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Supramolecular biovectors (SMBV): a new family of nanoparticulate drug delivery systems. Synthesis and structural characterization

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Summary

This paper describes a new drug delivery system, supramolecular biovectors (SMBV), and particularly the physical properties of the peripheral shells composed of fatty acids and phospholipids which permit the dispersion of SMBV in aqueous media. Size determination of SMBV prepared with various fatty acids (C8–C16) and two phospholipids (DMPC or DPPC) was compared to that of liposomes prepared with the same phospholipids, as fabrication controls. Theoretical calculations and experimental results confirmed the hypothesis that phospholipids are deposited as a monolayer around the acylated core of the SMBV. Polarization of the probe DPH included in the phospholipids gave information on the physical state of the phospholipid monolayer adsorbed on the surface of the SMBV and on the nature of the interactions between the phospholipids and the fatty acids.

Key words: Drug delivery system; Liposome; Fluorescence depolarization; Lipid phase transition temperature

Introduction

The therapeutic efficiency of numerous drugs is limited by their lack of specificity towards a cellular target. The major part of the drug is then not only lost for the intended therapeutic effect but also gives rise to unwanted and possibly damaging side effects elsewhere. These shortcomings could theoretically be offset by the use of a specific carrier to release the drug only upon contact with the intended target, i.e., the 'sick' cell. Some results have already been obtained with various carriers such as liposomes or nanoparticles but these systems are limited by a number of drawbacks such as poor drug entrapment capability or trapping by the reticuloendothelial system, so far preventing them from gaining wide acceptance by the pharmaceutical industry.

As a complex biochemical unit, the organism

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Abbreviations: SMBV, supramolecular biovector; C8, caprylic acid; C12, lauric acid; C16, palmitic acid; BV-C8, SMBV with a polysaccharide core acylated by caprylic acid; BV-C16/SA 75:25: SMBV with a polysaccharide core acylated by palmitic acid and succinic acid in an initial molar ratio of 75:25; DMPC, dimyristoylphosphatidylcholine; DPPC, dipalmitoylphosphatidylcholine; SUV, small unilamellar vesicles; DPH, 1,6-diphenyl-1,3,5-hexatriene.

is, however, already endowed with a number of existing delivery systems. Therefore, instead of trying to design artificial carriers, one could merely follow established delivery routes. Some of the most interesting natural carriers are the low density lipoproteins (LDL). These cholesterol carriers are small vesicles (20 nm in diameter) composed of an internal cholesterol core surrounded by a monolayer of phospholipids in which a specific protein, apolipoprotein B, is embedded. LDL have the ability to diffuse freely within the organism and the delivery of cholesterol to the cell is achieved through receptor-mediated endocytosis. Although LDL receptors are found on the surface of every cell, their expression differs widely and this differential expression can be used as a basis for cell targeting, to tumour cells in particular (Bijsterbosh and Van Berkel, 1990). The use of LDL as delivery systems is, however, restricted both by their ability to entrap only lipophilic drugs, most drugs used in therapeutics being hydrophilic, and also by the difficulties encountered in their preparation and handling.

The supramolecular biovectors (SMBV) (Samain et al., 1989) are new drug delivery systems which were built to mimic LDL. They are externally composed of a phospholipidic shell onto which proteins can be anchored. The lipoprotein lipidic core has been replaced by a cross-linked natural polysaccharide which has been regioselectively acylated on its surface with fatty acids to give it a peripheral hydrophobic character (Fig. 1). Polysaccharidic cores can be engineered to sizes from 10 nm to a few microns and they confer to SMBV particles a great stability. The structure of SMBV allows entrapment of various drugs, lipophilic, amphiphilic or hydrophilic. It can be adapted to a particular drug, by grafting of ionic ligands in the fatty acid layer or onto the polysaccharide nucleus thus permitting retention by an ion-exchange mechanism. Drug entrapment can be achieved with a high loading ratio and nearly quantitative yields. So, therapeutic applications of SMBV are very wide: cancer therapy, oral delivery of non-intestinally absorbed drugs, immunotherapy, etc.

