Biochimica et Biophysica Acta, 1106 (1992) 23–30

BBAMEM 75608

The stability and functional properties of proteoliposomes mixed with dextran derivatives bearing hydrophobic anchor groups

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(Received 18 November 1991)

Key words: Proteoliposome; Integral membrane protein; Dextran derivative; Hydrophobic anchor group

Liposomes composed of *Escherichia coli* phospholipid were coated with polysaccharides bearing hydrophobic palmitoyl anchors. The effect on the stability of liposomes without or with integral membrane proteins was investigated. A high concentration of hydrophobized dextrans protected the liposomes against detergent degradation, decreased the fluidity of the membranes, prevented fusion of the liposomes and enhanced their stability. Proteoliposomes containing beef heart cytochrome-*c* oxidase and the lactose transport carrier of *E. coli* were similarly affected by coating with the dextrans. Under these conditions both membrane proteins were still active. Long-term stability of the coated liposomes was obtained only in the absence of the integral membrane proteins.

Introduction

In bacterial cells the cytoplasmic membrane is covered with cell wall components which increase the mechanical stability of the cytoplasmic membrane and the cells. Such a stabilization would offer attractive experimental possibilities for artificial membranes or isolated biomembranes. Different approaches to stabilize these membranes have been described [1]. One approach has been the fixation of amphiphilic polymers via hydrophobic anchor groups to lipid bilayers [1,2].

The influence of amphiphilic polyvinylpyrrolidone and dextran derivatives on the morphology of giant unilamellar liposomes has been investigated [2]. Fixation of the polymers to the liposomal membranes was demonstrated with a fluorescent-labelled derivative of the dextrans. Exclusive interaction with the outer membrane of multilamellar liposomes was observed by freeze-fracture electron microscopy. Wolf et al. [3] studied the distribution and lateral diffusion of similar polymers in black lipid membranes composed of cholesterol and egg phosphatidylcholine. Coating of liposomes with polysaccharides was also used in targetting studies. Takada et al. [4] and Sunamoto et al. [5] observed an improved drug delivery to target specific organs after coating of liposomes. Liposomes (egg phosphatidylcholine/cholesterol, 3 : 1) coated with polysaccharides and labelled with [^{14}C]coenzyme Q_{10} showed increased lung uptake and a decrease in urinary excretion [4]. This observation indicated that the coated liposomes were more stable then the conventional ones. Further evidence for the increased stability of the coated liposomes was the reduced permeability for carboxyfluorescein of the coated liposomes and the increased resistance against enzymatic lysis by phospholipase D [5]. Increased long-term stability was also observed in black lipid membranes coated with polysaccharides [6]. Black lipid membranes composed of glycerol monooleate which are stable for about one hour, became stable for up to two days after coating with polysaccharides.

In our attempts to construct proteoliposomes with a greater chemical and mechanical stability we investigated the stability of liposomes composed of *Escherichia coli* lipids when coated with amphiphilic poly-

Abbreviations: PE, phosphatidylethanolamine; DEX-1, polysaccharide dextran esterified with palmitic acid anchors; R_{18}, octadecyl-rhodamine β chloride; DPH, 1,6-diphenyl-1,3,5-hexatriene; CF, 5,6-carboxyfluorescein; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine; TPP^+, tetraphenylphosphonium ion; decyl-PEG, decylpolyethylene glycol 3000; δp, transmembrane electrical proton gradient; $\Delta\psi$, transmembrane electrical potential; ΔpH, transmembrane pH gradient.

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mers. Liposomes composed of *E. coli* lipids are commonly used for the study of the function of reconstituted transport proteins. For these studies a linear polysaccharide dextran partially esterified with palmitic acid [2] was used (Fig. 1). When added to liposomes the hydrophobic anchors interact with the outer half of the phospholipid bilayer and the hydrophilic portion extends into the aqueous phase. In this way a two-dimensional network of polymers is formed around the liposomes.

