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INTERACTIONS OF LIPOSOMES WITH TRYPANOSOMA BRUCEI PLASMA MEMBRANE

J. Gruenberg[1], D. Coral[2], A.L. Knupfer[1] and J. Deshusses[1]

1) Department of Biochemistry and 2) Department of Pharmacy, University of Geneva, Quai Ernest-Ansermet 30, CH 1211 Geneva 4, Switzerland

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SUMMARY

Interactions of negatively-charged solid, and positively-charged fluid liposomes with Trypanosoma brucei were studied. Fluid, positive liposomes undergo fusion with the plasma membrane, while solid negative vesicles are only adsorbed to the membrane, as shown by trypsinization and temperature dependence. These results are consistent with the distribution profile of subcellular particles of cells pretreated with both types of liposomes. (^{3}H)concanavalin A and (^{35}S)diazobenzenesulfonate were used to label the plasma membrane.

INTRODUCTION

The possibility of therapy of mammalian parasitic diseases through liposome-encapsulated drugs has been reported for a trypanosomatidae flagellate, Leishmania donovani, the causative agent of visceral leishmaniasis, intracellular parasite of Küpfer cells (1-3).

Trypanosoma brucei, responsable for the African trypanosomiasis of cattle, belongs to the trypanozoon subgenus which includes the parasites of human sleeping sickness. There is double interest in investigation of liposome-encapsulated drugs regarding therapy of T.brucei infections: the hope to reach specific intracellular regions where the parasite is also found (4-5) and, more important, the decrease of toxicity of encapsulated drugs as compared to the free drugs, as shown already for anti-leishmanial antimonials (1-3). The aim of this study was to emphasize the nature of the interactions between the T.brucei plasma membrane and liposomes of different charge and phase transition.

MATERIAL AND METHODS

Organism: Trypanosoma brucei,strain STIB 478 TD, was cultivated on SDM-77 medium according to Brun and Jenni (6).

Liposomes and cell-liposome interactions Eggphosphatidylcholine: stearylamine (9:1 mol) vesicles will be referred to as fluid, positively-charged vesicles (phase transition around -10^{0} C (7)). Dipalmitoylphosphatidic acid:dipalmitoylphosphatidylcholine:distearylphosphatidylcholine (1:4.5:4.5 mol) vesicles will be referred to as solid, negatively-charged vesicles (the phase transition temperature being +38^{0} C, as shown by bichromate release). Vesicles were prepared by sonication for 45 min in a 100 W,MSE 20 KHz sonicator at ambient

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temperature for fluid vesicles and at 50° C for solid vesicles. Sonication was performed under Argon. Both types of liposomes were labelled by addition of trace amounts of [^{14}C]stearic acid to the lipids before sonication. The liposomes were always freshly prepared and submitted to centrifugation (1500 x g, 20 min) before the experiments, to remove aggregated particles. The buffer used throughout this study was Na_2HPO_4 42 mM, NaH_2PO_4 8 mM, NaCl 93 mM, KCl 3.6 mM pH 7.5. Incubations were performed for the indicated time at concentrations of 10^8 cell/ml and 8 mg/ml liposomal lipids. Trypanosomes were washed free of liposomes by 5 centrifugations (1500 x g, 5 min) in a Sorvall RC 2-B centrifuge. Trypsinization was performed by subsequent incubation at 37° C during 8 min in the presence of 0.015% (w/v)trypsin and was stopped by centrifugation and washing.

Labelling of membranes: Parasites (10^8 cells/ml) were incubated 30 min in the presence of 50 µM of p-diazobenzene-[^{35}S]sulfonate (4 mCi/µmole) according to Berg and Hirsch (8) or 15 min in the presence of 10 µg/ml [^{3}H]concanavalin A (491 µCi/mg).

