Liposome-Mediated Transfer of Macromolecules into Flagellated Cell Envelopes from Bacteria†

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ABSTRACT: We have studied the interaction between flagellated cell envelopes from *Escherichia coli* and liposomes. Oligolamellar liposomes of ca. 0.45-μm diameter, composed of azolectin, phosphatidylserine, and cholesterol at a molar ratio of 7:1:2, were prepared by freezing and thawing and subsequent extrusion through polycarbonate filters. These liposomes exhibited high entrapment capacity and low leakiness. Liposome–cell envelope interaction was monitored flow cytometrically in a fluorescence-activated cell sorter with a fluorescent aqueous space marker and by a filtration assay with radiolabels for the lipid phase and the liposomal aqueous space. Maximal association of liposomes with the envelopes was observed in both assays after ca. 25 min at 30 °C. After such period of time, it seems that up to 200 liposomes (depending on the liposome to envelope ratio) were associated with a single cell envelope, as calculated from the radiotracer studies. Fluorometric measurements of the transfer of liposomal contents and the intermixing of membrane lipids indicated that at least 20% of the envelope-associated liposomes had delivered their content into the envelopes, possibly by fusion. Electron microscopic observations confirmed the transfer of liposome-encapsulated ferritin molecules into the cell envelopes. Our data suggest that liposomal carriers might be employed to deliver cytoplasmic, chemotaxis-related macromolecules into bacterial cell envelopes.

Motile bacteria such as *Escherichia coli* are attracted by certain stimulating chemicals and repelled by others, a phenomenon known as chemotaxis. Gene products, involved in chemotaxis, have been identified and localized within the cytoplasm and the cytoplasmic membrane (Ridgway et al., 1977). On the basis of genetic, biochemical, and behavioral studies, the roles of a few of these proteins have been assigned. The role of most other chemotaxis proteins is still unknown [see Parkinson (1981) for review]. In order to elucidate the role of these proteins, a subcellular system consisting of cell envelopes with functional flagella, devoid of cytoplasm, has been recently isolated from bacteria (Eisenbach & Adler, 1981). These cell envelopes are not chemotactic, probably due to the loss of their cytoplasmic content (Ravid & Eisenbach, 1984). Reinsertion of cytoplasmic proteins involved in chemotaxis into these cell envelopes could provide an ideal system for assigning a role for these proteins.[1]

For the last decade, liposomes have been extensively investigated as a promising vehicle to insert exogenous macromolecules into cells [for review, see Pagano & Weinstein (1978) and Poste (1980)]. Recent improvements in liposome technology have led to the development of stable liposomes with relatively high entrapment efficiencies [for a review, see Szoka & Papahadjopoulos (1980)]. Some of these liposomes have already been used to introduce biologically active macromolecules into a variety of eukaryotic cells (Gregoriadis, 1979, 1980). Only a few studies have been published on the interaction of liposomes with prokaryotic cells. These include fusion of liposomes with the outer membrane of *Salmonella typhimurium* (Jones & Osborn, 1977 a,b) and transfer of liposome-entrapped plasmid into *E. coli* (Fraley et al., 1979) and *Mycoplasma capricolum* (Nicolau & Rottem, 1982). The aim of the present work is to characterize the interaction between liposomes and cell envelopes from *E. coli* and to assess the potential of this approach to deliver macromolecules into the cell envelopes.

Experimental Procedures

Chromatographically pure bovine brain phosphatidylserine (grade I) was purchased from Lipid Products (Nutfield, U.K.) and stored in sealed glass ampules under vacuum at −20 °C. Cholesterol (from Merck) recrystallized 2 times from ethanol and soybean lecithin fraction IV-S [azolectin (Miller & Racker, 1976)] (from Sigma) were dissolved in chloroform methanol (1:1 v/v) and stored at −20 °C. [^{14}C]DPPC[2] was from Amersham. [^{3}H]Inulin (M_r 5000) and [^{14}C]dextran (M_r 70 000) were obtained from New England Nuclear. Ferritin (6 times recrystallized, cadmium free) from horse spleen was obtained from Miles-Yeda (Rehovot, Israel). CF (Eastman Kodak, Rochester, NY) was purified by one-step elution on Sephadex LH-20 columns, as previously described (Lelkes, 1983). Stock solutions of CF (100 mM) were prepared in 10 mM HEPES, pH 7.5. Cellulose acetate membranes (0.45-μm pore size) were from Schleicher & Schuell, and Unipore polycarbonate membranes (0.4-μm pore size) were from Bio-Rad. The rest of the chemicals were of the highest purity commercially available.

