Plant Physiol. (1983) 72, 386–390
0032-0889/83/0386/05/$00.50/0

Targeting of Large Liposomes with Lectins Increases Their Binding to Plant Protoplasts[1]

Received for publication November 29, 1982 and in revised form February 11, 1983

RAYMOND E. SHEEHY AND PAUL F. LURQUIN
Program in Genetics and Cell Biology, Washington State University, Pullman, Washington

ABSTRACT

Soybean agglutinin, peanut agglutinin, and concanavalin A were covalently bound by condensation reaction to gangliosides and ceramides incorporated within the bilayer of multilamellar and unilamellar liposomes. These modified liposomes had a much higher affinity for carrot and tobacco protoplasts except when concanavalin A was used.

In addition, soybean agglutinin and concanavalin A were attached by ligand-specific binding to liposomes containing cholesterol molecules derivatized with each lectin-specific sugar. This procedure allowed efficient crosslinking of liposomes to protoplasts. The same effect was achieved with soybean agglutinin and peanut agglutinin when derivatized cholesterol was replaced by gangliosides. The implications of these findings for the liposome-mediated nucleic acid transfer into protoplasts are discussed.

Liposomes, artificial lipid vesicles, can be produced from a variety of phospholipids with different electric charges in conjunction with other molecules such as glycolipids (natural or artificial) and ceramides. Other molecules such as proteins in general and lectins in particular can then be associated with the lipid bilayer, giving an enormous range of liposome types (for review, see Ref. 18).

It has been shown recently that both RNA and DNA can be encapsulated into large liposomes without damage and subsequently transferred to the intracellular compartments of higher plant protoplasts (3–5, 9–12, 14–16, 20, 21, 25, 26). Moreover, it has been demonstrated that tobacco mosaic virus RNA (4, 5, 16, 26), turnip rosette virus RNA (21), and cowpea chlorotic mottle virus RNA (A. A. Christen and P. F. Lurquin, unpublished) encapsulated in negatively charged large unilamellar liposomes could very efficiently infect susceptible plant protoplasts. Similarly, turnip rosette virus sequestered in positively charged large multilamellar vesicles was also able to infect turnip protoplasts (21).

The fate of DNA after liposome-mediated transfer to protoplasts has been investigated largely by biochemical and microscopic techniques (9–12, 14, 15, 20, 25). However, there are preliminary indications that the *Agrobacterium tumefaciens* Ti plasmid can be transferred from liposomes into tobacco protoplasts, thereby effecting crown gall transformation of some of the treated cells (3).

Since Ti plasmid and some plant viruses are difficult to produce in large amounts, it is necessary to maximize protoplast-liposome interactions to optimize nucleic acid delivery and possible expression of the donor genomes. Parameters influencing liposome binding and nucleic acid delivery such as pH, composition of the osmoticum, Ca^{2+} ions, and polyethylene glycol have been studied (4, 9, 12, 14–16, 20).

Another possible way of enhancing liposomes binding to plant protoplasts is to target the lipid vesicles with lectin molecules. Indeed, lectins are able to bind specific carbohydrates or groups of carbohydrates and were shown to interact with liposomes containing natural or artificial glycolipids (1, 17, 22, 24, 27). Furthermore, lectins have also been shown to agglutinate or bind to plant protoplasts (2, 8).

These two sets of results indicate that liposome-protoplast interactions may be significantly enhanced through the use of lectins. In this study, three lectins, soybean agglutinin, peanut agglutinin, and concanavalin A, have been used in targeting experiments including covalent binding of lectin to the outside of liposomes as well as crosslinking of liposomes containing lectin receptors to protoplasts.

Two types of liposomes have been used in this study: (a) negatively charged unilamellar vesicles composed of phosphatidylserine and cholesterol and produced by the reverse evaporation technique (23). These liposomes were shown to allow efficient expression of viral RNA in protoplasts (4, 16, 21, 26). (b) We have also used positively charged multilamellar liposomes composed of phosphatidylcholine and stearylamine. Even though positively charged liposomes did not mediate infection of protoplasts with purified viral RNA (4; Christen and Lurquin, unpublished), they were able to produce infection of turnip protoplasts with intact turnip rosette virus (21).