Materials and Methods

Materials. L-α-Phosphatidylethanolamine (PE) from *Escherichia coli* (type IX) was obtained from Sigma Chem Co. (St Louis, MO, USA). The phospholipid preparation was further purified by acetone-ether extraction as described [7] and stored in chloroform under nitrogen at −20°C. The hydrophobized dextran (DEX-1) was synthesized at the Institute of Organic Chemistry, University of Mainz. [D-*glucose*-1-^{14}C]Lactose was from Amersham. *n*-Octyl β-D-glucopyranoside was from Boehringer (Mannheim Gmbh) and octadecyl rhodamine β chloride (R_{18}) and 1,6-diphenyl-1,3,5-hexatriene (DPH) were obtained from Molecular probes, Inc (Junction City, OR); 5,6-carboxyfluorescein (CF) was obtained from Eastman Comp (Rochester, NY). CF was purified by one-step adsorption chromatography on a Sephadex LH-20 column as described [8].

Preparation of liposomes. E. coli phospholipids were dried with a rotary evaporator and suspended in 50 mM K-phosphate (pH 7.5), at a concentration of 20 mg lipid/ml. Liposomes were obtained by sonication till clarity with a probe-type sonicator (MSE Scientific Instruments, West Sussex, UK), using intervals of 15 s sonication and 45 s rest, at 4°C under a constant stream of N_2. The preparation was then stored in liquid N_2. Before use a small sample was thawed at room temperature and sonicated for 5 s with a probe sonicator.

Isolation and reconstitution of cytochrome-c oxidase in proteoliposomes. Mitochondria were isolated from bovine heart as described [9]. Cytochrome-*c* oxidase was isolated according to the procedure of Yu et al. [10]. Reconstitution was according to the method of Driessen et al. [11].

Isolation and reconstitution of the lactose carrier of E. coli in proteoliposomes. The lactose carrier was isolated from *E. coli* strain T206, which harbors the *lacY* gene in a multicopy plasmid. Isolation and reconstitution were according to the method of Viitanen et al. [7]. The final preparation contained 110 μg lactose carrier protein/ml and 20 mg lipid/ml in 50 mM K-phosphate (pH 7).

Fusion of cytochrome-c oxidase proteoliposomes with lactose carrier proteoliposomes. Cytochrome-*c* oxidase proteoliposomes containing 4.5 nmol cytochrome-*c* oxidase and 20 mg lipid/ml were mixed with lactose carrier proteoliposomes in a 1:1 ratio. The suspension was then rapidly frozen in liquid N_2. Hybrid membranes were slowly thawed at room temperature and sonicated for 5 s with a probe-type sonicator.

Determination of fusion efficiency of coated liposomes. Fusion was quantitated with the R_{18} (octadecyl-rhodamine β chloride) fusion assay [12]. Liposomes (4 μg lipid) labelled with 9.4 mol% R_{18} were mixed with unlabelled liposomes (16 μg lipid) (in a ratio of 1:1) in a final volume of 2 ml in 50 mM Tris-HCl (pH 7). R_{18} fluorescence was continuously recorded at 25°C at an excitation and emission wavelengths of 560 and 590 nm, respectively. Measurements were performed with a Perkin-Elmer LS-50 spectrophotofluorometer. Fusion was induced by the addition of 12.5 mM Ca^{2+}. At the probe concentration used a linear relationship exists between the efficiency of self-quenching and the concentration of R_{18} in the phospholipid membrane [12]. The extend of fusion is therefore directly proportional to the extend of R_{18} fluorescence. The rate of fusion was measured during the first few seconds after the addition of Ca^{2+}. The fluorescence increase was than linear in time. The extend of fusion was measured after incubation of the mixture for 30 min at 25°C in the presence of 20 mM EDTA. The maximal level of fluorophore dilution was determined after the addition of 0.1% Triton X-100.