Liposomes were prepared from a mixture of azolectin, phosphatidylserine, and cholesterol at a molar ratio of 7:1:2 by freezing and thawing essentially as described by Pick (1981). In brief, the lipid mixture (occasionally containing [^{14}C]DPPC), dissolved in chloroform–methanol (1:1 v/v), was dried onto the wall of a glass vial under a stream of N_2.

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[1] In principle, any component present in the lysis medium is incorporated into the cell envelopes during the preparation. However, such an incorporation is impractical with regard to bacterial extract or isolated proteins.

[2] Abbreviations: CF, 5,6-dicarboxyfluorescein; [^{14}C]DPPC, di[^{14}C]-palmitoyl-L-α-phosphatidylcholine; FACS, fluorescence-activated cell sorter; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; S_9, *N*,*N*′-distearyldioxacyanine perchlorate; S_{11}, *N*,*N*′-distearyldithiocyanine perchlorate [S_9 and S_{11} refer to dyes III and I, respectively, in Kuhn et al. (1972)].

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Following removal of residual organic solvent under vacuum for 1 h, the aqueous solution to be entrapped was added, and freezing and thawing were carried out twice. The resulting liposomes were sonicated for 10–30 s in a Laboratory Supplies bath-type sonicator. To obtain uniform size distribution, these liposomes were extruded through a 0.4-μm polycarbonate filter (Olson et al., 1979) and then collected by centrifugation. For brevity, we shall call these freeze–thawed and extruded liposomes "liposomes". Unless otherwise mentioned, liposomes were prepared to a final lipid concentration of 45 mg/mL and were stored for not more than 72 h under N_2 at 4 °C. Immediately before use, they were separated from the nonentrapped marker by gel filtration: Sepharose 4B for dextran and ferritin; Sephadex G-50 for inulin and CF.

Cell envelopes were isolated by penicillin treatment and subsequent osmotic lysis as described by Eisenbach & Adler (1981), except that the concentration of $MgSO_4$ in the lysis medium was varied as indicated. The *E. coli* strain used for the preparation was RP487 (Goy et al., 1978), a K12 derivative, wild type for chemotaxis. It was a gift from Dr. J. Adler (University of Wisconsin—Madison). The protein concentration of the cell envelopes was determined as described by Bradford (1976), with lyophilized bovine γ-globulin as standard.

The interaction between liposomes and cell envelopes was studied by the following techniques. (a) Filtration assay (Schuldiner & Kaback, 1975): Liposome–cell envelope interaction was initiated by adding liposomes into a cell envelope suspension (25 μg of protein in 150-μL final volume) equilibrated at 30 °C and terminated by filtration through a cellulose acetate filter. In order to reduce nonspecific binding, the filter was washed 3 times with 2 mL of an ice-cold chasing medium containing a 10-fold excess of nonradioactive inulin, 0.2% bovine serum albumin, and a 3-fold excess of unlabeled liposomes. The chasing was effective as it reduced the radioactivity retained on the filter at $t = 0$ to 2–4% of the total. However, this nonspecific binding was still high in comparison to the observed effects and could amount to up to 50% of the effect. In order to increase our confidence in the results obtained with this assay, it was repeated many times until reliable reproducibility was obtained in spite of the high background. Radioactivity retained on the filter was counted in dpm in a PLD-Tricarb (Packard Instruments) scintillation counter. (b) Flow cytometry (Weinstein et al., 1977): Liposomes containing 100 mM CF (pH 7.6) were incubated at room temperature with cell envelopes in 2 mL of lysis medium (pH 7.6). The resulting change in fluorescence originating from the cell envelopes was monitored periodically for 60 min in a fluorescence-activated cell sorter (FACS II, Becton & Dickinson). Leakage of CF from the liposomes in the presence and absence of cell envelopes was measured continuously in a spectrofluorometer as described by Lelkes & Tandeter (1982). (c) Transfer of fluorescent markers (Pagano et al., 1981): Liposomes containing CF (100 mM) or a fluorescent lipid donor–acceptor pair, S_9 and S_{11}, were incubated with cell envelopes as described for the filtration assay. S_9 (donor) and S_{11} (acceptor) at a molar ratio of 1:10 were initially incorporated into the liposomal membrane (3% of the total lipid) to yield high efficiencies of energy transfer (>95%) [Figure 7.16 in Kuhn et al. (1972)]. Cell envelopes were separated from nonassociated liposomes by centrifugation on a Percoll gradient. For each time point, the fluorescence of liposome–cell envelope complexes and of the free liposomes in the presence and in the absence of 0.11% (v/v) Triton X-100 was measured. The ratio between fusion and adhesion was calculated as described (Pagano et al., 1981). Fluorescence was measured with Perkin-Elmer MPF 44 B or LS 5 spectrofluorometers.

