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Fluorescence Anisotropy and Light-Scattering Studies of the Interaction of Insulin with Liposomes

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The interaction between zinc-stabilized insulin and lecithin liposomal membranes was studied using DPH fluorescence anisotropy and light-scattering techniques. To ascertain a possible influence of a charge on the insulin molecule, experiments were performed at pH 4.5 (insulin possesses a positive charge) and at pH 7.4 (the charge of insulin is negative). Measurements at pH 4.5 revealed significant changes in scattered light intensity induced by the addition of insulin to lecithin liposomes. With increasing time of storage of liposomes the insulin effect became faster and more pronounced. At pH 7.4, significant changes in scattered light were registered only in the case of liposomes stored for 5 days. In these liposomes a peroxidation process of lecithin was revealed. No significant changes induced by insulin were observed in DPH fluorescence anisotropy either at pH 4.5 or at pH 7.4, which suggested the absence of an interaction of insulin with the hydrophobic core of liposomes. Thus, the observed changes in scattered light could be interpreted in terms of the insulin association to the liposomal surface in the case of phospholipid peroxidation and/or acidic pH.

KEY WORDS: Insulin; lecithin liposomes; light scattering; fluorescence anisotropy; 1,6-diphenyl-1,3,5-hexatriene.

INTRODUCTION

Although the application of lecithin liposomes in insulin therapy has been considered [1], a direct interaction of insulin with phospholipid bilayers has not yet been sufficiently investigated. Only a few articles deal with this problem [2–6]. Neither the extent nor the mechanism of insulin interaction with phospholipid membranes has yet been clarified. In the present work the interaction between zinc-stabilized insulin and soybean lecithin liposomes is studied under various pH and storage conditions of liposomes.

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MATERIALS AND METHODS

Soybean L-α-lecithin, a natural product, was purchased from Cal Biochem, AG. Liposomes were prepared by 40-min sonication of soybean lecithin suspensions in 5 m*M* Tris–HCl (pH 5.2). The pH of the suspensions was then adjusted with 1% HCl and 1% NaOH to 4.5 and 7.4, respectively. The final lecithin concentration in the sample was 1 mg/ml. Peroxidation of lecithin was detected by measuring absorption spectra on a SPECORD M40 (Carl-Zeiss) spectrophotometer. Zn-stabilized insulin (Spofa) was added from a stock solution (40 IU/ml) to lecithin suspensions to yield the final concentration of 0.20 IU/ml. 1,6-Diphenyl-1,3,5-hexatriene (DPH) from Kocht-Light Co. was used as a fluorescent probe at a concentration of 1×10^{-6} *M* in liposome suspensions. Spectrophotometer SPECORD M40 (Carl-

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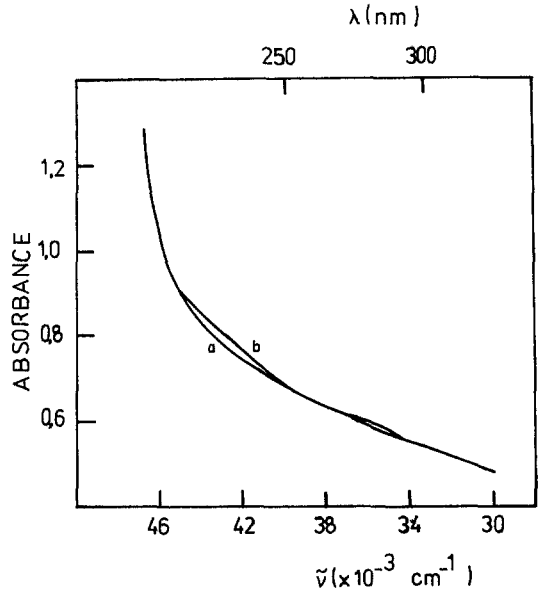

Fig. 1. Absorption spectra for soybean lecithin liposomes: (a) freshly prepared; (b) stored for 5 days at −18°C.

Zeiss) with standard fluorescence accessories was used for 450-nm light-scattering and 430-nm fluorescence anisotropy measurements. To evaluate the experimental data on both light scattering and fluorescence anisotropy, Student's paired t test was applied ($P<0.01$).

RESULTS AND DISCUSSION

The interaction of Zn-stabilized insulin with lecithin liposomes and its modification evoked by an insulin charge and liposome peroxidation were investigated. Our experiments were performed on liposomes freshly prepared or stored at −18°C for 1–5 days with the aim of elucidating the influence of storage and subsequent lipid peroxidation on the interaction between insulin and phospholipid bilayers. Lipid peroxidation was manifested by an increase in absorbance at 233 and at 280 nm, where peroxidation products have absorption peaks. No peroxidation was registered in the case of fresh liposomes and those stored for 1 day. Liposomes stored for a longer period showed the presence of peroxidation products (Fig. 1). To ascertain a possible influence of charge on the insulin molecule, experiments were performed at pH 4.5 (insulin possesses a positive charge) and pH 7.4 (the charge of insulin is negative). The effect of insulin on liposomes was pursued within the time interval of 0–160 min. Examples of the time course of scattered light intensity and DPH fluorescence anisotropy in lecithin bilayers are presented in Figs. 2 and 3. All experimental results are summarized in Table I. The addition of Zn-insulin to soybean lecithin liposomes (both fresh and stored) at pH 4.5 revealed significant changes in scattered light intensity. With increasing time of storage the insulin effect became faster and more pronounced. At pH 7.4, significant changes in scattered