This paper deals with the structural characterization of two types of SMBV, neutral SMBV described as type I and SMBV whose internal lipidic layer was grafted with both fatty acids and succinic acid as ionic ligand (type II). SMBV were first characterized with respect to their fatty acid, succinic acid and phospholipid contents and then studied for the properties of their phospholipid layer. Indeed, this external shell is essential for the dispersion of SMBV in aqueous media and thus it was important to determine its structural state. For this purpose, measurements of the polarisation P of a fluorescent probe, DPH (Shinitzky and Barenholz, 1978), included in the peripheral layer, were run on type I SMBV with different fatty acid compositions and on type II SMBV with various palmitic to succinic acid ratios. All experiments were performed in compari-

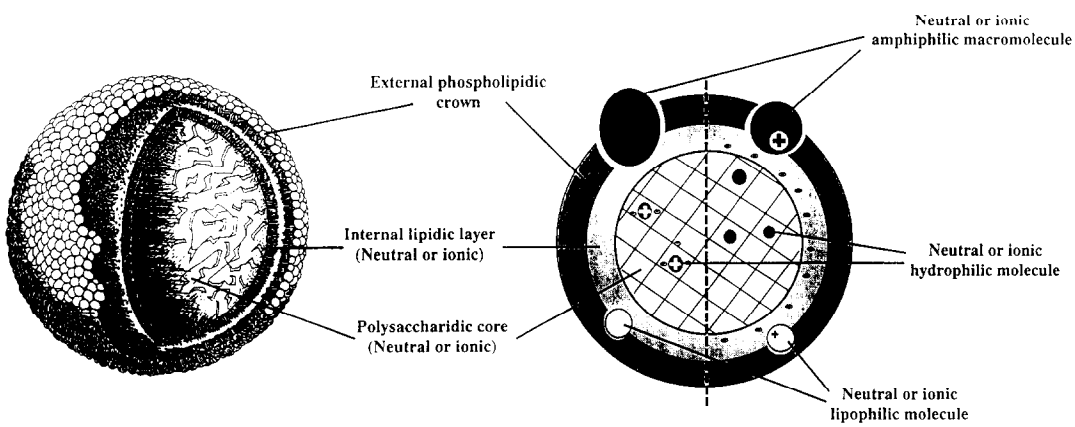

Fig. 1. Schematic representation of the structure of the SBMV.

son with liposomes (SUV) containing the same phospholipids, as a model system. In this way, we were able to demonstrate coupling between the acylated polysaccharide core and the peripheric phospholipid layer which was dependent on the fatty acid chain length and the nature of the phospholipids.

Materials and Methods

Chemicals

Dextran (Mol. Wt 229000), dimyristoylphosphatidylcholine (DMPC), dipalmitoylphosphatidylcholine (DPPC) and palmitic acid were purchased from Sigma Chemical Co. (St Louis, MO). Capryloyl, lauroyl and palmitoyl chlorides, epichlorhydrin, 4-dimethylaminopyridine, succinic anhydride, succinic acid, cupric nitrate trihydrate, triethanolamine, sodium diethylthiocarbamate, oxalyl chloride, ascorbic acid, ammonium molybdate and magnesium nitrate hexahydrate were obtained from Fluka (Büchs, Switzerland). 1,6-Diphenyl-1′,3′,5-hexatriene (DPH) was from Molecular Probes (Eugene, OR). [1-^{14}C]Palmitic acid (53.7 mCi/mmol) was purchased from CEA (Saclay, France). All other chemicals and solvents were commercially available and at least reagent grade.

Equipment

Radioactivity measurements were performed on a Beckman LS 1801 scintillation counter. The HPLC system was composed of a Model 302 Gilson pump, a Rheodyne Model 71-25 injector and a Model 802C Gilson manometric module. A Coulter N4MD nanoparticle analyzer (Coultronics, Margency, France) was used to determine the size of the liposomes and the SMBV. A statistical size distribution processor (SDP analysis) provided histograms of the size distribution of the particles in the sample studied.

Preparation of SMBV

SMBV were synthesized as described by Samain et al. (1989).