Table I: Entrapment Efficiency of Freeze-Thawed Liposomes after Extrusion

aqueous marker	M_r	entrapped vol ± SE (L/mol of lipid)[a]
CF	377	7.2 ± 0.4 (2)
inulin	5 000	6.0 ± 1.0 (4)
dextran	70 000	7.1 ± 1.2 (3)
ferritin	500 000	6.9 ± 0.4 (2)

[a] In parentheses are the number of determinations.

Quality control of the cell envelope preparations was routinely carried out on negatively stained specimens (DePamphilis & Adler, 1971). The liposomes were characterized by freeze–fracturing and by negative staining (Szoka & Papahadjopoulos, 1980). For freeze–fracturing, liposome suspensions were rapidly frozen in Freon and cooled in liquid nitrogen. Frozen specimens were freeze–fractured in a BAF 301 freeze–etch device (Balzers), deep etched at −100 °C for 30 s, undirectionally shadowed with platinum, and backed with carbon. For negative staining, 1 drop of a 1:1 (v/v) mixture of the liposome suspension and 4% ammonium molybdate (pH 7.4) was placed on a 400-mesh copper grid and the excess fluid removed with filter paper.

The transfer of liposome-encapsulated ferritin was vizualized in ultrathin sections of cell envelopes treated with liposomes, for 30 min. The incubation mixture was fixed in 2.5% formaldehyde and further processed as described (Eisenbach & Adler, 1981). Embedding was in Epon 812. Thin sections (400–500 Å thick) were cut with a Sorval MT2 ultramicrotome and collected on formvar-coated grids. All micrographs were taken in a JEOL JEM-7 electron microscope, operated at 80 kV.

Results

In choosing the appropriate delivery vehicle, we extended previously published work on the use of freeze–thawed liposomes for reconstitution of intrinsic membrane proteins (Kasahara & Hinkle, 1979; Pick, 1981). Freezing and thawing of sonicated liposomes provides a gentle technique to encapsulate labile biological macromolecules (Gurari-Rotman & Lelkes, 1982) and yields a large entrapped volume, independent of the size of the marker (Table I). Our standard liposomes were prepared from azolectin, phosphatidylserine, and cholesterol at a molar ratio of 7:1:2. Liposomes containing 10% phosphatidylserine and 20% cholesterol, as in this study, were previously shown to be optimally stable toward cell-induced leakage of their contents (Van Renswoude & Hoekstra, 1981). Electron micrographs of negatively stained liposomes and of a freeze–fractured sample of the same preparation are shown in A and B of Figure 1, respectively. With both techniques the liposomes appear to be homogeneous in size and uni- or oligolamellar (Figure 1B). The size distribution of negatively stained liposomes is plotted in Figure 2. The external diameters of these liposomes are centered rather narrowly around 0.45 μm.

Cell–liposome interactions can be enhanced by divalent cations (Poste, 1980). Since cell envelopes are commonly prepared in a medium that contains Mg^{2+} (Eisenbach & Adler, 1981), we chose this ion to enhance association between the liposomes and the envelopes. The Mg^{2+} concentration was kept below the threshold necessary to initiate liposome–liposome aggregation. This concentration was determined as shown in

