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Reconstitution of Na^+/K^+-ATPase into phosphatidylcholine vesicles by dialysis of nonionic alkyl maltoside detergents

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The reconstitution of Na^+/K^+-ATPase from outer medulla of rabbit kidney into large unilamellar liposomes was achieved through detergent removal by dialysis of mixed micellar solutions of synthetic dioleoyl phosphatidylcholine/octyl glucoside and Na^+/K^+-ATPase/decyl maltoside or decenyl maltoside. Tight, transport-active liposomes were formed when the lipid and the enzyme were solubilized separately in the nonionic detergents and mixed immediately before starting the dialysis. The two maltoside detergents with different structures of the hydrophobic part of the molecule proved to be well suited for the solubilization of Na^+/K^+-ATPase with high retention of enzyme activity; the inactivation of enzyme being evidently slower with the unsaturated decenyl maltoside. The diameters of the proteoliposomes, 110 and 170 nm, respectively, were also dependent on the structure of the maltoside detergent, the saturated decyl maltoside producing the bigger liposomes. After freeze-fracture, both preparations exhibited intramembranous particles as structural indicators of successful reconstitution. The electrogenic activity of the reconstituted enzyme was determined by fluorescence measurements with Oxonol VI and by tracer-flux measurements with $^{22}Na^+$.

Introduction

Incorporation of the purified Na^+/K^+-ATPase into closed artificial phospholipid vesicles has been accomplished mainly by detergent removal from mixed micellar suspensions of lipid, protein and detergent. In recent years reconstitution of Na^+/K^+-ATPase by cholate dialysis has been widely used [1,2]. A successful reconstitution of Na^+/K^+-ATPase has also been effected by detergent adsorption to polystyrene beads [3,4], freeze-thaw-sonication [5], detergent dilution [6], and by incubation of the enzyme with preformed vesicles [7].

Reconstitution of Na^+/K^+-ATPase by detergent dialysis has some advantages over other methods: (i) it is easy to accomplish; (ii) the system is not diluted and (iii) the resulting vesicles exhibit a narrow size distribution [2]. But some disadvantages should be noted too: (i) up to now the procedure has been restricted to the use of sodium cholate and other bile salts; (ii) cholate dialysis vesicles have comparatively small diame-

Abbreviations: DOPC, dioleoylphosphatidylcholine; DOPG, dioleoylphosphatidylglycerol; DOPS, dioleoylphosphatidylserine; DOPE, dioleoylphosphatidylethanolamine; $C_{12}E_8$, dodecyl octaethyleneglycol monoether; CMC, critical micelle concentration; HPLC, high-performance liquid chromatography.

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ters of 60–90 nm; and (iii) the protein is exposed to the detergent for a relatively long time.

Recent studies on the isolation of membrane proteins by use of nonionic detergents suggest that some compounds of this class of detergents are superior to the ionic bile salts because of their smaller denaturation effect [8]. But at the present time it is not possible to make any predictions from the structure of a detergent concerning its interaction with a given protein. The most suitable detergent must be determined by appropriate experiments.

The chemical structure of a detergent has a crucial influence on its physical properties. Hydrophobic-lipophilic balance (HLB number), critical micelle concentration (CMC), solubility above CMC, and micellar size control the interaction of the detergent with the protein and the lipid [9,10] and, in particular, the CMC is a determining factor for the strategy of detergent removal. The nonionic detergents $C_{12}E_8$ [3] and Triton X-100 [4] have been used with success for the reconstitution of Na^+/K^+-ATPase, but the detergent had to be removed by direct contact with polystyrene beads. Removal by dialysis is not possible with these detergents because of their small CMC (0.2 mM). Octyl glucoside, though well suited for removal by dialysis (CMC = 25 mM) and widely used for vesicle formation, proved to be unsuitable for reconstitution of Na^+/K^+-ATPase by dialysis [11], which is probably due to inactivation of the protein by high detergent concentrations during the reconstitution procedure [12].