Preparation of the cross-linked polysaccharide core Under vigorous shaking, dextran (150 g) was dispersed in 150 ml of 1 M NaOH to homogeneity. Then, epichlorhydrin (12 ml) was added for cross-linking. Shaking was stopped and the reaction was left to proceed at 80°C for 10 h. The gel obtained was diluted with water and mechanically ground in a Waring Blendor. Cross-linked dextran was treated either by sonication with a probe-type sonicator (Sonifier Branson 250), or by the extrusion technique with a French Press cell (Aminco, Silver Spring, MD) subjected to an internal pressure of 140 MPa, in order to provide sizes over a range of several nanometers. The nanoparticles so obtained were centrifuged at $6000 \times g$ to eliminate particles greater than 100 nm. The upper phase, containing a monodispersed population of nanoparticle cross-linked dextran cores, was spray-dried at an external temperature of 170°C and an internal temperature of 100°C (Laboratory spray-dryer 190, Büchi).

Synthesis of the first hydrophobic layer Cross-linked dextran nanoparticles (100 g) were dispersed in dichloromethane with 4-dimethylaminopyridine (75 g). Fatty acid chlorides were added to reach the ratio of 1 mole for 1 mole equivalent of glucose. When necessary, succinic anhydride was substituted to a fraction of fatty acid chloride in variable amounts to give type II SMBV. The reaction was carried out under anhydrous conditions at 37°C for 20 h. Dichloromethane was removed under vacuum and the particles were washed with ethanol by centrifugation at $1000 \times g$ for 5 min. Lipophilic nanoparticles were stored in ethanol at 4°C.

Synthesis of the external phospholipid layer Lipophilic cores and phospholipids dispersed in ethanol were injected into water at 50°C (above the phospholipid phase transition temperature) and kept for 30 min with vigourous stirring every 5 min. The suspension was extruded through the French press (internal pressure 140 MPa) and then centrifuged at $10000 \times g$ for 20 min to remove aggregates. SMBV were recovered in the upper phase and their diameter was measured.

Estimation of fatty acids bound to type I SMBV

Estimation of fatty acids bound using radiolabelled palmitic acid Titration of bound fatty acids by a radiolabelling technique needed the synthe-

sis of [1-^{14}C]palmitoyl chloride. 2.16 μCi of [1-^{14}C]palmitic acid and 10 g of palmitic acid were dissolved in diethyl ether under anhydrous conditions. 6 ml of oxalyl chloride were added and the reaction was carried out for 15 h under continuous magnetic stirring. The [1-^{14}C]palmitoyl chloride was obtained after evaporation under reduced pressure and stored at -20°C. It was used for synthesis of the fatty acid layer on cross-linked polysaccharide cores as described before. After acylation and centrifugation, lipophilic nanoparticles were hydrolyzed in a solution of 1 M KOH in methanol, at 90°C for 2 h, neutralized and after centrifugation, the upper phase was drawn off. Scintillation cocktail was added to the samples and radioactivity was counted in an LS1801 Beckman scintillation counter (Berkeley, U.S.A.). Measurements on the supernatants in ethanol gave the fraction of free [1-^{14}C]palmitoyl chloride and determinations on the hydrolyzed radiolabelled lipophilic particles provided the fraction of fatty acids bound to the particles.

Colorimetric estimation of bound fatty acids The level of fatty acids bound to the particles was estimated by colorimetric titration according to the method of Lauwerys (1969). Briefly, fatty acids bound to the polysaccharide core were extracted by hydrolysis with a solution of 1 M KOH in methanol at 90°C for 2 h, neutralized by a solution of 1 M HCl in methanol and the organic solvent was eliminated by evaporation under reduced pressure. Residues were redissolved in 0.01 M sulfuric acid and four successive extractions with chloroform/ether 3:1 were performed. After solvent evaporation, the free fatty acid fraction was dissolved in heptane. The reagent for colorimetric determination ('copper reagent') was prepared by dissolving 3.25 g of cupric nitrate trihydrate, 6.25 g of potassium sulfate, 17 g of triethanolamine and 0.3 ml of acetic acid in water (100 ml). 3 ml of the fatty acid extract in heptane were added to 3 ml of chloroform and 3 ml of copper reagent. After vigourous shaking for 5 min and centrifugation at $2500 \times g$ for 10 min, 3 ml of the chloroform/heptane upper phase received 0.5 ml of sodium diethylthiocarbamate (0.1% w/v) in *n*-butanol. After mixing, the absorbance was read at 435 nm against a reference solution of 3 ml of chloroform/heptane (1:1 v/v) and 0.5 ml of sodium diethylthiocarbamate reagent. A standard of palmitic acid subjected to the same procedure was used to construct the calibration curve.

