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Protective effect caused by the exopolymer excreted by *Pseudoalteromonas antarctica* NF_3 on liposomes against the action of octyl glucoside

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Abstract

The capacity of the glycoprotein (GP) excreted by *Pseudoalteromonas antarctica* NF_3, to protect phosphatidylcholine (PC) liposomes against the action of octyl glucoside (OG) was studied in detail. Increasing amounts of GP assembled with liposomes resulted for the same interaction step in a linear increase in the effective surfactant to PC molar ratios (Re) and in a linear fall in the surfactant partitioning between bilayer and the aqueous phase (partition coefficients K). Thus, the higher the proportion of GP assembled with liposomes the lower the surfactant ability to alter the permeability of vesicles and the lower its affinity with these bilayer structures. In addition, increasing GP proportions resulted in a progressive increase of the free surfactant concentration (S_W) needed to produce the same alterations in liposomes. The fact that S_W was always lower than the surfactant critical micelle concentration indicates that the interaction was mainly ruled by the action of surfactant monomers, regardless of the amount of assembled GP.

Keywords: New antarctica bacterial species *Pseudoalteromonas antarctica* NF_3 ; Exopolymer of glycoproteic character; Phosphatidylcholine liposomes; Octyl glucoside; Permeability alterations; Carboxyfluorescein release; Effective molar ratio of surfactant to phospholipid in bilayers; Surfactant partition coefficient

Abbreviations: PC, phosphatidylcholine; CF, 5(6)-carboxyfluorescein; cmc, critical micellar concentration; GP, glycoprotein; K, bilayer/aqueous phase surfactant partition coefficient; OG, octyl glucoside (*n*-octyl β-D-glucopyranoside); PI, polydispersity index; PIPES, piperazine-1,4 bis(2-ethanesulphonic acid); r^2, regression coefficient; Re, effective surfactant/lipid molar ratio; SW, surfactant concentration in the aqueous medium; TLC-FID, thin-layer chromatography/flame ionization detection system.

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1. Introduction

Prokaryotic organisms have developed in the course of evolution a broad spectrum of cell envelope structures. Despite this diversity, two separate surface enveloping structures can be distinguished, the plasma membrane and the associated cell wall proper (Beveridge, 1989; Beveridge and Graham 1991; Sleytr and Messner, 1992). Crystalline bacterial cell surface layers are a two-dimensional array of proteinaceous subunits termed S-layers (Sleytr, 1978; Sleytr and Messner, 1988; Austin et al., 1990). Isolated S-layer subunits from many organisms reassemble into regularly structured lattices in the presence or absence of supporting layers or interfaces (Pum et al., 1993; Sára and Sleytr, 1993). Recently, it was demonstrated that solubilized S-layer protein form of *Bacillus coagulans* E38-66 can recrystallize onto positively charged phosphatidylcholine (PC) liposomes. This characteristic feature is being exploited at present for coating and stabilizing liposomes in spite of the complex process of isolation of these crystalline cell surface layers (Sleytr and Sára, 1989; Küpcü et al., 1995). These recrystallized S-layers could subsequently be used as a regularly structured matrix for immobilizing functional molecules.

The use of liposomes as vehicles for drug delivery is limited because of their short survival time in blood. The effect of poly(ethylene glycol) in the fusion of phospholipid vesicles and to prolong their circulation time in blood has been recently studied (Yang et al., 1997; Edwards et al., 1997). Liposomes have been also used as membrane models to study the vesicle to micelle transformations that occurred in the interaction of surfactants with these bilayer structures (Paternostre et al., 1995; Polozava et al., 1995; Inoue, 1996; Cladera et al., 1997).

One of the most commonly used amphiphilic compounds in membrane solubilization and reconstitution experiments is the nonionic surfactant octyl glucoside, which is believed to be a 'mild' surfactant given its low denaturing effect on proteins (Vinson et al., 1989; Almog et al., 1990; Cully and Paress, 1991; Lummis and Martin, 1992; Oku et al., 1996; Tetlo et al., 1996; Seras et al., 1996).

