Biochimica et Biophysica Acta 862 (1986) 294–302
Elsevier

BBA 72968

Formation of large unilamellar vesicles using alkyl maltoside detergents

H. Alpes [a], K. Allmann [a], H. Plattner [a], J. Reichert [a], R. Riek [a] and S. Schulz [b]

[a] *Fakultät für Biologie, and* [b] *Fakultät für Physik, Universität Konstanz, Constance (F.R.G.)*

(Received 10 July 1986)

Key words: Vesicle formation; Alkyl maltoside; Dialysis kinetics

Several alkyl maltosides having different alkyl chain structures and dodecyl maltotrioside were synthesized. The detergent properties of these compounds were investigated with special emphasis on dialysis kinetics during liposome formation. The critical micelle concentration (CMC) and therefore the speed of detergent removal by dialysis mainly depended on the chain length of the hydrophobic part of the molecule, whereas the number of glucose residues in the polar headgroup had no effect on CMC. Liposome preparation was performed through detergent removal by dialysis combined with adsorption to Amberlite XAD-2 as described by Philippot et al. (Philippot, J.R., Mutaftchiev, S. and Liautard, J.P. (1983) Biochim. Biophys. Acta 734, 137–143), leading to a considerable reduction of dialysis volume. Decyl maltoside proved to be a suitable detergent in combination with mixtures of defined synthetic lipids (DOPC/DOPS and DOPC/DOPG). The presence of at least 10% of negatively charged lipid was essential for the formation of unilamellar liposomes.

Introduction

The formation of unilamellar artificial liposomes by removal of detergents from lipid detergent mixed-micellar suspensions is a well established method and the factors determining the properties of the resulting liposomes have been the subject of many investigations. Detergent removal was accomplished mainly by dialysis [2–4], by specific binding to amberlite XAD-2 [5,6], or by a combination of these two methods [1]. Ionic detergents, e.g., sodium cholate or cholic acid derivatives, as well as nonionic detergents of different chemical structure such as octyl glucoside, $C_{12}E_8$ and Triton X-100, were used. Depending on the method of formation and on the chemical structure of the detergent used, the diameters of the resulting liposomes varied from about 50 to 300 nm. Whereas cholic acid derivatives form small unilamellar vesicles (SUV), the size being determined by the molar ratio of lipid to detergent, nonionic detergents generally give large unilamellar vesicles (LUV) with diameters greater than 100 nm.

Nonionic detergents are of special interest with regard to reconstitution of membrane proteins into artificial liposomes. Considering denaturation problems, they seem to be less harmful than ionic detergents [7]. It has been recently shown that, from a series of several nonionic detergents (alkyl glucosides, maltosides, zwitterionic detergents and $C_{12}E_8$), lauryl maltoside had the lowest denatura-

Abbreviations: DOPC, dioleoylphosphatidylcholine; DOPS, dioleoylphosphatidylserine; DOPG, dioleoylphosphatidylglycerol; egg PC, egg phosphatidylcholine; CMC, critical micelle concentration; QELS, quasi elastic light scattering; HPLC, high-performance liquid chromatography.

Correspondence: Dr. H. Alpes, Fakultät für Biologie, Universität Konstanz, D-7750 Konstanz, F.R.G.

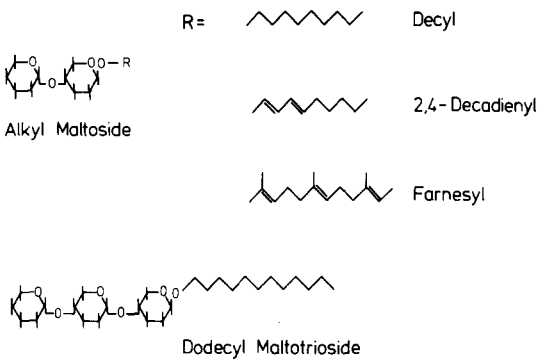

Fig. 1. Structure of alkyl maltosides.

tion effect on bovine heart cytochrome *c* oxidase [8]. The activity of the protein was dependent on the structure of the polar headgroup as well as on the length of the hydrophobic alkyl chain of the detergent molecule, But at the present time it is impossible to generalize these findings and to make any predictions from the chemical structure of the detergent about its physical properties.