Fluorescence polarization measurement. Fluorescence polarization of DPH (1,6-diphenyl-1,3,5-hexatriene) was measured according to Shinitsky and Barenholz [13]. For DPH labelling of the liposomes, 5 μl of DPH stock solution (4 mM in dimethylsulfoxide) was diluted in 245 μl of 50 mM K-phosphate (pH 7.5). Liposomes (30 mg lipid/ml) were mixed with the DPH solution in a 1:1 ratio, yielding a DPH/lipid ratio of 1:250 (mol/mol). The labeled liposomes were shaken for 1 h at room temperature. The steady state fluorescence polarization was measured at 25°C after dilution of 50 μl of the labelled liposomes in 1950 μl 50 mM K-phosphate (pH 7.5). The fluorescence intensity was measured parallel ($I_\parallel$) and perpendicular ($I_\perp$) to the emitted light at an excitation and emission wavelength of 360 and 430 nm, respectively. The polarization (P) was calculated according to the following equation [13–15]:

$$P = (I_\parallel - I_\perp)/(I_\parallel + 2I_\perp)$$

Release of entrapped 5,6-carboxyfluorescein. The integrity of the liposomes was measured with the use of the water soluble fluorescent dye 5,6-carboxyfluorescein (CF) according to Goessens et al. [16]. CF was entrapped into the liposomes by adding 100 mM CF to

the liposomes prior to sonication. External CF was removed by chromatography of the liposomes over a Sephadex G-50 column (coarse, 1 × 20 cm). The concentration dependent self-quenching of CF fluorescence permits leakage from liposomes to be monitored continuously. CF fluorescence was measured at excitation and emission wavelengths of 430 and 520 nm, respectively, in 50 mM K-phosphate (pH 7.5) at 25°C. The final concentration of the liposomes during the fluorescence measurements was about 10 μg lipid/ml. The maximal level of CF fluorescence was measured after the addition of 0.1% Triton X-100.

Oxygen consumption of cytochrome-c oxidase proteoliposomes The rate of oxygen consumption was measured using a Clark-type oxygen electrode. Cytochrome-c oxidase proteoliposomes were diluted to a final concentration of 0.2 mg lipid/ml in 50 mM K-phosphate (pH 7.5) at 25°C saturated with air. Oxygen consumption was initiated by the addition of 10 mM K-ascorbate, 200 μM TMPD and 10 μM cytochrome *c*.

Determination of the electrical potential in cytochrome-c oxidase proteoliposomes. The electrical potential across the membrane ($\Delta\psi$, interior negative) was measured from the distribution of the lipophilic cation tetraphenylphosphonium (TPP^+) with the use of a TPP^+-electrode. Proteoliposomes were diluted to a final concentration of 0.2 mg lipid/ml in 50 mM K-phosphate (pH 7.5) at 25°C saturated with air. The $\Delta\psi$ was generated by the addition of 10 mM K-ascorbate, 200 μM TMPD and 10 μM cytochrome *c*. The TPP^+-concentration was 4 μM. Nigericin was added to a final concentration of 10 nM. The magnitude of the membrane potential was calculated with the Nernst equation. A correction for concentration-dependent TPP^+ binding was applied [17,18], assuming symmetric binding of TPP^+.

Protonmotive force driven transport of lactose in the fused proteoliposomes. Lactose uptake driven by a proton motive force (Δp) generated by cytochrome-c oxidase activity was assayed as follows. Hybrid liposomes were diluted to a final concentration of 0.2 mg lipid/ml in 50 mM K-phosphate (pH 7) and 5 mM $MgCl_2$. K-ascorbate (10 mM), TMPD (200 μM) and cytochrome-c (10 μM) were added. At the start of the experiment 36 μM lactose (55.8 mCi/mmol) was added. The total assay volume was 1 ml. At different time intervals samples (100 μl) were taken, diluted in 2 ml LiCL (0.1 M) and filtrated over cellulose nitrate membrane filters with a pore size of 200 nm (BA 83, Schleicher and Schuell) and washed once with another 2 ml of 0.1 M LiCl. The filters were put into scintillation vials with 4 ml of Packard liquid scintillation TM299 and counted. Steady-state levels of lactose accumulation were converted into Δp_{lac} (in mV) using the following equation: Δp_{lac} (mV) $= -60 \log[\text{lactose}]_{in}/[\text{lactose}]_{out}$, where $[\text{lactose}]_{in}$ and $[\text{lactose}]_{out}$ denote internal and external lactose concentrations at the steady-state level of accumulation, respectively.

