Interaction of Insulin Receptors with Lipid Bilayers and Specific and Nonspecific Binding of Insulin to Supported Membranes†

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ABSTRACT: In the first part, we study the interaction of the insulin receptor with model membranes of dimyristoylphosphatidylcholine (DMPC) by various techniques, including calorimetry, densitometry, static light scattering, and electron microscopy. By analyzing the pronounced depression of the lipid chain melting transition in terms of the Van Laar–Hildebrand theory of regular dilute solutions, an (exothermic) interaction energy of W_p = 2000 kJ·mol^{-1} is found for the receptor and of W_L = 0.6 kJ·mol^{-1} for the lipid. This is interpreted in terms of an adsorption of the 2 hydrophilic head groups of the receptor to the membrane surface so that 1 protein interacts with about 2000 lipids. This number is verified by freeze–fracture electron microscopy. Binding of insulin induces a remarkable decoupling of the receptor head group from the membrane, pointing to a pronounced conformational change. In the second part, we introduce a simple fluorescence technique by which adsorption isotherms of water-soluble and fluorescent-labeled substrates, such as insulin, to membranes may be determined. It is based on the selective evanescent field excitation of ligands adsorbed to supported planar bilayers on argon-sputtered glass plates. These are deposited by the monolayer transfer technique or by vesicle condensation. The reconstituted receptor exhibits a weak (binding constant $K_w = 3 \times 10^9$ L·M^{-1}) and a strong (binding constant $K_s > 10^{10}$ L·M^{-1}) binding site. Insulin exhibits a weak but remarkable nonspecific binding to bilayers of pure DMPC and DMPC containing 20% positively charged lipid and a strong binding to DMPC containing negatively charged lipids such as phosphatidylserine or ganglioside (G_{T1b}). In the former three cases, the hormone can be detached by increasing the ionic strength but interestingly not in the latter case, which points to some specific binding.

The reconstitution of membrane proteins into lipid bilayers has become an important task of model membrane research for two reasons: First, calorimetric and spectroscopic studies of reassembled binary or ternary lipid–protein mixtures yield valuable information about selective lipid–protein interaction mechanisms [cf. Maksymiv et al. (1987) and Sackmann et al. (1987)]. Second, the development of powerful methods for the incorporation of enzymes, hormone receptors, or antibodies into vesicles or supported lipid lamellae is of primary importance for future progress in the field of biosensors or in order to study the competitive binding of hormones and their agonists or antagonists to receptors. There is some hope that such binding studies may become an important tool for drug research in order to study the side effects of drugs or to design new drugs (Johnson et al., 1987).

In the present work, we study first the interaction of the insulin receptor of human placenta with bilayers of dimyristoylphosphatidylcholine (DMPC)[1] in the limit of infinite dilution. By evaluation of the shifts in the phase transition temperature in terms of the Hildebrand–Van Laar regular solution theory, quantitative values of the excess free energy of lipid–protein interaction are obtained. Such measurements are thought to be helpful for the characterization of the lipid–protein interaction in a quantitative way or to gain insight into selective lipid–protein interaction mechanisms.

In the second part, we study the specific and nonspecific binding of insulin to DMPC bilayers containing (1) the receptor, (2) negatively or positively charged lipids, and (3) a ganglioside with three negatively charged sialic acid groups, namely, G_{T1b}. For that purpose, the bilayers of interest are deposited onto glass plates by the monolayer transfer technique or by a vesicle deposition technique (Watts et al., 1986) which enables the application of evanescent field techniques in order to distinguish between bound and nonbound fluorescent-labeled insulin. By insertion of the membrane-covered substrate into a fluorescence cuvette, the experiments may be performed in a conventional fluorescence spectrometer, and adsorption isotherms may be recorded by continuous variation of the bulk concentration. By argon sputtering of the substrate, the planar bilayers are separated from the glass surface by an ultrathin water layer (Schneider et al., 1986).

Materials and Methods

Materials. The phospholipids (DMPC, DMPS) are commercial products and are used without further purification. The ganglioside is a gift from Prof. Taglianolti (Milano, Italy).

FITC-labeled bovine insulin is purchased from Sigma Chemical Co. One mole of insulin is labeled with approximately 1.3 mol of FITC. The insulin receptor is isolated from human placenta membranes following Marshall et al. (1974). Fresh normal placentas from full-term deliveries (300–500 g wet weight) are washed free of clotted blood in ice-cold 0.25 M saccharose, 10 mM Tris buffer, 1 mM EDTA, and 0.01 mM PMSF (pH 7.6). Amnion and chorion are excised, and the tissue is homogenized in 500 mL of the same buffer with Ultra-turrax for 2 min at maximal speed. The homogenate

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[1] Abbreviations: DMPC, dimyristoylphosphatidylcholine; DMPS, dimyristoylphosphatidylserine; FITC, fluorescein isothiocyanate; OBG, *n*-octyl β-glucopyranoside.

