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Surface potential of liposomes with entrapped insulin

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Summary

From microelectrophoretic measurements on multilamellar liposomes as a function of lipid composition and pH of the medium, the pH dependence of the ζ-potential was found to be the same for liposomes in both the absence and presence of insulin. For liposomes without insulin, it is concluded that dicetyl phosphate (DCP) is incorporated at the external surface of bilayers consisting of phosphatidylcholine and cholesterol. Furthermore, the apparent pK_a for the $-PO_4H$ group of DCP in liposomes was 4.5. On the other hand, insulin entrapped in liposomes did not affect the ζ-potential of liposomes. The amount of entrapped insulin solution was the same as that of entrapped calcein solution, the latter being known to be distributed in the internal aqueous compartment of liposomes. By means of incubating the mixed solution of empty liposomes and insulin, the equilibrium constant for adsorption (k) and the amount of insulin adsorbed to liposomes at saturation (X_∞) could be determined from Langmuir plots. The present data suggest that the polar regions of the insulin molecule do not occur at the liposomal surface. This may be the result of the interaction between insulin and liposomes being hydrophobic in nature.

Introduction

Recently, interest has been increasing in the potential application of liposomes as a drug delivery system (Ostro, 1983). The use of liposomes as a transmucosal therapeutic system has many advantages, for example, protection of the drug from degradation by peptidase on the mucosa (Weingarten et al., 1985), maintenance of a high concentration at the site of administration and facilitation of their absorption from the mucosa.

The stability of insulin within liposomes is governed by its liposomal distribution. Therefore, the bioavailability of insulin may be influenced by the location of the drug in the liposome. Generally, water-soluble drugs and macromolecules are encapsulated in the internal aqueous compartment of liposomes. The incorporation of insulin, an amphiphile, into liposomes has been well established as a fact for several years. A number of investigators have demonstrated that charged liposomes with entrapped insulin can be administered to diabetic animals in vivo (Weingarten et al., 1981; Patel et al., 1982, 1985). However, the distribution of insulin in multilamellar liposomes remains to be reported, although the adsorption of bovine serum albumin (BSA) to liposomes has been studied on the basis of the surface potential of liposomes (Law et al., 1988).

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This paper is concerned with the distribution of insulin within phosphatidylcholine liposomes and examines the interactions of insulin with liposomes via the determination of the surface potential of liposomes in the absence and presence of insulin. Evidence is presented to demonstrate that the process of entrapment of insulin in liposomes is attributable to nonpolar forces.

Materials and Methods

Chemicals

L-α-Dipalmitoylphosphatidylcholine (DPPC), cholesterol (C), dicetyl phosphate (DCP) and crystalline bovine pancreas insulin (24.5 IU per mg, crystalline, zinc content, approx. 0.5%) were purchased from Sigma (St. Louis, MO, U.S.A.). DPPC was pure as judged on the basis of normal silica-gel thin-layer chromatography criteria. Calcein was purchased from Tokyo Kasei Kogyo (Tokyo, Japan). Other chemicals were all analytical grade and used as received. Water used throughout the experiments was membrane-filtered water (Milli-Q, Nihon Millipore Kogyo, K.K.).

Preparation of liposomes

Liposomes were prepared according to a standard method (Bangham et al., 1974). A mixed solution of DPPC (7 μmol), DCP (0–1.2 μmol) and cholesterol (1 μmol) in $CHCl_3$ (1–1.8 ml) was dried in an evaporator. After 15 h in vacuo, the dried lipid film was swollen in 500 μl of phosphate-buffered saline (1/10 dilution of the phosphate-buffered saline in distilled water: 137 mM NaCl/2.6 mM KCl/6.4 mM Na_2HPO_4/1.4 mM KH_2PO_4; pH 7.4). The pH of the liposome suspensions was adjusted to the required value by using hydrochloric acid and/or sodium hydroxide solution. The phosphate-buffered saline contained either zero or 1×10^{-4} M calcein, or either zero or 1.36×10^{-4} M insulin (20.0 IU/ml) depending on the purpose of the experiment. This solution containing swollen lipids was kept continuously under nitrogen, and was mixed by vortexing for 5 min followed by sonication for 5 min at 45°C in a bath-type sonicator (Honda Electronics W220R). 500 μl of the phosphate-buffered saline was added and the mixture allowed to stand for 16 h to hydrate liposomes. The average size of liposomes was determined to be 7–10 μm using a particle size analyzer (LA-500, Horiba Seisaku) and microscope. The liposomes with entrapped insulin were separated by passing 300 μl of liposome suspension over a column (1.8×35 cm) of Sephadex G-50 (Pharmacia) with the phosphate-buffered saline in all fractions. Each fraction contained 4.5 ml.