In this paper we report on the synthesis of some alkyl maltosides and maltotrioside (Fig. 1) and on their suitability for vesicle formation with special regard to detergent removal by dialysis. A further subject was the development of a dialysis method which allows the formation of liposomes in a minimum volume of buffer solution. In order to avoid autoxidation of lipids and to improve the reproducibility of experiments we mainly used synthetic lipids of defined structure such as DOPC, DOPS, and DOPG.

Materials and Methods

Materials. *n*-Octyl β-D-glucopyranoside (octyl glucoside) and *n*-dodecyl β-D-maltopyranoside (dodecyl maltoside) were purchased from Calbiochem (Frankfurt, F.R.G.).

Octyl, nonyl and decyl β-D-maltopyranoside (octyl, nonyl and decyl maltoside), *n*-2,4-decadienyl β-D-maltopyranoside (decadienyl maltoside) and *n*-dodecyl β-D-maltotriopyranoside (dodecyl maltotrioside) were synthesized from heptaacetobromomaltose or decaacetobromomaltotriose, respectively, and the corresponding alcohol as described in Ref. 9. The structure of the resulting compounds was analyzed by ^{1}H-NMR spectroscopy [19] and their homogeneity was checked by HPLC.

Dioleoyl-α-D-phosphatidylcholine (DOPC), egg phosphatidylcholine (egg PC), dioleoylphosphatidylserine (DOPS) and dioleoylphosphatidylglycerol (DOPG) were obtained from Avanti Polar Lipids (Birmingham, AL, U.S.A.).

Amberlite XAD-2 (XAD-2 beads) was purchased from Fluka AG (Buchs, Switzerland). The beads were activated by several washings with organic solvents and water according to Ref. 10. Dialysis tubings with 7 mm diameter and 0.05 and 0.01 mm wall thickness, respectively, were purchased from Serva (Heidelberg, F.R.G.).

Preparation of liposomes. Lipid-detergent mixtures were prepared from stock solutions of the appropriate lipids and detergents in methanol or chloroform, respectively. The organic solvent was evaporated under vacuum at 30°C and the remaining lipid-detergent film was kept under vacuum at 30–35°C for 30–45 min. The lipid-detergent mixture was dissolved in buffer A (100 mM potassium chromate/3 mM Tris/2 mM imidazole/2 mM histidine/1 mM EDTA (pH 7.4)). The final lipid concentration was 10 mg/ml and the lipid detergent mole ratio was 1 : 5 or 1 : 10. For equilibration, the micelle suspensions were kept at room temperature for several hours. 0.5–2 ml of the micelle suspension were transferred to a 7 mm wide dialysis tubing (wall thickness 0.01 mm) and dialysed for 60 h at 4°C against 4 ml buffer A and 1 g of XAD-2 beads. Dialysis tubing, buffer and XAD-2 beads were enclosed in a 10 ml test-tube and fastened to a device which rotated with a speed of 70–80 rpm. An air bubble in the dialysis tube and in the outer dialysis buffer caused mixing of the solutions during dialysis.

In comparison to other dialysis methods the procedure described here is inexpensive and well suited for serial batches with a very low consumption of materials.

Determination of the internal volume. The internal volume was determined by measuring the chromate concentration in the inner compartment of the liposomes by adaption of the method described in Ref. 11: 25 μl of the liposome suspension were applied to a 10 cm Sephadex G-50 column, which was connected to a Uvicord II

ultraviolet absorptiometer (LKB). The column was run with buffer B (150 mM KCl/0.02% sodium azide/3 mM Tris (pH 7.4)) at a flow rate of 25–30 ml/h. The liposome fraction which appeared after 2 min was collected and the spectrum was measured from 300 to 700 nm on a Lambda 5 UV/VIS spectrophotometer (Perkin Elmer). The 370 nm chromate absorption ($\epsilon = 3650\ mol^{-1} \cdot cm^{-1}$ (pH 7.4)) was corrected for light scattering and the internal volume was determined from the mesured chromate concentration.

