Biochimica et Biophysica Acta, 550 (1979) 464—473

BBA 78254

ON THE MODE OF LIPOSOME-CELL INTERACTIONS

BIOTIN-CONJUGATED LIPIDS AS ULTRASTRUCTURAL PROBES

EDWARD A. BAYER [a], BENJAMIN RIVNAY [b] and EHUD SKUTELSKY [c]

[a] *Department of Biophysics,* [b] *Department of Cell Biology and* [c] *Section of Biological Ultrastructure, The Weizmann Institute of Science, Rehovot (Israel)*

(Received July 5th, 1978)

Key words: Liposome-cell interaction; Biotin-lipid; Avidin; Ferritin

Summary

An efficient method for labeling and visualizing phospholipids at the ultrastructural level is described. Biotin was coupled to the amines of appropriate phospholipids via the *N*-hydroxysuccinimide ester. The biotinylated lipid could be specifically labeled by ferritin-avidin conjugates and detected by transmission electron microscopy. The lipid derivatives were analyzed and evaluated in terms of their resemblance to the original lipid. Although differing in some aspects from the parent lipid molecules, the biotinyl derivatives still retain the basic characteristics of lipids vis-a-vis their orientation and position in the membrane bilayer. The latter property renders the biotinylated lipid qualitatively suitable for tracing the fate of the lipid component(s) of liposomes during their interaction with biological membranes of various cell types. Using this system, we propose that the extent and pattern of the liposome-cell interaction depends, at least in part, on the cell type employed. This observation may be due to intrinsic variations in cell surface structure and properties relative to those of the liposome.

Introduction

In recent years artificially generated lipid vesicles (liposomes or phospholipid dispersions) have been used as an implement for probing cell surface membrane structure and function [1]. Such vesicles have been used as carriers for introducing membrane-impermeant substances into cells [2—10], as instruments for altering lipid composition of membranes in intact cells [9—14] and as inducers of cell fusion [15—19]. Many indirect methods have been attempted in order to elucidate the mechanism of the liposome-cell interaction,

Abbreviation used: BNHS, biotinyl-*N*-hydroxysuccinimide ester.

with particular emphasis on the difference between phagocytosis, adsorption and fusion phenomena. In one study, tissue culture cells treated with cyclic AMP-containing vesicles exhibited reduced growth rates [20]. Vesicle-cell fusion was assumed to be the cause of the apparent intracellular accumulation of the nucleotide, but direct analytical evidence was not presented. A double-radiotracer technique [21,22] and a fluorescence method [23,24] have also been described, in which more information was provided concerning the intracellular fate of incorporated material. In most cases, vesicle-cell fusion is considered the major mode of interaction, without, however, substantive corroboratory evidence. In the present study, we describe the ultrastructural visualization of the interaction between liposomes and cell surface membranes by use of the high-affinity avidin-biotin complex [25—27]. This approach comprises the following steps: (i) Biotin is covalently attached to the headgroups of appropriate lipids. (ii) Vesicles are prepared from characterized, biotinylated-lipids and are interacted with viable cells. (iii) After a desired time interval, the cells are washed, fixed and treated with ferritin-avidin conjugates. (iv) Samples are processed for electron microscopy and the membrane-based location of biotin-containing lipids is substantiated in thin sections by the presence of ferritin particles. The method affords high resolution and is applicable to kinetics and mechanistic studies. The rationale, validity and advantages of this approach have been discussed by us previously [28,29]. A somewhat similar but more complicated approach, using a double antibody labeling technique for the localization of dinitrophenylated-lipids on erythrocytes, was recently reported [30]. Part of this work was presented at the Annual Meeting of the Israel Biochemical Society, Tel Aviv, April, 1978 [31].

Materials and Methods

Phospholipids were purchased from Lipid Products (South Nutfield, England). Biotin, cholesterol and avidin were obtained from Sigma Chem. Co., St. Louis, MO. Biotinyl-*N*-hydroxysuccinimide ester (BNHS) was prepared as described previously [32]. Conjugation of avidin to ferritin was carried out as reported previously [28].