Subcellular fractionation: Parasites were disrupted by sonication in diluted buffer during 15 x 5 s at 1° C on a MSE 100 W sonicator using 20% of full sonication power. Then tonicity of the buffer was readjusted and unbroken cells were removed by centrifugation (1500 x g, 5 min). The homogenate was layered on the top of a continuous CsCl gradient and centrifuged at 28,000 rpm during 20 h (Beckman SW-41 rotor amd Sorvall OTD-2 centrifuge).

Proteins: Protein concentrations were determined according to Bradford (9).

Chemicals: N-[acetyl-^{3}H]acetylated concanavalin A, [^{35}S]-p-sulfanilic acid, [1-^{14}C]stearic acid and [^{3}H]inulin were obtained from the Radiochemical Center, Amersham (England). Trypsin (type I) was purchased from Sigma Chemical Co, St.Louis, Mo. (USA). Eggphosphatidylcholine was obtained from Lipid Products, South-Nutfield (England). All other chemicals were obtained from Merck, Darmstadt (FRG) and Fluka, Buchs (Switzerland).

RESULTS AND DISCUSSION

Mechanism of interaction

A first set of experiments was performed in order to compare time-dependent uptake of liposomes of various charges and fluidity (not shown). The negative solid, and positive fluid liposomes were shown to differ significantly, constant levels of uptake being, respectively, 18 µg/mg dry weight, reached in 5 min, and 250 µg/mg dry weight, reached in 30 min (Fig. 1).

Negatively-charged liposomes.

The evidence for adsorption with T.brucei cells as the major process comes from the decreased uptake when the temperature is raised from 1° C to 22° C (Fig. 2A), and from the release of the adsorbed liposomes by trypsin treatment, under conditions where the motility of the parasite is not impaired (Fig. 2A). The same effects of temperature and trypsin have been used as criteria for liposome adsorption to fibroblasts (10) and lymphocytes (11). These results rule out fusion or endocytosis as the major process since they increase with temperature (7). One can also exclude the transfer of lipids, which is not affected by tryp-

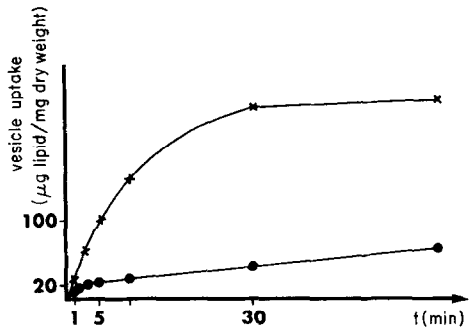

Fig. 1 Time dependence of cell-liposome interactions. The amount of vesicles associated with the cells at 22°C was measured with (x——x) positive, fluid liposomes and (●——●) negative,solid liposomes.

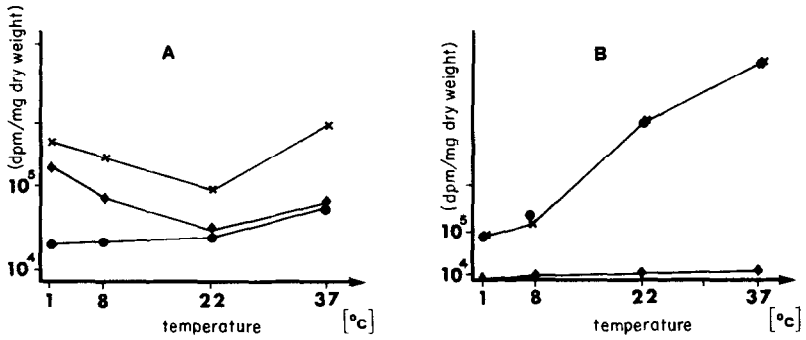

Fig. 2 Effects of temperature and trypsin. The parasites were incubated during 10 min with: A) negative, solid [^{14}C]liposomes. B) positive, fluid liposomes. (x——x)liposomes associated with the cells; (●——●)liposomes associated with the cells after trypsinisation; (◆——◆)liposomes released by trypsinisation.

sinisation. A mechanism different from adsorption should be partially involved since the amount of liposomes not released by trypsin increases with temperature (Fig. 2A). It has to be pointed out that the increased amount of liposomes associated with the parasite at 37° C compared to 22° C is due to the proximity to the transition phase of these liposomes (38° C), observations already reported for dimyristoyllecithine-vesicles-lymphocyte interaction (11). It is interesting to note that the rate of uptake of the same type of liposomes by 3T3 cells increases with temperature and the phenomenon has been interpreted as endocytosis (12).