The extent of liposome binding to protoplasts was followed by measuring the amount of [^{32}P]DNA initially sequestered in the vesicles which became tightly associated with protoplasts.

MATERIALS AND METHODS

Protoplasts. Tobacco (*Nicotiana xanthi*) cells were grown in suspension culture as described before (13, 14). Protoplasts were produced by incubating cells in high salt medium (2.5% KCl, 0.15% $CaCl_2$, 0.1% $MgCl_2$) containing 1% Cellulysin (Calbiochem) and 0.5% Macerase (Calbiochem) for 4 h at 29°C.

In some experiments, carrot (*Daucus carota* L.) cells grown in suspension culture (20) were used to produce protoplasts as described before (12, 20) except that high salt medium was used instead of mannitol.

All protoplasts were washed three times with high salt medium prior to use.

Liposomes. All lipids were obtained from Sigma, except for ManChol,[2] and NagChol which were kindly supplied by Dr. M.

[1] Supported by United States Department of Agriculture/Science and Education Administration Competitive Research Grant 59-2531-0-1-465-0 and by funds provided to Washington State University through the National Institutes of Health Biomedical Research Support Grant.

[2] Abbreviations: ManChol, 6-(5-cholesten-3β-yloxyl)hexyl-1-thio-α-D-mannopyranoside; NagChol, 6-(5-cholesten-3-yloxyl)hexyl-2-acetamido-2-deoxy-1-thio-α-D-galactopyranoside; SBA, soybean agglutinin; PNA, peanut agglutinin; Con A, concanavalin A; PC, L-α-phosphatidylcholine; PS, L-α-phosphatidylserine; SA, stearylamine; CH, cholesterol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

M. Ponpipom (Merck, Sharp and Dohme). These sugar-cholesterol derivatives bind to concanavalin A and soybean agglutinin, respectively (19, 27).

Positively charged multilamellar liposomes were generated by mechanical shaking as extensively described before (9, 12). They were composed of L-α-phosphatidylcholine and stearylamine in a 10:1 (w/w) ratio.

Negatively charged unilamellar liposomes were produced by a modified version of the reverse evaporation technique (14, 23). The modification consisted in emulsifying the aqueous and organic phases with a probe sonicator for 10 s as described before (14). These vesicles were composed of L-α-phosphatidylserine and cholesterol in a 2:1 (w/w) ratio.

Binding of lectins to liposomes was achieved after incorporation of 10% by weight gangliosides (type III from bovine brain, Sigma), ceramides (type III from bovine brain, Sigma), or sugar-cholesterol derivatives within the liposome bilayer.

Liposomes were labeled by sequestration of adenovirus 5 [^{32}P] DNA nick-translated to a specific radioactivity of about 10^8 cpm/μg with [^{32}P]dATP (3000 Ci/mmol, New England Nuclear) using a Bethesda Research Laboratories kit. Nick-translated DNA was separated from nonincorporated deoxynucleotides by molecular sieving on Sepharose 4B (Pharmacia) (14).

Covalent Binding of Lectins to Liposomes. Liposomes containing 10% by weight ceramides or gangliosides were coupled to concanavalin A, soybean agglutinin, or peanut agglutinin (all three from Vector Laboratories) by the method of Heath *et al.* (6). The hydroxyl groups supplied by ceramides and gangliosides incorporated within the liposome bilayer were first oxidized by 8 mM Na periodate in 20 mM Tris-HCl, 1 mM $MgCl_2$, pH 8.4, for 2 h at room temperature. Oxidized liposomes were then washed with the above buffer devoid of periodate and subsequently incubated with lectin in the presence of 10 mM Na cyanobrohydride in 20 mM Tris-HCl, 1 mM $MgCl_2$, pH 8.4, for 18 h at room temperature. This condensation reaction resulted in linkage between aldehyde groups on the liposome surface and amino groups in the lectin. Liposomes were then harvested and washed by centrifugation (12).

Typically, 600 μg of lectin were reacted with 1 to 2 mg lipids in liposome form resulting in the attachment of about 300 μg lectin/mg lipids.