Preparation of cells

A heterogeneous spleen cell population was obtained by gentle teasing of mouse (C3H/eb × C57Bl/6j) F1 spleens in phosphate-buffered saline, pH 7.2. The suspension was washed once in the same buffer and subjected to liposome interaction. No additional treatment was performed, in order to minimize structural damage and adsorption of nucleic acids expelled from lysed cells, which has been found by us to bind ferritin-avidin conjugates independent of biotin (unpublished results).

Human blood was collected by venipuncture from healthy donors (blood group A, Rh^+) into heparinized test tubes. Erythrocytes were separated by centrifugation and aspiration of the plasma and buffy coat, washed twice with Veronal-acetate-buffered saline, pH 7.4 and resuspended in the desired medium.

In order to immobilize biotinylated-liposomes, avidin-conjugated fixed erythrocytes were prepared as follows: Human erythrocytes (2 ml packed cells)

were fixed with 2% formaldehyde and treated with 20 ml 10 mM sodium *meta*-periodate for 1 h at room temperature. The cells were washed with water and a solution (5 ml) of avidin (1 mg/ml) was added. Immobilization of the avidin was checked by decrease in absorbance (280 nm) of the supernatant and by sequestration of erythrocytes on a biotin containing column.

Preparation of biotin-conjugated phospholipids

Two classes of phospholipids, phosphatidylethanolamine and phosphatidylserine, were derivatized with BNHS as shown schematically in Fig. 1. In a representative protocol, 30 mg of either phosphatidylethanolamine or phosphatidylserine were dissolved in 1 ml chloroform/methanol (2 : 1) containing 20 mg BNHS [32], and triethylamine (10 μl) was added. The reaction mixture was allowed to stand for 30 min at room temperature. The respective products were separated by preparative thin-layer chromatography on precoated silica gel 60 plates (0.25 mm thickness, Merck). Biotinylated phosphatidylethanolamine was developed using chloroform/methanol/water (80 : 25 : 2), and biotinylated phosphatidylserine using chloroform/methanol/acetic acid (30 : 4 : 3). Three different methods of detection were used in order to visualize components of the reaction mixture on the silica gel plates: (i) exposure to iodine vapors, (ii) a biotin-specific spray [33] and (iii) a phosphate-specific spray. The latter consisted of spraying sample areas of the plates with 1% ascorbic acid, 0.25% ammonium molybdate in 0.3 M H_2SO_4/3 M $HClO_4$, whereby phosphate-containing spots turned blue. The position of the respective biotinylated product (biotinylated phosphatidylethanolamine, $R_F = 0.65$; biotinylated phosphatidylserine, $R_F = 0.55$) was evident from its sensitivity to all three staining procedures. The desired product was scraped off the plate, extracted with chloroform/methanol (2 : 1), and stored at −20°C. In each case, only one spot was observed at the corresponding position, following rechromatography against the respective solvent system. Due to the possibility of dissociation of derivatized lipids, the freshly separated products were used within 24 h. No alteration of the chromatographic pattern of either biotinylated phosphatidylserine or biotinylated phosphatidylethanolamine was observed under the above conditions.

Differential scanning calorimetry

Differential scanning calorimetry-endotherms of lipid mixtures in 30%

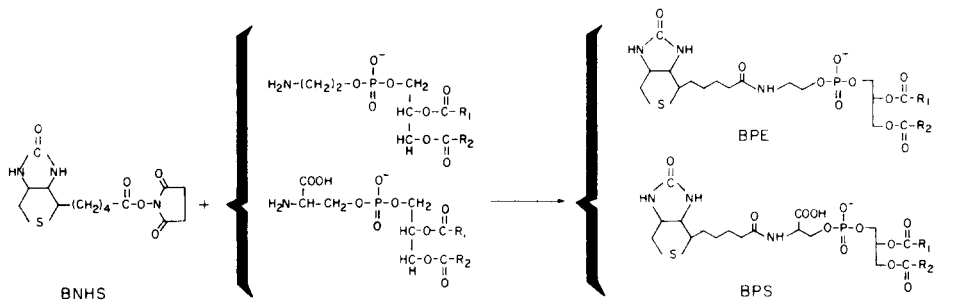

Fig. 1. Biotinylation of phosphatidylethanolamine and phosphatidylserine.

ethylene glycol in water were measured with a 990 Thermal Analyzer (Du Pont Instruments) against the heating direction only. Respective ΔH values represent a mean derived from 4—6 repeating runs at heating rates of either 5 or 2° C per min.