With unilamellar liposomes the apparent absorption by light scattering at 700 nm was small and ranged from 0 to 2% of the extinction at 370 nm. By taking into account the molecular weight of the lipid and the lipid concentration of the liposome suspension, the internal volume was expressed in l/mol, and the mean diameters of the liposomes were computed on the basis of the method given in Refs. 12 and 13.

HPLC procedures. A Waters HPLC system was used, consisting of a model 560 pump, a model 450 variable wave length detector for quantitative lipid determination, a RI 403 refractometer for quantitative detergent determination, and a Z-Modul with the appropriate RC/column.

Determination of detergent concentration. 100 μl of the detergent-lipid suspension were evaporated to dryness and the residue was dissolved in 300 μl of methanol/water (77.5 : 22.5). After centrifugation of precipitated material, 30–100 μl (depending on concentration) were applied to a C-18 reversed phase column RC Bondapak C18, which was run with methanol/water (77.5 : 22.5) at a flow rate of 2 ml/min. The refraction was detected and the detergent concentration of the respective sample was taken from a calibration curve. Concentrations down to 0.1 μmol/ml corresponding to about 0.1% of the initial detergent content could be determined.

Determination of DOPC concentration. 100 μl of the micelle or liposome suspension were evaporated to dryness and dissolved in 300 μl of methanol. After centrifugation of precipitated material, 30 μl of the solution were applied to an RC Porasil column. The solvent system was methanol/water (95 : 5) and the flow rate was 2 ml/min. The absorption was detected at 205 nm and the lipid concentration was taken from a calibration curve or was determined by comparison to a standard solution. The lipid concentrations of the standard solutions were determined by the method described in Ref. 14.

Ion flux measurements. Permeation of chromate ions was measured after exchanging the external chromate buffer for an iso-osmolar potassium chloride buffer: 300 μl of the liposome suspension were applied to a 30 cm Sephadex G-50 column which was connected to a Uvicord II ultraviolet absorptiometer (LKB). The column was run with buffer B (150 mM KCl/0.02% sodium azide/3 mM Tris (pH 7.4)) at a flow rate of 25–30 ml/h and the liposomal fraction was collected.

Then at certain times the decrease of the internal chromate concentration was determined. To remove the permeated chromate from the medium, 300 μl aliquots of the liposome fraction were rechromatographed and the internal chromate concentration was determined from the absorption peak at 370 nm and evaluated in percent of the initial concentration. The time constant ($t_{1/2}$) of the chromate efflux was obtained from semilogarithmic plots of the decrease of internal chromate concentration. Permeability coefficients (P) were obtained by using the relation $P = V/A\tau = r/3\tau$.

Determination of the critical micelle concentration (CMC). The critical micelle concentration (CMC) was determined using a fluorimetric assay [15]. Fluorescence measurements were carried out with a Perkin-Elmer 650-40 fluorescence spectrophotometer operating in ratio mode. The excitation wavelength was 358 ± 1 nm and the emission wavelength was 430 ± 20 nm. 5 μl of 1 mM 1,6-diphenyl-1,3,5-hexatriene dissolved in tetrahydrofuran was added to various amounts of detergent dissolved in a total volume of 1 ml buffer in plastic cuvettes. These were kept in the dark for 45 min at room temperature before the fluorescence of the samples was measured. Most of the experiments were made with multiple sets of samples.

The principle of the assay is that the fluorescence of 1,6-diphenyl-1,3,5-hexatriene will be greatly enhanced above the CMC due to its incorporation into the hydrophobic interior of the micelle. At low detergent concentrations the fluorescence is weak, but rises rapidly above the CMC. A measure for the CMC is the intersection of the

straight lines below and above the CMC.

Quasi elastic light scattering (QELS). The size of the liposomes was studied with QELS using a scattering photometer equipped with an argon ion laser (Spectra Physics; wavelength 488 nm). The fluctuations of the scattered light (scattering angle 90°) were detected and the autocorrelation function was computed with an ALV-correlator. The analysis of the deviation of the correlation function from a single exponential time dependence gives information about the size distribution of the liposomes.