Positively-charged fluid liposomes.

These vesicles undergo fusion as the main interaction process with T.brucei plasma membrane. Adsorption can be ruled out since trypsin cannot release the vesicles associated with the parasites (Fig. 2B), and the amount of vesicles

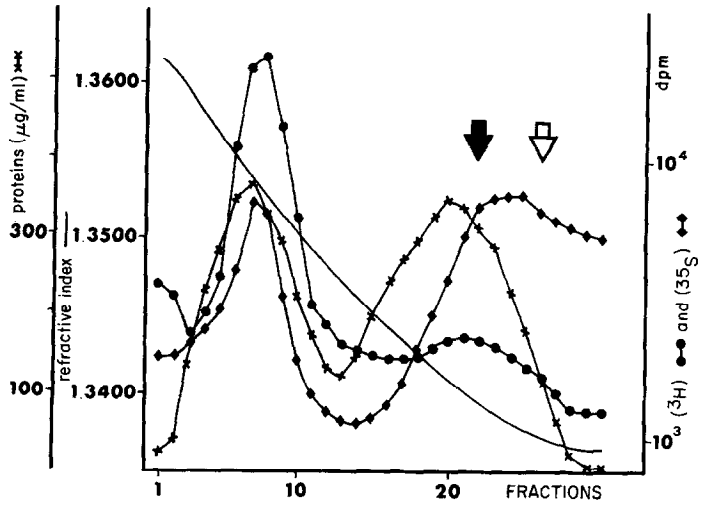

Fig. 3 Labelling of the plasma membrane. T.brucei cells were incubated 15 min at 22° C in the presence of (●——●) [3H]concanavalin A or (◆——◆) [35S]p-diazobenzenesulfonate before disruption. The homogenate was separated on a CsCl density gradient. (———) refractive index; (x——x)proteins. Equilibrium density of free concanavalin A and p-diazobenzenesulfonate are indicated by filled and open arrows respectively.

incorporated increases with temperature (Fig. 2B) indicating fusion or endocytosis (7). Endocytosis can be ruled out since no inhibitory effect at 4° C and 37° C could be detected in the presence of 10 mM KCN, NaF or NaN_3, reported to be inhibitors of endocytosis in T.brucei (13), or of 10 µM colchicine.

It has to be pointed out that at 37° C, lipid transfer is of minor importance (7), although at 2° C it may predominate (14).

Subcellular fractionation.

In order to investigate membrane-liposome interaction, chemical labelling of the plasma membrane and subcellular fractionation has been undertaken. When parasites, incubated in the presence of [3H]concanavalin A or [35S]diazobenzenesulfonate and subsequently disrupted, were layered on the top of a continuous CsCl density gradient, both [3H]- and [35S]-labelled material were recovered in the same peak, at the density of 1.20 g/cm^3 (Fig. 3). Under the same conditions free concanavalin A and diazobenzenesulfonate were recovered at lower densities, (1.06 and 1.02 g/cm^3 respectively). Since these reagents are non-permeant, it was concluded that the labelled fraction was the plasma membrane of the trypanosome; further characterisation would need marker enzymes which are not yet realiable for such organisms. If the cells are treated