We first studied the ability of the exopolymer of glycoproteic character excreted by *Pseudoalteromonas antarctica* NF_3, to coat phosphatidylcholine (PC) liposomes and to protect these bilayers against the action of various surfactants (de la Maza et al., 1998a,b,c). We also investigated the interaction of octyl glucoside with PC liposomes (de la Maza and Parra, 1994; de la Maza et al., 1998d). Here, we seek to extend these investigations by studying in detail the interaction of this nonionic surfactant with PC liposomes coated with increasing amounts of this exopolymer. To this end, we determined the effective molar ratio of surfactant to PC (Re) and in the surfactant partition coefficient between bilayers and water (K) as a function of the proportion of the exopolymer present in the system. This information may be useful to establish a criterion for the evaluation of the protective effect of this exopolymer on a simplified membrane model as PC liposomes.

2. Materials and methods

PC was purified from egg lecithin (Merck, Darmstadt, Germany) according to the method of Singleton et al. (1965) and was shown to be pure by TLC. Octyl glucoside (*n*-octyl β-D-glucopyranoside) (OG) was purchased from Sigma Chemicals Co. (St Louis, MO, USA). Piperazine-1,4 bis(2-ethanesulphonic acid) (PIPES) was obtained from Merck. PIPES buffer was prepared as 20 mM PIPES adjusted to pH 7.20 with NaOH, containing 110 mM Na_2SO_4. The starting material 5(6)-carboxyfluorescein, (CF) was obtained from Eastman Kodak (Rochester, NY, USA) and further purified by a column chromatography (Weinstein et al., 1986). Anti-freeze glycoproteins (AFGP) types I, II, and III, were suppied by AFP, A/F Protein, (Waltham, MA, USA). The glycoprotein produced by *P. antarctica* NF_3 (GP) was excreted into the culture medium and, consequently, did not form part of the bacterial cell wall (Bozal et al., 1994, 1997). The original isolate was obtained from a sludge sample collected at the bottom of a glacier in the region of Inlet Admiralty Bay (King George Island, South Shet-

land Islands) (Bozal et al., 1994). The purified glycoprotein is at the present available at laboratory scale (Bozal et al., 1996).

2.1. Preparation and characterization of GP/liposome systems

Unilamellar PC liposomes of about 200 nm were prepared by extrusion of large unilamellar vesicles obtained by reverse phase evaporation in PIPES buffer (de la Maza et al., 1998d). Liposomes were extruded through 800–200 nm polycarbonate membranes at 25°C using a thermobarrel extruder equipped with a thermoregulated cell compartment (Lipex Biomembranes, Inc. Vancouver, Canada). Liposomes were combined with GP aqueous dispersions to obtain different GP/liposome mixtures (PC/GP weight ratio 9:1, 8:2 and 7:3) (de la Maza et al., 1998a). The resulting GP/liposome aggregates were freed of the GP non-assembled with liposomes. To this end, the aggregates were sedimented at 140 000 × *g* at 25°C for 2 h and then resuspended in PIPES buffer. No PC was detected by thin-layer chromatography coupled to an automated flame ionization detection (TLC-FID) (Ackman et al., 1990) in any supernatant in spite of its opalescent aspect due to the presence of free GP. The percent of GP assembled with liposomes (vs PC concentration) was determined as the difference between the percentage of added GP and that remaining in the supernatant after sedimentation of the GP/liposome aggregates. This remaining percent (directly related to the opalescence of supernatants), was determined by measuring the static light scattering of the supernatants using the spectrofluorophotometer Shimadzu RF-540 at 25°C with both monochromators adjusted to 500 nm (de la Maza and Parra, 1994).

To study the permeability changes, vesicles containing CF were freed of the unencapsulated CF by passage through Sephadex G-50 medium resin (Pharmacia, Uppsala, Sweden) by column chromatography (de la Maza et al., 1998d). The PC concentration in liposomes was determined by TLC-FID (Ackman et al., 1990). The vesicle size distribution after preparation was determined with a Photon correlator spectrometer (Malvern Autosizer 4700c PS/MV) (de la Maza and Parra, 1994). After preparation the size of vesicles varied very little showing a similar value of about 200 nm (PI lower than 0.12), thereby indicating that the vesicle distribution was very homogeneous.