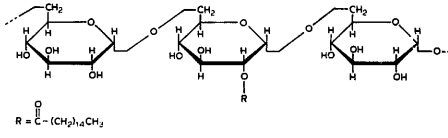
Results

Structure of DEX-1.

The structure and composition of the hydrophobized dextran (DEX-1) is presented in Fig. 1. When added to a liposome preparation, the hydrophobic anchor groups of DEX-1 insert spontaneously into the outer half of the phospholipid membrane [2]. Liposomes composed of *E. coli* phospholipid were coated with DEX-1 by adding increasing amounts of an aqueous DEX-1 solution to the liposome preparations. The effect on the fusion efficiency, fluidity of the membranes, as well as the stability of the liposomes was investigated and compared with the non-treated samples.

Fusion efficiency

Liposomes prepared in Tris-HCl (pH 7.5) were examined for their ability to fuse after coating with DEX-1. Fusion was quantitated with the R_{18} fusion assay as described in Materials and Methods. Liposomes without the fluorescence dye were incubated with increasing concentrations of DEX-1 before mixing with the R_{18}-labelled liposomes. Fusion was initiated by the addition of calcium (12.5 mM). The rate and



	molecular weight	$\bar{P}_n$	substitution [%]	anchor groups / polymer chain
DEX-1	32000 - 48000	245	5.5	14

Fig. 1. Structure and composition of the hydrophobized dextran.

extend of fusion at increasing DEX-1 concentrations were plotted as a percentage of the fusion measured in the absence of DEX-1 (Fig. 2). The rate of fusion was measured during the first seconds after the addition of calcium when the fluorescence increased linearly in time. A cloudy precipitate appeared due to the Ca^{2+}-induced fusion. This precipitate disappeared upon incubation in the presence of 20 mM EDTA. Therefore the extend of fusion was measured after incubation of the assay mixture for 30 min at 25°C with 20 mM EDTA. The extend of fusion in the absence of DEX-1 was 61% of the theoretically maximal level of fusion obtained when R_{18} is distributed randomly over the phospholipid present. DEX-1 itself did not induce fusion and the unsubstituted dextran (without hydrophobic anchors) did not influence the fusion efficiency (not shown).

In Fig. 2 the amount of DEX-1 is expressed relative to the amount of lipid present since the DEX-1 to lipid ratio appears to be the parameter that determines the action of DEX-1 rather than the absolute concentration. No significant difference in the fusion behaviour of the coated liposomes was observed between liposomes preincubated with DEX-1 at the final concentration of the fusion assay (8 μg lipid/ml) or at a 20-fold higher lipid concentration, as long as the DEX-1 to lipid ratio was the same. From the average molecular weight for DEX-1 of 40000, the substitution number of 14 anchors per polymer chain and the average molecular weight of 750 for *E. coli* phospholipid, it can be calculated that a DEX-1/lipid (w/w) ratio of 1 corresponds to about 0.25 hydrophobic anchors per phospholipid molecule, or 0.5 hydrophobic anchors per phospholipid molecule exposed to the outer surface of the liposomes. The anchor/phospholipid ratio that significantly inhibits the fusion efficiency ranges between 1:40 to 1:3 (Fig. 2).