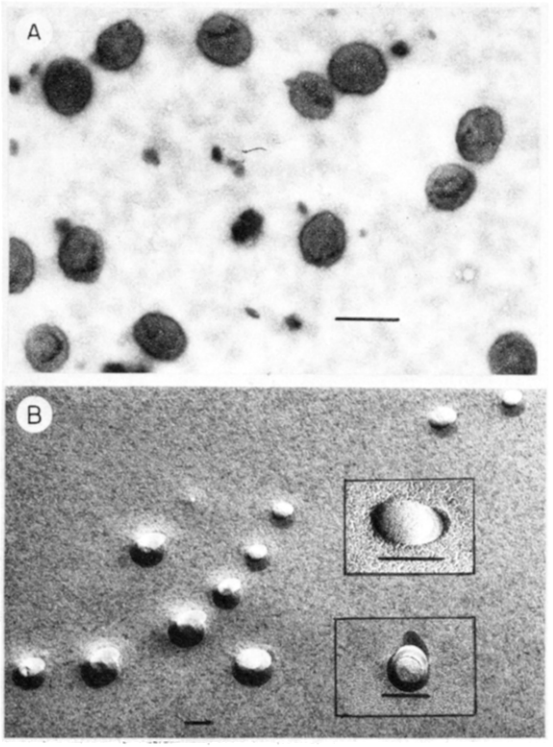

FIGURE 1: Electron micrographs of freeze–thawed liposomes after extrusion through polycarbonate filters (0.4-μm pore diameter). (A) Negative staining with 2% ammonium molibdate. (B) Freeze–fracturing. Bar = 0.5 μm.

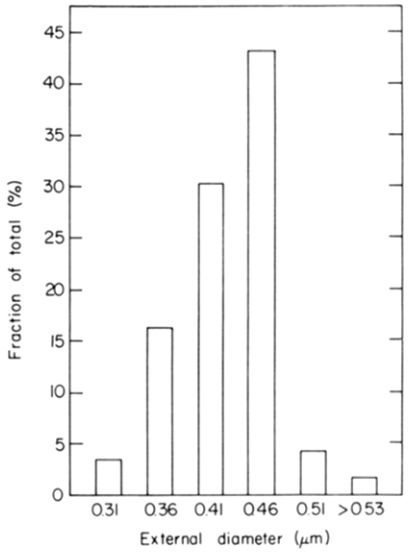

FIGURE 2: Size distribution of liposomes. The sizes were measured from negatively stained samples as in Figure 1 ($n = 246$). The narrow size distribution probably results from the experimental procedure: Following sizing of the liposomes, the suspension is centrifuged for 5 min in an Eppendorf microfuge, to remove larger liposomes. During the subsequent centrifugation of the supernatant (30 min at 50000g), small liposomes will not pellet down and are discarded. The pellet thus contains a rather homogeneous liposome population.

Figure 3 by measuring OD_{400nm} of liposomes incubated with increasing concentrations of Mg^{2+} for 40 min in isotonic lysis media (in the absence of cell envelopes). A clear break in the dose dependence occurs at ca. 14 mM $MgSO_4$, indicating increased interaction between liposomes. In the absence of liposomes, no change in OD_{400} was observable, indicative of the absence of MgP_i precipitate. In subsequent experiments, we used lysis media containing 20 mM KP_i and 12 mM $MgSO_4$, which is just below the break point in Figure 3.

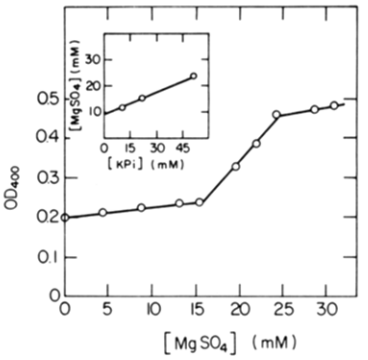

FIGURE 3: Liposome aggregation as a function of $MgSO_4$ concentration. Liposomes were incubated for 40 min in lysis media containing varying concentrations of $MgSO_4$, KCl (38 mM), KP_i (20 mM), and EDTA (0.1 mM) (pH 6.8; 30 °C). OD_{400} of the suspension was subsequently measured. The reference cuvette contained the same lysis media as the sample compartment. The break in the dose dependence of OD_{400} at ca. 14 mM $MgSO_4$ indicates the threshold for visible liposome aggregation. (Inset) Dependence of the threshold for Mg^{2+}-induced liposome aggregation on the KP_i concentration in different lysis media.