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is centrifuged for 15 min at 600*g*. The supernatant is successively centrifuged for 40 min at 8000*g* and then for 60 min at 40000*g*. This sediment is washed 3 times with 300 mL of 100 mM NaCl in 50 mM Tris buffer (pH 7.6) containing 0.01 mM PMSF. The final pellet is homogenized in 50 mM Tris buffer/0.01 mM PMSF (pH 7.6) at a concentration of about 20 mg of protein/mL and stored at −80 °C.

The insulin receptor from the 40000*g* microsomal fraction is solubilized by extraction with 2% (w/v) octyl β-glucopyranoside in a dilution of about 10 mg/mL protein for 20 min at 4 °C. After centrifugation at 130000*g* for 60 min, most of the insulin binding activity is contained in the supernatant. The insulin binding activity is determined following Williams et al. (1979) using the specific binding of ^{125}I-insulin and dextran-coated charcoal for separation of the unbound ligand. The insulin receptor is finally partially purified on a 80 × 5 cm column containing Sepharose CL-6B equilibrated with 50 mM Tris/0.05% (w/v) octyl β-glucopyranoside. The fractions containing the insulin binding activity are combined and concentrated to about 2 mL with an Amicon Diaflo ultrafiltration membrane.

Reconstitution of Receptor into DMPC Vesicles by Dialysis. Various amounts (depending on the desired protein molar fraction) of the solution of the receptor–detergent complex in Tris buffer (concentration 6.6 μg/μL) are mixed with 3 mL of a well-mixed solution of DMPC (1.5 mM) and the detergent (25 mM) in the same Tris buffer and stirred at 30 °C for 10 min. This solution is filled into a homemade dialysis cell, and detergent is removed by perfusion with the buffer for 48 h at 37 °C. Vesicles of an average diameter of some micrometers are obtained. In a separate experiment, it is shown that the residual detergent amounts to less than 1% of the phospholipid content (F. J. Heiszler, unpublished results).

Preparation of Supported Bilayers and Record of Adsorption Isotherms. In order to prepare supported bilayers on glass which are separated from the surface of the substrate by an ultrathin water layer, it is essential to treat the substrate in such a way that it is highly hydrophilic and that a strong disjoining pressure arises between the solid surface and the adjacent monolayer. In the present work, this is achieved by sputtering the substrates with argon in a high frequency plasma cleaner. Prior to this treatment, the glass plates are carefully cleaned by first boiling in Hellmanex cuvette cleaner and subsequent extensively washing with distilled water. The substrates are then dried in a vacuum chamber at 180 °C for some hours.

Pure lipid bilayers are deposited by subsequent transfer of two monolayers from the air–water interface [cf. Tamm and McConnell (1985)]. Bilayers with integral receptors are directly deposited by dipping the pretreated substrate into a suspension of reconstituted vesicles. An advantage of the former technique is that asymmetric bilayers may be deposited in order to optimize the repulsion of the bilayer from the substrate. A drawback is that only receptors with partially penetrating hydrophobic parts could be incorporated into the outer monolayer. The second technique is due to Dr. H. Gaub of this laboratory, who observed for the first time that vesicles fuse spontaneously at glass plates which have been pretreated as described above and who provided evidence that the repulsion is due to a charging of the substrate. For the present binding studies, it is only important that the total area from which the fluorescence is monitored is covered by the bilayer, which need not necessarily be completely homogeneous.

For the present study of the specific and nonspecific binding of insulin, the following method is adopted: A fluorescence

