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Entrapment of plasmid DNA into niosomes: characterization studies

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Previous studies (Gregoriadis et al 1996; Gregoriadis et al 1997; Perrie & Gregoriadis 1997; Gregoriadis 1998) have shown that plasmid DNA can be quantitatively entrapped into phospholipid-based liposomes by the dehydration – rehydration procedure (Kirby & Gregoriadis 1984). The procedure, employing mild conditions to produce dehydration-rehydration vesicles (DRV) of submicron and micron size, has been now applied to entrap plasmid DNA into non-anionic surfactant-based vesicles (niosomes). These were supplemented with 3β-(N,N-dimethylaminoethane)carbamoyl cholesterol (DC-Chol; generously donated by Dr C. Kirby) and, in some experiments, with dioleyl phosphatidylcholine (DOPE). Both agents are known (Gregoriadis et al 1997; Gregoriadis 1998) to contribute considerably to the induction by plasmid DNA-containing liposomes of humoural and cell-mediated responses to the encoded antigen.

pRc/CMV HBS (10 or 50 μg and ^{35}S-labelled tracer of the same DNA) encoding the S region of HBsAg (subtype ayw) was incorporated into niosomes composed of 16μmoles mono-palmytal-rac-glycerol (Monopal) or sorbitan monostearate (Span 60) and DOPE, cholesterol and DC-Chol (molar ratios 16:8:4:4). In some experiments DOPE was omitted from the formulations (molar ratios 16:4:4) and in others, DNA was complexed with preformed empty niosomes. Entrapment (56-65%) and complexing (44-60% of the DNA used) values were independent of the original amount (10 or 50 μg) of DNA and the composition of niosomes the size of which, as measured by photocorrelation spectroscopy, was 9-20μm in diameter. Agarose gel electrophoresis of niosome-incorporated DNA revealed no change in its structural integrity which was also retained after brief (2-5 seconds) probe sonication to obtain smaller (1-7μm) vesicles. Moreover, most of the niosome-entrapped (72-90%) or complexed (31-85%) DNA was not degraded on exposure to deoxyribonuclease. These findings, also observed with phospholipid liposomes (Gregoriadis et al 1996), can be attributed to the inaccessibility of entrapped DNA to the enzyme and, in the case of DNA complexed with the vesicle surface, to its condensed state assumed (Gregoriadis 1998) on interaction with cationic charges.

A net cationic surface charge of liposomes (and presumably niosomes) following DNA entrapment or complexing, is considered (Gregoriadis 1998) instrumental to their ability to mediate DNA transfection. Therefore, the zeta potential (zP) of all preparations was measured by micro-electrophoresis. Results showed that (a) the zP of DNA-free (empty) niosomes was, as expected, reduced by the presence of the anionic DNA whether entrapped or complexed; (b) Monopal liposomes had a much greater (e.g. +25.6 mV) zP than those incorporating Span 60 (e.g. +1.6 mV), regardless of the presence or absence of DNA; (c) the zP of both Monopal and Span 60 niosomes was significantly reduced by the presence of DOPE. The way(s) by which Span 60 and DOPE reduce the overall cationic surface charge of the vesicles is unknown at present. Animal experiments are in progress to establish whether niosomes can act similarly to liposomes in promoting immune responses to the antigen encoded by their DNA content.

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