Ligand-Specific Binding of Lectins to Liposomes. Gangliosides are glycolipids containing sugar groups recognizable by a variety of lectins. In addition, ManChol and NagChol carry sugar residues specifically recognized by concanavalin A and soybean agglutinin, respectively. Therefore, liposomes formed in the presence of 10% by weight of each of those glycolipids (about 1 mg total lipid) were simply incubated for one h at room temperature in the presence of 600 μg of their cognate lectin in 20 mM Tris-HCl, 1 mM $MgCl_2$, pH 8.4, and then harvested and washed by centrifugation. The amount of lectin bound to liposomes was about 400 μg/mg lipids.

Incubation of Protoplasts with Liposomes. Protoplasts (about 10^6/ml in the case of tobacco and 5×10^6/ml in the case of carrot) were incubated with liposomes at a nontoxic concentration (0.1 mM lipid) for 1 h at 29°C. All incubations were performed in high salt medium. The affinity of targeted liposomes for protoplasts was measured by adding a large excess (5 mg/ml) of lectin-specific sugar, *i.e.* *N*-acetylgalactosamine in the case of soybean agglutinin and β-D-galactosyl in the case of peanut agglutinin.

After incubation, protoplasts were washed three times with high salt medium, resuspended in 1 ml of water, and lysed with 2% final SDS, and radioactivity was determined by liquid scintillation counting.

Results are expressed as percentage of input radioactivity bound to protoplasts. All data points represent the average of triplicate samples. The effect of lectins on protoplast viability was determined by incubating 10^6 tobacco protoplasts in 1 ml high salt medium in the presence of 800 μg Con A or SBA for 1 h. Protoplast viability was estimated by fluorescein diacetate staining (7). It was found that those extremely high lectin concentrations reduced viability by about 25%. Lectin concentrations in liposome targeting experiments never exceeded 60 μg/ml.

Isolation of Nuclei. Tobacco protoplasts (50×10^6 in 10 ml high salt medium) were incubated for 1 h at 29°C in the presence of 0.07 mM liposomes loaded with [^{32}P]DNA. Protoplasts were then washed three times as above, resuspended at a concentration of 10^6 protoplasts/ml in culture medium containing 0.45 M mannitol and incubated for 18 h at 29°C. After incubation, protoplasts were washed three times and regenerating cell wall was removed by incubating protoplasts for 1.5 h at 29°C with 0.5% Macerozyme, 0.5% Cellulase R-10 (both from Yakult Honsha), 0.5% Macerase, 0.5% Cellulysin (from Calbiochem) and 0.05% Pectolyase Y 23 (Seishin Pharmaceuticals). After washing, protoplasts were lysed for 15 min on ice with 5% (v/v) Triton X-100 (Sigma) dissolved in 0.15 M sucrose, 5 mM $MgCl_2$, 1 mM DTT, 1 mM EGTA, 0.1 mM Spermine HCl, and 5 mM morpholinoethanesulfonate buffer, pH 6.1. Salmon sperm DNA (Sigma) was present at a concentration of 100 μg/ml to minimize adsorption of donor DNA to the nuclear membrane (9). Nuclei were then washed three times in the above solution devoid of Triton X-100 and used for radioactivity determination by liquid scintillation counting.

Statistical Analysis of Liposome Binding Results. Upper and lower confidence limits were determined by $S\bar{y} \times 100$/input (per cent SE). These limits are indicated in the figure legends for each experiment.

RESULTS

Lectins Covalently Bound to Liposomes. Figure 1A describes the binding of PC/SA positively charged multilamellar liposomes to tobacco protoplasts. It can be seen that the incorporation of SBA into liposomes (achieved simply by generating liposomes in the presence of the lectin) significantly increased their binding to protoplasts as we reported earlier (14). However, if liposomes were preformed in the presence of gangliosides or ceramides and subsequently condensed with SBA, it was observed that binding was further enhanced (more than doubled) as compared with PC/SA liposomes. Similar results were obtained with SBA covalently bound to liposomes containing either gangliosides or ceramides. Covalently binding Con A to liposomes containing gangliosides did not result in increased interactions with protoplasts.