Analysis of lipid and insulin

The lecithin concentration was measured using a commercially available kit (Phospholipids B-test; Wako Pure Chemical Co.). The insulin concentration was determined by an enzyme immunoassay method, with a commercially available kit (Insulin EIA kit, Dainabot). 10 μl of the stock solution of insulin standard was used for the insulin EIA kit. To obtain a working calibration curve, aliquots of 0, 0.1, 0.3, 0.7, 1.5 and 3 mIU/ml of the stock insulin standard solution were pipetted into separate test tubes (standard sample). To ascertain whether Triton X-100 affects the activity of insulin, 10 μl of empty liposomes (22.1 mg/dl for DPPC) and 3.0 μl of Triton X-100 solution (1%) were added to aliquots of 0–3 mIU/ml of stock solution and then mixed. The concentration of the test sample was found to show the same values as the standard in the range 90.7–106.8%.

Measurement of the amount of calcein entrapped in liposomes

The amount of calcein was determined using a fluorophotometer (excitation at 490 nm and emission at 520 nm; Hitachi 650-10S fluorescence spectrophotometer, Hitachi, Tokyo, Japan) as described previously (Oku et al., 1982). The fluorescence intensity of calcein entrapped in liposomes was measured after consecutive addition of cobalt chloride and Triton X-100. In preparation of liposomes (7 μmol of DPPC, 1 μmol of C and 0–0.75 μmol of DCP), the dried lipid film was swollen in 500 μl of phosphate-buffered saline containing 1×10^{-4} M calcein. After preparing the calcein-entrapping liposomes, 500 μl of buffer was added. 20 μl of the liposome suspension was diluted by adding 1 ml of buffer. The fluorescence of this liposome suspension was measured before and

after the addition of 2 μl of $CoCl_2$ (10 mM), respectively. Subsequently, 25 μl of 10% Triton X-100 solution was added and the fluorescence was measured again. The trapped volume was calculated from these fluorescence values.

Measurement of the amount of insulin entrapped in liposomes

Entrapped insulin in liposomes was released from the liposomes by disruption with a surfactant. Triton X-100 solution (1%) was added to 10 μl of the eluted solution (diluted to 100 × volume with phosphate-buffered saline) in the proportion of lecithin concentration (3 μl for 22.1 mg/dl lecithin) which had been determined by Phospholipids B test.

Measurement of amount of insulin adsorbed to liposomes in incubation

The binding of insulin to liposomes was performed by incubating a liposome suspension containing 1.65 μmol/ml lipids (DPPC : C : DCP = 7 : 1 : 0.25) with insulin at various temperatures (20, 28.5 and 37 °C) for 10 min. After incubation, these samples were kept at 20 ± 1 °C and their electrophoretic mobilities were subsequently measured. To obtain the adsorption isotherm for insulin on liposomes, the concentration of insulin was varied from 1.36×10^{-5} to 1.22×10^{-4} M, and the amount of insulin bound to liposomes at saturation was determined.