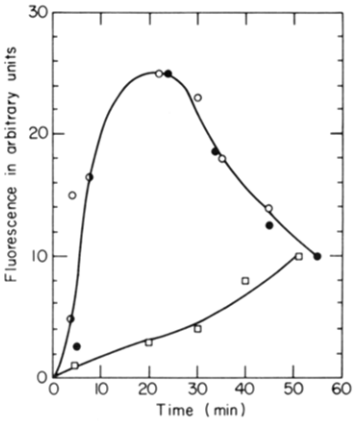

FIGURE 4: Kinetics of liposome-mediated transfer of CF into cell envelopes as monitored with the FACS. The fluorescence intensities, obtained from the peak positions of the FACS histograms, were normalized for the different experiments and expressed in arbitrary units. (●, ○, and ◑) Three independent experiments using a liposome to cell envelope ratio of protein to lipid of 1:1 (w/w); (□) liposome to cell envelope ratio of 1:10. The incubation medium contained 38 mM KCl, 20 mM KP_i, 12 mM $MgSO_4$, and 0.1 mM EDTA (pH 7.5). Temperature was 25 °C.

The interaction of CF-containing liposomes with the cell envelopes was monitored fluorometrically. Leakage of CF from the liposomes at 30 °C was 9 and 15% of their total contents per hour in the absence and presence of envelopes, respectively. In order to discriminate between fluorescence originated from dilution of the liposomal contents into the cell envelopes and that arising from leakage of the dye into the external medium, we used a fluorescence-activated cell sorter (Bonner et al., 1972). The time course of the interaction was monitored at several liposome–cell envelope ratios. Two of them are shown in Figure 4. Cell envelope associated fluorescence increased transiently for ratios of 1 (Figure 4) and above (not shown in the figure), reaching a maximum at approximately 25 min. For the ratio 0.1, the fluorescence increased throughout the time course of the experiment (Figure 4). For the liposome to envelope ratios studied (0.1–10), the intensity of the fluorescence in the cell envelopes at 25 min was proportional to the concentration of the liposomes in the suspending medium (not shown in the figure). This indicates that the subsequent decrease in fluorescence was not the consequence of self-quenching due to increasing CF concen-