Preparation of liposomes

Liposomes containing cholesterol : phospholipid (1 : 1 molar ratio), the latter comprising freshly-purified biotinyl-lipid derivatives (usually 10%) in lecithin (phosphatidylcholine), were prepared as follows: Lipid mixtures in chloroform/methanol (2 : 1) were taken to dryness under nitrogen, vacuum-dried, suspended to a concentration of 1 mg/ml with phosphate-buffered saline, pH 7.2, and sonicated under nitrogen in an ice-cooled chamber for 10 min in a Branson Sonifier Model 130. The suspension was centrifuged for 20 min at 10 000 rev./min. Liposomes (supernatant fraction) were used within 24 h after preparation. In order to ascertain whether biotinylated-lipids were still accessible to avidin after incorporation into the liposome bilayer, the liposomes were immobilized onto avidin-conjugated, fixed erythrocytes. The residual biotin label on the liposomes could then be determined by either radioactive avidin, fluorescent-avidin or with ferritin-avidin conjugates. Using the above lipid mixtures, satisfactory binding of all avidin probes was observed. Hence, this ratio was found suitable for further studies.

Liposome treatment of cells

Cell samples (approx. $3 \cdot 10^7$/ml) were incubated for varying time periods at 37° C (5% CO_2 in air) in RPMI 1640 tissue culture medium (GIBCO) containing labeled liposomes (about 0.5 mg phospholipids/ml). Cells were washed once in Veronal-acetate-buffered saline, fixed with Karnovsky's fixative [34], treated with ferritin-avidin conjugates and processed for electron microscopy as previously reported [28]. Controls consisted of similar treatment using underivatized liposomes.

Results

Characterization of biotinyl-lipids

The purified phospholipid derivatives were characterized by two-dimensional thin-layer chromatography using appropriate standard markers. The normal pattern of distribution is given in Fig. 2. Further analysis of the physical properties of biotinylated-lipids was achieved using differential scanning calorimetry in order to examine the modulative effect of derivatization on the thermal properties which characterize native phosphatidylserine [35]. The differential scanning calorimetry-endotherms (Fig. 3) reveal that the normal thermal transition, typical of pure phosphatidylserine (Curve B) is obliterated in biotinylated phosphatidylserine (Curve A). The nature of this effect was further assessed by calorimetric scanning of biotinylated phosphatidylserine/phosphatidylserine lipid mixtures (Curve C). Only minor changes were observed in the transition temperature, whereas the normal ΔH value for phosphatidylserine evinced a decrease, proportional to the molar fraction of biotinylated phosphatidylserine in the mixture. The latter result was apparently a function