2.2. Parameters involved in the interaction of OG with coated liposomes

In the analysis of the equilibrium partition model proposed by Schurtenberger et al. (1985) for bile salt/lecithin systems, Lichtenberg (1985) and Almog et al. (1990) have shown that for a mixing of lipids (at a concentration L (mM)) and surfactant (at a concentration S_T (mM)), in dilute aqueous media, the distribution of surfactant between lipid bilayers and aqueous media obeys a partition coefficient K, given (in mM^{-1}) by:

$$K = S_B/[(L + S_B)\cdot S_W] \quad (1)$$

where S_B is the surfactant concentration in the bilayers (mM) and S_W is that in the aqueous medium (mM) (de la Maza and Parra, 1994). For $L >> S_B$, the definition of K, as given by Schurtenberger, applies:

$$K = S_B/(L\cdot S_W) = Re/S_W \quad (2)$$

where Re is the effective surfactant to lipid molar ratio in the bilayers ($Re = S_B/L$). Under any other conditions, Eq. (2) has to be employed to define K; this yields:

$$K = Re/S_W[1 + Re]. \quad (3)$$

The determination of Re, S_W and K was carried out on the basis of the linear dependence existing between the surfactant concentrations needed to reach the interaction steps studied and the PC concentration in liposomes, which can be described by the equation:

$$S_T = S_W + Re\cdot L \quad (4)$$

where Re and S_W are in each curve respectively the slope and the ordinate at the origin (zero PC conc). Permeability changes caused by OG in liposomes coated with increasing GP amounts were determined by monitoring the rise in the fluorescence intensity of liposomes due to the CF released from the interior of vesicles to the bulk

aqueous phase. Fluorescence measurements were made with a Shimadzu RF-540 spectrofluorophotometer (de la Maza et al., 1998d).

3. Results and discussion

In preliminary studies we studied the ability of some anti-freeze glycoproteins (AFGP) to coat PC liposomes. To this end, we investigated different AFGP found in Antarctic fish and northern col species. Although different in structure, these compounds were not able to coat PC liposomes and, consequently, did not protect these vesicles against the action of surfactants. This fact contrasts with the reported ability of the glycoprotein (GP) excreted by *P. antarctica* NF_3 to coat PC liposomes and to protect these bilayers against the action of various surfactants (de la Maza et al., 1998a,b,c). The specific behaviour of this compound could be attributable, in part, to its capacity to form aqueous dispersions at very low concentrations (de la Maza et al., 1997). This characteristic was not found in the other AFGP tested.

In the present study, we first determined the proportion of GP assembled with these liposomes for the different amount of GP added to the system (PC concentration 10.0 mM). The percentages of GP remaining in the supernatants after sedimentation of the GP/liposome aggregates (determined by static light-scattering) were 6, 9, and 17 for the systems formed by the PC/GP weight ratios 9:1, 8:2 and 7:3 respectively. As a consequence, the weight percentages of assembled GP (with respect to the PC) were 9.4, 18.2 and 24.9%, respectively.

3.1. Interaction of OG with coated PC liposomes

It is known that, in surfactant/lipid systems, complete equilibrium may take several hours (Inoue, 1996; Almog et al., 1990). However, in subsolubilizing interactions a substantial part of the surfactant effect takes place within approx. 30 min after its addition to the liposomes (Ruiz et al., 1988). In order to determine the time in which the leakage ceased, a kinetic study of the interaction of OG with coated liposomes was carried out (PC concentration ranging from 1.0 to 10.0 mM). Coated vesicles were treated with OG at subsolubilizing concentrations and subsequent changes in CF release were studied as a function of time. The CF release showed always a transient state of enhanced permeability of the liposomal bilayers, in which about 40–60 min was needed to achieve CF release plateaux for the PC/GP weight ratio ranging from 9:1 to 7:3. This behaviour was attributed to the incorporation of OG monomers into coated membranes, which facilitated the release of the flourescent dye encapsulated into the vesicles. It is noteworthy that this effect took place in all cases regardless of the amount of GP present in the system. The only difference was the fact that the time needed to achieve the aforementioned CF release plateaux increased with the percentage of GP in the system. Hence, permeability alterations were studied 60 min after the addition of surfactant to the systems at 25°C. The CF release of these systems in the absence of OG 60 min after preparation was negligible.

To determine the partitioning of OG between lipid bilayers and the aqueous phase we first studied the validity of the equilibrium partition model proposed by Almog et al. (1990), Lichtenberg (1985), based on Eq. (1) for the sytems studied. This equation may be expressed by: $L/S_B = (1/K)(1/S_W) - 1$. Hence, this validity requires a linear dependence between L/S_B and $1/S_W$; this line should have a slope of $1/K$, intersect with the L/S_B axis at -1 and intersect with the $1/S_W$ at K.