Similar experiments performed with PS/CH unilamellar reverse evaporation vesicles are described in Figure 1B. Here also, incorporation of gangliosides within the liposome bilayer and condensation with SBA or PNA resulted in a very significant increase in binding to tobacco protoplasts. Interestingly, it was observed that the presence of a large excess of competing sugar in the medium resulted only in a small decrease in binding to protoplasts. This indicates that liposomes coated with lectins have a much higher affinity for receptor sites located on the protoplast membrane than for their specific sugars.

Figure 2 describes the experiment in which control PC/SA positively charged multilamellar liposomes as well as PC/SA liposomes containing gangliosides or ceramides covalently bound to SBA were incubated with tobacco and carrot protoplasts. It can be seen that modified liposomes bound much better to both tobacco and carrot protoplasts than control liposomes. In both cases, the modification brought to the liposome surface more than doubled the amount of liposome binding.

Ligand-Specific Binding of Lectins to the Liposome Surface. Another way of targeting liposomes with lectins is to include lectin receptors in the bilayer. One may then envision that lectin molecules will act as crosslinkers between liposomes and protoplasts.

Figure 3A shows that the incorporation of NagChol or Man-

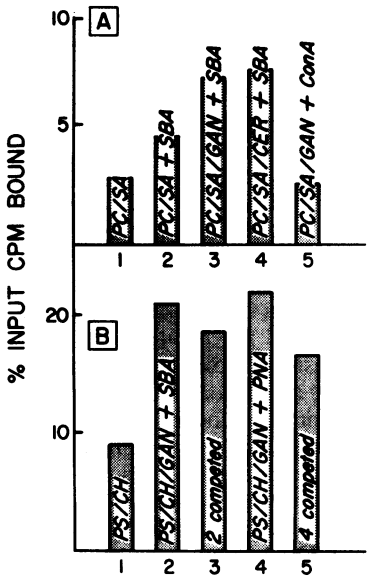

FIG. 1. A, Effect of lectins on the binding of multilamellar PC/SA positively charged liposomes to tobacco protoplasts. 1, PC/SA liposomes without lectin; 2, PC/SA formed in the presence of SBA (lectin added to the dry lipid film); 3, PC/SA liposomes formed in the presence of gangliosides (GAN) and then covalently condensed with SBA; 4, PC/SA liposomes formed in the presence of ceramides (CER) and then covalently condensed with SBA; 5, PC/SA liposomes formed in the presence of gangliosides and then covalently condensed with Con A. The per cent SE values are (lanes 1–5): ±0.3, 0.2, 1.1, 0.5, and 0.1. B, Effect of lectins on the binding of unilamellar negatively charged PS/CH reverse evaporation vesicles to tobacco protoplasts. 1, PS/CH liposomes without lectin; 2, PS/CH liposomes formed in the presence of gangliosides and then covalently condensed with SBA; 3, same as 2 except that liposome binding was performed in the presence of 5 mg/ml *N*-acetylgalactosamine; 4, PS/CH liposomes formed in the presence of gangliosides and then covalently condensed with PNA; 5, same as 4 except that liposome binding was performed in the presence of 5 mg/ml β-D-galactosyl. The per cent SE values are (lanes 1–5): ±0.7, 1.0, 0.7, 0.8, and 1.3.

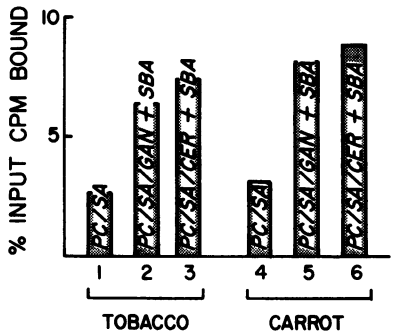

FIG. 2. Effect of lectins covalently bound to liposomes on their attachment to tobacco and carrot protoplasts. 1 and 4, binding of control PC/SA multilammelar liposomes; 2 and 5, binding of PC/SA liposomes containing gangliosides (GAN) and condensed with SBA; 3 and 6, binding of PC/SA liposomes containing ceramides (CER) and condensed with SBA. The per cent SE values are (lanes 1–6): ±0.2, 0.4, 0.1, 0.1, 1.1, and 0.5.