The first two cumulants [16] were obtained by fitting the mesured curve with a sum of exponentials and a constant [17]. From the first cumulant the average translational diffusion coefficient, D, of the vesicle can be determined. Assuming a spherical shape, the average liposome radius, r, can be calculated using the Stokes-Einstein relation $D = kT/6\pi\eta r$ (k, Boltzmann's constant; T, absolute temperature; η, viscosity of the medium). The second cumulant is a measure for the variance, σ, of the distribution of the liposome radii.

Electron microscopy. Negative staining was carried out with unfixed liposomes using 2% ammonium heptamolybdate solution. The electron microscope grids were coated with collodium and enforced by carbon evaporation.

For freeze-fracturing (Balzers BAF 300 device equipped with electron beam evaporators for 45° Pt/C shadowing and 90° carbon enforcement) a rapid freezing method was used [22,23].

Electron micrographs were taken in a Siemens-Elmiskop 101 at instrumental magnification from 20000 to 100000, operating at 60 and 80 kV acceleration voltage and equipped with 30 and 50 μm objective apertures. The instrument magnifications were calibrated with latex standards.

Results and Discussion

Influence of molecular structure on the critical micelle concentration (CMC)

The physical properties of a detergent are controlled by several factors such as hydrophobic-lipophilic balance (HLB number), critical micelle concentration (CMC), solubility above CMC, micelle size [18,19]. The question is, how these factors are influenced by alterations of the chemical structure of the detergent. In view of liposome formation and reconstitution experiments, the kinetics of detergent removal by dialysis are of special interest.

The CMC and micellar weight of some alkyl glucosides and of dodecyl maltoside are known [20]. Octyl glucoside (CMC = 22.3 mM) can be removed from micellar suspensions by dialysis within a few hours, whereas the removal of dodecyl maltoside (CMC = 0.16 mM) under comparable conditions takes 6–8 days [20]. This leads to the assumption that the kinetics of detergent removal by dialysis are ruled mainly by the CMC.

Table I shows that in a homologous series of alkyl oligoglucosides the CMC is exclusively given by the chain length of the hydrophobic part of the molecule. The dodecyl derivatives of glucose, maltose and maltotriose have nearly identical CMCs of about 0.2 mM. The number of glucose residues in the polar headgroup obviously has no effect on CMC, but it has considerable effect on the solubility of the detergent in water and therefore on the formation of micelles: decyl glucoside [20] (CMC = 2.19 mM) is nearly insoluble above the CMC (no micelle formation), whereas decyl maltoside (CMC = 2.0 mM) is readily soluble.

The conjugated double bonds in 2,4-decadienyl maltoside and the branched methyl groups and isolated double bonds in farnesyl maltoside have only a small effect on CMC, which is not markedly increased compared to the unbranched saturated compounds with the same chain length. The good solubility of farnesyl maltoside, with 15 carbon atoms in the hydrophobic chain, is remarkable.

Fig. 2 shows a HPLC separation of a mixture of several alkyl glucosides and maltosides on a C-18 reversed-phase column. The retention times are chiefly influenced by hydrophobic binding of the solutes to the C-18 matrix of the column material. Thus they give information on the relative hydrophobicity of the individual detergent: with increasing retention times the detergents behave more hydrophobically.

Detergent removal by dialysis

The kinetics of octyl glucoside and Triton X-100 removal in direct contact with XAD-2 beads and with dialysis against buffer containing the beads have been investigated [1]. Octyl glucoside (1 μmol

TABLE I

CRITICAL MICELLE CONCENTRATION (CMC) OF ALKYL GLUCOSIDES

Compound	Number of C-atoms in alkyl chain	Number of glucose residues	CMC (mM) [a]	Solubility (aqueous med.)	Refs.
Octyl glucoside	8	1	23.2	+	20
Octyl maltoside	8	2	26.5	+	this paper
Nonyl maltoside	9	2	6.3	+	this paper
Decyl glucoside	10	1	2.19	–	20
Decyl maltoside	10	2	2.20	+	this paper
2,4-Decadienyl maltoside	10	2	0.62	+	this paper
Dodecyl glucoside	12	1	0.19	–	20
Dodecyl maltoside	12	2	0.16	+	20
Farnesyl maltoside	12 [b]	2	0.26	+	this paper
Dodecyl maltotrioside	12	3	0.20	+	this paper

[a] In 150 mM KCl, 20°C.
[b] Without branching methyl groups.

detergent/9 mg beads) in direct contact with the beads can completely be removed in less than 1 h, whereas when the beads are added to the dialysis medium the removal takes 20–25 h.