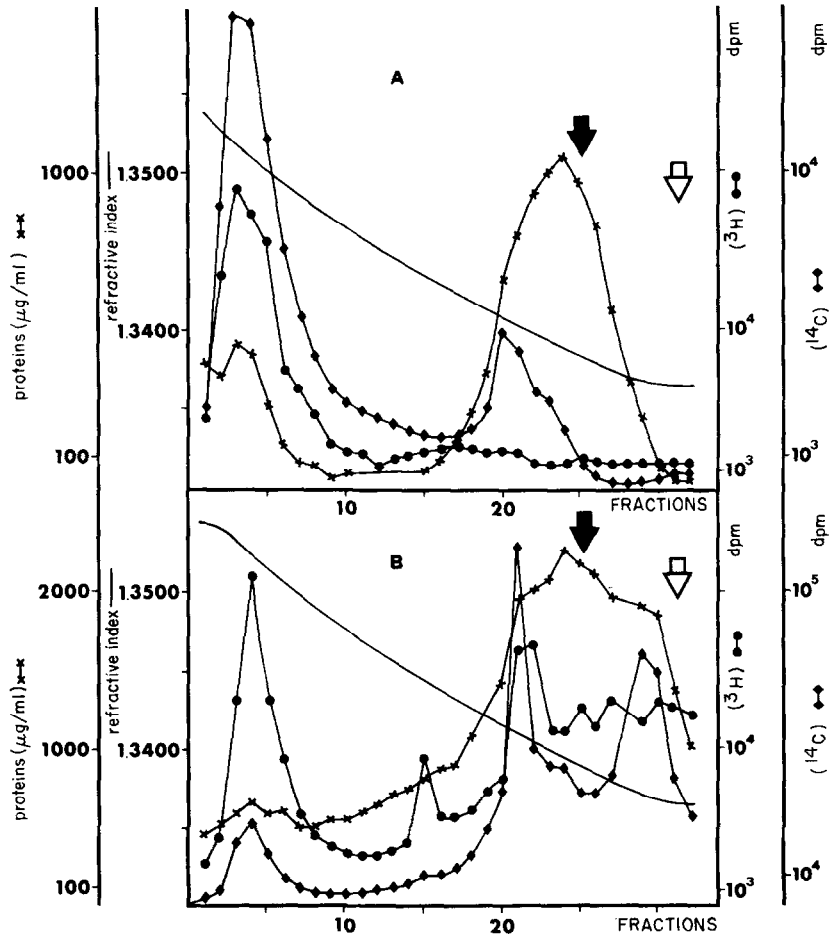

Fig. 4 Interaction of positive,fluid liposomes with T.brucei. Incubation (15 min at 22° C) in the presence of ^{3}H/concanavalin A and positive, fluid liposomes: A) before cell disruption, B) after cell disruption. CsCl density gradient of the homogenate: (●——●) ^{3}H/concanavalin A; (■—■) ^{14}C/liposomes; (——) refractive index; (x——x)proteins. Equilibrium density of free concanavalin A and free positive, fluid liposomes indicated by filled and open arrows respectively.

with ^{3}H/concanavalin A and ^{14}C/fluid liposomes, the profiles of both isotopes superimpose perfectly, indicating that fluid liposomes interact mainly with the plasma membrane (Fig. 4A). Free fluid liposomes remained at the top of the gradient. Additional peaks appeared when the cell homogenate was treated under the same conditions with positive, fluid liposomes and concanavalin A (Fig. 4B). More than 90% of the ^{14}C-label of fluid vesicles equilibrates at the same density as the plasma membrane; a minor peak was detected at a lower density (d=1.08) indicating that liposomes were also associated with some other cel-

