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Short Communications

The electrokinetic behavior of liposomes adsorbed with bovine serum albumin

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Numerous studies dealing with the interaction of serum albumin with liposomes have been published (Hoekstra and Scherphof, 1979; Jonas, 1975, 1976; Kitagawa et al., 1976; Papahajopoulos et al., 1975; Zborowski et al., 1977; Law, 1986). Among these reports, the adsorption of serum albumin on liposomes has been shown (Hoekstra and Scherphof, 1979; Law, 1986). However, in some cases, adsorption was not found (Jonas, 1975, 1976; Kitagawa et al., 1976; Zborowski et al., 1977).

A previous study showed that adsorption took place by incubating serum albumin with a high concentration of liposomes (Law, 1986). The present study attempts to look at the alteration of the surface properties of the liposomes due to the presence of bovine serum albumin using a microelectrophoretic technique and attempts to confirm the occurrence of adsorption of bovine serum albumin on the surface of liposomes.

Crystallized and lyophilized bovine serum albumin (Sigma Co., U.S.A.), dicetyl phosphate (P.L. Chemicals), stearylamine (P.L. Chemicals) and phosphatidylcholine purified from egg yolk were used as previously described (Law, 1986). The polystyrene latex particles were prepared by the first-stage method of Chung-Li et al. (1976).

The previously documented method for adsorption of bovine serum albumin on liposomes was used (Law, 1986). A chloroform mixture of phosphatidylcholine, cholesterol and either dicetyl phosphate or stearylamine at the desired molar ratio was dried to a thin film under reduced pressure at 37 °C in a rotary evaporator. Multilamellar liposomes were prepared by constant vortexing for 5 min in a 10^{-3} M sodium chloride solution to give a lipid concentration of 13.6 mmol. The liposome preparation was hydrated at 37 °C for 2 h. Equal volumes of liposome dispersion and bovine serum albumin solution were mixed and equilibrated in a constant-shaking water bath (100 throws/min) at a temperature of 37 °C. The final concentration of the bovine serum albumin was 2.25 mg/ml. The time necessary to establish equilibrium was found to be 90 min. The pH of the dispersions was adjusted to the required value by using hydrochloric acid and/or sodium hydroxide solution.

Microelectrophoretic mobility measurements were carried out using a Rank MK II Microelec-

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trophoretic Apparatus (Rank Bros., Cambridge, U.K.) as previously described (Law, 1984). A flat-cell assembly and platinum electrodes were used. The location of the stationary levels was obtained according to the procedures of Shaw (1969). Liposomes focused by a microscope at the stationary level were detected. Ten liposomes were timed over a fixed distance on the calibrated eyepiece. Each liposome was measured in alternate directions to eliminate errors due to the effect of drift and polarization of electrodes, i.e. for each measurement 20 timings were obtained. The voltage was adjusted to give timings of about 10 s. The electrophoretic mobility was calculated by the electrophoretic velocity (the graticule distance divided by the time of passage) divided by the field strength (the applied voltage divided by the distance between electrodes). All measurements were made at 25°C and at ionic strength in 10^{-3} M sodium chloride solution in order to give a solution of suitable conductance and to keep the ionic strength, as far as possible, constant.

The pH versus electrophoretic mobility plots of positively-charged, negatively-charged and neutral liposomes and negatively-charged polystyrene latex particles are shown in Fig. 1.

The pH–electrophoretic mobility profile of polystyrene latex particles is similar to that obtained previously showing a typical curve for negatively-charged polystyrene latex particles (Law, 1984). The positively-charged and negatively-charged liposomes resulted in positive and negative mobility curves, respectively. The neutral liposomes should have shown no mobility; however, a slightly negative mobility was obtained. This may be due to preferential negative adsorption of hydrogen ions (Shaw, 1969). The result of negative mobility of neutral liposomes obtained from this study is in good agreement with that given by Bangham (1958) in the presence of 0.145 M sodium chloride solution.

The effects of pH on the electrophoretic mobility of positively-charged, negatively-charged and neutral liposomes and polystyrene latex particles after incubation with bovine serum albumin are shown in Fig. 2.

The use of polystyrene latex particles as an adsorbent for serum albumin has been reported by