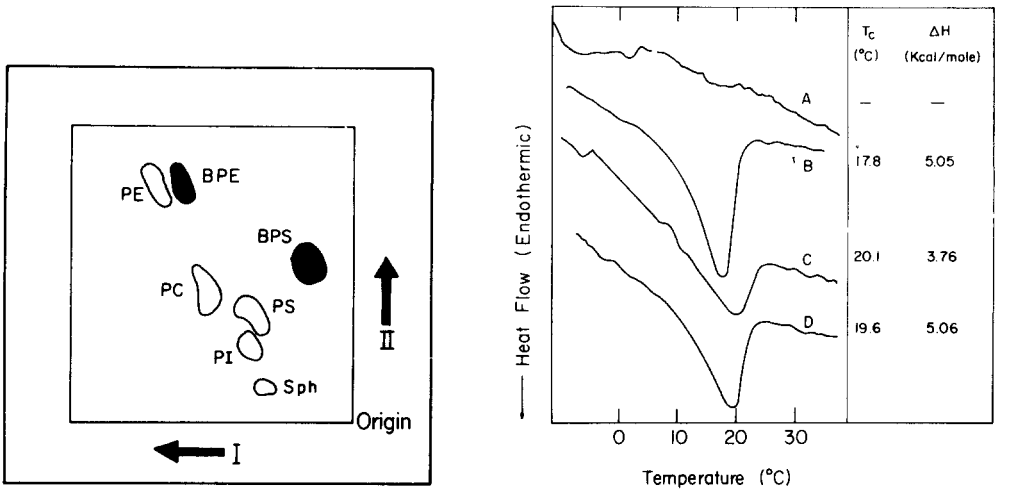

Fig. 2. Two dimensional thin-layer chromatography of modified lipids (biotinylated phosphatidylethanolamine and biotinylated phosphatidylserine) and a mixture of natural phospholipids. PC, PE, PI, PS and Sph represent the positions of phosphatidyl-choline, -ethanolamine, -inositol, -serine, and sphingomyelin, respectively. The plates (10 cm × 10 cm) were chromatographed against Solvent I: chloroform/methanol/water (80 : 25 : 2) followed by Solvent II: chloroform/methanol/acetic acid (30 : 4 : 3).

Fig. 3. Differential scanning calorimetry-endotherms; Curve A: biotinylated phosphatidylserine alone (in 30% ethylene glycol); Curve B: phosphatidylserine alone; Curve C: 30% (mol/mol) biotinylated phosphatidylserine in phosphatidylserine; Curve D: phosphatidylserine with free biotin (23% mol/mol, i.e. molar equivalent of that in Curve C). Endotherms B—D were performed in 50% ethylene glycol. 600—800 nmol phospholipids were used for measurements.

of the covalent binding of the vitamin, since free biotin has no effect on ΔH (Curve D).

From the above analyses, it seems that biotinylated phosphatidylserine behaves differently from phosphatidylserine, and at this point, phase separation between the native and derivatized phosphatidylserine cannot be excluded. However, these results are also consistent with a homogeneous lipid distribution, wherein the size of the lipid flow unit [36] is reduced, due to a weaker packing of hydrophobic chains, presumably induced by the biotinylated head groups.

Visualization of liposome-cell interactions

The interaction between biotinylated phosphatidylserine-containing liposomes and the human erythrocyte membrane is shown in Fig. 4. The gaps, which seemingly perforate the trilamellar structure of the erythrocyte membrane, are distinguished by the direct concurrence of ferritin label. These gaps, which measure up to 120 nm in diameter, did not disappear upon incubation nor was further incorporation of biotinyl-lipids into native membrane components observed, as manifested by spreading of the ferritin label. On the other hand, no gaps were observed upon interaction of biotinylated phosphatidylserine-containing vesicles with the lymphocyte cell membrane. Following an incubation period of 30 min, the ferritin label was found to encompass the surface membrane; i.e. biotinylated phosphatidylserine molecules were incorporated into the lymphocyte membrane (Fig. 5). It was observed that the

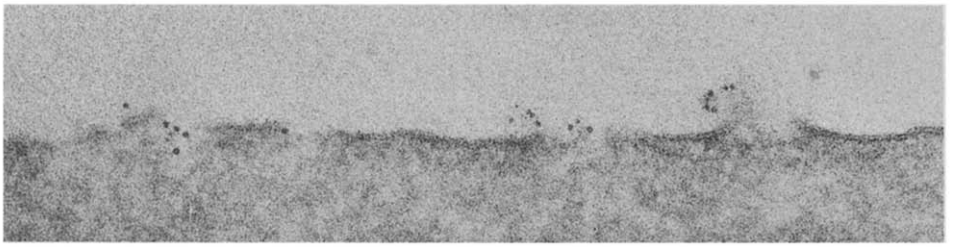

Fig. 4. Human erythrocyte, incubated for 1 h with biotinylated phosphatidylserine-containing liposomes, fixed with aldehydes and labeled with ferritin-avidin conjugates. Note the association of ferritin particles with the gaps in the membrane (X100 000).

interaction between biotinylated phosphatidylserine-liposomes and lymphocytes was apparently restricted to selected fractions of the lymphoid cell population, since some lymphocytes were heavily labeled with ferritin whereas other individual cells were totally void of the label.