Microelectrophoresis

Electrophoretic mobilities were evaluated using an ultramicroelectrophoresis apparatus (Mitamura Riken Kogyo). A rectangular quartz cell (Briggs cell, 75 × 25 × 3 mm) was used with two electrodes; the electrodes were mercury pools across which a constant potential was applied. Under these routinely employed conditions, an electric field of 1.3–6.0 V/cm was achieved. As the light source, an He-Ne laser lamp was used. The liposomes were viewed with a long working range objective at right angles to the direction of the incident light beam by using dark-field illumination. Electrophoretic mobilities were determined at 20 ± 1 °C and the values (expressed in $cm^2\ V^{-1}\ s^{-1}$) represent the average of about 40 measurements. In order to minimize electrode polarization, successive measurements were taken in the opposite direction. Measurements carried out in both the upper and lower stationary layers agreed within the limits of experimental error. The electrophoretic mobility of the particle (μ) was determined from the particle velocity at the stationary level (v) using the equation:

$$\mu = v/E = vkA/I \qquad (1)$$

where E denotes the electric field, A is the cross-sectional area of the cell, I is electric current and k is the specific conductance of the solution, which was separately measured using a conductivity me-

TABLE 1

Characterization of multilamellar liposomes

Liposome DCP (X)	DPPC : C : DCP (7 : 1 : X)				
	0	0.1	0.25	0.5	0.75
Encapsulation efficiency (%) [a]	8.9 ± 1.3	12.4 ± 0.4	11.9 ± 5.9	12.4 ± 4.8	17.7 ± 5.5
Captured volume (μl/mg) [b]	8.05	11.11	10.51	10.69	14.90

The diameter of liposomes (DCP (X) = 0–0.75) was determined using a laser diffraction size distribution analyzer. The measured size was 7.64–9.62 μm.

[a] Encapsulation efficiency for calcein, i.e. the ratio of the amount of calcein remaining in the liposomes determined by the method of Oku et al. (1982). Values are means ± S.D. for at least three experiments.

[b] Captured volume per mg lipid (DPPC, C, DCP), i.e. the ratio of the equivalent volume of aqueous space containing the encapsulated calcein to the weight of lipid in the preparation.

ter (TOA Electronics, type CM-40S) immediately after the particle velocity measurements. The pH values were measured using a pH meter (Central Kagaku, type HG-3).

Results

Our system produces large (7.64–9.62 μm) multilamellar liposomes. They are composed of DPPC, C and DCP and have a high capture efficiency for calcein and insulin. A summary of the size ranges, encapsulation efficiencies and internal volumes for various liposomes is given in Table 1.

Typical patterns obtained from gel-filtration chromatography of liposomes (DPPC : C : DCP = 7 : 1 : 1) with entrapped insulin and free insulin are shown in Fig. 1. As determined from the absorbance (turbidity) at 450 nm, the fraction which contained liposomes was that of the void volume (fraction no. 4). This fraction showed low activity of insulin (7.0 mIU/ml), indicating that insulin was enclosed in the liposomes. The analytical procedure employed to determine the total and encapsulated insulin activities in liposomes is depicted in Fig. 2. The total insulin activity was determined using the EIA kit after rupturing the liposomes with Triton X-100. The liposomes (DPPC : C = 7 : 1) containing entrapped insulin displayed low insulin activity of 14.6 ± 12.7

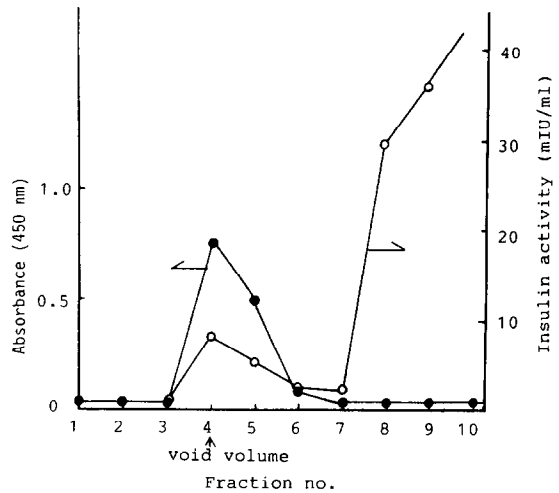

Fig. 1. Sephadex G-50 elution profiles of liposomes (DPPC : C : DCP = 7 : 1 : 1). (●) Liposomes (450 nm), (○) insulin (EIA).