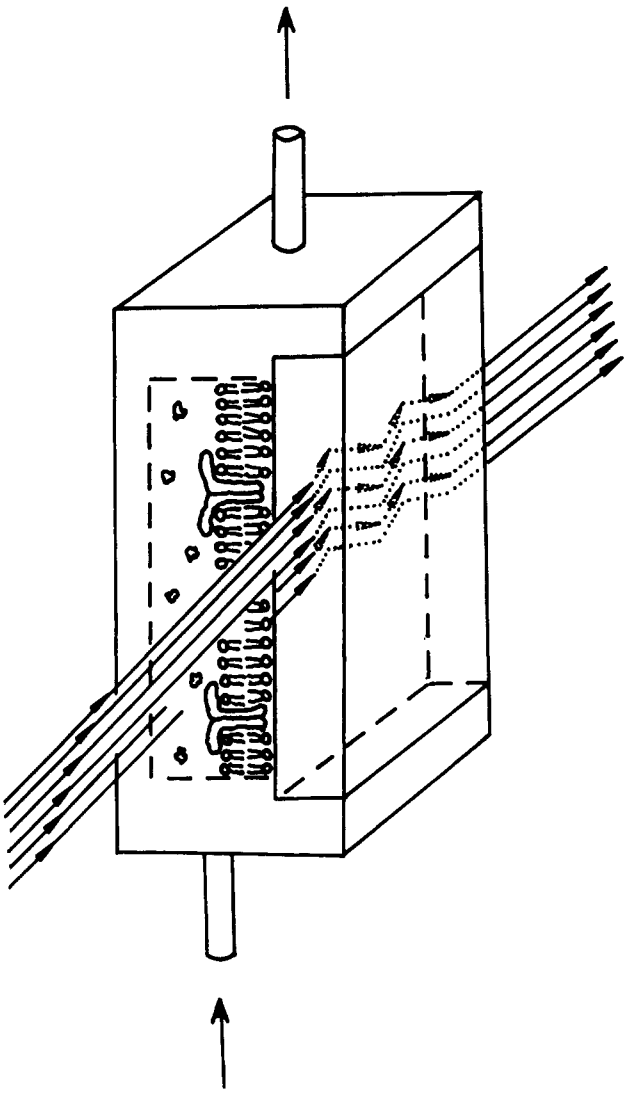


FIGURE 1: Cuvette used for the determination of binding isotherms of fluorescent-labeled proteins to supported bilayers. The side wall on the right side consists of a 2-mm-thick quartz plate with polished front faces. The irradiating light enters through this side wall in a direction parallel to its plane. Through the tubes at the top and the bottom of the cuvette, the bulk solution can be exchanged continuously in order to determine adsorption isotherms.

cuvette (cf. Figure 1) fitting into the holder of the spectrometer is assembled in such a way that one of the side walls (parallel to the direction of incident light) consist of a 2-mm-thick quartz plate with optically polished front faces. The cuvette is fixed in the measuring chamber of the fluorescence spectrometer (Schöffel R1000) in such a way that the irradiating light passes through the thick quartz plate. Provided the cross section of the irradiating light beam is smaller than the thickness of the plate, it is totally reflected along the whole pathway through the plate. Therefore, only fluorescent molecules which are adsorbed to the quartz plate or reside within a distance of less than 150 nm (Sui, 1988) from its surface are excited.

The procedure of the cuvette assembly depends on the type of supported bilayer studied. In the case of the receptor-doped membranes, the cuvette is first assembled in clean air with a freshly argon-sputtered quartz side wall. The bilayer is deposited by first filling the cuvette with the vesicles suspension which is then replaced by pure buffer or the insulin solution after an incubation time of 30 min. In the case of the monolayer deposition technique, the bilayer is deposited onto the pretreated quartz plate. The cuvette is then assembled below

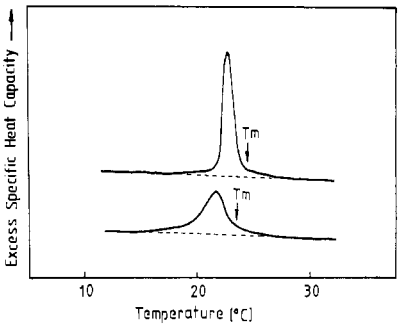


FIGURE 2: Comparison of the temperature dependencies of the specific heat of pure DMPC (upper curve) and of the same lipid containing insulin receptors at a molar fraction of 10^{-4} (lower curve). The area beneath the peaks, ΔA, which is a measure for the heat of transition, is $\Delta A = 350$ for pure DMPC and $\Delta A = 265$ in the presence of receptor. The base line is marked by dashed lines.

water and transferred into the measuring chamber of the spectrometer.

Calorimetry, Densitometry, and Electron Microscopy. The calorimetric studies are performed with a high-sensitivity microcalorimeter (MC-2, Microcal; Amherst, MA) interfaced to an IBM AT computer. The total lipid concentration is kept small (0.3 mM) in order to avoid vesicle aggregation. The endotherms are taken at a rate of 60 °C/h.

The setup for the densitometric measurement includes two external measuring cells (DMA 602 HP, Paar-Heraeus, Graz, Austria) which are connected to the DMA60 densitometer. Both cells are temperature controlled by the same thermostat (Haake, Model F3C). Typically, density data are taken at increasing and decreasing temperatures between 8 and 40 °C with scanning rates of 0.14 °C/min. The temperature of the sample cell is measured with a tiny PT 100 sensor connected to a digital thermometer (Lauda, R 42/D) with a relative sensitivity of 0.01 °C. Densitometer readings are taken every 0.1 °C and stored together with the temperature of the sample cell by the microcomputer (Apple 2). For each sample, at least three temperatures cycles are taken. The curves of the second and the third cycle agreed very well at all protein concentrations while in some cases the first run deviated slightly from the following ones.

The freeze–fracture studies are performed as described earlier (Rüppel et al., 1983). For the estimation of the receptor concentration, gold-labeled Con A (Polysciences Inc.), which is known to bind selectively to glycosylated proteins, is added to the vesicle suspension after preparation. A small droplet is deposited onto carbon-covered electron microscope grids dried and inspected by a Philips EM 400 T microscope. The vesicles appear as a weak dark shadow.

RESULTS

Thermodynamic Properties. A primary aim was to study the interaction of the insulin receptor with the lipid at very low protein concentration (molar fractions $x_p < 10^{-3}$) and a high excess of water in order to avoid interferences with protein–protein interaction effects. The shift of the chain-melting transition is measured by calorimetry, densitometry, and static light scattering.

Figure 2 shows a result obtained by calorimetry. Even at concentrations of 1 protein per 10^4 lipid molecules, the onset of the chain-melting transition is shifted to lower temperatures by $\Delta T = -1.8$ °C. The transition is strongly broadened, and the area beneath the transition curve which is a measure for the heat of transition is reduced by about 30% (cf. Table I). It should be noted that a broad background is situated beneath the main transition band, in particular in the case of the reconstituted vesicles. This background is most probably due to very small vesicles. The integrated heats of transition may thus only be determined to an accuracy of about 10%.