To test the validity of this model for the systems investigated, coated liposomes (at various PC/GP weight ratios) were mixed with varying sublytic OG concentrations (S_T). The resultant surfactant-containing vesicles were then spun at $140\,000 \times g$ at 25°C for 2 h to remove the vesicles. No PC was detected in the supernatants by (TLC-FID) (Ackman et al., 1990). The OG concentration in the supernatants (S_W) was determined by HPLC (Seino et al., 1984) and its concentration in the lipid bilayers was calculated ($S_B = S_T - S_W$). The S_B and S_W values obtained (at the same range of PC and OG concentrations used to determine K) were plotted in terms of the dependence of L/S_B on $1/S_W$. Straight lines were obtained for

each system tested ($r^2 = 0.991$, 0.990, and 0.992 for the PC/GP weight ratios 9:1, 8:2, and 7:3, respectively). These straight lines were dependent on L and intersected with the L/S_B axis always at -0.97 ± 0.12. Both the linearity of these dependences and the proximity of the intercept to -1 support the validity of this model to determine K for these surfactant/liposome systems.

To determine the Re, S_W, and K values, a systematic study of permeability changes of CF-containing liposomes was performed for liposomes coated with increasing GP amounts (PC/GP weight ratio ranging from 9:1 to 7:3). In each case the concentration of PC in liposomes varied from 1.0 to 10.0 mM. The CF release curves for the PC/GP weight ratio 7:3 as a function of the OG concentration are given in Fig. 1 (the curves for the other systems are not shown). The surfactant concentrations producing different percentages of CF release were graphically obtained and plotted vs the PC concentration. An acceptable linear relationship was established in each case. The straight lines obtained corresponded to Eq. (4) from which Re and K were determined. The Re, K, and S_W values as well as the regression coefficients (r^2) of the straight lines for the PC:GP weight ratios 9:1, 8:2 and 7:3 are given in Table 1.

The Re values always increased as the release of the trapped dye increased. Furthermore, rising GP proportions in the system led (for the same interaction step) to a progressive increase in Re. As for K, these values decreased as the release of the trapped dye increased in all cases. Increasing GP proportions resulted in a slight decrease of this parameter. In addition, the S_W values increased as the percentage of CF release increased in all cases. These findings are in line with the data reported for the interaction of pure PC liposomes with OG (de la Maza and Parra, 1994; de la Maza et al., 1998d). Increasing GP proportions in the system resulted in a progressive increase of S_W. The fact that more free OG concentration was needed to reach the same liposome alterations suggests that the GP coating structure acts as a barrier that protects progressively these bilayers, in agreement with our previous TEM observation (de la Maza et al., 1998a,b,c). Furthermore, the fact that the S_W values were always lower than the surfactant critical micelle concentration (18.0 mM) (de la Maza and Parra, 1994; de la Maza et al., 1998d) indicates that the interaction was mainly ruled by the action of surfactant monomers in all cases.

Fig. 2 shows the variation of Re versus the release of the trapped dye when varying the PC/

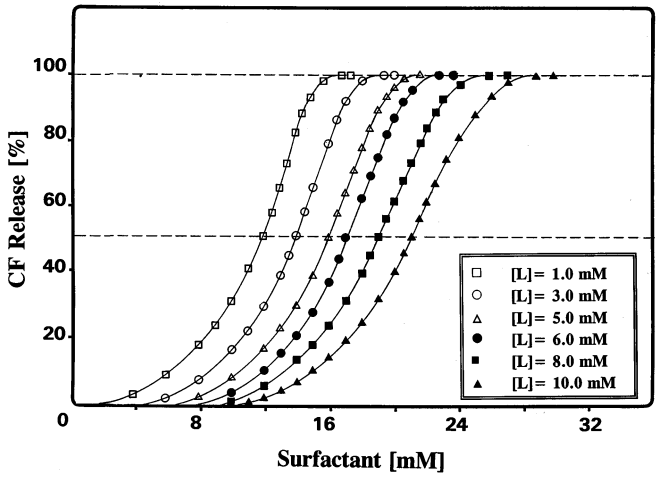

Fig. 1. Percentage changes in CF release induced by OG in PC liposomes (PC concentration ranging from 1.0 to 10.0 mM) coated with GP at the PC/GP weight ratio 7:3. $[L] = 1.0$ mM (□), $[L] = 3.0$ mM (○), $[L] = 5.0$ mM (△), $[L] = 6.0$ mM (●), $[L] = 8.0$ mM (■), $[L] = 10.0$ mM (▲).

