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Effect of Salts on the Entrapment of Calf Thymus DNA into Liposomes

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Abstract □ To correlate the conformational changes of DNA (Calf Thymus) with entrapment of DNA into lipsomes, the effect of ions(Na^+, Mg^{++}) on the entrapment of calf thymus DNA into liposomes was investigated. The effect of divalent ion(Mg^{++})on the structural changes of DNA indicated by decrease of observed ellipticity at 274 nm and nonspecific binding of DNA to lipid bilayers was greater than monovalent ion(Na^+). But the efficiency of DNA encapsulated was not altered. These results show that entrapment of DNA into liposomes is not due to nonspecific binding and structural changes because of electrostatic forces but to mechanical capture of DNA by the internal aqueous space of liposomes although divalent ion contributes large structural changes and more nonspecific association of DNA with liposomes due to strong charges.

Keywords □ Liposomes-large unilamellar vesicles, phosphatidylcholine, lipid bilayer, conformational changes of calf thymus DNA, circular dichroism, entrapment of DNA into liposomes.

Liposomes have been used in recent years to entrap a wide spectrum of molecules, ranging from small molecules to macromolecules including several enzymes[1-3], polyinosinic: polycytidylic acid[4], viruses[5,6], high molecular-weight RNA[7-9], methaphase chromosomes[10] and high molecular-weight DNA[11-21]. Liposomes containing DNA could be useful as carriers for genetic exchanges in recombinent DNA technology[14,15,17-21] and could also be resistant to external degradative enzymes which are present in the medium[12,13,17,18].

In the nature of DNA, DNA structure may be dynamically folded and extended by salts due to its conformational versatility[22-31] For the entrapment of DNA in liposomes, effect of salts on the structural changes of DNA must be considered.

We here attempted to correlate structural changes of DNA with electrostatic binding of DNA to lipid bilayers and efficiency of DNA encapsulated varying ionic conditions.

EXPERIMENTAL METHODS

Materials

Egg yolk lecithin (phosphatidylcholine) was purchased from Sigma Co. LTD. and purified by alumina column chromatography. The purity was checked by thin layer chromatography. Calf thymus DNA, initially containing 6.8% Na^+ and 5.3% H_2O when assayed, was purchased from Sigama Co. Ltd. and used without further purification. All other chemicals were of reagent grade. Solutions were prepared in double distilled water.

Preparation of liposomes

Large unilamellar vesicles composed of lecithin only were prepared by the modification of method of Szoka and Papahadjopoulos[32]. The schematic diagram of preparation was shown in Fig. 1.

Determination of structural changes of DNA in solutions

Conformational changes of DNA varying salt concentrations at fixed DNA concentration (88μg/m*l*) have been monitored by circular dichroism spectra obtained with a JASCO Model J-20C spectropolarimeter.

Determination of efficiency of DNA encapsulated

After formation of liposomes in each solutions, the liposomes were sedimented by ultracentrifugation at 100,000 *g* at 5 °C for 20 min in Beckman 75 Ti rotor and washed 3 times with TEN solution (composed of 0.02 M Tris-HCl, 0.1 mM EDTA and

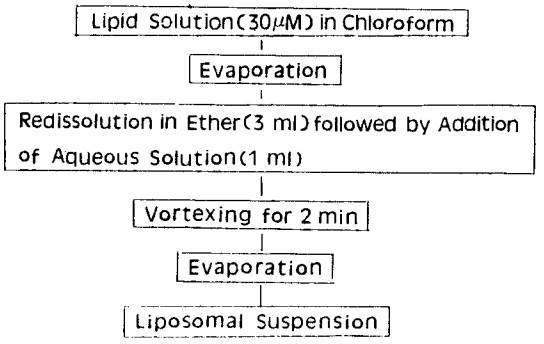

Fig. 1. Schematic diagram of preparation of liposomes by reverse phase evaporation method.

0.15 M NaCl). The resulting pellet or supernatant, added 27 v/v% propyl alcohol, was analyzed by LKB UV spectrophotometer.

To determine any nonspecific association of DNA with liposomes, mixture of empty liposomes with DNA was analyzed as same procedure. Calculation of the amount of DNA encapsulated and associated was input (total) DNA minus free (supernatant) DNA separated from liposome by ultracentrifugation.