Table I: Summary of Shift and Broadening of the DMPC Phase Transition Caused by Receptor As Obtained by Calorimetry, Densitometry, and Static Light-Scattering Experiments[a]

receptor weight fraction	0	4.2×10^{-2}	8.1×10^{-2}	3×10^{-1}
receptor mole fraction	0	10^{-4}	2×10^{-4}	10^{-3}
T_L (°C)	24	22.4	21.8	18
T_s (°C)	21.7	18.5	16.5	7.3
ΔH (kJ·mol^{-1})	30	21		

[a] T_L, liquidus line = onset of transition from above; T_s, solidus line (cf. Figures 2 and 3 for definition).

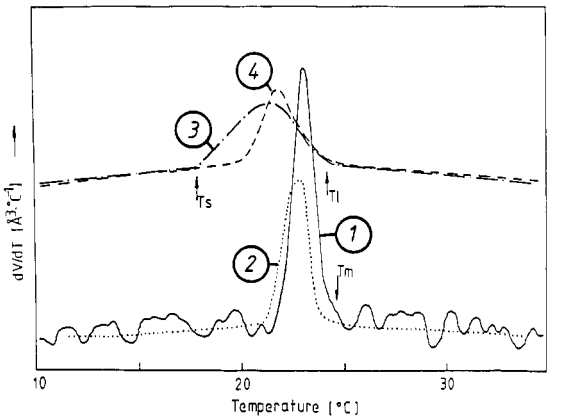
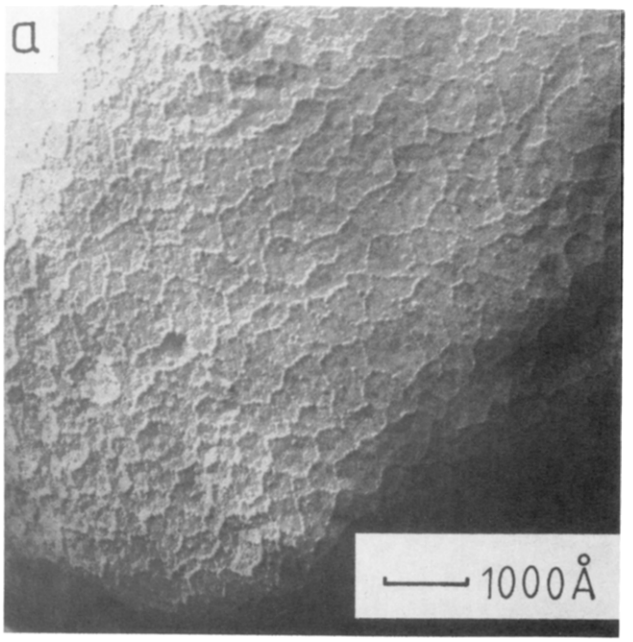


FIGURE 3: Mass densitometric study of the effect of insulin on the DMPC phase transition in the absence and presence of insulin receptor. (Curve 1) First derivative of molar volume of DMPC (dV/dT, corresponding to relative thermal expansivity) as a function of temperature. (Curve 2) dV/dT versus T curve of the same preparation after addition of 0.2 mol % insulin with respect to DMPC. (Curve 3) Temperature dependence of thermal expansivity of DMPC in the presence of insulin receptor (10^{-4} molar fraction). (Curve 4) Effect of insulin on dV/dT versus T curve of the above reconstituted system. Note in particular the pronounced decrease in the width of the transition.

Figure 3 exhibits results obtained by densitometry. The temperature dependence of the first derivative of the molar volume of the lipid bilayer with respect to temperature, dV/dT, corresponding to the thermal expansivity, is given for four systems: (1) pure DMPC (curve 1); (2) the same preparation after addition of insulin (0.2 mol % with respect to lipid (curve 2); (3) DMPC containing 1 receptor per 10^4 lipid molecules (curve 3); (4) the same preparation as in curve 3 after addition of 0.2 mol % insulin with respect to DMPC (curve 4). In agreement with the calorimetric result, the very small amount of receptor leads to a drastic broadening of the transition and a remarkable shift of the melting transition by −1.5 °C. Insulin causes a slight shift of the transition of pure DMPC to lower temperatures. However, in the case of the reconstituted bilayer, insulin cancels part of the low-temperature shift of the transition caused by the receptor. Moreover, it leads to a pronounced sharpening of the transition. This effect of insulin is observed with all three techniques. The effect on the pure lipid points to a substantial nonspecific binding of the insulin whereas that on the reconstituted membranes strongly suggests a conformational change of the receptor.