If using a combined dialysis/bead method, the properties of the dialysis membrane influence the speed of detergent removal. We tested dialysis tubings with a wall thickness of 0.05 and 0.01 mm and found that detergent removal with a 0.01 mm tubing was about 2.5-times faster. Therefore we used mainly the thinner tubings.

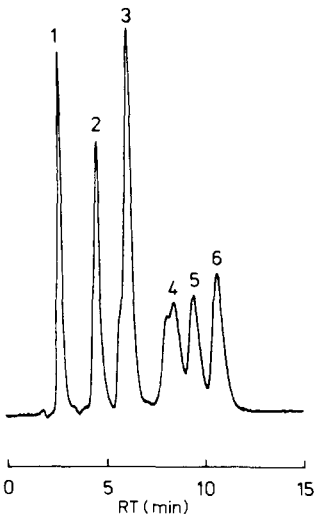

Fig. 2. HPLC separation of a mixture of six alkyl glycosides with different structure of the polar headgroup and hydrophobic alkyl chain. The compounds are, with increasing retention times (RT): (1) octyl glucoside (RT = 2.8); (2) 1,4-decadienyl maltoside (4.7); (3) decyl maltoside (6.3); (4) farnesyl maltoside (8.8); (5) dodecyl maltotrioside (9.8); (6) dodecyl maltoside (11.0).

Fig. 3 shows the kinetics of detergent removal during liposome formation. Among the synthesized and tested alkyl maltosides and maltotrioside, only the octyl, nonyl and decyl maltoside (saturated and unsaturated) could be removed by dialysis within a suitable time. Farnesyl maltoside and dodecyl maltotrioside needed 5–6 days at 20°C, like dodecyl maltoside.

Formation and characteristics of liposomes

Liposomes made by octyl glucoside dialysis are well established [4]. The high CMC of this detergent leads to its fast and complete removal in about 20 h. On the other hand, its high CMC leads to a high consumption of the detergent during chromatographic purification procedures. In view of this, a detergent with a lower CMC but a sensible dialysis time is a good compromise. Considering the above, we tested the alkyl maltosides concerning their applicability. Table II shows the size characteristics of liposomes made from mixtures of neutral lipid (DOPC) and negatively charged lipids (DOPS, DOPG) using alkyl maltosides as detegents. The presence of at least 10% of negatively charged lipid was essential for liposome formation. With neutral lipids alone (DOPC, egg PC) mainly multilamellar material with small in-

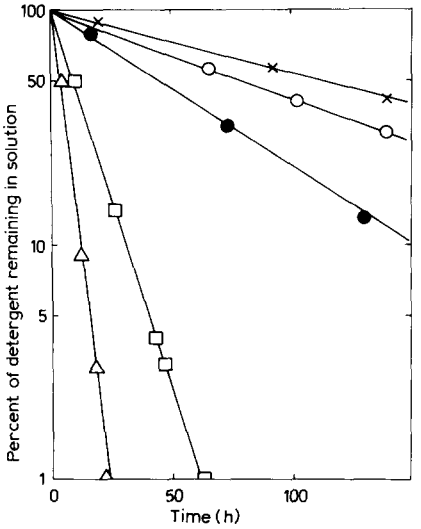

Fig. 3. Time-course of detergent removal during dialysis at 4°C using a dialysis tubing with a wall thickness of 0.01 mm. The dialysis was performed with mixed micellar suspensions containing 10 mg/ml of total lipid and a lipid to detergent molar ratio of 1:5. The detergents were: nonyl maltoside, △; decyl maltoside, □; farnesyl maltoside, ●; dodecyl maltotrioside, ○; and dodecyl maltoside, ×. The relative amount of detergent of the samples was determined with HPLC (see Materials and Methods).

ternal volume was formed. This effect could not be improved by enlarging the detergent-to-lipid molar ratio as was reported for octyl glucoside [5].