Chol into the liposome bilayer also significantly enhanced liposome binding to protoplasts in the presence of their specific lectin, namely SBA and Con A, respectively.

The presence of NagChol and ManChol did not increase binding relative to control; neither did Con A when liposomes contained cholesterol instead of ManChol. These observations indicate that a lectin receptor must be present on the liposome surface

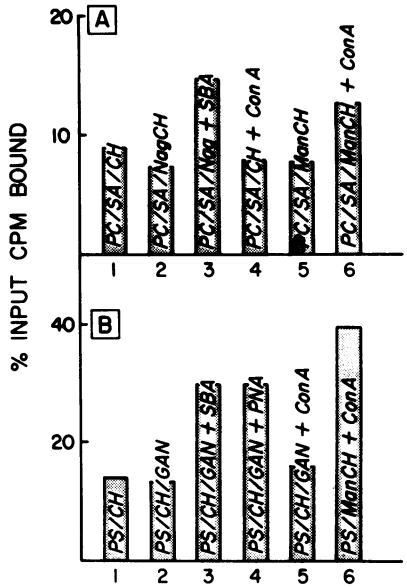

FIG. 3. A, Effect of lectins on the binding of multilamellar PC/SA liposomes to tobacco protoplasts. Sugar-derivatized cholesterol molecules are included in the liposome bilayer allowing ligand-specific binding of lectin molecules to the liposome surface. 1, control PC/SA liposomes containing cholesterol; 2, PC/SA liposomes containing NagChol; 3, liposomes as in 2 reacted with SBA; 4, liposomes as in 1 reacted with Con A; 5, PC/SA liposomes containing ManChol; 6, PC/SA liposomes as in 5 reacted with Con A. The per cent SE values are (lanes 1–6): ±0.8, 0.7, 0.7, 1.3, 0.9, and 0.8. B, Effect of lectins on the binding of unilamellar PS/CH liposomes to tobacco protoplasts. Sugar-derivatized cholesterol molecules in the bilayer as above. 1, control PS/CH liposomes; 2, PS/CH liposomes containing gangliosides; 3, PS/CH liposomes as in 2 reacted with SBA; 4, PS/CH liposomes as in 2 reacted with PNA; 5, liposomes as in 2 reacted with Con A; 6, PS liposomes containing NagChol instead of cholesterol and reacted with Con A. Here also, lectin binding to liposomes occurs via ligand-specific reaction. The per cent SE values are (lanes 1–6): ±0.4, 0.4, 0.3, 0.3, and 0.6, 0.1.

in order to achieve crosslinking with tobacco protoplasts.

Figure 3B shows that unilamellar reverse evaporation vesicles composed of PS and ManChol could also be crosslinked to tobacco protoplast through Con A. Here again, a large increase in binding was observed relative to control PS/CH unilamellar reverse evaporation liposomes.

Gangliosides are also known to contain lectin receptors. Figure 3B shows that the sugar derivatives of cholesterol used above can be replaced by gangliosides and still be efficiently crosslinked to tobacco protoplasts through the action of SBA and PNA. However, Con A did not display a significant increase in binding when ManChol was substituted by gangliosides.

Our previous studies and others (9, 14, 15) showed that PEG also enhanced liposome binding to protoplasts. We tested the effect of 20% PEG 6000 (final concentration) on the binding of PC/SA multilamellar liposomes containing SBA covalently bound to gangliosides. In addition, we also determined the effect of PEG 6000 on the binding of PS/NagChol reverse evaporation vesicles coupled to SBA. In both cases, PEG increased the amount of liposome binding by a factor of approximately 2 (data not shown).

Donor DNA Associated with Nuclei. Figure 4 shows that, as expected, Con A strongly increased the binding of PS reverse evaporation vesicles containing ManChol. Moreover, increased binding to protoplasts was accompanied by a proportional increase in radioactivity found associated with a nuclear fraction. Sucrose gradient analysis showed that this radioactivity corresponded to donor DNA and not to endogenous DNA (not shown).