RESULTS AND DISCUSSION

Determination of minimal concentration of propyl alcohol on liposomal solubility and DNA structure

To complete solubilization of liposomes containing DNA without structural changes of DNA, we chose the propyl alcohol as solvent to measure

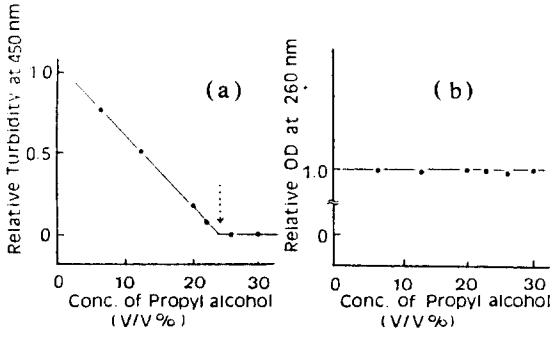

Fig. 2. Effect of propyl alcohol on liposomes and DNA. Relative trubidity of liposome at 450 nm and relative optical density of DNA at 260 nm were measured as a function of propyl alcohol concetration. A dotted arrow indicates minimal concentration of propyl alcohol to solubilize liposomes completely. The concentration of lipid and the amount of DNA were 30 μM and 88 μg/ ml, respectively.

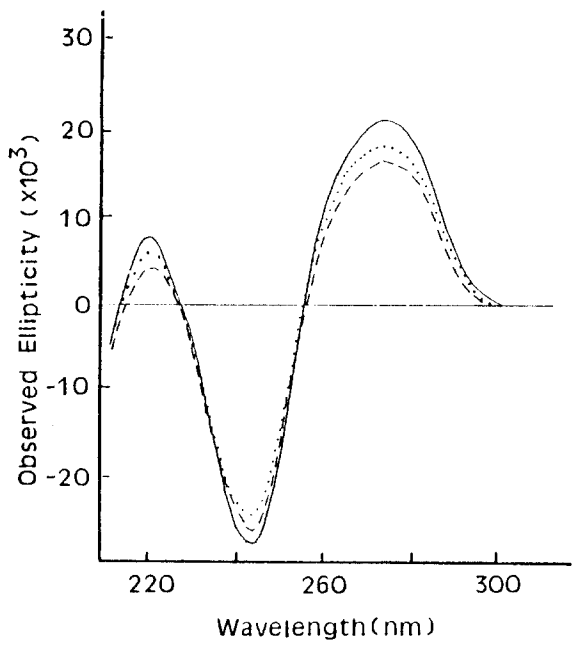

Fig. 3. Circular dichroism spectra of DNA at pH 7.4 (25°) in aqueous solutions of varying concentration of $MgCl_2$.
— 0.0002 E, ... 0.02 E, ---- 2 E

efficiency of DNA encapsulated by UV spectrophotometry. Fig. 2a shows that relative turbidity of liposomes at 450 nm was linearly decreased and completely solubilized liposomes at about 25 v/v% (indicated by dotted arrow). Also, relative optical density of DNA, reflecting denatured properties of DNA, was not changed (Fig. 2b) and circular dichroism spectra of DNA with or without propyl alcohol (within 30 v/v%) did not reveal any structural denaturation of DNA (this spectra were not shown).

These results indicate that DNA structure is not affected within this established concentration of propyl alcohol. In addition, propyl alcohol can be used as solvent in this experiment.

Determination of structural changes of DNA in salt solution

The structure of DNA may be folded and extended dynamically due to its conformational flexibilities[22-25]. Therefore, we first investigated DNA conformation by circular dichroism to assure whether these conformational changes could affect the efficiency of DNA encapsulated.

Fig. 3 and 4 show that positively observed ellipticity is decreased gradually as concentration of salt is increased. Table I indicates that effect of divalent

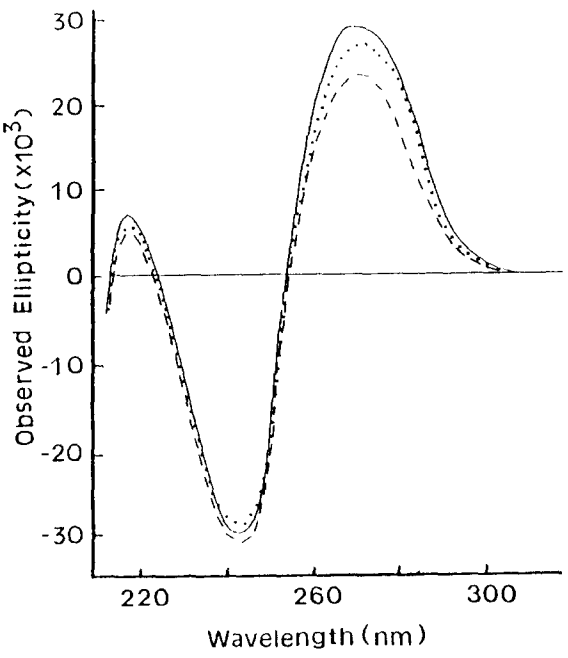

Fig. 4. Circular dichroism spectra of DNA at pH 7.4 (25°) in aqueous solutions of varying concentration of NaCl.
— 0.0002 E, ... 0.15 E, ---- 2 E,

salt(Mg^{++}) on DNA conformation, although in lower concentrations, was greater than that of monovalent salt(Na^{+}).