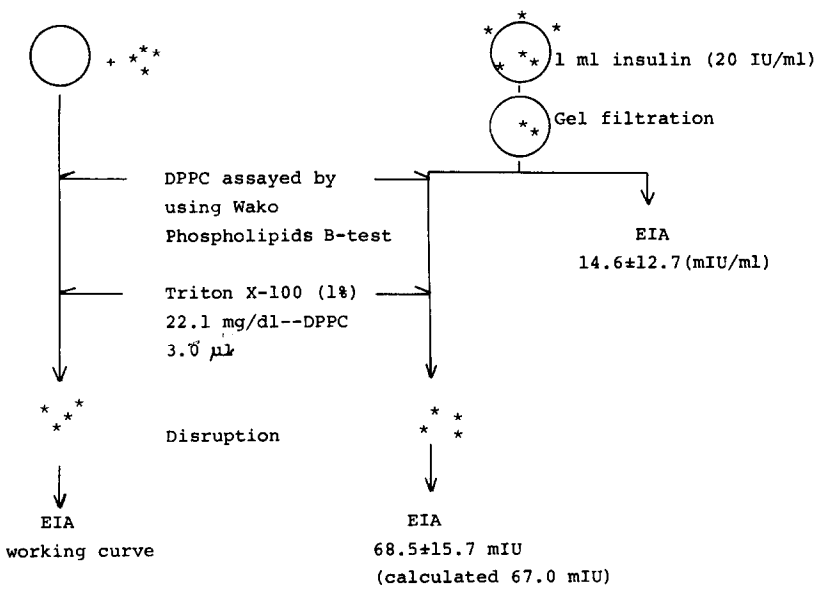

Fig. 2. Schematic representation of analysis procedure for total and encapsulated insulin liposomes (DPPC : C = 7 : 1). (○) Liposomes, (*) insulin. Values are expressed as means ± S.D. ($n = 4$).

mIU/ml after gel filtration, however, enhancement of the activity to reach a value of 68.5 ± 15.7 mIU/ml resulted from the disruption of liposomes.

Effect of DCP concentrations on ζ-potential of liposomes

The electrophoretic mobility of liposomes containing various concentrations of DCP was measured in the absence and presence of insulin. The ζ-potentials were calculated from the observed mobility, μ, using the Helmholtz-Smoluchowski equation (in SI units):

$$\zeta = \eta\mu/\epsilon_0 D \tag{2}$$

where ϵ_0, D and η denote the dielectric constant of a vacuum, relative dielectric constant and viscosity of the medium, respectively.

According to the Gouy-Chapman theory, the relation between the surface potential, which is approximated by the ζ-potential, and the surface charge density, σ, of the liposomal surface is expressed as (Nakagaki, 1968):

$$\sigma\ (C\,\mathrm{cm}^{-2}) = -5.8906 \times 10^{-6} \sqrt{\sum_{i=1} \left(C_i\{\exp(-0.03957\, z_i\zeta) - 1\}\right)} \tag{3}$$

where 80.36 is taken as the value of the relative dielectric constant of water at 20 °C, C the concentration of electrolyte (mol dm^{-3}), ζ the potential (in V) and z_i the valence of ion i. Taking the experimental values of ζ in Eqn 3, we can calculate the charge per unit area, i.e. σ values (in $C\ \mathrm{cm}^{-2}$). The effect of the concentration of DCP on the electrophoretic mobility (μ) of liposomes in the absence and presence of calcein is shown in Fig. 3. The electrophoretic mobility of liposomes containing egg phosphatidylcholine and DCP has been reported previously by Hauser et al. (1979). Their data are represented by the solid line [$(-0.22 \pm 0.02) \times 10^{-4}$ $\mathrm{cm}^2/(\mathrm{V\ s})$ per mol%] in

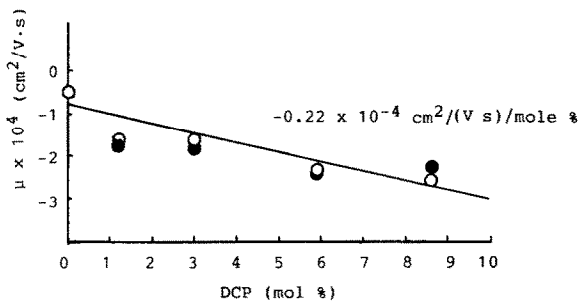

Fig. 3. Effect of DCP on the electrophoretic mobility of liposomes in the absence and presence of calcein at pH 7.4. (○) Empty liposome, (●) calcein-entrapping liposomes.