Table 1
Re, K and S_W parameters as well as the regression coefficients (r^2) resulting in the interaction of OG with PC liposomes coated with increasing proportions of GP[a]

CF release%	Re			K			S_W			r^2		
	PC:GP weight ratio			PC:GP weight ratio			PC:GP weight ratio			PC:GP weight ratio		
	9:1	8:2	7:3	9:1	8:2	7:3	9:1	8:2	7:3	9:1	8:1	7:3
10	0.22	0.24	0.25	0.093	0.092	0.090	1.94	2.10	2.20	0.990	0.998	0.990
20	0.44	0.47	0.50	0.083	0.081	0.080	3.67	3.94	4.15	0.993	0.997	0.992
30	0.66	0.70	0.75	0.069	0.067	0.066	5.72	6.15	6.50	0.995	0.994	0.997
40	0.88	0.93	1.0	0.060	0.057	0.056	7.77	8.35	8.90	0.993	0.996	0.995
50	1.10	1.18	1.25	0.053	0.051	0.050	9.83	10.5	11.1	0.998	0.997	0.994
60	1.15	1.24	1.31	0.049	0.047	0.046	10.8	11.6	12.2	0.991	0.993	0.990
70	1.21	1.29	1.37	0.046	0.044	0.043	11.9	12.7	13.4	0.995	0.991	0.999
80	1.25	1.34	1.42	0.042	0.041	0.040	13.0	13.9	14.6	0.994	0.992	0.991
90	1.28	1.37	1.45	0.040	0.038	0.037	14.0	15.0	15.8	0.998	0.999	0.995
100	1.30	1.39	1.47	0.037	0.036	0.035	15.0	16.1	16.9	0.992	0.991	0.989

[a] PC/GP weight ratios: 9:1, 8:2 and 7:3.

GP weight ratio from 9:1 to 7:3. The Re values reported for pure PC liposomes (●) are also included (de la Maza and Parra, 1994). A linear relationship was established up to 40% CF release between Re and the release of the trapped CF in all cases. The presence of increasing GP amounts of led (for the same interaction step) to a rise in the Re values. Given that the surfactant capacity to alter the permeability of liposomes is inversely related to the Re values, the increasing presence of GP reduced this capacity. Hence, the protection of liposomes against OG increased with the proportion of GP in bilayers. The fact that the Re curves showed a similar trend than that exhibited by the curve for pure PC liposomes (●) suggests that the presence of increasing amounts of GP almost did not affect the mechanism of interaction between surfactant and PC bilayers. However, this increasing presence progressively reduced the surfactant activity on these bilayer structures.

Fig. 3 shows the variation in the partition coefficients (K) vs Re for liposomes varying the PC/GP weight ratio from 9:1 to 7:3. The K values reported for pure PC liposomes (●) are also included (de la Maza and Parra, 1994). A linear decrease in K occurred as the Re rose regardless of the GP proportion in the system. The parallelism between the curve of pure PC liposomes (●) and those for coated vesicles suggests that the mechanism of surfactant partitioning between bilayers and water was almost unaffected by the presence of increasing GP amounts in the system. However, this increase slightly reduced the surfactant affinity with liposomes (directly related to the K values, see Table 1).

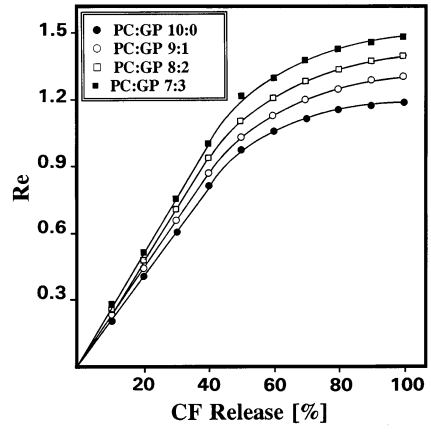

Fig. 2. Variation in the effective surfactant to PC molar ratio (Re) due to the action of OG vs the percentage of CF release of liposomes coated with increasing proportion of GP. PC/GP weight ratios: 10:0 (●), 9:1 (○), 8:2 (□) and 7:3 (■).