Estimation of fatty acids bound to type II SMBV by an HPLC method

Acylated and succinylated polysaccharide cores dispersed in ethanol were suspended in 2 M NaOH/0.03% sodium dodecyl sulfate and kept at 65°C for at least 48 h, with continuous stirring. Subsequently, they were acidified with a solution of 5 M H_2SO_4 to pH 4–5. An aliquot was diluted with methanol, centrifuged for 30 min at $5000 \times g$ and analyzed by HPLC. Chromatographic analysis was performed on a reverse-phase column (Nucleosil-C18, 10×0.4 cm i.d., d_p 5 μm) with methanol/0.1% trifluoroacetic acid. A light diffraction apparatus (DDL11, Cunow) was used for detection. Chromatographic conditions were as follows: flow rate, 0.8 ml/min; injection volume, 20 μl; detection temperature, 40°C; detector pressure, 2.3 bar. Standards of fatty acids were subjected to the same procedure.

Determination of succinic acid bound to type II SMBV

Methanolic aliquots of acylated and succinylated polysaccharide cores were submitted to the treatment described before. The succinic acid content was measured by HPLC on a Spherisorb SAX, 5 μm–25 cm (Chromato-Sud, Bordeaux, France). Trifluoroacetic acid (0.04% in water) was used as eluent. Analyses were run at a flow rate of 1 ml/min and detection was performed at 210 nm (UV-Visible detector SPD-2A Shimadzu Co, Kyoto, Japon).

Estimation of phospholipid concentration

Colorimetric determination of phospholipids The amount of phospholipid around the SMBV was estimated by phosphorus determination according to the method of Ames and Dubin (1960).

Enzymatic determination of phospholipids The amount of phospholipid surrounding the SMBV was estimated using an enzymatic determination kit (Bio-Mérieux, France). The enzyme prepara-

tion consisted of choline oxidase (> 2000 IU/l), phospholipase D (> 600 IU/l), peroxidase (> 1000 IU/l) and amino-4-antipyrine (0.5 mmol/l), dissolved in a Tris pH 7.8 (20 mmol/l)/surfactant (3 mmol/l)/phenol (10 mmol/l) buffer. Various amounts of SMBV dispersed in 10 ml of water were added to 1 ml of enzyme preparation. After 10 min incubation at 37°C, samples were centrifuged at $15000 \times g$ for 15 min and the absorbance of the upper phases was read at 505 nm. DPPC liposomes were used as standard.

Phospholipid dispersion

Dried lipids (DMPC and DPPC, 5 mg/ml) were suspended in a 150 mM NaCl solution by gentle vortex agitation for 30 min at a temperature above the lipid transition temperature (50°C). Liposomes were then sonicated in a bath for 60 min until a clear solution was obtained.

DPH incorporation into liposomes and SMBV

The fluorescent probe DPH was incorporated into the liposomes and SMBV by simple diffusion. Its partition coefficient is greatly favourable to the lipid phase. In order to obtain a good signal and a very small disturbance from the probe, a DPH/phospholipid ratio of 5:1000 was used for the systems.