Considerable differences were observed in the extent of the biotinylated phosphatidylethanolamine-liposome binding with cells, according to different cell types and/or degree of maturation. Reticulocytes, which were present as a subpopulation in spleen cell preparations, were apparently the most reactive cell type with respect to binding (Fig. 6). The reticulocyte cell membrane was generally associated with intact vesicular structures, the latter being heavily labeled with ferritin particles. From the micrographs, the vesicles were determined to range in size between 40—60 nm in diameter. In most instances, unlike the biotinylated phosphatidylserine-containing liposome-erythrocyte interaction, the reticulocyte membrane remained intact directly beneath the vesicles. On the other hand, lymphoid cells and the mature erythrocyte were very poorly labeled in general; although, in rare instances, individual lymphocyte cells were heavily labeled with the characteristic, intact, biotinylated phosphatidylethanolamine-vesicular structure (Fig. 7). No spreading was observed upon additional incubation, indicating that (in contrast to the biotinylated phosphatidylserine-liposome interaction in Fig. 5) biotinylated phosphatidylethanolamine-liposomes bound to the lymphocyte membrane, but no further incorporation of biotinylated phosphatidylethanolamine took place.

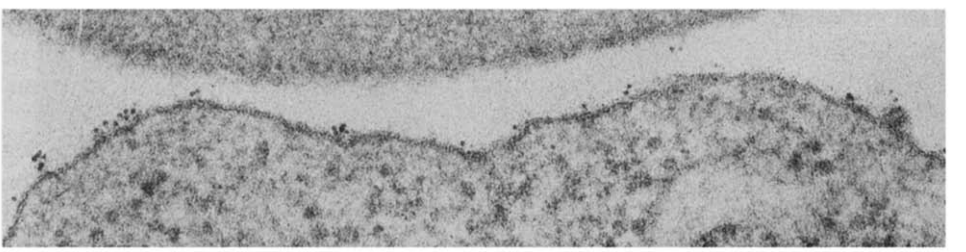

Fig. 5. Mouse lymphocyte, treated as in Fig. 4 (X 100 000).

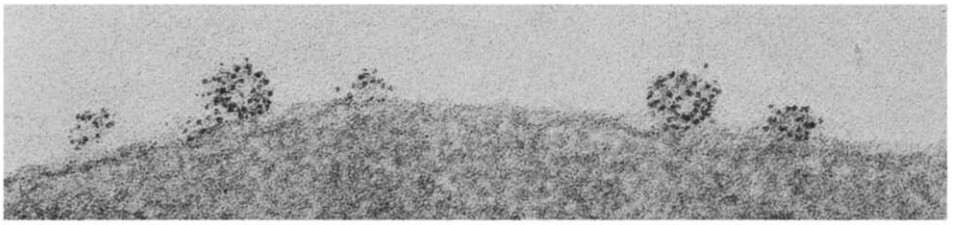

Fig. 6. Mouse reticulocyte, incubated for 1 h with biotinylated phosphatidylethanolamine-containing liposomes, fixed with aldehydes and labeled with ferritin-avidin conjugates. The intact structure of the vesicle is apparently maintained upon further incubation. (× 100 000).

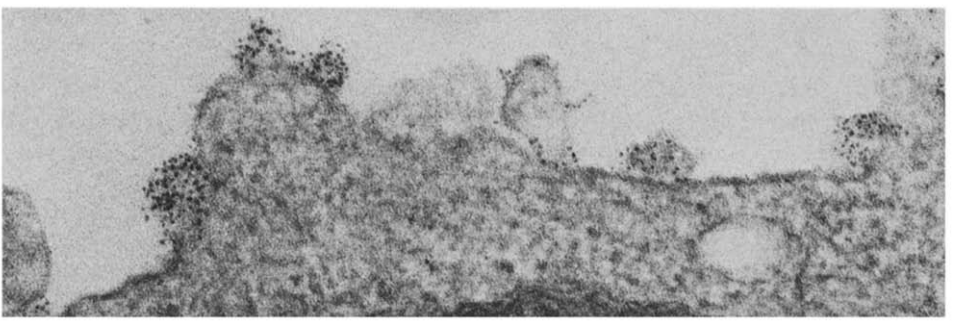

Fig. 7. Mouse lymphocyte treated with biotinylated phosphatidylethanolserine-containing liposomes as in Fig. 6. Most spleen cells, however, were unreactive with biotinylated phosphatidylethanolserine-vesicles, indicating that the above cell might represent a specific subpopulation of splenocytes. (× 100 000).