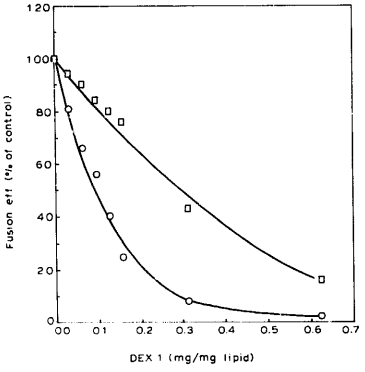


Fig. 2. Effect of DEX-1 on Ca^{2+} induced fusion of liposomes. Fusion efficiency was measured in liposomes with the fluorescent probe R18 as described in Materials and Methods. Unlabelled liposomes (16 μg of lipid) were preincubated at room temperature with increasing concentrations of DEX-1 before mixing with the R_{18} labeled liposomes. The rate (○) and extend (□) of fusion were plotted as a percentage of the control without DEX-1.

Membrane fluidity

Fluorescence polarization of DPH can be used as an indicator for the relative viscosity of the phospholipid membrane [13]. An increase in DPH-polarization reflects a decrease in membrane fluidity. The liposomes were labelled with the fluorescent probe DPH prior to coating of the membranes with increasing amounts of the hydrophobized dextran. The fluorescent polarization was determined as described in Materials and Methods. Concentrations of DEX-1 which almost completely inhibit fusion, did not effect DPH-polarization. Even at concentrations of 4 mg DEX-1/mg lipid (2 anchors per outer phospholid molecule) no effect on the membrane fluidity could be detected. Further increases of the DEX-1 concentration led to an linear increase of DPH-polarization with the DEX-1 concentration. The DPH-polarization value increased from 0.198 at 4 mg DEX-1/mg lipid to 0.235 at 26 mg DEX-1/mg lipid (13 anchors per outer phospholipid molecule). This observation suggests that the movement of the probe in the membrane becomes restricted at very high DEX-1 concentrations.

DPH-fluorescence was not quenched upon the addition of the high DEX-1 concentrations. Furthermore, the same dextran concentration in the absence of the palmitic anchors did not increase DPH-polarization.

Effect of DEX-1 on the stability of liposomes and proteoliposomes

The relative stability of the liposomes was measured from the fluorescence of the water soluble fluorescent dye 5,6-carboxyfluorescein (CF), encapsulated in the interior of the liposomes during sonication. External CF was removed by chromatography of the liposomes over Sephadex. Leakage of CF from the liposomes can be detected as a fluorescence increase due to a relief of self-quenching of the probe at the high concentration in the liposomes.

Addition of the detergent decyl-polyethylene glycol 300 (decyl-PEG) caused a rapid release of CF, indicating that the integrity of the liposomes was lost. Coating of the liposomes with DEX-1 severely inhibited the rate of detergent-induced CF release (Fig. 3A). Relative protection against detergent degradation started at slightly lower DEX-1/lipid ratios than those which decreased the fluidity of the membranes. At two hy-

