects participating in the interactions between chitosan and the lipids is problematic, even though the deacetylation degree of chitosan and the number of lipid molecules deposited are known. But unknown remains how much chitosan comes up to the monolayer to possibly be incorporated therein. However, considering our results and the supporting literature we may tentatively speculate that in our system the lipid molecules interact with chitosan in two steps. In the first step the molecules get anchored to chitosan chains through interactions between their headgroups and the specific functional groups of chitosan, i.e., between the carboxylic groups of fatty acids and $-NH_3^+$ groups of chitosan by electrostatic interactions, and between the hydroxyl groups of cholesterol and of chitosan by hydrogen bonding, which makes lipid molecules localized. Importantly, the bulkiness of the unsaturated fatty acid molecules allows for looser packing at the air−water interface than stearic acid and cholesterol. In the second step, hydrophobic interactions between the lipid tails and chitosan take place allowing chitosan to come up to the monolayer and fill in the empty space, this apparently resulting in the higher expansion of the monolayers observed for the unsaturated fatty acids than for stearic acid and cholesterol. On increasing chitosan concentration in solution, the expansion of the monolayers increases to finally reach the point of saturation. It can further be viewed that chitosan filling the space between the molecules of the unsaturated acids imparts rigidity to the monolayers, which was observed. This proposed interpretation of our results that in addition to lipid−chitosan electrostatic interactions and hydrogen bonding includes hydrophobic lipid−chitosan interactions, however, needs further research to be carried out for the mechanism to be fully resolved.

Conclusions

This study used the Langmuir monolayer technique to characterize the interactions between chitosan and lipids. Studied were cholesterol and fatty acids, saturated (stearic) and unsaturated (oleic, linoleic, and α-linolenic). The monolayers of the lipids were formed on the subphase containing different concentrations of chitosan, and their isotherms were measured. The results showed that chitosan significantly modified the monolayers. Their expansion provided evidence for chitosan binding to the lipids. The expansion was found to reach the saturation with increasing chitosan concentration. For the fatty acids, overall, it may be stated that the degree of saturation of the acid hydrocarbon chain had a significant impact on the architecture of the acid complexes developed, namely, the higher was the number of the double bonds, the higher the monolayer expansion at saturation took place. This effect was correlated with the molecular geometry of the acids. Electrostatic interactions, hydrogen bonding, and hydrophobic interactions were considered as possibly participating in the overall mechanism of chitosan−lipid interactions at the air−water interface. Thus, the results of this study confirm the formation of lipid−chitosan complexes and also provide particulars to the understanding of the interactions that give rise to these complexes. They may prove useful in validating chitosan as a dietary antilipidemic supplement.

References and Notes

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