Discussion

As described in the Results Section, the conjugation of biotin to the headgroups of phospholipids alters the properties of the modified lipid compared to those of the parent compound. The addition of the relatively bulky biotinyl moiety increases the molecular weight of the lipid by about 30%. Furthermore, as a result of the formation of the amide bond, the charge on the amine of the native lipid is neutralized. The modified lipid is thus more acidic than the underivatized lipid. It is therefore stressed that biotinyl lipid is an entirely different entity, as exemplified by the altered differential scanning calorimetry pattern in the case of biotinylated phosphatidylserine versus that of phosphatidylserine. However, the biotinylated molecule, albeit chemically altered, maintains the fundamental properties of a lipid; since it is fully extractable in organic solvents, forms liposomes and mixed liposomes, and is properly orientated in the latter (i.e., the fatty acid components comprise the interior of the bilayer and the biotinyl headgroups are exposed to the aqueous environment of the solvent).

In order to obviate latent ambiguities arising from the inclusion of reactive biotinyl reagents into liposomes, it was decide to approach this problem via the initial synthesis of purified biotinyl-lipids rather than direct biotinylation of liposomes. This experimental tactic was employed in order to preclude nonspecific, residual labeling of surface components by unreacted reagent. The

validity of this argument is supported by the exclusive association of ferritin-avidin conjugates with intramembranous gaps in the erythrocyte (Fig. 4) and with the vesicular structures on the reticulocyte membrane (Fig. 6). In addition, the liposome composition can be more precisely controlled.

Since no detectable signs of lysis nor changes in the bidiscoid shape of the erythrocyte were observed, the appearance of the gaps in the biotinylated phosphatidylserine-treated erythrocyte membrane apparently arises as a function of lipid depletion, due to the effect of dehydrating agents used in the preparation of samples for electron microscopy [37]. The intra-membranous gaps in Fig. 4 were thus interpreted as sites of liposome fusion with the cell surface membrane. The joint presence of ferritin at the site of fusion supports this contention. The appearance of gaps seems to be characteristic of the erythrocyte membrane. In the case of the lymphocyte, no gaps were observed upon incubation with biotinylated phosphatidylserine-liposomes, and exposed biotinylated lipids were found dispersed within the lipid matrix of the membrane — apparently following fusion.

Unlike the biotinylated phosphatidylserine-containing liposome-erythrocyte interaction, the sole appearance of intact labeled biotinylated phosphatidylethanolamine vesicles on the reticulocyte membrane may implicate a different mode of interaction. Again, the ferritin-avidin conjugates label is restricted to anomalous membrane-associated structures. This could indicate a lack of further incorporation of biotinylated lipids into the native reticulocyte membrane. Consequently, under the conditions used in this study, the major biotinylated phosphatidylethanolamine-liposome-cell interaction seems to be an adsorption rather than fusion of the vesicles.

Both biotinylated lipids used in this study are negatively charged molecules. It is interesting and somewhat surprising to note that the more negative of the two (biotinylated phosphatidylserine) seems to undergo more extensive interaction with membrane constituents.

Morphological evidence indicated differences in the pattern or extent of the liposome-cell interaction associated with cell type and/or degree of maturation. The latter phenomenon may be related to differential surface properties between the cell and liposome. This is further suggested by the previous demonstration that electrostatic forces may account for liposome attachment to cell surfaces [38] in conjunction with known cell-specific variations in composition of surface anionic groups [39,40].

The use of biotinylated lipids in affinity cytochemistry for the study of liposome-cell interactions provides several advantages over past techniques. The probe described in this report is lipid-specific and results obtained are directly relevant to the membrane lipid component. Biotinylated lipid derivatives provide us with a non-toxic, lipid-bound hapten, either as an antigenic determinant if used in conjunction with antibiotin antibodies [41] or as a non-antigenic determinant if combined with avidin.