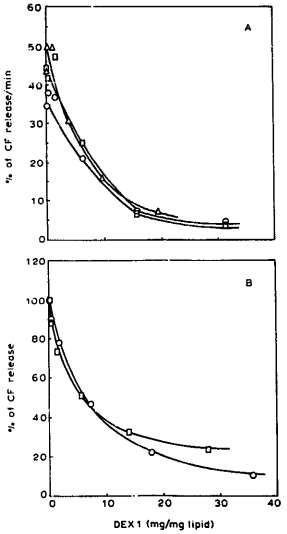


Fig. 3. Release of carboxyfluorescein after detergent treatment or freeze-thawing of liposomes and proteoliposomes. (A) CF-loaded liposomes and lactose carrier proteoliposomes were preincubated with increasing concentrations of DEX-1 for 3 or 40 min. CF fluorescence was continuously registered and decyl-PEG ($5 \cdot 10^{-3}$% v/v) was added. The rate of CF release at different DEX-1 concentrations was plotted as a percentage of CF release per min in the absence of DEX-1. The maximum fluorescence level (100%) is obtained after the addition of 0.1% Triton X-100. Liposomes preincubated with DEX-1 for 3 min (○) or 40 min (□). Proteoliposomes preincubated with DEX-1 for 3 min (△). (B) The preparations of CF-loaded liposomes and lactose carrier proteoliposomes were diluted in 50 mM K-phosphate (pH 7.5) to a final concentration of 0.2 mg lipid/ml in the presence of increasing concentrations of DEX-1. Samples were rapidly frozen in liquid N_2 and diluted slowly at room temperature. CF fluorescence was measured immediately. The maximum fluorescence level (100%) is determined by the addition of 0.1% Triton X-100.

drophobic anchors per outer phospholipid molecule a significant protection was already obtained. Prolonged incubation with DEX-1 did not influence its effect on the liposomes. No difference in efflux rate of CF was observed when DEX-1 was added either 3 or 40 min prior to decyl-PEG (Fig. 3A).

Proteoliposomes containing the lac carrier protein of *E. coli* were also examined for their resistance against detergent degradation after coating with the dextrans. No significant difference could be measured between the CF release from liposomes and proteoliposomes tested (Fig. 3A).

Freezing of liposomes in liquid nitrogen, and subsequent thawing at room temperature, normally leads to a collapse of the liposomes and induces fusion of membranes due to dehydration. The integrity of the liposomal membrane can than only be restored by sonication of the membrane preparation. Consequently, freeze-thawing of liposomes results in a complete release of the internal contents of the preparation. Coating of liposomes with DEX-1 protected against the loss of membrane integrity by freeze/thawing (Fig. 3B). The uncoated liposomes lost all the internal CF after freeze/thawing of the liposomes (100% value of Fig. 3B). At the highest DEX-1 concentration (about 18 anchors per outer phospholipid

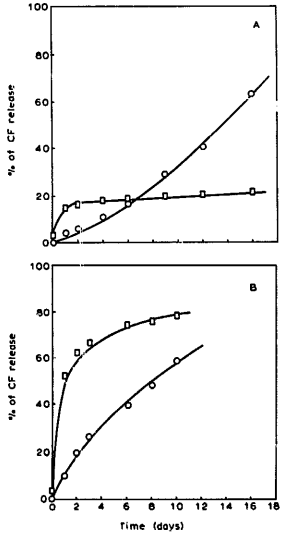


Fig. 4. Release of carboxyfluorescein from liposomes and proteoliposomes in the presence and absence of DEX-1. CF-loaded liposomes or proteoliposomes were diluted in 50 mM K-phosphate (pH 7.5), to a concentration of 9 μg lipid/ml and incubated at room temperature in the absence (○) or in the presence (□) of DEX-1 (30 mg/mg lipid). The release of CF was measured during 16 days at indicated times. The maximum fluorescence level (100%) is determined by the addition of 0.1% Triton X-100. (A) Liposomes, (B) lactose carrier proteoliposomes.

molecule) 90% of the liposomes were intact after freezing and thawing. The same concentration of unsubstituted dextrans did not give any protection to the liposomes (not shown). Again no significant difference was observed between liposomes and lactose carrier containing proteoliposomes (Fig. 3B).