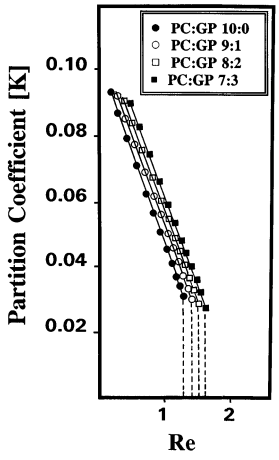

Fig. 3. Variation in the surfactant partition coefficients (K) between bilayers and the aqueous phase vs the effective surfactant to PC molar ratio (Re). PC/GP weight ratios: 10:0 (●), 9:1 (○), 8:2 (□) and 7:3 (■).

The variations of the Re and K values versus the percentage of GP assembled with liposomes are plotted in the Fig. 4(a) and (b) respectively. A linear relationship was established between the Re and K values and the percentage of assembled GP. Hence, both the surfactant ability to alter the permeability of liposomes and its affinity with these bilayer structures showed an inverse linear dependence with the amount of assembled GP in the range of PC/GP weight ratios investigated. These finding underlines the progressive protective effect caused by this exopolymer on PC liposomes.

The fact that the surfactant always showed at 100% CF release lower K values than those for 50% could be attributed to the progressive saturation of the bilayers by the surfactants (the amounts of surfactants in the aqueous phase increased more than in the bilayers). This behaviour is in line with that reported by Paternostre et al., when studying the interaction of OG with PC liposomes (Paternostre et al., 1995) and with our previous studies on the effects of alkyl glucosides of different alkyl chain lenght with these bilayer structures (de la Maza and Parra, 1994; de la Maza et al., 1998d). It is noteworthy that this effect occurred regardless of the GP assembled with liposomes and that the difference between the K values for 50 and 100% CF release was similar in all cases. Hence, the process of saturation of bilayers by surfactant was almost unaffected by the presence of increasing amounts of GP assembled with liposomes.

From these findings we may conclude that the presence of increasing amounts of GP assembled with liposomes resulted in the formation of a coating structure which acted as a physical barrier. This barrier hampered the action of OG against the coated PC vesicles reducing its sublytic activity as well as its affinity with these bilayer structures. However, the mechanisms of surfactant interaction with liposomes were almost unaf-

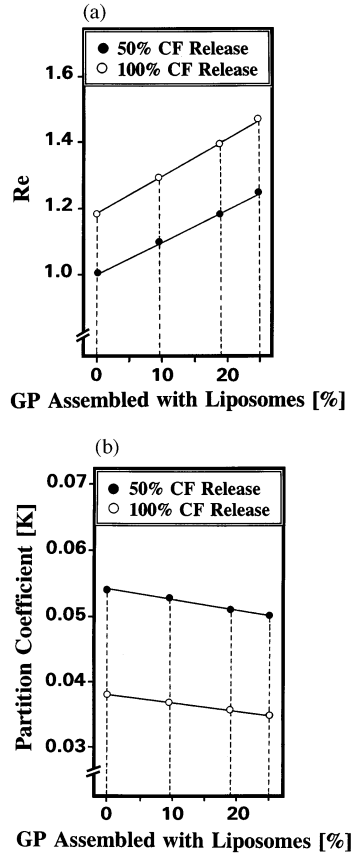

Fig. 4. (a) Variation in the effective surfactant to PC molar ratio (Re) vs the percentage of GP assembled with liposomes. 50% CF release (●), 100% CF release (○). (b) Variation in the surfactant partition coefficients (K) between bilayers and the aqueous phase versus the percentage of GP assembled with liposomes. 50% CF release (●), 100% CF release (○).

fected by the increasing presence of this glycoprotein.

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References

Ackman, R.G., Mc Leod, C.A., Banerjee, A.K., 1990. An overview of analyses by chromarod-Iatroscan TLC-FID. J. Planar Chrom. 3, 450–490.

Almog, S., Litman, B.J., Wimley, W., Cohen, J., Wachtel, E.J., Barenholz, Y., Ben-Shaul, A., Lichtenberg, D., 1990. States of aggregation and phase transformations in mixtures of phosphatidylcholine and octyl glucoside. Biochemistry 29, 4582–4592.

Austin, J.W., Stewart, M., Murray, R.G., 1990. Structural and chemical characterization of the S-layer of a *pseudomonas*-like bacterium. J. Bacteriol. 172, 808–817.