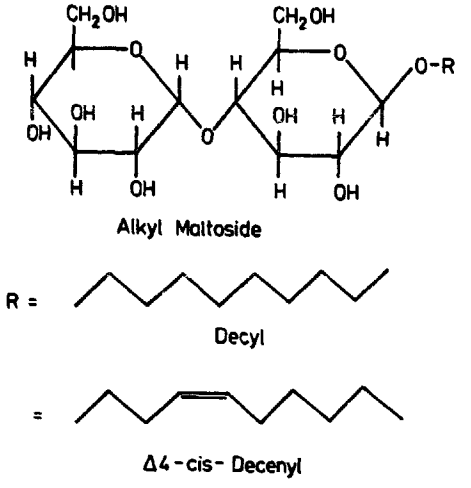

Fig. 1. Structure of alkyl maltoside detergents.

In the present paper we describe a dialysis method for the reconstitution of Na^+/K^+-ATPase using a mixed system of nonionic detergents. Synthetic dioleoylphosphatidylcholine and kidney Na^+/K^+-ATPase are solubilized separately in different detergents: the lipid in octyl glucoside and the enzyme in decyl maltoside or decenyl maltoside (Fig. 1). By this method, tight vesicles can be formed which exhibit a high transport activity.

Materials and Methods

Materials. *n*-Octyl β-D-glucopyranoside (octyl glucoside) was purchased from Calbiochem (Frankfurt, F.R.G.) DOPC was obtained from Avanti Polar Lipids (Birmingham, AL, U.S.A.). *n*-Decyl β-D-maltopyranoside (decyl maltoside) and Δ^4-*cis*-decenyl β-D-maltopyranoside (decenyl maltoside) were synthesized from heptaacetobromomaltose and the corresponding alcohol, as described earlier [13]. *n*-Decan-1-ol and Δ^4-*cis*-decen-1-ol were from Alfa Products (Danvers, MA, U.S A.).

Oxonol VI (bis(3-propyl-5-oxoisoxazol-4-yl) pentamethinoxonol) was from Molecular Probes (Junction City, OR, U.S.A.), ATP (Sonderqualität) from Boehringer (Mannheim, F.R.G.), vanadate from Ventron (Karlsruhe, F.R.G.) and ^{22}Na from Amersham International. All other reagents (analytical grade) were obtained from Merck (Darmstadt, F.R.G.). High-speed Spectra/Pore 2 dialysis tubings with 6.5 mm diameter were purchased from Spectrum Medical Industries (Los Angeles, U.S.A.) and ion exchange resin (Bio-Rex 70) from Bio-Rad (Munich).

Lipid solutions and buffer media. Lipid/detergent mixtures were prepared from stock solutions of DOPC and octyl glucoside in methanol. 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 H containing 30 mM imidazole/1 mM L-cysteine/1 mM EDTA/5 mM $MgSO_4$/70 mM K_2SO_4/5 mM Na_2SO_4. The final lipid concentration was 25 mg/ml and the lipid/detergent weight ratio was 1 : 3. Stopping and flushing buffer for the tracer flux experiments contained 267 mM saccharose to maintain isoosmolarity.

Preparation of membrane-bound and solubilized Na^+/K^+-ATPase. Purified membrane-bound enzyme was prepared from outer medulla of rabbit kidney following procedure C of Jørgensen [14]. The specific activity at 37°C was 1100–1300 μmol P_i/mg per h. The membrane-bound enzyme (2 mg/ml) was centrifuged for 15 min at 130000 × *g* at 20°C in a Beckman airfuge. The protein-free supernatant was discarded and replaced by an equal volume of buffer H containing 6 mg/ml of the nonionic detergent decyl maltoside or decenyl maltoside. The final detergent/protein weight ratio was 3, based on the initial amount of membrane-bound protein. After an incubation time of 10 min at 20°C, the mixture was centrifuged for 15 min at 130000 × *g* in a Beckman airfuge at 20°C. The solubilized enzyme which was collected from the supernatant had a specific activity of 600–900 μmol P_i/mg protein per h at 37°C. The final protein concentration was 0.8–1.1 mg/ml.