Long-term stability of the liposomes was examined by measuring the spontaneous release of encapsulated CF as a function of time at room temperature (Fig. 4A). Liposomes composed of *E. coli* lipid appeared to be rather stable in the absence of DEX-1. After 6 days of incubation about 82% of the CF was still inside the liposomes. In the next 10 days liposomes degraded more rapidly leading to 65% leak after 16 days. Coating of the liposomes with DEX-1 (30 mg DEX-1/mg lipid or 15 anchors per outer phospholipid molecule) increased the initial leakage of CF. After an initial rapid loss of about 18% in the first day, the liposomes were stabilized and lost only a few % of the label in the next 15 days (Fig. 4A). Proteoliposomes containing the lactose carrier protein of *E. coli* were tested for long term stability in an analogous manner (Fig. 4B). Proteoliposomes lost their internal CF content more rapidly than liposomes without reconstituted proteins (compare Figs. 4B and 4A). After 6 days 40% of the CF was released from the liposomes and in 10 days this value increased to about 60%. In the presence of 30 mg DEX-1/mg lipid a dramatic loss of CF occurred during the first 24 h. After 2 days the leakage rate decreased and like observed in liposomes the proteoliposomes tended to stabilize, but at this stage the majority of the liposomes were already damaged (Fig. 4B). The same phenomenon was observed using cytochrome-*c* oxidase liposomes instead of lactose carrier proteoliposomes (not shown).

The effect of DEX-1 on the biologic activity of integral membrane proteins

Cytochrome-*c* oxidase from beef heart mitochondria can be used as a Δp generator in liposomes and other membrane systems [11]. To investigate whether coating of the liposomes influences cytochrome-*c* oxidase activity both the oxygen consumption rate and the generation of a $\Delta\psi$ by cytochrome-*c* oxidase were measured at increasing DEX-1 concentrations (Table I). Coating of liposomes with DEX-1 inhibited the rate of oxygen consumption by reconstituted cytochrome-*c* oxidase. At the highest DEX-1 concentration (25 mg/mg lipid) 47% of the initial oxygen consumption rate was present. The $\Delta\psi$ generated by the activity of cytochrome-*c* oxidase was only slightly affected. The $\Delta\psi$ changed from -166 mV in the absence of DEX-1 to -133 mV at the highest DEX-1 concentration. In the presence of nigericin, which dissipated the ΔpH, the $\Delta\psi$ was slightly increased due to a partial conversion of the ΔpH into a $\Delta\psi$. The nigericin-induced increase of $\Delta\psi$ was more pronounced at higher DEX-1 concentrations. Therefore in the presence of nigericin the $\Delta\psi$ in the coated liposomes varied from -168 to -153 mV only (Table I). The time course of TPP^+ uptake in the coated vesicles was not visibly effected, indicating that the TPP^+ permeability was not decreased (not shown).

TABLE I

The effect of DEX-1 on oxygen consumption and the generation of a membrane potential by cytochrome-c oxidase reconstituted in liposomes

DEX-1 (mg/mg lipid)	Rate of oxygen consumption (nmol O_2/min per mg lipid)	G	$\Delta\psi$ (mV)	
			− nig	+ nig
0	28.6	100	−166	−168
2	17.1	60	−137	−149
5	16.8	59	−121	−147
10	18.3	64	−125	−152
25	13.3	47	−133	−153

The effect of DEX-1 on the activity of the lactose transport protein of *E. coli* was investigated in proteoliposomes containing both beef heart cytochrome-*c* oxidase and the lactose carrier. The proteoliposomes were obtained by fusing cytochrome-*c* oxidase proteoliposomes with lactose carrier proteoliposomes. Lactose transport was driven by a Δp generated by cytochrome-*c* oxidase activity. Both the initial rate of lactose transport and the steady-state level of lactose accumulation decreased slightly in the presence of DEX-1 (Table II). At the highest DEX-1 concentration (25 mg/mg lipid) the initial rate of lactose transport was about 60% of the original activity. The steady-state accumulation level of lactose decreased from 99 mV in the absence of DEX-1 to 75 mV at the highest DEX-1 concentration. Transport of lactose only takes place in liposomes containing both cytochrome-*c* oxidase and the lactose transport carrier. A similar decrease of the Δp as observed for the proteoliposomes after coating with DEX-1 (Table I) will probably occur in the hybrid membranes of Table II, since the cytochrome-*c* oxidase concentrations in both preparations are the same. Therefore, a decrease in transport activity proportional

TABLE II

The effect of DEX-1 on proton motive force driven lactose transport in fused liposomes containing cytochrome-*c* oxidase and the lactose carrier

DEX-1 (mg/mg lipid)	V_i lactose (nmol/min per mg lipid)	G	Δp_{bet} (mV)
0	0.29	100	99
2	0.37	127	99
10	0.24	83	94
25	0.18	62	75

to the decrease in driving force as listed in Table I is to be expected.