Fig. 3. The agreement between their data and ours indicates that our experimental procedures are correct and reliable.

The ζ-potential is calculated by using Eqn 2 and the surface charge density, σ, Eqn 3. The σ value is plotted vs concentration of DCP in Fig. 4. One observes only very slight differences in ζ-potential and in surface charge density of liposomes between the presence and absence of insulin or calcein. The surface charge density of liposomes became more negative with increasing concentration of DCP.

Effect of pH on the ζ-potential of liposomes

The ζ-potential of liposomes containing various concentrations of DCP, with or without insulin, is shown in Fig. 5 as a function of pH. The pH of the liposome solution was adjusted using HCl and NaOH solution for acidic and alkaline pH ranges,

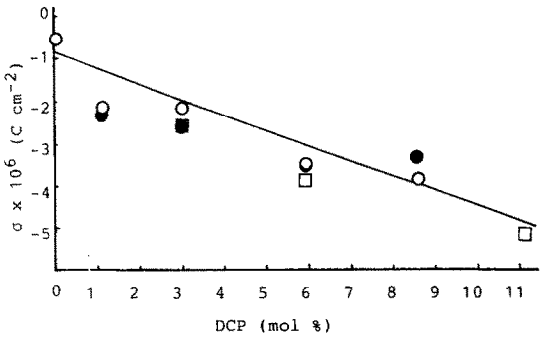

Fig. 4. Surface charge density (σ) of liposomes containing DCP at pH 7.4. (○) Empty liposomes, (●) liposomes with entrapped calcein, (□) liposomes with entrapped insulin.

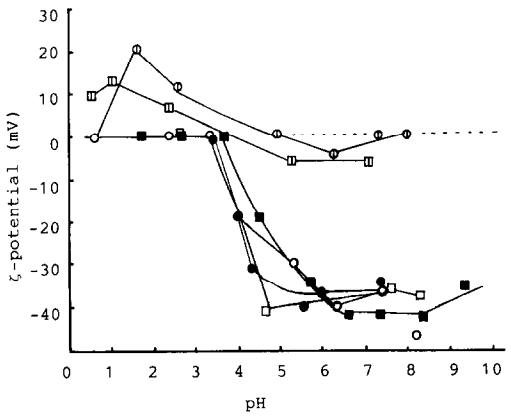

Fig. 5. pH dependence of the ζ-potential of liposomes (DPPC:C:DCP = 7:1:X). Insulin-encapsulating liposomes: (Φ) $X = 0$, (●) $X = 0.25$, (○) $X = 0.5$; empty liposomes: (▣) $X = 0$, (■) $X = 0.25$, (□) $X = 0.5$.

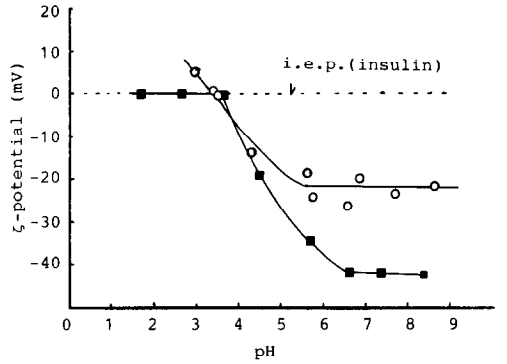

Fig. 7. pH vs ζ-potential plots of liposomes (DPPC:C:DCP = 7:1:0.25) after incubation with insulin solution at 20 °C. The concentration of the insulin solution was 4.90×10^2 μg/ml. (■) Empty liposomes, (○) empty liposomes after incubation.

respectively. Irrespective of the presence or absence of entrapped insulin, the plots of the ζ-potential exhibit closely similar patterns in the pH regions.

