LIPOSOMES WITH A DOUBLE BILAYER – A STUDY ON STABILITY

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There are no methods described for the preparation of vesicles with a double phospholipid bilayer, except for vesosomes as multicompartmental aggregates of tethered vesicles encapsulated within a large bilayer vesicle [1]. They are tethered to the aggregate by biotin-streptavidin coupling. The yield of this process is about 5%.

The modified reverse phase evaporation method was used to prepare two new kinds of vesicle [2]. With the use of this technique, it is possible to obtain intermediate unilamellar vesicles with a double bilayer (IUV-DB) or multi-vesicular vesicles (MVV-DB), that is a few vesicles, coated with a common bilayer. The membrane of the vesicles and the coating membrane have different phospholipid compositions. In this investigation, an attempt has been made to apply fluorescence resonance energy transfer to count the stability of the coating membrane over time. The intermediate unilamellar vesicles, about 100 nm in diameter, were labelled with N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (NBD-PE) and coated with an additional membrane formed of dipalmitoylphosphatidylcholine and N-(Lissamine rhodamine B sulfonyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine triethylammonium salt (N-Rh-PE). The fluorescence label NBD from the outer surface of the coated and non-coated vesicles was reduced with sodium dithionite to its non-fluorescent form after and before coating. From the fluorescence data, the degrees of coating were calculated. The highest degree of coating was above 50%.

The significant fluorescence resonance energy transfer (FRET) from the NBD label of the vesicles to the N-Rh of the coating membrane was registered. The reduction FRET effect with time was a measure of the stability of the coating membrane with time**.** We found that the decrease in the FRET effect followed a zero-order rate of process with a correlation coefficient R^2=0.9343. The rate constant of the stability of the coating membrane was equal to $k=2.3\times10^{-7}$ [mol×min^{-1}], with respect to the quantity of DPPC coating used. The half-life of the coating membrane was equal to $t_{1/2}$=16.5 days.

REFERENCES

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