Beveridge, T.J., 1989. A fundamental design of bacteria. In: Poindexter, J.S., Leadbetter, E.R. (Eds.), Bacteria in Nature, Structure, Physiology, and Genetic Adaptability, vol. 3. Plenum Press, New York, pp. 1–65.

Beveridge, T.J., Graham, L.L., 1991. Surface layers of bacteria. Microbiol. Rev. 55, 684–705.

Bozal, N., Manresa, A., Castellvi, J., Guinea, J., 1994. A new bacterial strain of antarctica, *Alteromonas* sp, that produces a heteropolymer slime. J. Polar Biol. 14, 561–567.

Bozal, N., Tudela, E., Rosello-Mora, R., Lalucat, L., Guinea, J., 1997. *Pseudoalteromona antarctica* sp. nov.; isolated from an antarctic coastal environment. Int. J. Syst. Bacteriol. 47, 345–351.

Bozal, N., Guinea, J., Tudela, E., Congregado, F., Parra, J.L., de la Maza, A., Mercadé, M.E., Reque, M., 1996. Use of a glycoprotein for liposome protection. Spanish patent, 9.700.784.

Cladera, J., Rigaud, J.L., Villaverde, J., Duñach, M., 1997. Liposome solubilization and membrane protein reconstitution using Chaps and Chapso. Eur. J. Biochem. 243, 798–804.

Cully, D.F., Paress, P.S., 1991. Solubilization and characterization of a high affinity ivermectin binding site from *Caenorhabditis Elegans*. Mol. Pharmacol. 40, 326–332.

de la Maza, A., Parra, J.L., 1994. Structural phase transitions involved in the interaction of phospholipid bilayers with octyl glucoside. Eur. J. Biochem. 226, 1029–1038.

de la Maza, A., Parra, J.L., López, O., Congregado, F., Bozal, N., Guinea, J., 1997. Assembly properties of a glycoprotein produced by *Pseudoalteromonas antarctica* NF_3. J. Colloid Interface Sci. 192, 286–293.

de la Maza, A., Parra, J.L., Sabés, M., Congregado, F., Bozal, N., Guinea, J., 1998a. Glycoprotein excreted by *Pseudoalteromonas Antarctica* NF_3, as a coating and protective agent of liposomes against sodium dodecyl sulfate. Langmuir 14, 42–48.

de la Maza, A., Lopez, O., Parra, J.L., Sabés, M., Guinea, J., 1998b. Aggregation state of the glycoprotein excreted by *Pseudoalteromonas antarctica* NF_3, on a support of phosphatidylcholine liposomes. Langmuir 14, 5680–5684.

de la Maza, A., Parra, J.L., Congregado, F., Bozal, N., Guinea, J., 1998c. Interaction of the glycoprotein excreted by *Pseudoalteromonas antarctica* NF_3, with phosphatidylcholine liposomes. Colloids Surfaces A. 137, 181–188.

de la Maza, A., Coderch, L., Gonzalez, P., Parra, J.L., 1998d. Subsolubilizing alterations caused by alkyl glucosides in phosphatidylcholine liposomes. J. Control. Release 52, 159–168.

Edwards, K., Johnsson, M., Karlsson, G., Silvander, M., 1997. Effect of polyethyleneglycol-phospholipids on aggregate structure in preparation of small unilamellar vesicles. Biophys. J. 73, 258–266.

Inoue, T., 1996. Interaction of surfactants with phospholipid vesicles. In: Rosoff, M. (Ed.), Vesicles. Marcel Dekker, New York, pp. 151–195.

Küpcü, S., Sára, M., Sleytr, U.B., 1995. Liposomes coated with crystalline bacterial cell surface protein (S-layer) as immovilization structures for macromolecules. Biochim. Biophys. Acta 1235, 263–269.

Lichtenberg, D., 1985. Characterization of the solubilization of lipid bilayers by surfactants. Biochim. Biophys. Acta 821, 470–478.

Lummis, S.C.R., Martin, I.L., 1992. Solubilization, purification and functional reconstitution of 5-hydroxytryptamine$_3$ receptors from N1E-115 neuroblastoma cells. Mol. Pharmacol. 41, 18–26.