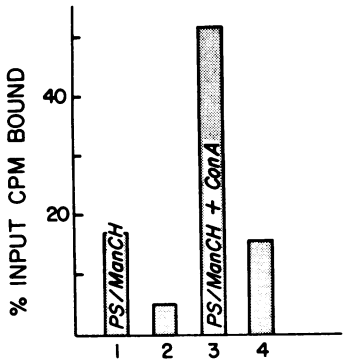

FIG. 4. Effect of Con A on the binding of PS/ManChol unilamellar liposomes to tobacco protoplast and transfer of encapsulated DNA to protoplast nuclei. 1, binding of PS/ManChol liposomes to intact protoplasts; 2, radioactivity associated with the nuclear fraction isolated from protoplasts treated as in 1; 3, binding of PS/ManChol liposomes reacted with Con A to intact protoplasts; 4, radioactivity associated with the nuclear fraction isolated from protoplasts treated as in 3.

DISCUSSION

This study shows that liposomes can be targeted with lectins either by covalent binding or reaction with specific sugar groups present in the liposome bilayer. This targeting results in a significant enhancement of modified liposome affinity for plant protoplasts. Our previous results (14) showed that liposome targeting could be achieved simply by mixing lectins and lipids prior to the production of liposomes. However, enhancement of liposome binding to protoplasts was observed only with SBA and not with Con A. It was then assumed that SBA had the ability to cross the lipid bilayer whereas Con A either could not cross the lipid membrane or did not extend far enough from the membrane to interact with receptor sites on the protoplast plasmalemma. Such steric inhibition of Con A has been discussed recently (22).

This report shows that Con A can be used to target liposomes provided it is bound to the exterior of the liposome via its interactions with a specific sugar-cholesterol derivative. However, covalent binding of Con A to gangliosides did not result in an increased binding to plant protoplasts, indicating that the active site of Con A may have been destroyed by the condensation reaction. In contrast, SBA and PNA caused increased liposome binding to protoplasts when either the condensation reaction or the ligand-specific reaction were used for liposome targeting. These experiments also indicated that a covalent binding between the lectin and the liposome surface was not required to ensure a stable association between targeted liposomes and protoplasts. The fact that Con A did not affect the binding of ganglioside-containing liposomes to protoplasts may indicate that these gangliosides did not contain adequate Con A binding sites or that steric hindrance prevented targeting (22). Therefore, lectins such as SBA and PNA seem to have a wider range of application than Con A.

In general, the nature of the liposomes used did not influence their targeting; in other words, multilamellar PC/SA liposomes responded in much the same way as PS/CH unilamellar liposomes when reacted with lectins either by condensation or by ligand-specific binding.

The observation that lectin-specific sugars compete only slightly with lectin-targeted liposome binding to protoplasts seems to indicate that this binding is either not totally ligand-specific (*i.e.* parts of the lectin molecules other than the active site may interact with noncarbohydrate moieties present on the protoplast surface) or else that a mechanism such as fusion or endocytosis makes liposome binding irreversible. We also observed that PEG had a significant effect on the binding of targeted liposomes to protoplasts. Therefore, it may still be necessary to include this treatment in cases where internalization of the liposome contents is to be achieved. All the binding studies reported here were performed with protoplasts incubated in high salt medium in order to prevent interference from sugars in the ligand-specific reactions. We have shown that the binding of positively charged liposomes to protoplasts is strongly impaired by high salt concentrations (14). Therefore, the experimental conditions used in this study were suboptimal as far as PC/SA liposomes are concerned. On the other hand, we showed that the binding of PS/CH reverse evaporation vesicles to protoplasts was much less sensitive to ionic strength (14), making this type of liposome better suited for targeting experiments and DNA or RNA transfer experiments as already stated in the Introduction.

Liposomes targeted with lectins could be used to study the properties of the plant protoplast membrane or to investigate the transfer and expression of viral particles and nucleic acids.