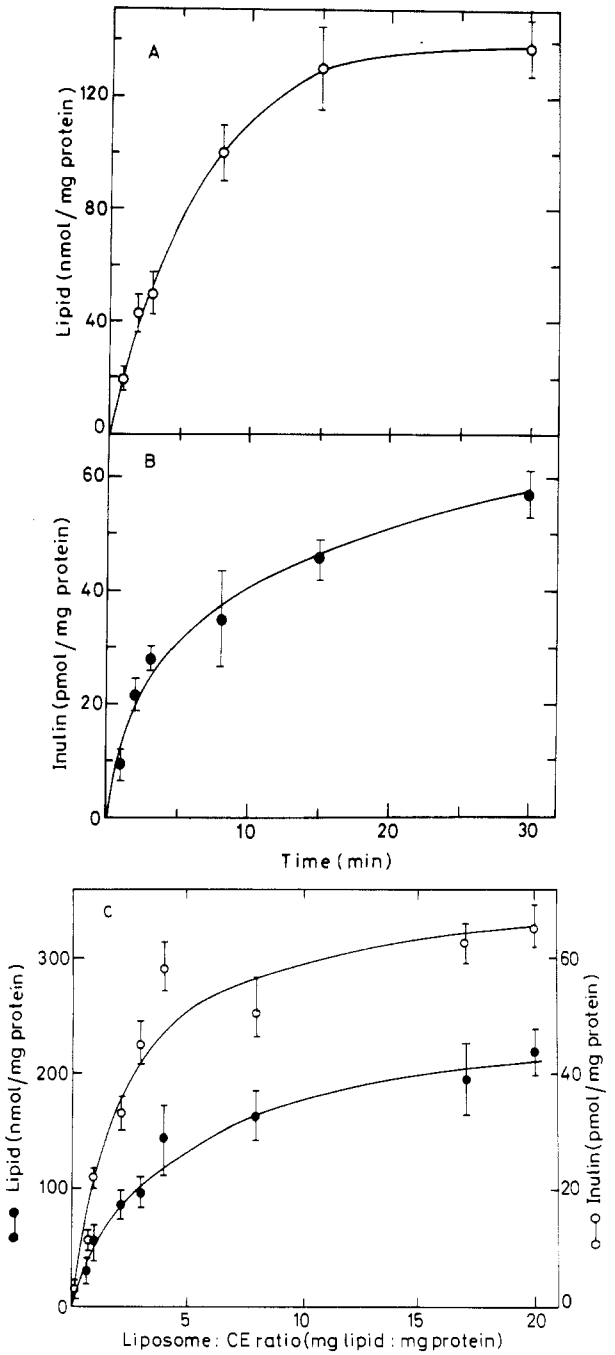

FIGURE 5: Association of liposomes with cell envelopes, studied by radiotracer techniques. Time dependence of the association, measured at a liposome to cell envelope ratio of 4:1 (mg of lipid to mg of protein) and expressed as moles of lipid per milligram of administered protein (A, open circles) and picomoles of inulin per milligram of administered protein (B, closed circles). Dose dependence of the association derived from cummulative data of several independent experiments (C). The cell envelopes (between 20 and 35 μg of protein in each experiment) were incubated for 25 min at 30 °C with various amounts of double-labeled liposomes (2–500 μg), and the association was measured by the filtration assay. The results were normalized and expressed as moles of lipid per milligram of protein (closed circles) and moles of inulin per milligram of protein (open circles). All data were corrected for zero-time (nonspecific) adhesion. To further reduce nonspecific binding, extensive chasing was performed as described under Experimental Procedures. The bars represent the standard error (three determinations for each point).

tration in the envelopes but that it was rather due to the burst of envelopes resulting from extensive interaction with liposomes. Supporting evidence for the latter was obtained from electron micrographs showing the burst of cell envelopes after

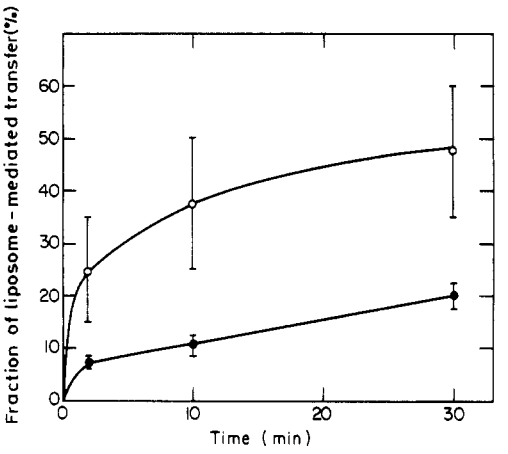

FIGURE 6: Fraction of liposomal markers transferred to cell envelopes as a function of the incubation time. The distinction between markers transferred apparently by fusion and those adsorbed to the envelopes was achieved as described in the text. The markers for the liposomal membrane were the fluorescent lipid analogues S_9 and S_{11} (○), and the marker for the aqueous space, in separate preparations, was CF (●). Fluorescence wavelengths: S_9, 360/412 nm; CF, 460/520 nm. The experimental points represent cummulative data from two independent experiments, measured in triplicate. The bars represent maximal error.

prolonged incubation times with liposomes. Similar observations were reported for synaptosomes (Crosland et al., 1983). In all the subsequent experiments we, therefore, followed the interaction between liposomes and envelopes for up to 30 min. CF-containing liposomes did not contribute to the fluorescence signal when analyzed in the absence of cell envelopes at identical settings of the photomultiplier and the light-scattering windows of the FACS. In addition, incubation of cell envelopes with free CF at a concentration that would result if all the liposomes had released their contents into the medium did not yield significantly increase in the cell envelope associated fluorescence. This observation, as well as the transient nature of the fluorescence in the cell envelopes, was confirmed with a fluorescence microscope.

The association between the cell envelopes and the liposomes was further investigated by radiotracer studies with [^{14}C]-DPPC and [^{3}H]inulin as markers for the lipid membrane and the aqueous phase of the liposomes, respectively (Figure 5). Maximal association (corresponding to 130 nmol of lipid/mg of protein and 60 pmol of inulin/mg of protein) was obtained after 20–30 min. As before, envelope lysis was observed after longer incubation times. At 30 min, the association was saturable at high liposome to envelope ratios (Figure 5C). The efficiency of the association, as calculated from these data for both the lipid and aqueous space markers, increased with decreasing liposome to envelope ratio: from 1% of the markers' amount in the liposomes at a ratio of 20 to 5% at a ratio of 1.

