BINDING AND ENTRAPMENT OF HIGH MOLECULAR WEIGHT DNA BY LECITHIN LIPOSOMES

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1. Introduction

Closed lipid vesicles (liposomes) have been widely used in recent years for introducing biologically active substances into cells. The spectrum of substances which have been thus entrapped ranges from small ions and molecules to large macromolecules including polyinosinic–polycytidylic acid [1] and 4 S–23 S RNA [2,3]. However up to now no attempts to incorporate DNA into liposomes have been reported in the literature. Such DNA-containing liposomes could be potentially useful as carriers for introducing foreign DNA into cells and could also serve as models for the study of membrane interactions.

We demonstrate here that high molecular weight DNA of more than 10 000 base-pairs can be associated with egg lecithin and entrapped in liposomes. Such DNA becomes DNase-resistant. Incorporation of DNA into the liposomes seems to be the result of both electrostatic binding and mechanical entrapment of macromolecules.

2. Materials and methods

DNA was extracted from Ehrlich ascites cells, prelabeled with [^{3}H]thymidine (spec. act. 8900 dpm/μg) and from SV40 virus, grown in cells labeled with the same nucleotide (spec. act. 9000 dpm/μg). DNA from λ phage was prepared as described in [8] and restricted by *Eco*RI endonuclease as in [9]. Commercially available calf thymus DNA was also used in some experiments.

Liposomes were made of chromatographically pure egg lecithin [4]. Lecithin in benzene was evaporated on a rotatory evaporator, mixed with DNA in different proportions in buffer and extensively shaken for 90 min.

Routinely liposomes were prepared in buffered solutions of Tris–HCl (0.02 M; pH 7.5) with 1 mM $MgCl_2$. After formation liposomes were sedimented for 10 min in a Janetzky cytocentrifuge (16 000 rev./min), washed with Tris buffer, resuspended and sedimented once more. Liposomal suspension, 100 μl, was applied to a filter (Whatman GF/C), dried and the radioactivity counted in a scintillation counter.

In some experiments liposomes were treated with DNase. Liposomes were washed 3 times by the sedimentation–resuspension procedure and finally resuspended in Tris–HCl buffer with 1 mM $CaCl_2$, containing 3 μg/ml micrococcal nuclease. After incubation for different periods at room temperature, aliquots were applied to GF/C-filters prewetted with 5 μl 5 mM EDTA to stop the reaction. Filters were washed with trichloroacetic acid and ethanol and the radioactivity measured.

For DNA extraction liposomes were resuspended in 0.4 M NaCl. The lipids were extracted with chloroform–methanol (2:1, v/v). The size of DNA was

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measured by gel electrophoresis in 1% agarose using DNAs of defined molecular weight as markers as described in [5]. DNA was detected with ethidium bromide.

3. Results and discussion

After shaking mouse ascites DNA with lecithin (1:2000 w/w) in Tris–HCl + 1 mM $MgCl_2$ practically all the DNA co-sedimented with the liposomes. Even after repeated washings 95% of the DNA remained associated with the lipid vesicles. These results contrast with the data of similar experiments in which inulin and glucose were incorporated into liposomes [6,7]. In those experiments less than 1% of the carbohydrates were associated with the liposomes and these molecules were incorporated only into the inner space of the liposomes. Hence the high association of DNA with lecithin could be the result of lipid–DNA interaction.

DNA association with liposomes is saturable. When the lecithin/DNA (w/w) ratio is less than 500, the DNA bound to lecithin diminishes (fig.1). Increase of the ionic strength of the media inhibits the formation of the DNA–lecithin complex. When 0.14 M NaCl was added to the Tris–HCl + $MgCl_2$ buffer, only 8% of