ManChol and NagChol are presently not commercially available. Therefore, the easiest way to produce targeted liposomes for use in protoplast experiments is to include gangliosides in the liposome bilayer and simply incubate the vesicles in the presence of SBA or PNA, thus allowing binding of the lectin to sugar groups present in gangliosides. Alternatively, liposomes can be formed in the presence of ceramides and subsequently covalently coupled to SBA or PNA (but not to Con A) by condensation. However, such targeting involves organic reactions which considerably increase the time and manipulations required to produce such liposomes without enhancing their protoplast binding ability as compared to liposomes targeted through ligand-specific reaction.

The enhanced binding of liposomes to protoplasts does not necessarily mean that the liposome contents will be transferred in a biologically active form. Indeed, positively charged multilamellar and unilamellar liposomes containing stearylamine were shown to interact strongly with plant protoplasts (10, 12) but were unable to deliver functional viral RNA (4; Christen and Lurquin, unpublished). However, autoradiographic experiments (20) as well as positively charged liposome-mediated transfer of whole viral particles (21) have indicated that delivery from these liposomes did occur. These observations suggest that stearylamine may form a stable complex with RNA (and DNA) which would prevent its expression in protoplasts. Therefore, targeting positively charged liposomes with lectins may not increase their capacity to deliver nucleic acids but may enhance their ability to deliver intact viral particles. On the other hand, it has been shown that the expression of tobacco mosaic virus RNA after PS-based liposome-mediated transfer was clearly dose-dependent (4, 5, 26). Hence, increasing interactions between such liposomes and protoplasts can be justified on the basis that higher binding values may lead to similar infectivities at a lower liposome/protoplast ratio. Such higher binding values are conveniently displayed by targeted PS reverse evaporation liposomes as shown in this study.

The problem of liposome-mediated DNA transfer into protoplasts is more difficult to address inasmuch as convenient plant genetic transformation systems do not exist at the present time. It has been reported (3) that *A. tumefaciens* pTi DNA encapsulated in PS/CH reverse evaporation vesicles could effect crown gall transformation of *N. xanthi* protoplasts used in this study. However, the low frequency of transformation observed precludes this system from being used in the evaluation of the effectiveness of DNA transfer. Therefore, an estimation of donor DNA transfer to recipient nuclei presently has to rely on autoradiographic or biochemical techniques (9, 12, 15, 20), even though the results of such experiments are not absolutely quantitative (10). Our results showed that nuclei isolated from protoplasts incubated with DNA-loaded targeted liposomes contained much more donor DNA than if control unmodified liposomes were used. In this experiment, a

1000-fold excess of salmon sperm DNA was added to the nuclei isolation buffer to minimize donor DNA redistribution after protoplast lysis (9). The association of donor DNA with protoplast nuclei after liposome-mediated transfer has also been reported by others (15). It will be possible to quantitatively assess the biological activity of donor DNA transferred by means of liposomes when convenient vectors and adequate markers selectable in plant cells are developed.

Acknowledgment—We gratefully thank Elizabeth LaCrosse for competent technical assistance.