Fluorescence polarisation measurements

Experiments were carried out with a T-format automatic apparatus of our fabrication connected to a microcomputer. The excitation channel consisted of a light source (mercury or xenon arc lamp), a shutter, a monochromator, and a rotating polarizer (Glan prism) polarizing the incident light alternatively either vertically (fluorescence polarization measurements) or horizontally (determination of the relative sensitivity of the two detection systems). The detection system comprised two independent channels measuring simultaneously, the vertical I_V and the horizontal I_H components of the fluorescence emission. Samples were placed in a closed, stirred and thermostated housing system, the temperature of which was monitored (Peltier element) from 4 up to 60°C. A quartz optic was used allowing experiments to be carried out in the UV. Fluorescence polarization P is given as $=(I_V - I_H)/(I_V + I_H)$. In these experiments, the absorbance of the solutions (UV 350 nm) was never greater than 0.1.

Results and Discussion

Synthesis of SMBV: type I

As previously described (Samain et al., 1989), cross-linking of dextran was achieved by use of epichlorhydrin, which reacts on the hydroxyl groups of saccharides. It gave rise to a gel which, after mechanical crushing, provided particles about 10 μm in diameter. High-pressure fragmentation (French press) led to particles a few nanometers in size. In order to obtain a homoge-

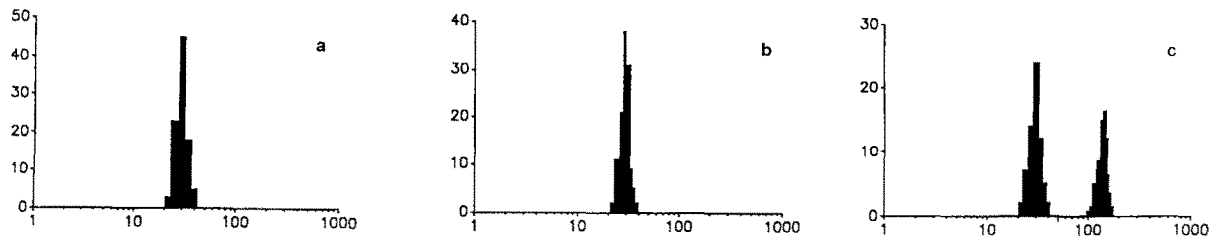

Fig. 2. Size histograms of (a) polysaccharide cores, (b) SBMV and (c) SBMV preparation containing liposomes. SMBV used were SMBV-C16-DPPC.

neous population in size, a centrifugation step was needed to discard any aggregates formed. Then, and as shown in Fig. 2a, a suspension of solid cross-linked dextran cores with the desired diameter was obtained (30 nm for this study).

In order to synthesize the first lipophilic layer on the surface, the polysaccharide cores were first completely dried. Then, the binding reaction of the fatty acids was carried out in an aprotic solvent in which saccharides are insoluble. Thus, fatty acid chlorides react only with the saccharide hydroxyl groups located at the core periphery. This process allowed us to preserve an internal hydrophilic core and to maintain the structure of the particles. The particles then bear an externally lipophilic layer which permits their further coating with a phospholipid layer.

The synthesis of the phospholipidic shell uses methods derived from liposome technology. For this purpose, we made the assumption that the phospholipids were organized as a monolayer around the acylated cores, conferring to the particles the hydrophilicity required for their dispersion in water. Moreover, it was necessary to devise a synthesis method yielding liposome-free SMBV suspensions. Binding of the phospholipid layer around the acylated cores was achieved by injection of an organic solution of phospholipids into an aqueous medium as described previously for liposomes (Batzri and Korn, 1973). Phospholipids and acylated cores were suspended in ethanol and completely dispersed by sonication. Subsequently, they were injected into water brought to a temperature above that of the gel-to-liquid phase transition of the lipids (50°C). The preparation of perfectly dispersed SMBV needed a high-energy process: high-pressure extrusion in a French press. As can be seen in Fig. 2b, we obtained a homogeneous population of SMBV with a defined size: 32 nm. The amount of phospholipid necessary for complete coating of the acylated cores but without liposome formation in the medium was first estimated by theoretical calculation integrating the diameter and the volumic mass (approximated to unity) of the polysaccharide cores and the average molecular area of the phospholipids. For example, for a 15 nm core radius, the calculated proportion of phospholipid is 50% by weight of acylated cores. The best experimental conditions were determined by modifications of the phospholipid amounts around this value of 50%. A lack of phospholipid was immediately apparent on aggregation of incompletely coated acylated cores. Conversely, an excess of phospholipids with regard to the acylated cores induced the formation of unwanted liposomes. The presence of these vesicles was readily identified by size analysis distribution: Fig. 2c shows the existence, beside SMBV, of a 100–200 nm population which corresponds to liposomes. Experimentaly, it was determined that the optimal level of phospholipids which was required to cover the acylated cores of 30 nm diameter was 75% by weight of acylated cores. The discrepancy observed between the theoretical (50%) and experimental values (75%) may be explained by the use of average values for some parameters. For example, a molecular area of 75 $Å^2$ was used for the phospholipids. In fact, as has been described for liposomes (Huang and Mason, 1978), this value may vary according to the localisation of the lipids inside or outside the vesicle and to its curvature radius.