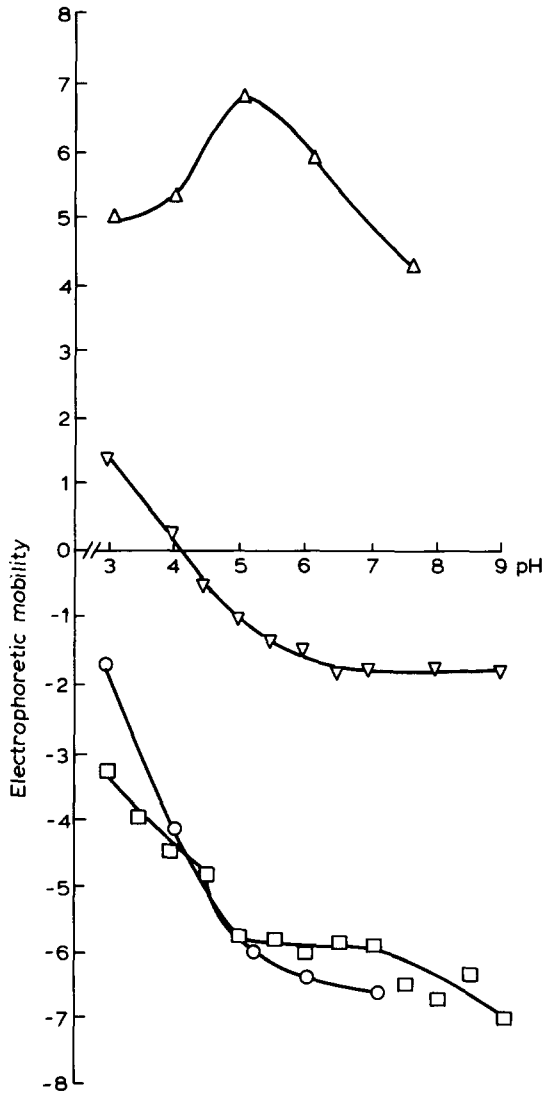

Fig. 1. pH–electrophoretic mobility plots of liposomes and polystyrene latex particles. △, positively charged liposomes (PC/C/S 1.6 : 1 :0.15); ○, negatively charged liposomes (PC/C/DP 1.6 : 1 : 0.15); ▽, neutral liposomes (PC); □, polystyrene latex particles. PC = phosphatidylcholine; C = cholesterol; DP = dicetyl phosphate; S = stearylamine.

a number of researchers (Dezelic et al., 1971; Edwards and Rutter, 1980; Norde and Lyklema, 1978; Suzawa and Murakami, 1980). It has been shown that serum albumin molecules formed a monolayer coating on the surface of polystyrene latex particles after adsorption. The pH–mobility property of polystyrene latex particles absorbed

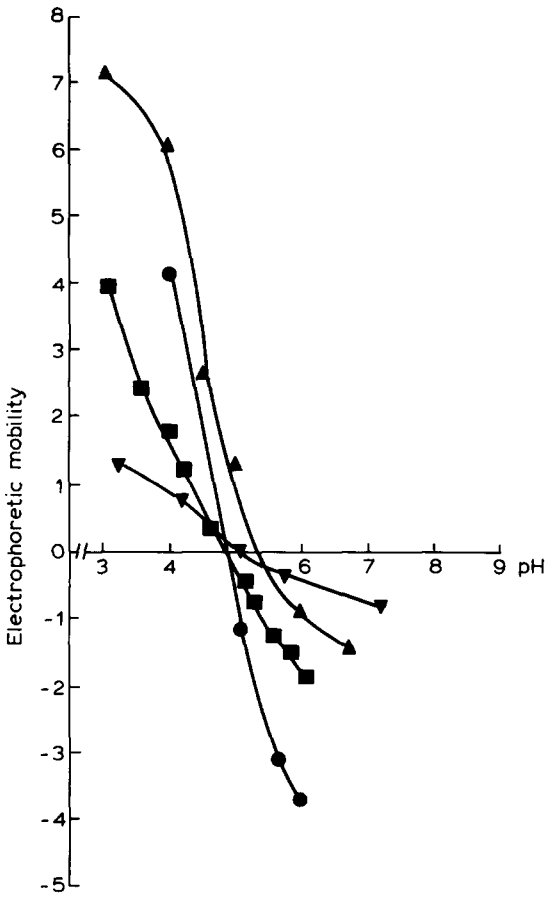

Fig. 2. pH–electrophoretic mobility plots of liposomes and polystyrene latex particles after incubation with bovine serum albumin, ▲, positively charged liposomes (PC/C/S 1.6 : 1 : 0.15); ●, negatively charged liposomes (PC/C/DP 1.6 : 1 : 0.15); ▼, neutral liposomes (PC); ■, polystyrene latex particles.

with serum albumin was similar to that of dissolved serum albumin (Norde and Lyklema, 1978). The pH–mobility patterns of positively-charged, negatively-charged and neutral liposomes after incubation with bovine serum albumin are similar to that of polystyrene latex particles adsorbed with bovine serum albumin. In view of this evidence and the fact that the properties of bovine serum albumin are imposed on liposomes as bovine serum albumin becomes incorporated into the surface of liposomes, the adsorption of bovine serum albumin molecules on the surface of liposomes is confirmed. A previous report suggests that the adsorption of bovine serum albumin on the phospholipid bilayers of liposomes may be driven by the hydrophobic effect, despite the charge characteristics of liposomes (Law, 1986). The bovine serum albumin molecules may partially penetrate (anchor) into the phospholipid bilayer. The non-penetrated moiety of the bovine serum albumin protrudes outside the phospholipid bilayer and coats on the surface of the liposomes.

Due to the evidence that the permeability of the entrapped contents of the liposomes can be enhanced due to its interaction with serum albumin, it has been proposed that serum albumin may penetrate into the phospholipid bilayers of the liposomes (Kitagawa et al., 1976; Papahadjopoulos et al., 1975; Zborowski et al., 1977). Experiments showed that the interaction of proteins with the phospholipid bilayers can also increase the pressure and expand the area of the phospholipid films which may result from the penetration of the proteins (Papahadjopoulos et al., 1975). Further experiments are necessary in order to investigate the occurrence of the penetration or partial penetration of the bovine serum albumin into the phospholipid bilayers.

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