Adsorption of insulin to liposomes on incubation

The interaction of insulin with liposomes may be investigated by incubating a mixed solution of empty liposomes and insulin. After incubation at various temperatures (20, 28.5 and 37 °C) for 20 min, the samples were kept at 20 °C and then subjected to ultramicroelectrophoresis at this temperature. The incubation period of 20 min was chosen based on the prior confirmation that equilibrium was attained in less than 20 min in the present adsorption measurements (data not shown). As shown in Fig. 6, adsorption reached saturation above an insulin concentration of 200 μg/ml and the ζ-potential of liposomes shifted to a higher value (i.e. less negative) on adsorption of insulin.

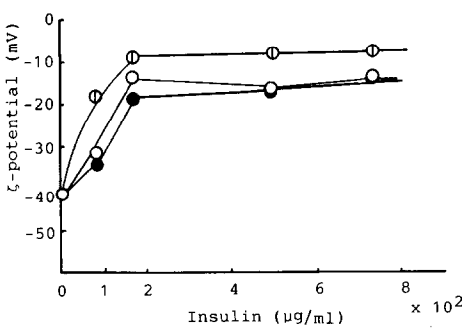

Fig. 6. Saturation curve for binding of insulin to liposomes (DPPC:C:DCP = 7:1:0.25) by incubation at pH 7.4. The binding of insulin with liposomes was performed by incubation of liposomes containing 100 μl of 1.65 μmol/ml lipids with various amounts of insulin in a total of 1 ml of buffer for 10 min. (○) 20 °C, (●) 28.5 °C, (Φ) 37 °C.

The ζ-potential of liposomes which adsorbed insulin externally was measured and is plotted in Fig. 7 as a function of pH for liposomes containing 1.65 μmol/cm³ of lipid (DPPC:C:DCP = 7:1:0.25) and 8.16×10^{-5} M insulin. The ζ-potential of liposomes onto which insulin was adsorbed shifted to a high value, about 20–25 mV, on adsorption of insulin in the region pH 6–9. The isoelectric point of insulin occurs at pH 5.1–5.3, but no shift was observed for the ζ-potential-pH curve of liposomes at this pH.

Discussion

Characterization of liposomes

As shown in Table 1, the efficiency of encapsulation of calcein was raised by increasing the concentration of DCP in liposomes. This may result from the repulsion due to DCP in liposomes (Cohen et al., 1976). In Table 1, the calculated

values fall within the range of 8.9–17.7% encapsulation efficiency of calcein solution. As demonstrated in Fig. 2, following gel filtration, 300 μl of the insulin-entrapping liposome suspension (DPPC, 14 μmol; C, 2 μmol) exhibited insulin activity before the disruption of liposomes. This signifies that insulin was adsorbed on the liposomal surface. Alternatively, this insulin activity might be caused by disruption during the process of operating the insulin EIA kit. After disruption of liposomes with Triton X-100, the insulin activity in the liposomal suspension increased to 68.5 mIU/ml. For the case of 10% of an insulin suspension (20 IU/ml) being entrapped in liposomes, the insulin activity in the liposomal suspension (fraction nos 4 and 5) following gel filtration of 300 μl of the liposome suspension would amount to 67.0 mIU/ml. This value is almost identical with that determined experimentally.

Therefore, it is concluded that about 10% of the insulin solution became encapsulated within liposomes during preparation of the liposomes, this encapsulation efficiency equalling that for calcein reported in Table 1 (DPPC : C = 7 : 1). In other liposomes (DPPC : C : DCP = 7 : 1 : 0.25, 0.5), the values were found to be almost the same as in the case of calcein.