The postulated structure of SMBV, composed of a phospholipid monolayer assembled around a peripherally acylated core, led us to propose different patterns of phospholipid organization according to the various fatty acids grafted on the polysaccharide core. For this purpose, we synthesized type I SMBV with different grafted fatty acids: caprylic, lauric and palmitic acids.

Characterization of type I SMBV

Estimation of the level of fatty acid binding

Two methods were used to determine the degree of acylation: radioactivity measurement and colorimetric assay according to Lauwerys (1969).

Pure [^{14}C]palmitoyl chloride used to acylate the polysaccharide core was obtained by reaction of oxalyl chloride on [^{14}C]palmitic acid. Under these conditions, the level of fatty acid binding, estimated by the difference between the initial quantity of ^{14}C used and the quantity of fatty acid present in the supernatant after washing the acylated cores, was 39%. The proportion of fatty acid binding was evaluated for various fatty acids

TABLE 1

Fatty acid binding rates, expressed by weight of fatty acids bound by weight of polysaccharidic cores and by the molar ratio fatty acid / glucose equivalent

Fatty acid	Fatty acid/PS core (w/w) (%)	Fatty acid/glucose (mol/mol)
Caprylic (C8)	26	0.29
Lauric (C12)	38	0.31
Palmitic (C16)	44	0.28

Values obtained by colorimetric titration as described in Materials and Methods.

(caprylic, lauric and palmitic acid) by colorimetric determination and was expressed as weight of fatty acids bound per weight of polysaccharide cores and as the molar ratio of fatty acid/glucose (Table 1). The levels of fatty acid binding were found to be approximately of the same order for the various fatty acids tested. When expressed as a molar ratio between the number of fatty acid molecules and that of glucose molecules, the results showed that, irrespective of the fatty acid used, the number of hydroxyl groups located at the periphery of the core and reacting with the fatty acid chloride was always the same (around 0.3 mol of fatty acid per mol of glucose). The theoretical proportion of fatty acid covering a particle of 30 nm in diameter, calculated according to the molecular area of the fatty acid, always gave greater values than those found experimentally. For example, palmitic acid binding was theoretically 51% by weight of the polysaccharide core as compared to 44 % experimentally. These differences may be explained by the reticulation of the core which is not accounted for by the theoretical calculation.

These results suggest that there is no peracylation (which would give higher binding values) and that only the hydroxyl groups located at the surface of the polysaccharide core are acylated. As postulated in the design of the SMBV, acylation in a heterogeneous phase allows the particles to be uniformly covered with fatty acids.

Estimation of the proportions of phospholipid binding As described in Materials and Methods, after checking for the absence of liposomes in the medium, the proportion of phospholipids covering the particles was determined according to Ames and Dubin (1960). For DPPC, 74% binding per weight of acylated core was measured. This corresponds to the quantity necessary to cover the acylated cores (see SMBV synthesis). This value was confirmed by an enzymatic assay using the reaction of DPPC with the phospholipase D (results not shown).

Type II SMBV: synthesis and characterization

In order to prepare SMBV which may encapsulate drugs charged positively at physiological pH, type II SMBV were synthesized, in which the internal lipid leaflet carried negative charges. In this case, during the acylation reaction, succinic anhydride was added to react with the hydroxyl groups of the polysaccharide core. Succinic anhydride is biocompatible but its presence could modify the overall hydrophobicity of the acylated particles and thus, the subsequent fixation of phospholipids.