Preparation of proteoliposomes. Immediately before starting the dialysis, 100 μl of ice-cold protein solution were mixed with 20 μl of ice-cold lipid solution and transferred to a 6.5 mm wide high-speed dialysis tubing Spectra/Pore 2. The dialysis was performed for 60 h at 4°C against 200 ml of buffer H, changing the buffer twice at 20-h intervals.

Analytical procedures. DOPC concentrations in micelle and liposome suspensions were determined by the enzymatic phospholipid B test [15]. Determination of detergent concentrations were performed by HPLC as described in a previous paper [13]. Protein concentrations were determined by the method of Lowry et al. [16] or by the Bio-Rad protein assay [17] using bovine serum albumin as a standard. Enzyme activities were determined by the pyruvate kinase/lactate dehydrogenase test [18].

Tracer-flux experiments. To determine active or passive sodium flux through vesicle membranes, tracer-flux experiments were performed. Vesicles were incubated with or without 20 mM Mg-ATP in buffer H containing 5 mM K_2SO_4/70 mM Na_2SO_4 and up to $3.7 \cdot 10^4$ Bq ^{22}Na. After a certain incubation time, aliquots of the vesicle suspension were taken and added into ice-cold stopping buffer of equal osmolarity. The aliquots (volume V_a) contained approx. 18 μg lipid and 900 Bq ^{22}Na. The external radioactivity was removed by a method derived from Cornelius and Skou [19]. Vesicles and stopping buffer were flushed for 30 s through a microcolumn filled with 400 μl cation exchange resin Bio-Rex 70 with 1 ml of isoosmolar saccharose solution into scintillation vials containing 5 ml scintillation fluid (Aqualuma Plus, Baker). All radioactivity values obtained from the same incubation were referred to the radioactivity of an equal volume (V_a) of the incubation buffer ('external' ^{22}Na).

Determination of membrane potentials. Since Na^+/K^+-ATPase is an electrogenic transport protein, determination of the membrane potential yields information about the activity of the reconstituted protein. A convenient method for detecting membrane potentials is the use of potential-sensitive fluorescence dyes. Oxonol VI is a well-understood and successfully applied potential probe [20]. The partition coefficient γ of the dye between aqueous and lipid phase for the above-characterized vesicle preparation was determined to be 15200; it differs slightly from that of reconstituted vesicles prepared by cholate dialysis (γ = 19000). According to Apell and Bersch [21], calibration curves of fluorescence vs. membrane potential can be evaluated and used to transform fluorescence signals into potential curves. The initial slope of the potential change after starting the pump by ATP addition is proportional to the product of pumping rate and amount of transport active Na^+/K^+-ATPase, averaged over all vesicles [21].

Electron microscopy. For rapid freezing, samples sandwiched between copper and plastic sheets (Thermanox, Lux Sci.) [22] were dipped at a speed of 2 m/s into heavily stirred propane at its melting point, the copper part facing the stirring direction of propane. The frozen samples were freeze-fractured in a Balzers 360 M device at 173 K and Pt/C shadowed at 45°C. Intact replicas were obtained by dissolving the copper [23]. Electron micrographs were taken in a Siemens Elmiskop 101 at 80 kV. The magnification was checked using a grating replica.

Results and Discussion

Optimal detergent / protein weight ratio

The inactivation of Na^+/K^+-ATPase by ionic and nonionic detergent has been the subject of several investigations [12,24–26]. An optimal solubilization of the purified membrane-bound protein by detergents has to take into account the yield of protein as well as the conservation of enzyme activity. For the nonionic detergent $C_{12}E_8$ an optimal detergent/protein weight ratio of 2–4 was found. At this ratio the irreversible inactivation is very slow: after 100 h at room temperature, about 90% of the enzyme activity is retained [27]. With higher detergent/protein ratios and at higher temperatures, a rapid irreversible inactivation is observed.