Radiotracer studies can be informative as to the extent of the association between liposomes and cell envelopes but cannot distinguish between true transfer of liposomal contents (with concomitant merging of the membrane constituents) and passive adsorption of the liposomes to the surface. Such distinction was accomplished by employing fluorescent assays recently developed by Pagano et al. (1981). In separate experiments, we used liposomes that contained a self-quenched concentration of fluorescent markers of the aqueous contents (CF) and of the lipid membrane (the donor–acceptor pair S_9–S_{11}), respectively. The time course of the fraction of the envelope-associated liposomes that, according to these assays, has interacted, possibly by fusion with the cell envelopes, is

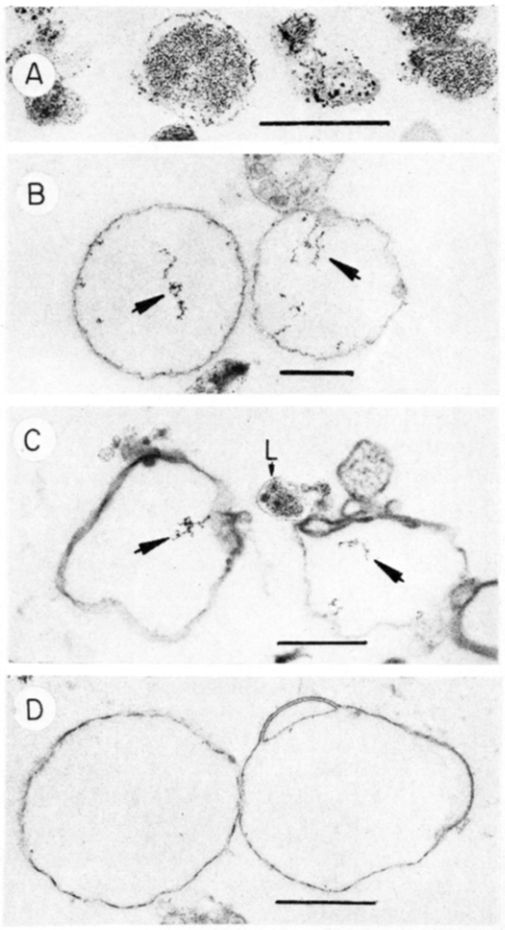

FIGURE 7: Liposome-mediated insertion of ferritin into cell envelopes. (A) Electron micrograph of ferritin-containing liposomes, prefixed with gluteraldehyde (2.5%). (B and C) Electron micrographs of cell envelopes after 25-min incubation (30 °C) with ferritin-containing liposomes and subsequent fixation with formaldehyde as described under Experimental Procedures. Clumps of ferritin particles are marked with arrows. A ferritin-containing liposome in conjuction with a cell envelope is marked with an L in (C). (D) Electron micrograph of cell envelopes after 25-min incubation with free ferritin (as a control) at a concentration that would result if all the liposomes had released their content. Similar micrographs were obtained for a ferritin concentration 4-fold higher. Each bar = 0.5 μm.

shown in Figure 6. As assessed from the lipid analogues, ca. 50% of the cell envelope associated liposomes appear to be fused after 30 min of incubation, whereas under the same incubation conditions only ca. 20% of the liposomes seem to have transferred their contents. The apparent discrepancy in the transfer of the two probes is probably due to the envelope-induced leakage of CF from the liposomes (cf. Poste, 1980). From these data, it appears that at least 50% of the envelope-associated liposomes do not transfer their contents and/or membrane lipids but are adsorbed to the cell surface.

Liposome-mediated transfer of macromolecules was visualized electron microscopically. Representative electron micrographs of ferritin-containing liposomes are shown in Figure 7A and of cell envelopes after 25-min incubation (30 °C) with these liposomes in Figure 7B,C. Unlike Figure 7A (see legend), these figures were obtained with samples fixed with formaldehyde, which essentially dissolved the liposomal membrane. With this fixative, very few intact liposomes could be observed, most of which were in association with the cell envelopes (see, e.g., in Figure 7C). Ferritin inside the cell envelopes was seen only after incubation with the liposomes. Incubation of cell envelopes with free ferritin and empty liposomes did not result in uptake of ferritin (Figure 7D).