Table 2 shows the proportion of palmitic acid and succinic acid bound to the polysaccharide core (w/w), vs their initial molar ratio in the mixture. It is clear that, when the quantity of succinic acid was increased, the rate of palmitic acid binding decreased drastically (from 46 to 5% with 0 to 40% succinic acid, respectively). An explanation may be that the anionic group (succinate) increased the polarity of the core which then became less accessible to the fatty acids.

TABLE 2

Palmitic and succinic acid binding rates, expressed by the weight of each acid bound by weight of polysaccharidic cores and by the molar ratio acid / glucose equivalent

Palmitic/succinic initial molar ratio	Palmitic acid bound per polysaccharidic core		Succinic acid bound per polysaccharidic core	
	w/w	mol/mol	w/w	mol/mol
100:0	46%	0.29		
95:5	39%	0.25	0.63%	0.01
75:25	17%	0.11	1.5%	0.024
60:40	5%	0.032	4.5%	0.07

Values obtained by HPLC analysis as described in Materials and Methods.

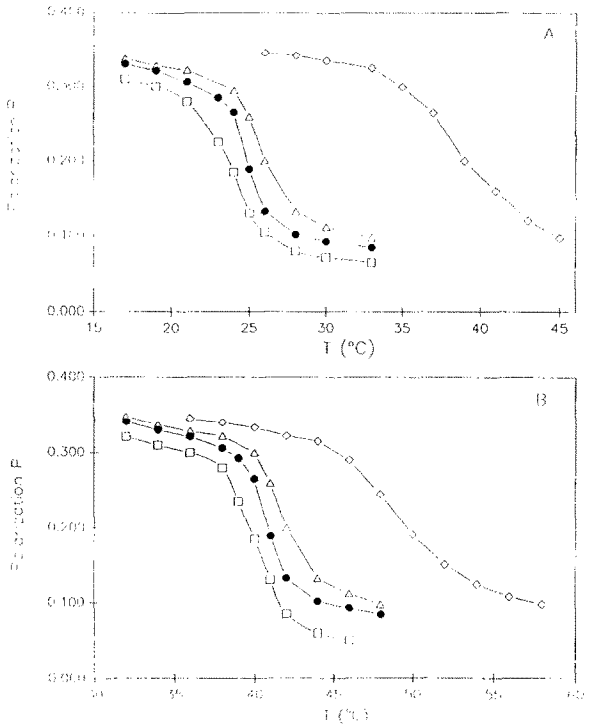

Fig. 3. Fluoresence polarisation (P) of DPH in SUV (●) and type I SMBV composed of DMPC (A) or DPPC (B) in the peripheral layer. Polysaccharide cores of SBMV were acylated with C8 (□), C12 (△) or C16 (◇) fatty acids.

The quantity of phospholipid strictly necessary to cover the acylated cores was estimated at 75% by weight of the acylated cores.

Organization of the phospholipidic layer around the acylated cores

Fig. 3 shows the polarization P of the fluorescent probe DPH introduced into liposomes or into type I SMBV constituted with DMPC (A) or DPPC (B) surrounding acylated polysaccharide cores previously grafted with various fatty acids (C8, C12, C16). Liposomes were used as a reference to localize the gel-to-liquid phase transition of the lipids.

The first observation which could be made was that in SMBV acylated with short-chain fatty acids (C8, C12), the transition temperature of DMPC (theoretically 24°C) was slightly modified as compared to liposomes. This strongly suggests the existence of a continuous and homogeneous external phospholipid layer which behaved thermodynamically as if it was not energetically coupled to the supporting fatty acid hydrophobic layer. This is an interesting observation which strongly supports the contention that in phospholipid bilayers, each leaflet behaves independently of the other (Tocanne, 1992).