Decyl maltoside yielded the largest liposomes, whereas the use of nonyl or octyl maltoside yielded smaller ones. The shorter alkyl chain lengths of the octyl and nonyl derivative are obviously responsible for this decrease. By using the unsaturated 2,4-decadienyl maltoside as detergent also smaller unilamellar liposomes were formed which may be explained by a decrease of hydrophobicity compared to the saturated decyl derivative.

The electron micrographs (Fig. 4) show that the preparations mainly exist of unilamellar liposomes with a fairly homogeneous size distribution. Representative fields of the micrographs were taken for the determination of average diameters (Table II). The different methods for determining the liposomes' diameters show a good agreement. The average values obtained by the internal volume determination fit well to the QELS and electron microscopy data. The variance of the size distributions ranges from 0.2–0.4. Similar values were reported using octyl glucoside for liposome formation [4]. The results show further that mixtures of defined synthetic lipids (DOPC/DOPS and DOPC/DOPG) with exclusively oleic acid in the hydrophobic part of the lipid are well suited for the formation of large unilamellar liposomes.

Ion permeability

Fig. 5 shows the time-dependence of chromate

TABLE II

SIZE CHARACTERISTICS OF LIPOSOMES

A Calculated from internal volum, V_i.

B By quasi elastic light scattering.

C From electron microscopy, negative stain technique. About 500 vesicles were analyzed, particles with a diameter of less than 50 nm were not taken into account.

D From electron microscopy, freeze-fracture technique. Equatorially broken vesicles were selected and analyzed according to the method given in Ref. 21.

Lipid [a]	Detergent [b]	V_i (l/mol)	Vesicle diameter (nm)			
			A	B	C	D
DOPC/DOPS	decyl maltoside	7.6 ± 0.29	230 ± 62	228 ± 62	194 ± 73	215 ± 45
DOPC/DOPG	decyl maltoside	7.4	227	226 ± 78	–	–
DOPG/DOPS	2,4-decadienyl maltoside	1.4	48	–	–	–
DOPC/DOPS	nonyl maltoside	2.6 ± 0.04	89 ± 4	–	–	–
DOPC/DOPS	octyl maltoside	1.1 ± 0.11	36 ± 4	–	–	–
DOPC/DOPS	octyl glucoside	6.4	198	–	–	–

[a] The molar ratio of neutral to charged lipids was 1:1.

[b] The molar ratio of total lipid to detergent was 1:5.