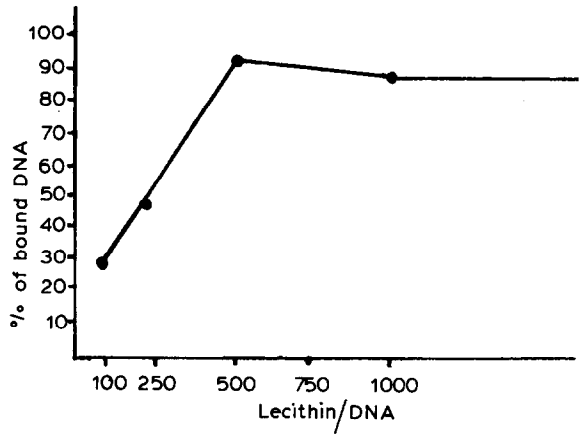

Fig.1. Association of DNA with liposomes at different lecithin–DNA ratios. DNA-containing liposomes were made with mouse Ehrlich ascites DNA and lecithin in 0.02 M Tris–HCl buffer + 1 mM $MgCl_2$ as described in section 2. The liposomes were washed 3 times and the amount of DNA in them calculated as % bound to input DNA.

the DNA became associated with the liposomes, 92% remained in the supernatant after sedimentation of the liposomes. Thus the DNA–lecithin interaction seems to depend on electrostatic forces.

When washed DNA-containing liposomes were transferred to media of high ionic strength, ~50% of the DNA dissociated from the DNA–liposome complex. Thus association of 50% of the DNA in such a complex does not depend on electrostatic forces. We supposed that this fraction of DNA is entrapped in the inner space of the liposomes and hence should be resistant to DNase treatment. We tested this hypothesis.

DNase treatment of the DNA-containing liposomes resulted in degradation of 60% of the linear ascites DNA and 70% of the circular SV40 DNA. In control experiments DNase completely degraded free DNA in the presence of 'empty' liposomes. Thus only DNA which had been present during liposome formation becomes DNase resistant. We then tested the DNase resistance of the DNA which remains associated with the liposomes after their transfer to a solution of high ionic strength. Only 20% of this DNA fraction was degradable by the enzyme. In control experiments DNase completely degraded free DNA under the same conditions. It follows from these experiments that DNA associates with liposomes in low ionic strength media and that part of this DNA remains liposome-associated in high ionic strength solution. This fraction of DNA becomes DNase resistant giving proof of DNA entrapment into the closed lipid vesicles.

Further support for this suggestion comes from experiments on osmotic lysis of liposomes. DNA-containing liposomes were prepared in 0.13 M sucrose. As this medium was too viscous for liposome sedimentation, the liposome suspension was diluted with Tris-buffer containing 0.13 M NaCl. Non-bound DNA was washed off by the sedimentation–resuspension procedure and the DNA-containing liposomes were resuspended in distilled water. This resulted in release of 32% of the DNA into the medium. We think that after transfer of the DNA-containing liposomes into hypotonic solution (water) DNA is released into the medium due to liposome lysis. Resealing of the liposomes prevents complete release of DNA.

We attempted to measure the size of entrapped DNA. Preliminary experiments have shown that the

molecular weight of calf thymus DNA did not change when associated with and extracted from lecithin vesicles as proved by electrophoresis in 1% agarose gels. We measured the size of the fraction of mouse ascites DNA associated with the liposomes and resistant to DNase treatment. The result of electrophoresis of this DNA, shown in fig.2a, demonstrated that liposomes contained a DNase-resistant fraction, corresponding to the molecular weight of untreated DNA (*E. coli* plasmid pMB9 was used as a marker).

We also measured the size of DNA which remained associated with the liposomes after their transfer to high ionic strength media. As shown in fig.2b this

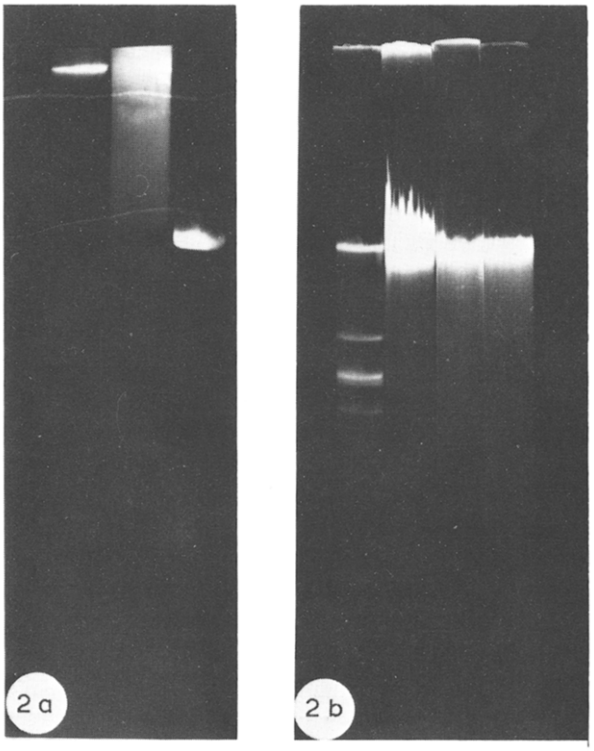

Fig.2. Molecular weight of mouse Ehrlich ascites and λ phage DNA extracted from liposomes as determined by electrophoresis on 1% agarose gels.