In contrast, when the particles were acylated with long-chain fatty acids (C16), a considerable increase in the transition temperature of the phospholipid was observed for DMPC (from 25 to 35°C) as well as for DPPC (from 42 to 50°C) (Fig. 3A and B). This corresponds to a marked rigidification of the external phospholipid layer, as indicated by the absence of mobility of the probe DPH. It is clear that in this case, the phospholipid layer and the supporting fatty acid layer interacted together. Such an increase in the phase transition temperature of DPPC indicates that the lipid molecules were more tightly packed. This might originate from an anchoring of the palmitoyl chain into the phospholipid layer, giving rise to a molecular structure similar to the interdigitated phase described for various phospholipids (DPPC, DPPG) in the gel state (Ranck and Tocanne, 1982a,b; Slater and Huang, 1988). Such an interdigitation would not occur if the supporting hydrophobic layer was composed of shorter chain fatty acids (C8, C12).

When the acylated cores were synthesized in the presence of increasing quantities of succinic

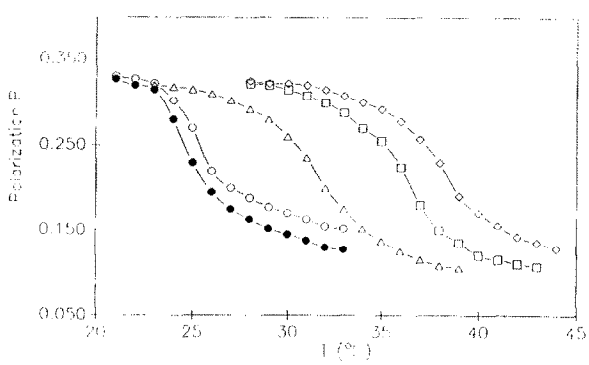

Fig. 4. Fluoresence polarisation (P) of DPH in DMPC/SUV (●) and ionic DMPC/SMBV. Polysaccharide cores of SBMV were acylated with C12 (◇) or C16/succinic acid with initial molar ratios of 95:5 (□), 75:25 (△) or 60:40 (○).

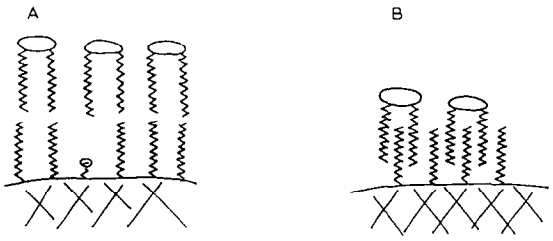

Fig. 5. Two models proposed for the peripheral organization of fatty acids and phospholipids around the polysaccharide core of SMBV. (A) SMBV with short-chain fatty acids (C8, C12). (B) SMBV with long-chain fatty acids (C16).

anhydride, 95:5, 75:25 or 60:40 (Fig. 4), the corresponding type II SMBV returned to a more liposomal behaviour and the transition temperature values indicated a progressive return to a fluid state for the phospholipid layer. It may be assumed that the ionic character of the internal layer due to the presence of succinate could induce repulsive forces between fatty acid chains. This would not favour the ordered state of the rigidified layer as described for interdigitation (Slater and Huang, 1988).

On the basis of these preliminary results, we can propose two models of organization of the particles (Fig. 5):

(i) A first model for SMBV synthesized with short-chain fatty acids (C8, C12), where acyl chains behave independently from each other.
(ii) A second model for SMBV synthesized with long-chain fatty acids (C16), where the lipid molecules are in a more tightly condensed state.

Conclusion

In this paper, we have described the synthesis of a new drug delivery system, the supramolecular biovector, using techniques which allowed us to obtain populations with homogeneous size and good dispersibility. Moreover, we were able to propose a description of the organization of the phospholipid monolayer surrounding the particle, depending on the fatty acid grafted onto the polysaccharide core and on the phospholipid chosen for the outer shell. It appeared that the use of a long-chain fatty acid, irrespective of the phospholipid used, induced intense rigidification of the phospholipid shell, which would give rise to greater retention for drugs entrapped in the SMBV. Derivatization of the internal lipid layer with succinic acid reduced the rigidification but the decrease was compensated by the presence of ionic functions allowing the incorporation and retention of basic drugs.

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