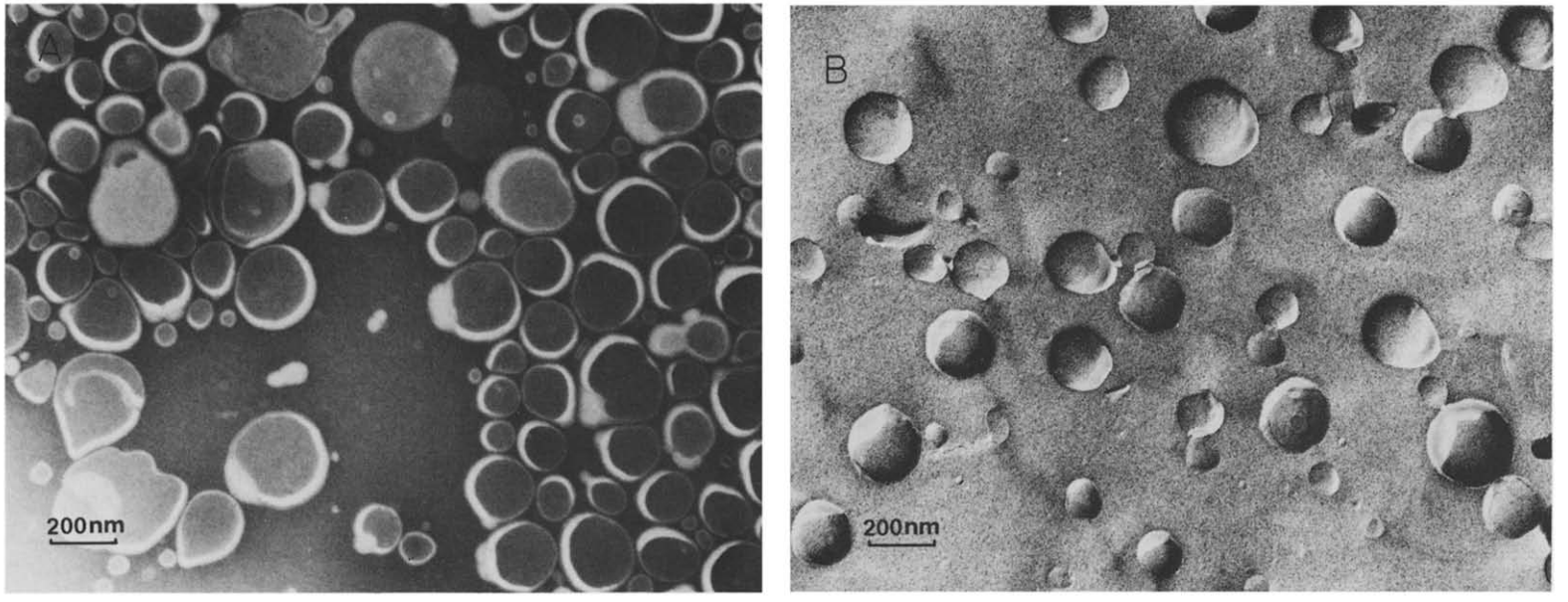

Fig. 4. Electron microscopy. The vesicles were prepared by dialysis from mixtures of decyl maltoside and DOPS/DOPS. (A) Negative staining with 2% ammonium heptamolybdate. The liposomes contain chromate in the inner compartment. Original magnification 84000×. (B) Freeze-fracturing. Same liposome preparation as in (A). A rapid freezing method was used [22,23]; shadowing with Pt/C (45°) and 90° carbon enforcement. Original magnification 84000×. The bar represents 200 nm.

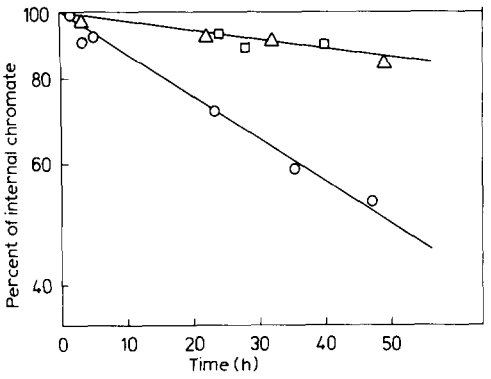

Fig. 5. Time-course of chromate efflux at 20°C. The vesicles were prepared by dialysis for 70 h at 4°C from mixtures of DOPC/octyl glucoside (○), DOPC/DOPS/octyl glucoside (□) and DOPC/DOPS/decyl maltoside (△). The lipid-to-detergent molar ratio was 1 : 5 in each case.

efflux from liposomes prepared by dialysis of octyl glucoside or decyl maltoside, respectively. Chromate efflux is noticeably slower with liposomes containing the negatively charged lipid, which is probably caused by repulsion of the negatively charged CrO_4^{2-} ion. A comparison of liposomes with a 1 : 1 ratio of DOPC to DOPS shows (Table III that octyl glucoside and decyl maltoside produce liposomes of comparable characteristics. As seen from Fig. 5, the decrease in internal chromate concentration obeyed first-order kinetics within experimental error limits.

Conclusions

Alkyl maltosides with 8 to 12 carbon atoms in the alkyl chain prove to be suitable detergents for the solubilization of phospholipids. Removal of detergent from mixed micellar suspensions containing negatively charged lipids results in the formation of unilamellar vesicles. The speed of detergent removal is ruled by the permeability of the dialysis membrane and by the CMC of the detergent. Attempts to influence the CMC by structural variation of the detergent molecule show that the CMC is governed mainly by the length of the alkyl chain, whereas the number of glucose residues in the polar headgroup has little effect.