Discussion

Coating of liposomes composed of *E. coli* lipids with hydrophobized dextrans affect the properties of the liposomal membranes. A concentration of 0.1 to 0.65 mg DEX-1 per mg lipid almost completely inhibits Ca^{2+}-induced fusion of the liposomes. Fusion efficiency measurements with R_{18} is based on the rapid distribution of the probe over the phospholipid membranes. Kinetic studies of Ca^{2+} induced fusion have indicated that the mixing of bilayer lipids occurred with the same rate as the mixing of the aqueous content of the vesicles [19]. However, we cannot exclude that in the presence of the hydrophobized dextrans lateral diffusion of R_{18} might be restricted.

The fluidity and stability of the liposomes was affected only at DEX-1 concentrations at which the hydrophobic anchors outnumber the number of phospholipid molecules (5–30 mg DEX-1/mg lipid, which corresponds to 2.5–15 hydrophobic anchors per outer phospholipid molecule). It is unlikely that at this high concentrations all hydrophobic anchors are in contact with the liposomal membrane. Recently, Ringsdorf et al. [20] (1991) determined the amount of hydrophobic anchors of octadecyl-pyrene coupled to poly *N*-isopropylacrylamide with the use of fluorescence spectroscopy of the pyrene label. In small unilamellar liposomes of dimyristoyllecithin (DMPC) the upper level of the inserted anchor groups appeared to be 1 per 110 lipids. The excess of polymer adopts a micellar structure identical to that of pure polymer in water. Nevertheless in our hands both the viscosity and the stability of the liposomal membranes increased with increasing DEX-1 concentrations from 2 to 15 hydrophobic anchors per phospholipid molecule. Probably the space between the liposomes is completely substituted by the hydrophobized dextrans under these conditions. At the highest DEX-1 concentrations, the liposomes turned out to be remarkable stable. Even after a rigorous treatment as freeze-thawing at least 90% of the liposomes remained intact. The accumulation of hydrophobic anchors forming micelles between the liposomes could be responsible for the stabilization of the membranes, since the same concentration of unsubstituted dextrans did not cause stabilization of the liposomes.

Also in proteoliposomes the fluidity decreased and the stability increased with the DEX-1 concentration The biological activities of both beef heart cytochrome-*c* oxidase and the lactose transport carrier of *E. coli* are only slightly affected by the highest DEX-1/lipid ratio. This indicates that the diffusion of substrates to the transport carriers was not limited under these conditions. However, long term stability of the proteoliposomes was not retained in the presence of the high DEX-1 concentrations. Long term stability is restricted in the first place by the intrinsic instability of the membrane proteins itself. The lactose carrier completely desintegrates within 1 week after reconstitution. There is also a direct effect of DEX-1, which causes destabilization of the proteoliposomes when stored at room temperature. This is probably the consequence of the more rigid structure of the lipids at the high DEX-1 concentrations. The presence of membrane proteins might be responsible for defects in packing at the protein/lipid interphase due to the restricted movement of the phospholipids in the presence of the hydrophobic anchors.

In summary, liposomes as well as proteoliposomes can be stabilized by coating with hydrophobized dextrans. However, the coated proteoliposomes are restricted in repair of packing defects, which causes a decrease in the long-term stability of the proteoliposomes.

Acknowledgement

This research was supported by the Foundation for Technical Sciences (STW) with financial aid from the Netherlands Organization for Scientific Research (NWO).

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