ζ-potential of liposomes entrapping calcein or insulin

The ζ-potentials (surface charge density) of liposomes containing internally entrapped and unentrapped calcein or insulin decreased, showing a similar dependence on DCP concentration (Fig. 4). Calcein is a water-soluble compound whereas insulin is more hydrophobic. However, the surface charge density vs DCP curves of the insulin-entrapping liposomes are almost identical with those for entrapped calcein, the latter being known to be distributed in the internal aqueous compartment of liposomes. Lai et al., (1988) reported on the mechanism of liposome fusion with entrapped insulin. One of the factors involved in the fusion of these liposomes is the binding of insulin to the liposomal surface (Wiessner et al., 1986; Lai et al., 1988). However, the results in Fig. 4 indicate that insulin, as well as calcein, did not appear on the surface of liposomes.

Degree of dissociation (α) of DCP

The relationship between the surface charge density, σ, and area per unit elementary charge, A (cm^2 $molecule^{-1}$), is expressed as:

$$A = e/|\sigma| \tag{4}$$

where e denotes the elementary charge. If the degree of dissociation and the mole fraction of the electrically dissociating species (DCP in this case) are represented by α and X_{DCP}, respectively, then,

$$A = A_0/\alpha X_{DCP} \tag{5}$$

where A_0 is the molecular area of lipid. According to Eqns 4 and 5

$$|\sigma| = (e\alpha/A_0)\, X_{DCP} \tag{6}$$

is obtained.

From the plot of $|\sigma|$ vs X_{DCP} (mol%), which was determined by linear regression analysis, it was confirmed that $|\sigma|$ decreased with increasing mole fraction of DCP, X_{DCP}, as shown in Fig. 4.

$$\sigma = -3.5 \times 10^{-5} X_{DCP} \tag{7}$$

From the slope (-3.5×10^{-5}), the degree of dissociation, α, of liposomes was calculated as 0.874 from Eqn, 6, for an estimated area A_0 of 40 $Å^2$ (Hauser et al., 1976). This result indicates that DCP is distributed on the external membrane of liposomes and is dissociated to almost the same extent as DCP in the solution.

Apparent pK_a of DCP

The decrease in ζ-potential with increasing pH in the range 3.5–6 is due to the dissociation of the $-PO_4H$ group of DCP, the process of dissociation reaching completion at pH ≃ 5. The plateau region above pH 6 (Fig. 5) represents the pH range over which the DCP molecule has one net negative charge due to a fully ionized phosphate group. DPPC is known to be electrically neutral based on the intramolecular neutralization of charges between the ammonium cation and phosphate anion groups of the lipids in the pH range 5.5–7.0 (Seimiya and Ohki, 1973; Noda, 1987). The rise in

ζ-potential at pH < 3 is due to the $-\overset{+}{N}-(CH_3)_3$ group producing a positive charge. The apparent pK_a value of the $-PO_4H$ group of DCP, as derived from the variation in ζ-potential with pH shown in Fig. 5, was found to be 4.5. This is almost equal to that of 4.7 derived from the data on $^{45}Ca^{2+}$ adsorption on monolayers (Hauser et al., 1976). The close agreement between the pK_a values determined from the ζ-potential and $^{45}Ca^{2+}$ adsorption suggests that the phosphate group of DCP is fully ionized at pH > 6.

pH dependence of liposomes with insulin adsorption

Liposomes with entrapped insulin display virtually the same pK_a value as that of empty liposomes (Fig. 5). The pK_a values of DCP in empty liposome and liposome containing entrapped insulin are quite similar. This result probably stems from an effect on the dissociation properties of the phosphate group on the outer surface of the liposome rather than from the presence of entrapped insulin within the liposomes.