The results obtained with the three techniques are summarized in Table I, together with the heats of transitions which are obtained from the areas beneath the calorimetric transition curves. The values of the onsets and of the end points of the transitions are averages as obtained by the three techniques. The former corresponds to the liquidus line (at the temperature



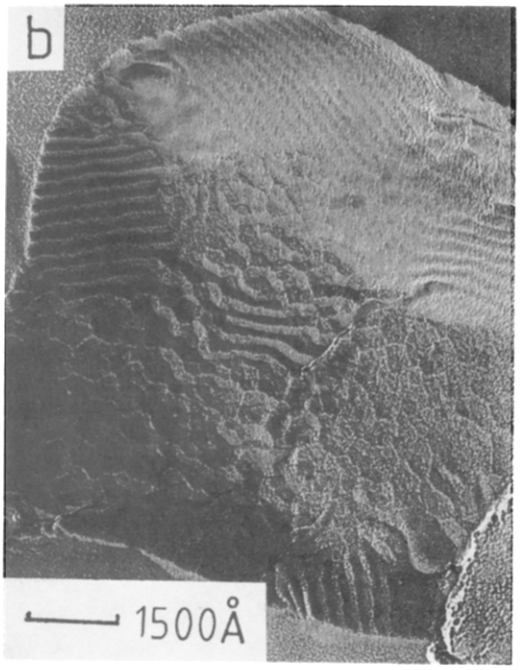


FIGURE 4: Freeze–fracture electron micrographs of DMPC vesicle containing insulin receptor at a molar fraction of $x_p = 10^{-3}$. (a) Sample frozen from 17 °C; (b) same vesicle preparation frozen from 4 °C.

T_l) and the latter to the solidus line (at temperature T_S) of the lipid–protein mixture.

Freeze–Fracture Electron Microscopy. As shown previously (Rüppel et al., 1982), information on the degree of perturbation of the lipid bilayer by the proteins can also be obtained from characteristic modifications of the surface texture of freeze–fracture micrographs taken from the $P_{\beta'}$ and $L_{\beta'}$ phases of lecithins. This technique is therefore also applied in the present work. As shown below, it provides further evidence for the interaction of the insulin receptor with a large number (~1000) of lipids. Figure 4 shows freeze–fracture electron micrographs of DMPC vesicles in the presence of a 10^{-3} molar fraction of receptor. The vesicle dispersions are frozen from temperatures between the main and the pretransition (17 °C; Figure 4a) and below the latter (4 °C; Figure 4b).

If the vesicles are frozen from the $P_{\beta'}$ phase, the regular ripple structure is completely abolished and changed into an irregular arrangement of wormlike protrusions. Interestingly, it appears that the threadlike protrusions often form closed loops, and the areas enclosed are all of about the same size (diameter of 300–400 Å) corresponding to 2000 lipid molecules. In the center of many of the loops, one can clearly observe particles which are attributed to the proteins.

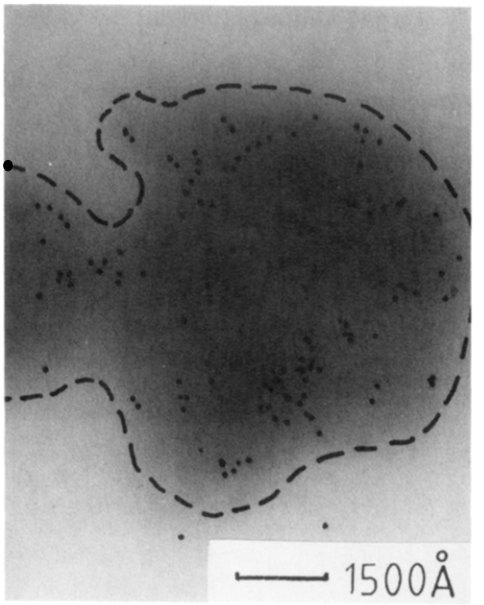


FIGURE 5: Electron micrograph of DMPC vesicle with reconstituted receptor prepared by dialysis to which gold-labeled Con A was added. The initial protein to lipid ratio is $1:10^3$. The slightly darkened background which is encircled by a dashed line corresponds to the vesicles and the dark spots to the gold label.

At 4 °C, a mosaic-like texture is observed which consists of domains exhibiting (1) a regular corrugated structure which is typical for a nonequilibrated $L_{\beta'}$ phase (Sackmann et al., 1980) and (2) a wavy appearance. It is intriguing to estimate the number of lipid molecules interacting with one protein by assuming that the domains with the wavy texture are due to perturbed lipid. Inspection of a number of images of the type of Figure 4b shows that for $x_p = 10^{-3}$ about 60% of the total area exhibits the wavy texture. This would correspond to about 800 lipid molecules. This value is certainly a lower limit for the number of lipid molecules perturbed by the receptor and holds only for the $L_{\beta'}$ phase.

Measurement of Receptor Concentration by Gold Labeling. The concentration of reconstituted receptor is estimated by electron microscopy by labeling of the receptor with Con A–gold particles which are known to bind selectively to glycosylated membrane proteins. Figure 5 shows an electron micrograph of dried reconstituted vesicles (dialyzed in the presence of 10^{-3} mole fraction of receptor) on an electron microscope grid. The dark shadowed area which has been encircled by a dashed line corresponds to the vesicles and the dark spots to the gold label. The latter are randomly distributed. From such micrographs, the number density of receptors can be estimated. By analysis of about five vesicles, we arrive at a value of 4×10^{-4}; that is, about 50% of the receptor has been inserted into the bilayer.