Our previous experience using the avidin-biotin complex in affinity cytochemical studies has indicated that this system provides a highly reliable, highly versatile, general probe for a variety of cell surface receptors and interactions. To date, we have used the avidin-biotin complex for the analysis of cell surface sialic acid sites in the developing erythroid line in the rabbit [42] and for

evaluating the distribution of these cell surface determinants in diseased cells [43—45]. Furthermore, we have extended the universality of the technique to apply to cell surface antigens and lectin receptor sites [28]. It should be mentioned that the avidin-biotin complex has recently been used to analyze the effect of the state of membrane lipids upon the distribution of protein constituents in *Acholeplasma laidlawii* [46,47]. In the latter studies, endogenous membrane proteins were biotinylated and temperature-induced modulations were visualized in the electron microscope via ferritin-avidin conjugates.

In the present study, we have demonstrated the delivery of biotinylated lipids to the cell surface via liposomes. The procedure can be extended to protein constituents by the biotinylation of lipophilic proteins. The latter can be incorporated into liposome membranes and after interaction with cells, their fate can be followed by ferritin-avidin conjugates. The method is applicable to thin sections or deep-etched freeze fracture techniques and permits specific localization of lipids at the electron microscope level of resolution. Additional versatility is inherent in this method, since fluorescent-avidin may be substituted for ferritin-avidin. Consequently, the liposome-cell interaction may also be analyzed in a fluorescent microscope. Moreover, a cell sorter may be used to separate cell populations according to the extent of their interaction with liposomes.

Fixation can be performed either before or after staining with ferritin-avidin conjugates. Different types of liposome-cell interactions, including adsorption, fusion or phagocytosis, may thus be distinguished. By analysis of the interaction at varying time periods, kinetics, turnover and reconstitution studies can be performed. In our laboratory, we are particularly interested in liposome-cell interactions from the mechanistic viewpoint. In this context we are presently studying the kinetics of the above processes in various cell types, including the erythrocyte and its precursors, macrophages and lymphocytes.

Acknowledgments

The authors are indebted to Dr. Diana Bach for help in obtaining and analyzing the differential scanning calorimetry data, to Professor Meir Wilchek for his advice and critical reading of the manuscript and to Mrs. Ora Asher and Mr. Stanley Himmelhoch for their excellent technical assistance.

References

1 Poste, G. and Allison, A.C. (1973) Biochim. Biophys. Acta 300, 421—465
2 Gregoriadis, G. and Ryman, B.E. (1972) Biochem. J. 129, 123—133
3 Gregoriadis, G. and Buckland, R.A. (1973) Nature 244, 170—172
4 Rahman, Y.E., Rosenthal, M.W. and Cerny, E.A. (1973) Science 180, 300—302
5 Allison, A.C. and Gregoriadis, G. (1974) Nature 252, 252
6 Gregoriadis, G. and Neerunjun, E.D. (1975) Biochem. Biophys. Res. Commun. 65, 537—544
7 Poste, G. and Papahadjopoulos, D. (1976) Proc. Natl. Acad. Sci. U.S. 73, 1603—1607
8 Gregoriadis, G. (1976) New Engl. J. Med. 295, 704—777
9 Tyrell, D.A., Heath, T.D., Colley, C.M. and Ryman, B.E. (1976) Biochim. Biophys. Acta 457, 259—302
10 Poste, G., Papahadjopoulos, D. and Vail, W.J. (1976) in Methods in Cell Biology (Prescott, D.M., ed.), Vol. 14, pp. 34—41, Academic Press, New York
11 Bruckdorfer, K.R., Demel, R.A., de Gier, J. and van Deenen, L.L.M. (1969) Biochim. Biophys. Acta 183, 334—345