In view of vesicle diameter and speed of detergent removal by dialysis, decyl maltoside is the most favorable detergent among the synthesized and tested compounds. Compared to the widely used octyl glucoside it requires a 3-times longer dialysis time, but the 10-fold lower CMC of decyl maltoside allows the use of much smaller amounts of detergent, e.g., in chromatographic purification procedures. A further advantage of decyl maltoside might be the markedly higher hydrophobicity, which possibly causes a lower denaturation effect during isolation and purification of membrane proteins [8].

Acknowledgements

The authors wish to thank Prof. P. Läuger for many helpful discussions. We are also indebted to Dr. W. Kinzi for the interpretation of ^{1}H-NMR-spectra, to Mrs. C. Wolf and R. Hildebrand for excellent technical assistance in electron microscopy and to Mrs. M. Roudna for preparing the drawings. This work was financially supported by Deutsche Forschungsgemeinschaft (Sonderforschungsbereich 156).

TABLE III

ION-FLUX MEASUREMENTS

At 20°C, 100 mM K_2CrO_4 inside, 150 mM KCl outside.

Lipid	Detergent [a]	Vesicle diameter (nm) [b]	CrO_4^{2-} efflux $t_{1/2}$ (h)	P (10^{-11} cm/s)
DOPC	octyl glucoside	204	48.3	1.96
DOPC/DOPS, 1 : 1	octyl glucoside	198	293	0.31
DOPC/DOPS, 1 : 1	decyl maltoside	237	289	0.38

[a] Used for preparation of vesicles.
[b] From internal volume.

References

1 Philippot, J.R., Mutaftchiev, S. and Liautard, J.P. (1983) Biochim. Biophys. Acta 734, 137–143
2 Milsmann, H.W., Schwendener, R.A. and Weder, H.G. (1978) Biochim. BIophys. Acta 512, 147–155
3 Rhoden, V. and Goldin, S.M. (1973) Biochemistry 18, 4173–4176
4 Mimms, L., Zampigi, G., Nosaki, Y., Tanford, C. and Reynolds, J.A. (1981) Biochemistry 20, 833–840
5 Ueno, M., Tanford, C. and Reynolds, J.A. (1984) Biochemistry 23, 3070–3076
6 Philippot, J.R., Mutaftchiev, S. and Liautard, J.P. (1985) Biochim. Biophys. Acta 821, 79–84
7 Lichtenberg, D., Robson, R.J. and Dennis, E.A. (1983) Biochim. Biophys. Acta 737, 285–304
8 Robinson, N.C., Neuman, J. and Wiginton, D. (1985) Biochemistry 24, 6298–6304
9 Rosevear, P., Van Aken, T., Baxter, J. and Ferguson-Miller, S. (1980) Biochemistry 19, 4108–4115
10 Holloway, P.W. (1973) Anal. Biochem. 53, 304–308
11 Schieren, H., Rudolph, S., Finkelstein, M., Coleman, P. and Weissmann, G. (1978) Biochim. Biophys. Acta 542, 137–153
12 Huang, C. and Mason, J.T. (1978) Proc. Natl. Acad. Sci. USA 75, 308–310
13 Enoch, H.G. and Strittmatter, P. (1979) Proc. Natl. Acad. Sci. USA 76, 145–149
14 Bartlett, G.R. (1959) J. Biol. Chem. 234, 466–468
15 Chattopadhyay, A. and London, E. (1984) Anal. Biochem. 193, 408–412
16 Koppel, D.E. (1972) J. Phys. Chem. 57, 4814–4820
17 Povencher, S.W. (1976) J. Chem. Phys. 64, 2772–2777
18 Helenius, A., McCaslin, D.R., Fries, E. and Tanford, C. (1979) Methods Enzymol. 56, 734–749
19 Helenius, A. and Simons, K. (1975) Biochim. Biophys. Acta 415, 29–79
20 De Grip, W.J. and Bovee-Geurts, P.H.M. (1979) Chem. Phys. Lipids 23, 321–325
21 Van Venetie, R., Leunissen-Bijvelt, J., Verkleij, A.J. and Ververgaert, P.H.J.Th. (1980) J. Microscopy 118, 401–408
22 Pscheid, P., Schudt, C. and Plattner, H. (1981) J. Microscopy 121, 149–167
23 Knoll, G., Oebel, G. and Plattner, H. (1982) Protoplasma 111, 161–176