Binding of Insulin to DMPC Bilayer Containing Insulin Receptor, Charged Lipids, and the Ganglioside G_{T1b}. The construction of the cuvette according to Figure 1 allows one to vary the concentration of the fluorescence-labeled solute (FITC–insulin in present work) continuously by exchange of the solution. The relative amount of adsorbed FITC–insulin can thus be measured as a function of the bulk concentration. By comparison with the fluorescence intensity of a deposited

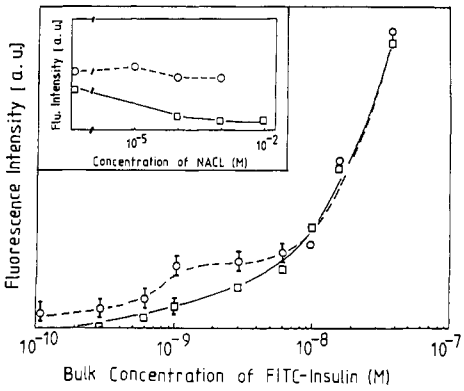


FIGURE 6: Comparison of binding curves (adsorption isotherm) of fluorescence-labeled insulin to supported bilayer of DMPC (□) and of DMPC containing 10^{-4} molar fraction of receptor (○). The bilayer is prepared by the vesicle deposition technique. Insert: Effect of salt (NaCl) on fraction of adsorbed insulin onto pure DMPC bilayers (□) and onto receptor-containing bilayer (○), respectively.

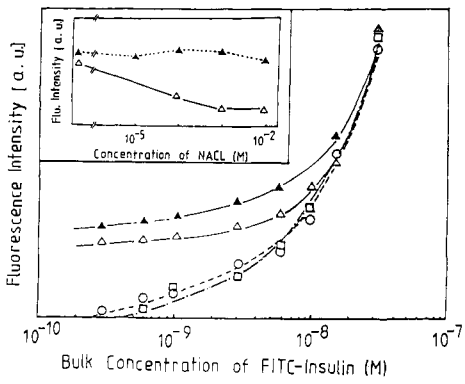


FIGURE 7: Comparison of binding curves of FITC–insulin onto supported lipid bilayers which are deposited by the monolayer transfer technique and which contain various guest lipids in the outer monolayer. (□) Pure DMPC; (○) DMPC with 30% positively charged ammonium lipid in outer monolayer; (△) DMPC with 30% DMPS in the outer monolayer; (▲) DMPC with 20% ganglioside G_{T1b} (with three sialic acid residues) in the outer monolayer. Insert: Effect of increase of ionic strength on fraction of adsorbed FITC–insulin onto bilayers of a 3:1 DMPC/DMPS mixture (△) and of DMPC containing 20 mol % ganglioside (▲).

monolayer containing a known amount of fluorescence-labeled lipid, it is even possible to determine the absolute surface concentration of the adsorbed solute.

As a first example, Figure 6 shows binding curves of FITC–insulin onto supported DMPC bilayers in the absence and presence of receptors. The fluorescence intensity at 518 nm is plotted as a function of the bulk concentration of insulin. In the former case, one obtains a smoothly ascending curve which below $C_I = 5 \times 10^{-9}$ M insulin shows no appreciable fluorescence. In the presence of the receptor, however, the isotherm exhibits a finite fluorescence even at $C_I = 10^{-10}$ M insulin and a stepwise increase at 10^{-9} M. At $C_I \geq 5 \times 10^{-8}$ M, the two curves coalesce. This is due to the fact that above this limit the fluorescence is dominated by the dissolved protein residing within a distance of 150 nm from the quartz surface. The present technique can thus only be applied at small concentrations of the adsorbed species.

In the insert of Figure 6, we demonstrate that insulin binds also appreciably to pure DMPC bilayers. The fluorescence intensity is plotted as function of the ionic strength of the solvent for the case of a bulk insulin concentration of $C_I = 10^{-9}$ M. Clearly, insulin is detached from the supported DMPC membrane in the absence of the receptor but is not salted out in its presence.

In Figure 7, we present binding curves of insulin to supported asymmetric bilayers which contain three types of charged lipids in the outer monolayer while the inner monolayer is pure DMPC: (1) negatively charged DMPS; (2) a positively charged two-chain amphiphile; and (3) a ganglioside, namely, G_{T1b} with three negatively charged sialic acid residues. It is seen that insulin binds strongly to negatively charged lipid (both DMPS and the ganglioside) but very slightly to positively charged membranes. A remarkable effect is shown in the insert of Figure 7. It is found that insulin may be detached from DMPS-containing bilayers by the addition of salt to the bulk solution but is not salted out in the presence of the ganglioside. This suggests that the binding of insulin to the ganglioside is controlled also by a nonelectrostatic interaction mechanism.