12 Inbar, M. and Shinitzky, M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 2128—2130
13 Shinitzky, M. and Rivnay, B. (1977) Biochemistry 16, 982—986
14 Rivnay, B., Globerson, A. and Shinitzky, M. (1978) Eur. J. Immunol. 8, 185—189
15 Papahadjopoulos, D., Poste, G. and Schaeffer, B.E. (1973) Biochim. Biophys. Acta 323, 23—42
16 Poole, A., Howell, J. and Lucy, J. (1970) Nature 227, 810—813
17 Ahkong, Q.F., Cramp, F.C., Fisher, D., Howell, J.I., Tampion, W., Verrinder, M. and Lucy, J.A. (1973) Nature New Biol. 242, 215—217
18 Martin, F. and MacDonald, R. (1974) Nature 252, 161—163
19 Martin, F.J. and MacDonald, R.C. (1976) J. Cell Biol. 70, 506—514
20 Papahadjopoulos, D., Poste, G. and Mayhew, E. (1974) Biochim. Biophys. Acta 363, 404—418
21 Huang, L. and Pagano, R. E. (1975) J. Cell Biol. 67, 38—48
22 Pagano, R.E. and Huang, L. (1975) J. Cell Biol. 67, 49—60
23 Weinstein, J.N., Yoshikami, S., Henkart, P., Blumenthal, R. and Hagins, W.A. (1977) Science 195, 489—491
24 Blumenthal, R., Weinstein, J.M., Sharrow, S.O. and Henkart, P. (1977) Proc. Natl. Acad. Sci. U.S. 74, 5603—5607
25 Green, N.M. (1975) Adv. Prot. Chem. 29, 85—133
26 Heitzmann, H. and Richards, F.M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3537—3541
27 Bayer, E.A., Skutelsky, E., Wynne, D. and Wilchek, M. (1976) J. Histochem. Cytochem. 24, 933—939
28 Bayer, E.A., Wilchek, M. and Skutelsky, E. (1976) FEBS Lett. 68, 240—244
29 Bayer, E.A., Skutelsky, E. and Wilchek, M. (1978) Methods Enzymol. 62D, in the press
30 Martin, F.J. and MacDonald, R.C. (1976) J. Cell Biol. 70, 515—526
31 Bayer, E.A., Rivnay, B., Wilchek, M. and Skutelsky, E. (1978) Israel J. Med. Sci., in the press
32 Bayer, E.A. and Wilchek, M. (1974) Methods Enzymol. 34, 265—267
33 McCormick, D.B. and Roth, J.A. (1970) Methods Enzymol. 18A, 383—385
34 Karnovsky, M.J. (1965) J. Cell Biol. 27, 137a—138a
35 Papahadjopoulos, D. (1977) J. Coll. Interface Sci. 58, 459—470
36 Shinitzky, M. and Inbar, M. (1976) Biochim. Biophys. Acta 433, 133—149
37 Stein, O. and Stein, Y. (1971) Adv. Lipid Res. 9, 1—72
38 Martin, F.J. and MacDonald, R.C. (1976) J. Cell Biol. 70, 494—505
39 Skutelsky, E. and Danon, D. (1970) J. Membrane Biol. 2, 173—179
40 Skutelsky, E. and Farquhar, M.G. (1976) J. Cell Biol. 71, 218—231
41 Berger, M. (1975) Biochemistry 14, 2338—2342
42 Skutelsky, E., Danon, D., Wilchek, M. and Bayer, E.A. (1977) J. Ultrastruct. Res. 61, 325—335
43 Kahane, I., Polliack, A., Rachmilewitz, E.A., Bayer, E.A. and Skutelsky, E. (1978) Nature 271, 674—675
44 Skuteslky, E., Bayer, E.A., Wilchek, M., Matzner, Y., Kahane, I. and Polliack, A. (1978) Proc. 12th Int. Leuk. Cult. Conf., BeerSheba, in the press
45 Skutelsky, E., Hoglund, S., Morein, B. and Bayer, E.A. (1978) Proc. 9th Int. Cong. Elect. Microsc., Toronto, in the press
46 Wallace, B.A., Richards, F.M. and Engelman, D.M. (1976) J. Mol. Biol. 107, 255—269
47 Wallace, B.A. and Engelman, D.M. (1978) Biochim. Biophys. Acta 508, 431—449