2a. Extracted Ehrlich ascites DNA after treatment of DNA-containing liposomes with micrococcal nuclease: Left gel, control DNA; center gel, extracted DNA; right gel, plasmid pMB9 DNA, M_r 3.5 × 10^6.

2b. Extracted DNA after treatment of DNA-liposomes with 0.13 M NaCl in 0.02 M Tris–HCl. From left to right: phage λ DNA restricted with *Eco*RI; mouse ascites DNA; ^{3}H-labeled mouse ascites DNA; Ehrlich ascites DNA extracted from [^{3}H]DNA-containing liposomes after transfer to high ionic strength medium.

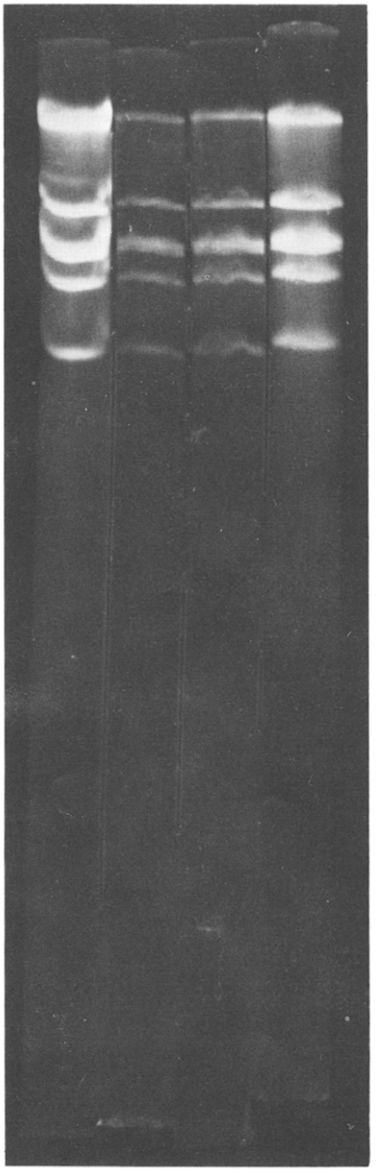

Fig.2c

2c. Extracted *Eco*RI-restricted λ phage DNA after treatment of DNA-liposomes with micrococcal nuclease. From left to right: gel 1, control DNA; gels 2–4, extracted DNA in order of increasing concentration.

DNA migrated as a sharp band of molecular weight $10–20 \times 10^6$. Similar experiments were conducted with λ phage DNA that had been restricted by *Eco*RI endonuclease into discrete fragments. Liposomes were made with the restricted fragments and treated with DNase in a high ionic strength solution, as described above. Electrophoresis in 1% agarose gel showed that each fragment was protected by the liposomes (fig.2c). Thus the DNA entrapped in liposomes is of high molecular weight. Assuming liposomes to be of 10^9 daltons one can conclude that every liposome is associated with 1–10 molecules of DNA.

Summarizing, the present work shows that DNA associates with lecithin vesicles due to both electrostatic forces (probably interaction between positively charged choline residues and negatively charged phosphates of the DNA) and mechanical entrapment into the inner space of the vesicles. The entrapment makes DNA resistant to dissociation from the lipid–DNA complex in high ionic strength media. This DNA also becomes resistant to DNase treatment. DNA comes out of the liposomes after lysis in hypo-osmotic media. Association of DNA with liposomes is saturable. The saturation may be caused by the limited accessibility of charged groups on the surface of liposomes as well as by the size of the inner space of the liposomes. The size of the DNA entrapped in liposomes is large enough to include several genes. Possibly such liposomes could be used as vehicles for the introduction of new genes into cells.

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