LITERATURE CITED

1. BOLDT DH, SF SPECKART, RL RICHARDS 1977 Interactions of plant lectins with glycolipids in liposomes. Biochem Biophys Res Commun 74: 208–214
2. BURGESS J, PJ LINSTEAD 1976 Ultrastructural studies of concanavalin A to the plasmalemma of higher plant protoplasts. Planta 130: 73–79
3. DELLAPORTA SL, RT FRALEY 1981 Delivery of liposome-encapsulated nucleic acids into plant protoplasts. Plant Mol Biol Newslett 2: 59–66
4. FRALEY RT, SL DELLAPORTA, D PAPAHADJOPOULOS 1982 Liposome-mediated delivery of tobacco mosaic virus RNA into tobacco protoplasts: a sensitive assay for monitoring liposome-protoplast interactions. Proc Natl Acad Sci USA 79: 1859–1863
5. FUKUNAGA Y, T NAGATA, I TAKEBE 1981 Liposome-mediated infection of plant protoplasts with tobacco mosaic virus RNA. Virology 113: 752–760
6. HEATH TD, RT FRALEY, D PAPAHADJOPOULOS 1980 Cell specificity obtained by conjugation of F(ab')2 to vesicle surface. Science 210: 539–541
7. LARKIN PJ 1976 Purification and viability determinations of plant protoplasts. Planta 128: 213–216
8. LARKIN PJ 1978 Plant protoplast agglutination by lectins. Plant Physiol 61: 626–629
9. LURQUIN PF 1979 Entrapment of plasmid DNA by liposomes and their interactions with plant protoplasts. Nucleic Acids Res 6: 3773–3784
10. LURQUIN PF 1981 Binding of plasmid loaded liposomes to plant protoplasts: validity of biochemical methods to evaluate the transfer of exogenous DNA. Plant Sci Lett 21: 31–40
11. LURQUIN PF, RE SHEEHY, NA RAO 1981 Quantitative aspects of nucleic acids sequestration in large liposomes and their effects on plant protoplasts. FEBS Lett 125: 183–187
12. LURQUIN PF, RE SHEEHY 1982 Binding of large liposomes to plant protoplasts and delivery of encapsulated DNA. Plant Sci Lett 25: 133–146
13. LURQUIN PF, A KLEINHOFS 1982 Effects of chloramphenicol on plant cells: potential as a selectable marker for transformation studies. Biochem Biophys Res Commun 107: 286–293
14. LURQUIN PF, RE SHEEHY 1982 Effects of conditions of incubation on the binding of DNA-loaded liposomes to plant protoplasts. Plant Sci Lett. 28: 49–61
15. MATTHEWS BF, DE CRESS 1980 Liposome-mediated delivery of DNA to carrot protoplasts. Planta 153: 90–94
16. NAGATA R, K OKADA, I TAKEBE, C MATSUI 1981 Delivery of tobacco mosaic virus RNA into plant protoplasts mediated by reverse-phase evaporation vesicles (liposomes). Mol Gen Genet 184: 161–165
17. ORR GA, RR RANDO, BANGERTER 1979 Synthetic glycolipids and lectin-mediated aggregation of liposomes. J Biol Chem 254: 4721–4725
18. PAGANO RE, JN WEINSTEIN 1978 Interactions of liposomes with mammalian cells. Annu Rev Biophys Bioeng 7: 435–468
19. PONPIPOM MM, RL BUGRANESI, TY SHEN 1980 Cell surface carbohydrates for targeting studies. Can J Chem 58: 214–220
20. ROLLO R, MG GALLI, B PARISI 1981 Liposome-mediated transfer of DNA to carrot protoplasts: a biochemical and autoradiographic analysis. Plant Sci Lett 20: 347–354
21. ROLLO R, R HULL 1982 Liposome-mediated infection of turnip protoplasts with turnip rosette virus and RNA. J Gen Virol 60: 359–363
22. SUNDLER R 1982 Agglutination of glycolipid-phospholipid vesicles by concanavalin A. Evidence for steric modulation of lectin binding by phospholipid head groups. FEBS Lett 141: 11–13
23. SZOKA F, D PAPAHADJOPOULOS 1978 Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc Natl Acad Sci USA 74: 4194–4198
24. SZOKA F, K MAGNUSSON, J WOJCIESZYN, Y HOU, Z DERZKO, K JACOBSON 1981 Use of lectins and polyethylene glycol for the fusion of glycolipids containing liposomes with eukaryotic cells. Proc Natl Acad Sci USA 78: 1685–1689
25. UCHIMIYA H, H HARADA 1981 Transfer of liposome-sequestering plasmid DNA into *Daucus carota* protoplasts. Plant Physiol 68: 1027–1030
26. WATANABE Y, T OHNO, Y OKADA 1982 Virus multiplication in tobacco protoplasts inoculated with tobacco mosaic virus RNA encapsulated in large unilamellar vesicle liposomes. Virology 120: 478–480
27. WU PS, GW TIN, JD BALDESCHWIELER, TY SHEN, MM PONPIPOM 1981 Effects of surface modification on aggregation of phospholipid vesicles. Proc Natl Acad Sci USA 78: 6211–6215