Oku, N., Tsudera, J., Kurohani, K., Okada, S., 1996. Effect of freeze-thawing on phospholipid/surfactant mixed bilayers. Chem. Pharm. Bull. 44, 1928–1930.

Paternostre, M., Meyer, O., Madelmont, C.G., Lesieur, S., Ghanam, M., Ollivon, M., 1995. Partition coefficient of a surfactant between aggregates and solution: Application to the micelle-vesicle transition of egg phosphatidylcholine and octyl β-D-glucopyranoside. Biophys. J. 69, 2476–2488.

Polozava, A.I., Dubachev, G.E., Simonova, T.N., Barsukov, L.I., 1995. Temperature-induced micellar-lamellar transformations in binary mixtures of saturated phosphatidylcholines with sodium cholate. FEBS Lett. 358, 17–22.

Pum, D., Weinhandel, M., Hödel, C., Sleytr, U.B., 1993. Large-scale recrystallization of the S-layer of *Bacillus coagulans* E38-66 at the aie/water interface and on lipid films. J. Bacteriol. 175, 2762–2766.

Ruiz, J., Goñi, F.M., Alonso, A., 1988. Surfactant-induced release of liposomal contents. A survey of methods and results. Biochim. Biophys. Acta 937, 127–134.

Sára, M., Sleytr, U.B., 1993. Relevance of charged groups for the integrity of the S-layer lattice from *Bacillus coagulans* E38-66 and for molecular interactions. J. Bacteriol. 175, 2248–2254.

Schurtenberger, P., Mazer, N., Känzig, W., 1985. Micelle to vesicle tansition in aqueous solutions of bile salt and lecithin. J. Phys. Chem. 89, 1042–1049.

Seino, H., Uchibori, T., Nishitani, T., Inamasu, S., 1984. Enzymatic syntesis of carbohydrate esters of fatty acid 1. Esterification of sucrose, glucose, fructose and sorbitiol. J. Am. Oil Chem. Soc. 61, 1761–1765.

Seras, M., Edwards, K., Almgren, M., Carlson, G., Ollivon, M., Lesieur, S., 1996. Solubilization of nonionic monoalkyl amphiphile-cholesterol vesicles by octyl glocoside; cryo-transmission electron microscopy of the intermediate structures. Langmuir 12, 330–336.

Singleton, W.S., Gray, M.S., Brown, M.L., White, J.L., 1965. Chromatographically homogeneous lecithin from egg phospholipids. J. Am. Oil Chem. Soc. 42, 53–57.

Sleytr, U.B., Messner, P., 1992. Crystlline bacterial cell surface layer (S-layers). In: Lederberg, J. (Ed.), Encyclopedia of Microbiology, vol. 1. Academic Press, San Diego, CA, pp. 605–614.

Sleytr, U.B., 1978. Regular arrays of macromolecules on bacterial cell walls: structure, chemistry, assembly and function. Int. Rev. Cytol. 53, 1–64.

Sleytr, U.B., Messner, P., 1988. Crystalline surface layers on bacteria. In: Sleytr, U.B., Messner, P., Pum, D., Sára, M. (Eds.), Crystalline Bacterial Cell Surface Layer. Springer, Berlin, pp. 160–186.

Sleytr, U.B., Sára, M., 1989. Use of structure with membranes having continuous pores. United States of America Patent 4.849.109.

Tetlo, I.J., Bowsher, C.G., Emes, M.J., 1996. Reconstitution of the hexose phosphate translocator from the envelope membranes of wheat endosperm amyloplasts. Biochem. J. 319, 717–723.

Vinson, P.K., Talmon, Y., Walter, A., 1989. Vesicle-micelle transition of phosphatidylcholine and octyl glucoside elucidated by cryo-transmission electron microscopy. Biophys. J. 56, 669–681.

Weinstein, J.N., Ralston, E., Leserman, L.D., Klausner, R.D., Dragsten, P., Henkart, P., Blumenthal, R., 1986. Self-quenching of carboxyfluorescein fluorescence: uses in studying liposome stability and liposome cell interaction. In: Gregoriadis, G. (Ed.), Liposome Technology, vol. III. CRC Press, Boca Raton, FL, pp. 183–204.

Yang, Q.-L., Guo, Y., Li, L., Hui, S.W., 1997. Effect of lipid headgroup and packing stress on poly(ethylene glycol)-induced phosphilipid vesicle aggregation and fusion. Biophys. J. 73, 277–282.