These dynamic variations are presumed to reflect structural changes of DNA probably resulted from some types of interactions between counter ion and sugar-phosphate backbone[26] and solvation of DNA in the vicinity of cationic binding site[27]. The phosphate groups of DNA have a rather low pK and are fully ionized at any pH above 4. These groups are located on the outer periphery of the double helix and are more effectively screened by divalent cations such as Mg^{++} and Ca^{++} as well as the polycationic amines than by Na^{+} in the same geometrical position. The binding of cations in the groove of double helix both stabilizes DNA molecules and makes it more flexible.

Table I. Comparison of observed ellipticity ($\times 10^3$) of DNA at 274 nm as a function of electrolyte concentration

	Conc. (E)	Observed Ellipticity
$MgCl_2$	0.0002	22.2
	0.02	18.8
	2.0	17.3
NaCl	0.0002	29.4
	0.15	28.0
	2.0	23.4

Table II. Comparison of percentage of DNA associated with liposomal bilayers at salt solutions

	Conc. (E)	Suspending Solution	
		Isotonic	TEN
$MgCl_2$	0.0002	19.5 ± 2.4	ND
	0.002	81.7 ± 3.5	ND
	0.02	92.4 ± 8.9	ND
NaCl	0.0002	ND	
	0.15	ND	

The various salt solutions containing DNA were added to empty liposomes prepared by method shown in Fig. 1 and washed 3 times with isotonic or TEN solution. The amount of DNA nonspecifically bound was determined. Results are expressed as mean ± SD. ND; Not Detectable

Determination of efficiency of DNA encapsulated

Nonspecific binding of DNA to liposomal bilayers was investigated because this fraction was not regarded as efficiency of DNA encapsulated.

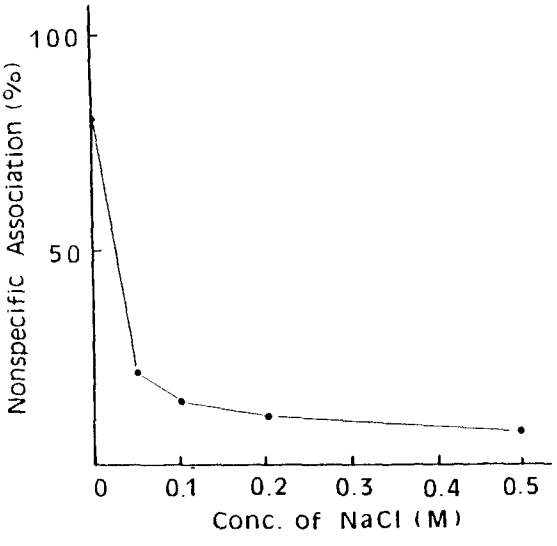

Fig. 5. Effect of NaCl on the nonspecific association of DNA with liposomal bilayers.
NaCl solutions were added to empty liposomes highly associated with DNA in 2 mE $MgCl_2$ solution. The liposomes were washed 3 times and the amount of DNA nonspecifically bound was determined.

Table III. Effect of salts on the efficiency of DNA encapsulated

	Conc. (E)	Percentage
$MgCl_2$	0.0002	8.6 ± 2.6
	0.02	5.8 ± 1.2
NaCl	0.0002	7.7 ± 2.4
	0.15	6.6 ± 2.1

The liposomes were washed 3 times with TEN solution and the efficiency of DNA encapsulated was compared. The concentration of lipid and the amount of DNA were invariably 30μM and 176μg, respectively. Results are expressed as mean ± SD

More association of DNA with liposomes in $MgCl_2$ solutions was observed although not detected in NaCl solution. However, when this highly associated fraction was transfered to TEN medium (composed of Tris-HCl, EDTA and NaCl), it was not detected any nonspecific association of DNA to liposomal bilayers (Table II). Fig.5 shows that nonspecifically associated fractions of DNA with liposomes in $MgCl_2$ solutions can be largerly hindered by addition of NaCl (*i.e.*, increased ionic strength) although 9% of DNA still become associated with liposomal bilayers nonspecifically.

It is assumed that these association may be derived from electrostatic binding among ionized phosphate groups of DNA, divalent ions and liposomal choline residues in lecithin. That is, three charged species are electrostatically combined together but these coordinations can be competitively hindered by ionic strength and removed by chelation of divalent ions. Nonspecifically associated fractions of DNA are not entrapped in the inner space of liposomes and hence should be degradable to DNase treatment.

After removal of nonspecific binding, efficiency of DNA encapsulated was compared. Table III shows that efficiency of DNA encapsulated as a function of salts is not altered although divalent ion contributes large structural changes and more nonspecific association of DNA with liposomal bilayers due to their strong charges.

These results indicate that entrapment of DNA in liposomes is not due to nonspecific binding and structural changes because of electrostatic forces but to mechanical sequestration into the aqueous space of liposomes. Finally, proper selection of medium compositions containing salts to enhance affinity for cell surface during cell-cell interaction and fusion, and stability of DNA structure[33] must be considered.

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