Discussion

In this study we demonstrate the feasibility of liposomal carriers to transfer their contents, including macromolecules, into flagellated cell envelopes from bacteria. This conclusion is based on several independent lines of evidence. (A) The kinetics of the cell-associated fluorescence, monitored with the FACS, demonstrate qualitatively the time- and dose-dependent transfer of the liposomal contents. A contribution to the fluorescence signal from noninteracting liposomes was eliminated by appropriate control experiments and proper setting of the lower cut-off limit of the light scattering detection (Weinstein et al., 1977). A contribution from liposomes adsorbed onto the cell envelopes was minimized by encapsulating a high enough concentration of CF (100 mM), so that even leakage of the liposomal contents, much above what we have observed, would not significantly relieve the self-quenched fluorescence of the dye retained in the liposomes. Furthermore, if the interaction between the envelopes and the liposomes were adsorption with subsequent leakage of the liposomal contents, one would expect the fluorescence to increase during the time period of the experiment. The transient nature of the fluorescence (Figure 4) excludes this explanation. (B) The association between radiolabeled liposomes and cell envelopes was irreversible: even after extensive chasing with nonradioactive liposomes, inulin, and bovine serum albumin, the markers of both the liposomal membrane and contents were still associated with the cell envelopes (Figure 5). (C) The fluorescent assays involving markers for the lipid membrane[3] and the liposomal contents indicate that a significant fraction of the envelope-associated liposomes had possibly fused with their targets (Figure 6). The apparently higher extent of fusion obtained with the lipid analogues (ca. 50%) could reflect accumulative contributions of fusion and of lipid exchange (Pagano & Huang, 1975). On the other hand, transfer of the liposomal contents (ca. 20%) is possibly underestimated due to the leakage of the small marker CF (Szoka et al., 1979; Poste, 1980). The true value is probably between these limits. (D) Incorporation of ferritin into cell envelopes was documented electron microscopically following incubation with ferritin-containing liposomes (Figure 7).

From this study we can assess quantitatively the efficacy of the liposomal transfer. The number of molecules delivered will depend on the entrapment efficiency of the liposomes (Table I) and on the degree of fusion with the envelopes (Figures 5 and 6). For liposomes as large as in this study, the entrapment efficiency does not depend on the size of the molecules to be entrapped (Szoka & Papahadjopoulos, 1980). The number of molecules inside the liposomes thus depends on the original solute concentration, as confirmed by the similarities in the entrapment efficiencies (1/mol of lipid) for molecules ranging in molecular weight from 400 to 500 000 (Table I). A simple calculation based on Figures 5 and 6 indicates that, e.g., for a ratio of 1:1 liposome to cell envelope (25 μg of lipid/25 μg of protein), ca. 50 liposomes are associated with a single envelope, of which 10–25 liposomes transfer their content to the envelope after 25-min incubation. Thus, for an initial solute concentration of 20 mg/mL, we estimate that a total of 2600–5200 molecules of ferritin, 38 000–76 000 molecules of dextran, or 260 000–520 000

[3] Similar degree of lipid transfer upon interaction with the envelopes was found between the fluorescent lipid analogues, used in this study, and head group labeled fluorescent phosphatidylethanolamines (S. Ravid and P. I. Lelkes, unpublished results). The latter are considered to be nonexchangeable with mammalian plasma membranes (Struck & Pagano, 1980).

molecules of inulin can be transferred into a single cell envelope. Under saturating conditions (Figure 5), these numbers will be 4-fold larger, provided that the efficiency of transfer is unchanged. Strong support for the validity of our calculation is obtained from the transfer of ferritin (Figure 7). A random count in ca. 200 cell envelopes indicates that there are some 75 ± 20 ferritin molecules/μm^2. From the average thickness of an ultrathin section (ca. 50 nm) and the cell envelope volume (2.3×10^{-18} m^3), we can estimate that ca. 5000 ferritin molecules have actually been transferred.

These quantities seem to suffice for insertion of all the known *che* gene products, ca. 6×10^3 molecules in total,[4] into the cell envelope. Furthermore, since the highest molecular weight of the *che* gene products is 76 000 (Ridgway et al., 1977), the number of molecules transferred is expected to be more than 50 000. Whether the insertion of these proteins (in the form of bacterial extract) indeed suffices to restore chemotactic competence remains to be seen and is currently under study. This delivery technique by liposomes may be useful not only for studies with cell envelopes but also for insertion of membrane-impermeant molecules (e.g., *S*-adenosylmethionine or cGMP) into the recently developed system of cytoplasm-containing chemotactic spheroplasts (Ravid & Eisenbach, 1984).

Acknowledgments

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Registry No. Cholesterol, 57-88-5; magnesium, 7439-95-4.

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[4] This number is based on the known amount of molecules of the *tar* gene product in a single bacterial cell (Hazelbauer et al., 1982) and the known stoichiometries between them and the *che* gene products (Ridgway et al., 1977; Smith & Parkinson, 1980; DeFranco & Koshland, 1981).