Discussion

Thermodynamics of Lipid–Protein Interaction. The remarkably strong depression of the DMPC chain melting transition points to a strong interaction of the receptor with the lipid bilayer. From the concentration dependence of the depression, $\Delta T(x_p)$, at very small protein molar fractions, x_p, one obtains a quantitative measure for the strength of the lipid–protein interaction by application of the classical rules of thermodynamics of solutions. According to the familiar Van Laar relationship (Sanfeld, 1971), the activity coefficient (fugacity), f_L, of the lipid is related to the depression ΔT according to

$$\ln f_L = \frac{\mu_L - \mu_L^\circ}{k_B T_{m,0}} = \frac{\Delta H_0}{k_B T_{m,0}^2} \Delta T \qquad (1)$$

In this equation, ΔH_0 is the heat of chain melting (per molecule) of pure DMPC, and $T_{m,0}$ is the melting temperature of the hypothetical ideal solution which in our case is practically equal to the transition temperature of the pure DMPC since $x_p \sim 10^{-4}$. $\Delta\mu_L = \mu_L - \mu_L^\circ$ is the excess chemical potential of the lipid and is a measure for the work needed to transfer a lipid molecule from a (hypothetical) ideal solution (the reference state) to the real solution. The excess free energy, $\Delta\mu_p$, of a protein molecule is then obtained via the Gibbs–Duhem relationship according

$$\frac{d\Delta\mu_p}{dx_L} = k_B T \frac{x_L}{x_p} \frac{d}{dx_L}(\ln f_L) \qquad (2)$$

Thus, $\Delta\mu_p$ is obtained from the concentration dependence of the freezing point depression of the lipid.

Approximate numerical values of $\Delta\mu_L$ and $\Delta\mu_p$ are obtained in a straightforward way from the experimental data summarized in Table I:

$$\Delta\mu_L = 0.6 \text{ kJ·M}^{-1}; \quad \Delta\mu_p = 2400 \text{ kJ·M}^{-1} \qquad (3)$$

$\Delta\mu_p$ corresponds to a very large binding energy of the protein to the lipid bilayer. Concerning a molecular interpretation of the large free binding energy of the protein, we postulate that it is mainly due to an adsorption of the hydrophilic head group to the membrane surface whereas the hydrophobic interaction is of minor importance. This is first of all suggested by the finding that 1 protein molecule interacts with some 1000 lipids as follows, for instance, from the freeze–fracture electron micrographs. It should be noted that the excess entropy of mixing is negligible since the area (A_p) occupied by each of the two α-helical chains of the receptor penetrating the bilayer is only by about a factor of 3 larger than the area per lipid molecule (A_L) [cf. Sanfeld (1971)]. The excess entropy of mixing is, however, of the order of $kTx_p \cdot A_p/A_L \ll kT$ [see

chapter 21 of Lewis and Randall (1961)].

Some further evidence for the interpretation of the receptor–DMPC interaction in terms of the protein head-group adsorption comes from the finding that the number of lipids, N_L, interacting with the protein is roughly proportional to the number of monomers, M, per protein head group. Thus, it is $N_L = 300$ for glycophorin compared to $N_L = 2000$ for the insulin receptor whereas the corresponding number of monomers are $M = 121$ and $M = 1339$, respectively.

The above interpretation suggests that the binding of insulin to the receptor leads to a pronounced conformational change of its head group. The partial reversal of the low-temperature shifts of the liquidus and solidus line by insulin (cf. top trace of Figure 3) points to a decoupling of the receptor head group from the lipid–water interface in such a way that it extends more into the third dimension.

Insulin Binding to Reconstituted Membrane. The binding of insulin to the receptor containing planar bilayers may be described in terms of an adsorption–desorption equilibrium. The appearance of two steps in the adsorption isotherm of insulin shown in Figure 6 suggests that the receptor has a weak (w) and a strong (s) binding site which are characterized by binding constants K_w and K_s, respectively. These constants are obtained from the adsorption isotherms by considering the well-known relationship for the fraction, α, of bound insulin:

$$\alpha_i = \frac{K_i C_I}{1 + K_i C_I} \tag{4}$$

where i denotes the type of binding site (s or w) and C_I is the bulk insulin concentration. K_i is then easily obtained from the half-concentrations $[I]_{1/2}$ where $\alpha_i = {}^1/_2$. From Figure 6, we find $K_w = 3 \times 10^9$ L·M^{-1}. Unfortunately, the sensitivity of our spectrometer is not sufficient to measure also K_s which is smaller than $K_s \leq 10^{10}$ L·M^{-1}.

Conclusions

One primary aim of the present work is to show that the excess free energy of solution of a protein in a lipid bilayer, as obtained from the depression of the chain-melting transition, is a suitable physical measure to characterize the strength of the lipid–protein interaction in a quantitative way. As shown previously, this concept may be extended to binary lipid mixtures in order to obtain information about specific lipid–protein interaction mechanisms (Maksymiv et al., 1987).

A somewhat different approach was adopted by Morrow et al. (1985) in order to characterize the strength of the lipid–protein interaction in terms of thermodynamic observables. These authors treat the lipid–protein system as a regular solution, and their model is certainly more appropriate to treat the cases of high peptide concentrations ($x_p > 10^{-3}$) and of peptides which interact primarily via their hydrophobic part with the lipid bilayer than the present consideration.