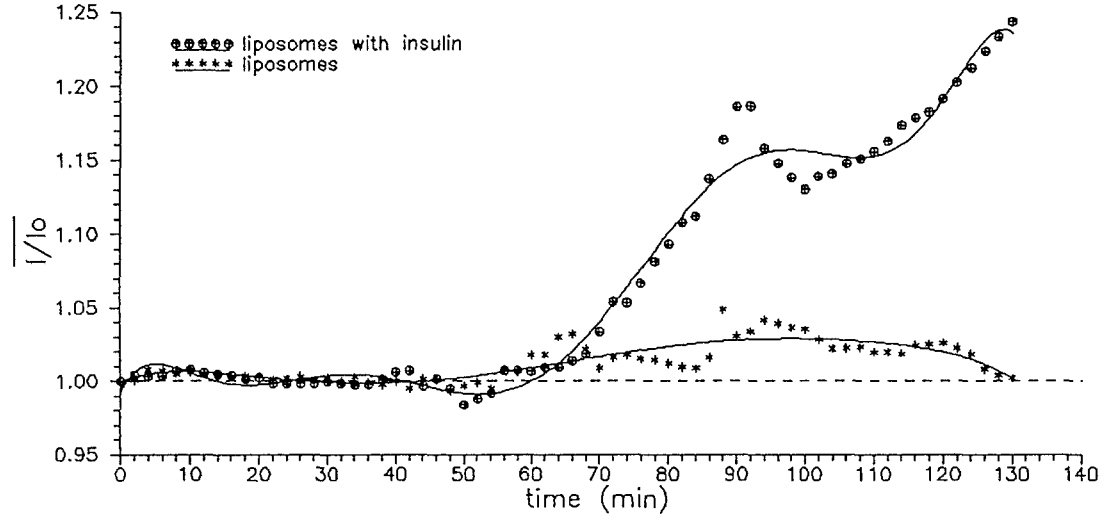

Fig. 2. The time course of the relative intensity I/I_0 of light scattered on lecithin liposomes stored for 5 days (pH 4.5). Individual points represent the average of four independent determinations.

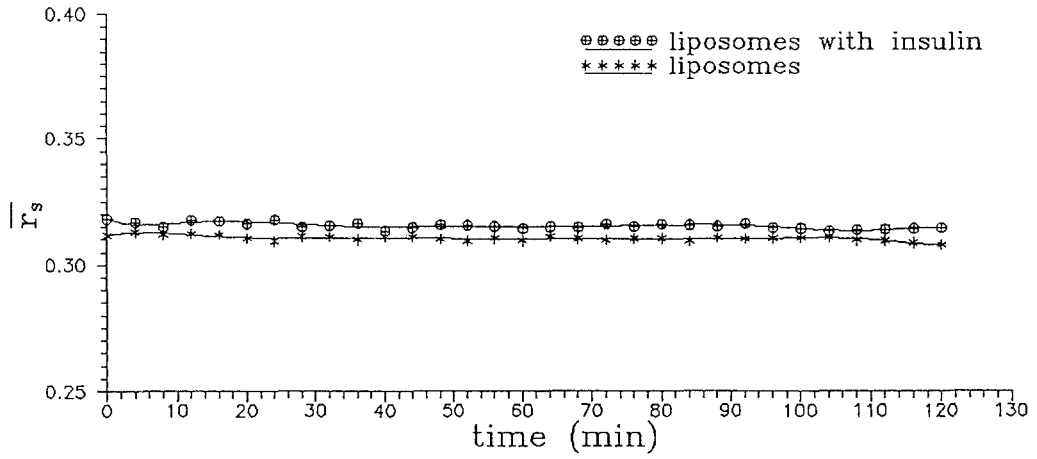

Fig. 3. The time course of DPH fluorescence anisotropy $r_s=(I_{II}-I_I)/(I_{II}+2I_I)$ after 60-min preincubation in lecithin liposomes stored for 5 days (pH 7.4). Individual points represent the average of four independent determinations.

Table I. Summary of the Data on Association of Insulin to Lecithin Liposomes[a]

Sample				
Storage time (days)	pH	Light scattering		Fluorescence anisotropy,
		t_s (min)	$(I-I_0)/I_o$	t_F (min)
0	4.5	148	0.021	ns
1	4.5	90	0.033	ns
5	4.5	80	0.155	ns
0	7.4	ns	—	ns
1	7.4	ns	—	ns
5	7.4	74	0.032	ns

[a] t_s and t_F are the values for the beginning of significant changes induced by insulin ($P<0.01$) in scattered light intensity and fluorescence anisotropy, respectively. $(I-I_O)/I_O$ is the insulin-induced change in the relative intensity of light scattered on liposomes at t_s+10 min.

light were registered only in the case of liposomes stored for 5 days. No significant changes induced by insulin were observed in DPH fluorescence anisotropy at either pH 4.5 or pH 7.4 in either fresh or stored liposomes.

The observed changes in scattered light induced by the addition of insulin to lecithin liposomes supported the idea of insulin interaction with phospholipid bilayers. However, our DPH fluorescence anisotropy data showed no significant changes after the addition of insulin, corresponding to an absence of changes in the hydrophobic core of bilayers. Our data could be interpreted in terms of the insulin association to the liposomal surface in the case of phospholipid peroxidation and/or acidic pH. This conclusion is in agreement with reports by Wiesner and Hwang [5] and Lai *et al.* [6], who found binding of insulin to the liposomal surface.

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