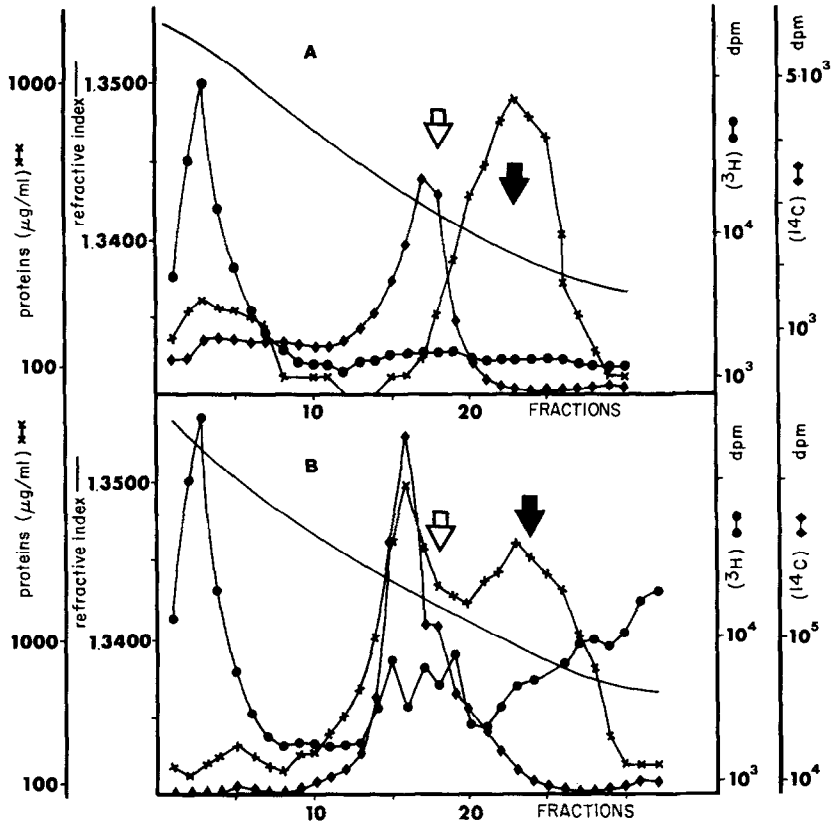

Fig. 5 Interaction of negative, solid liposomes with T.brucei. Incubation (15 min at 22° C) in the presence of [3H]concanavalin A and [14C] negative, solid liposomes: A) before cell disruption, B) after cell disruption. CsCl density gradient. (●——●) [3H]concanavalin A; (■——■) [14C]liposomes; (————) refractive index; (x————x)proteins. Equilibrium density of free concanavalin A and negative, solid liposomes indicated by filled and open arrows respectively.

lular material (Fig. 4A). No free, fluid vesicles were found at their equilibrium density, showing their absence in the cytosol (Fig. 4A). This implies that fluid liposomes are homogenously integrated within the plasma membrane, although vesicles are most likely of various sizes, in contrast to observations made by Huang & Pagano (15). They reported that 50% of dioleylllecithin, fluid, unilamellar vesicles involved in a fusion process with Chinese hamster fibroblasts was found free in the cytosol, and only 20% of the vesicles was integrated within the plasma membrane.

The same experiments were performed on cells with [14C] negative, solid liposomes and [3H]concanavalin A. No [14C] label could be detected in the plasma membrane fraction (Fig. 5A). The [14C] label equilibrates always at the same

density as the free liposomes after being incubated with intact cells or with homogenate (d=1,092) (Fig. 5A-B). Such vesicles adsorbed on membranes are released by the high saline concentration of the CsCl gradient. It has been demonstrated that increasing NaCl concentration disminishes liposome adhesion to erythrocytes (16).

CONCLUSION

The pharmacological utilization of liposomes depends upon cell-vesicle interactions. Therefore it is of interest to determine the mechanisms involved. As shown here, positive, fluid vesicles undergo, nearly exclusively, a fusion process with T.brucei plasma membrane. This result is of importance regarding the application to parasite chemotherapy by the use of liposome-encapsulated drugs or, even more promising, the possibility to induce specific modifications of the plasma membrane which would lead to cell impairment.

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