The amount of insulin bound to liposomes

The surface charge density, σ, of the liposomal surface was evaluated from Eqn 3. The variation in σ with respect to insulin concentration is very similar to that of the ζ-potential as a function of concentration as shown in Fig. 6. In order to study the binding of insulin to the external surface of liposomes, the difference in surface charge density between empty and insulin-binding liposomes is determined from:

$$|\Delta\sigma| = |\sigma - \sigma_0| \tag{8}$$

where σ_0 represents the surface charge density of empty liposomes and σ is that of liposomes with bound insulin. The change in $|\sigma|$ for liposomes with increasing concentration of insulin is directly related to the adsorption of insulin. The assumption of monolayer adsorption of insulin occurring on the liposomal surface leads to the expression:

$$c/X = 1/X_\infty k + c/X_\infty \tag{9}$$

where X and X_∞ are related to the decrease in and the saturation value of the surface charge density on the adsorption of insulin, according to the following equation:

$$X = |\Delta\sigma|/eN_a \tag{10}$$

where N_a is Avogadro's number, k the equilibrium constant for adsorption, and c the concentration of insulin. Eqn 9 is well known as Langmuir's equation. Plots of c/X vs c are shown in Fig. 8, remaining linear with correlation coefficients of 0.988 for insulin at 20 °C. Using the least-squares method, we can determine the values of k and X_∞. Taking into consideration the area occupied by one insulin molecule, A_i, the following relation is yielded:

$$X_\infty = X'_\infty/m = 1/(A_i N_a m) \tag{11}$$

where X'_∞ is the amount of insulin adsorbed at saturation and m the number of insulin molecules required to prevent the dissociation of one DCP molecule from the liposomal surface. Since the value of A_i is approx. 290 Å^2/molecule for insulin (Benhamou and Guastalla, 1960), the value of m can be determined. The parameters values for k,

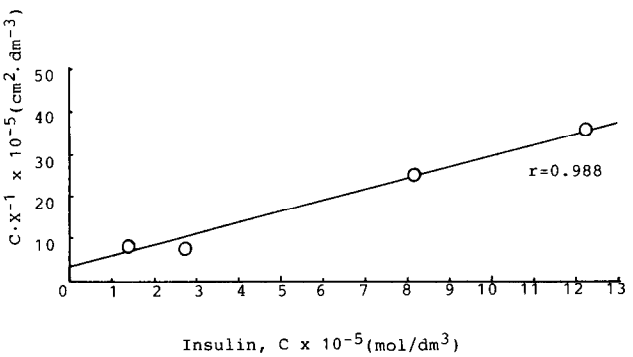

Fig. 8. Langmuir plots for insulin at 20 °C obtained using the electrokinetic method.

TABLE 2

Parameters for adsorption of insulin to liposomes (7:1:0.25) at 20 °C

	X_∞ (mol/cm^2)	k (dm^3/mol)	m
Insulin	3.82×10^{-11}	8.19×10^5	1.5

X_∞ and m are listed in Table 2. From the value of m, one can deduce that three molecules of insulin occupy the equivalent of two molecules of DCP in the liposomes.

The adsorption of BSA to liposomes and of insulin to small unilamellar liposomes has been reported previously. From the former, it was concluded that the amount of BSA adsorbed at saturation was not influenced by the liposomal charge and that the driving force for adsorption may be dominated by a hydrophobic effect (Law et al., 1986). From the latter, the amount of insulin adsorbed at saturation to liposomes on incubation of small unilamellar liposomes with insulin solution was determined as 48–50 μg insulin/μmol DPPC (Wiessner et al., 1982). In our experiments we determined a value of 828 μg insulin/μmol DPPC. Nevertheless, since both insulin and liposomes are supposed to possess a negative charge at pH 7.4, it was concluded that insulin was immediately adsorbed to liposomes on incubation of the mixed solution of insulin and liposomes probably through a hydrophobic effect. Insulin adsorbed to liposomes appeared to have no electrical charge. Therefore, the surface charge density of liposomes, which arises due to DCP, decreased when the charge of DCP was shielded on adsorption of insulin.

The data on the ζ-potential of liposomes with entrapped insulin suggest that the polar regions of the insulin molecule were not located at the surface of the liposomes. This may be a consequence of the interaction between insulin and liposome being hydrophobic in nature.

The liposomes containing entrapped insulin will be stable in the suspension, since the entrapped insulin does not affect the surface charge of liposomes. In addition, it is expected that the liposomes should be able to protect the intact insulin from degradation by peptidase in the nasal mucosa when administered intranasally.

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