The finding of the present work that one insulin receptor molecule interacts with some thousand lipids is typical for receptors with large glycosylated head groups such as glycophorin (Rüppel et al., 1982). The present interpretation in terms of an adsorption of the protein head group to the lipid–water interface is one likely explanation. It must be pointed out, however, that this finding can in principle also be explained in terms of an elastic deformation of the bilayer which is a long-range effect and would also affect many lipid molecules simultaneously. Such a theoretically predicted effect (Sackmann, 1984; Mouritsen & Bloom, 1984) has indeed been observed experimentally (Riegler & Möhwald, 1986). However, the finding of a (roughly linear) correlation between the size of the head group and the number of lipids affected by the protein provides strong evidence for the former explanation.

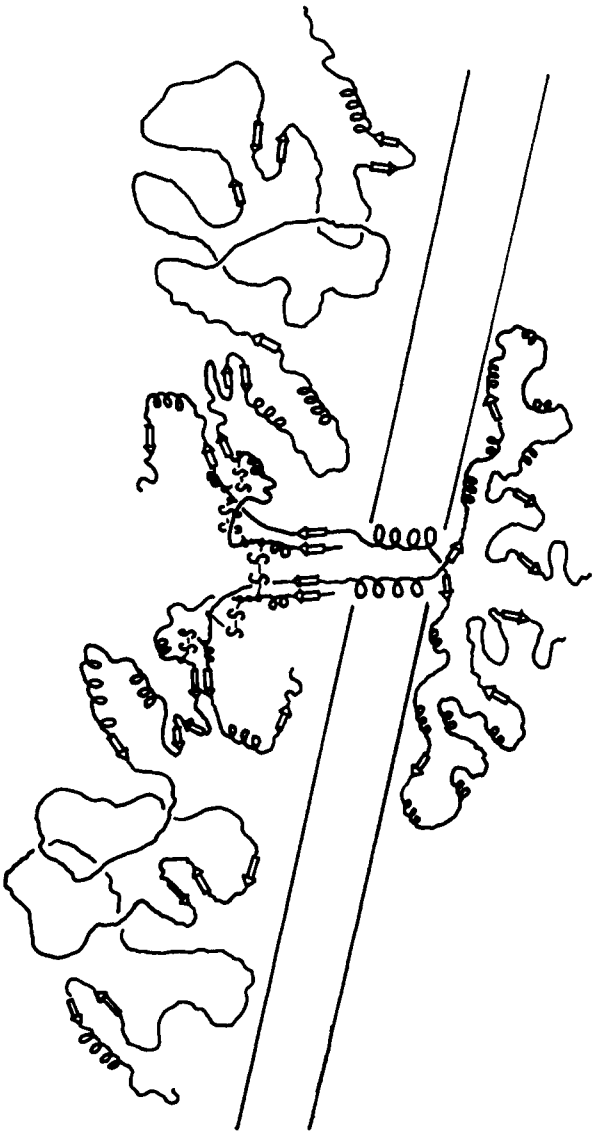


FIGURE 8: Schematic view of insulin receptor showing distribution of domains of α-helical, β-sheet, and random-coil structure as predicted by the probabilistic method.

In Figure 8, we present a schematic view of the domain structure of the insulin receptor as predicted by the probablistic method (Garnier et al., 1978; Chou et al., 1978). Both the head groups of the α-chain (pointing into extracellular space) and of the β-chain (pointing mainly to the cytoplasmic side) exhibit large stretches of random-coil structure and are thus expected to be rather flexible. On the other side, about 38% of the residues of the head groups of both chains are hydrophobic (Ullrich et al., 1985). Since these are arranged in uninterrupted sequences of up to four monomers, it is well conceivable that they penetrate into the hydrophobic core of the bilayer.

An intriguing suggestion of the present study is the decoupling of the receptor from the bilayer after insulin binding which points to an induced conformational change. A conformational change of the receptor after insulin binding has indeed been reported by Pilch et al. (1980) and Juul et al. (1986). In the presence of ATP, this conformational change may trigger the phosphorylation of the cytoplasmic β-subunit (Kasuga et al., 1982). In view of the model presented in Figure 8, the effect of insulin binding could consist of a detachment of the head groups from the bilayer which could also help to expose the phosphorylation site.

The evanescent field technique introduced in order to distinguish between specific and nonspecific binding of insulin to receptor-containing membranes is restricted to small concentrations of the fluorescence-labeled ligand ($<10^{-7}$ M). However, in combination with the evanescent field photobleaching technique (Tamm & McConnell, 1985), it can be extended to higher concentrations, since the fluorescence of the substrate in the bulk will recover much faster than that of the adsorbed fraction. The present binding study to pure lipid bilayers shows that water-soluble polypeptides may adsorb remarkably to lipid bilayers in particular in the presence of charged lipids. Of particular interest is the strong adsorption of insulin in the presence of the ganglioside even at high salt concentration. In vivo, this could help to accumulate insulin at the cell surface which would greatly increase the effective hormone concentration.

Finally, we point out that the evanescent field technique may be helpful in order to study the competitive interaction of hormones with their agonists and antagonists as well as other drugs to hormone receptors. Such studies are supposed to be helpful for the search for new drugs and in order to test possible side effects of a given drug (Johnson, 1987).

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