CELL-LIPOSOME INTERACTION IN A PROTOZOAN

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Two mechanisms, endocytosis and fusion with the cell membrane, may be involved in the uptake of liposomes by cells (Tyrrell et al 1976). Tetrahymena pyriformis provides a useful cell model for the uptake of liposomes and other drug carriers since this ciliate can internalize materials by pinocytosis and phagocytosis and discrimination between these uptake mechanisms is possible by cytochalasin B (CCB) inhibition of the latter (Nilsson 1977). We have investigated the mode of uptake of liposomes by T. pyriformis using nitroblue tetrazolium (NBT) as a marker. Dried lipid films, composition: dipalmitoyl-L-α phosphatidylcholine, cholesterol, dicetylphosphate (molar ratio 7:2:1), were hydrated (47° under N_2) with NBT 2mg ml^{-1} solution or 2mg ml^{-1} NBT plus 10mM reduced nicotinamide adenine dinucleotide solution to produce NBT and diformazan liposomes respectively. Solutions throughout are in 6.67mM pH7.2 phosphate buffered 0.3% $^{w}/_{v}$ saline. Before sonication (3 min, 47° under N_2, MSE 150W Sonicator, 19mm probe, 20ml tube) 0.1ml 10mM phenazine methosulphate solution was added to the diformazan liposomes to reduce the pale-yellow water soluble NBT to dark blue insoluble diformazan. 2ml aliquots of liposome suspensions were fractionated on Sepharose 6B and 1ml of the pooled 5ml liposome fractions mixed with 1ml starved cell suspensions of T. pyriformis, GL strain, to give a final cell density of 6×10^{-4} ml^{-1}. In NBT liposome experiments the cells were pretreated with 1mM phenazine methosulphate. Cells were sampled after 30, 60, 90, 120 and 150 min exposure to the liposomes, immediately fixed in 2.5% $^{w}/_{v}$ glutaraldehyde and examined by phase contrast microscopy (160xmagnification).

Table 1 Food vacuole formation in T. pyriformis and exposure time to diformazan liposomes

Time (min)	No. of cells counted	Total no. of food vacuoles	Mean no. of food vacuoles/cell
30	280	265	1.0
60	280	485	1.7
90	140	600	4.2
120	187	935	5.0
150	91	648	7.1

In cells exposed to diformazan liposomes dark blue food vacuole formation, at a maximum after about 150 min (Table 1), was almost completely inhibited by 40μg ml^{-1} CCB in 0.8% $^{v}/_{v}$ DMSO solution (mean vacuole no. of 0.5/CCB treated cell). Food vacuole formation in cells exposed to NBT liposomes was not as obvious, and rather a pervasive cytoplasmic staining (dark blue) was observed. It would appear that phagocytosis with food vacuole formation (shown by accumulation of insoluble diformazan) represents the main uptake mechanism of these large liposomes by this eukaryotic cell; soluble liposome contents (NBT) rapidly diffusing from the vacuoles. Liposome mode of entry into cells has significance for drug delivery to specific (target) intracellular sites and may be studied using this organism.

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Tyrrell, D.A. et al (1976) Biochim. Biophys. Acta 457: 259-302