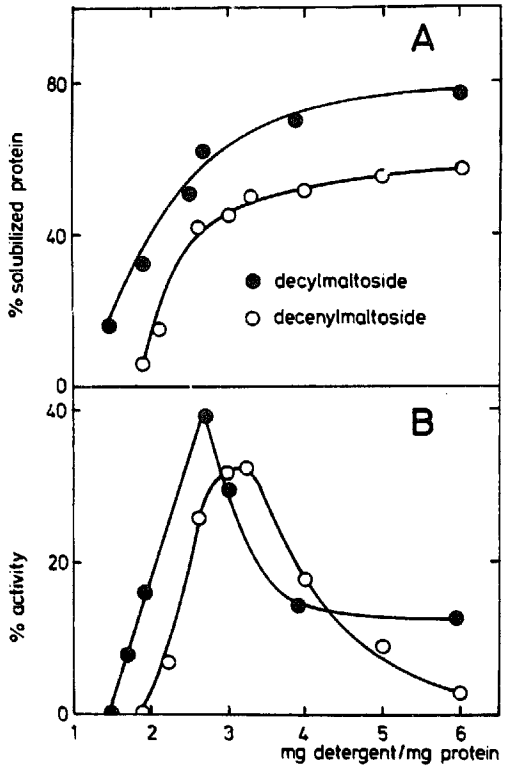

Fig. 2. Optimum detergent/protein weight ratio. Purified membrane-bound kidney Na^+/K^+-ATPase was solubilized in increasing amounts of detergent in buffer H. After centrifugation of undissolved membranes, aliquots of the supernatant were taken for the determination of protein content and specific enzyme activity, the amount of detergent in the assay medium remaining below the CMC. (A) % yield of solubilized protein referred to the protein content prior to solubilization; (B) total activity of solubilizate, referred to total activity prior to solubilization (equal to the product of relative protein yield times the relative yield of specific activity, in percent). Detergents: ●, decyl maltoside; ○, decenyl maltoside.

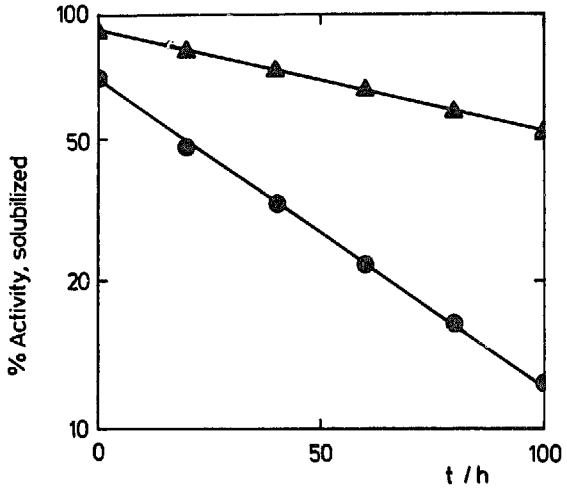

Fig. 3. Inactivation of Na^+/K^+-ATPase solubilized at optimum detergent/protein weight ratio (2.5 for decyl and 3.0 for decenyl maltoside). The protein concentration was kept at 2 mg/ml in buffer H. The solubilized enzyme was stored at 0 °C, and aliquots were taken for the determination of activity as described in the legend to Fig. 2B. Detergents: ●, decyl maltoside and ▲, decenyl maltoside.

In Fig. 2A the amount of the solubilized protein is plotted as a function of the detergent/protein weight ratio. It is seen that almost 80% of the total content of enzyme can be recovered with decyl maltoside and almost 60% of the enzyme content in decenyl maltoside.

In order to be suitable for reconstitution experiments, not only must a detergent give a high yield of solubilized membrane protein, but it must also preserve the specific activity. In Fig. 2B the product of specific activity (referred to the initial specific activity of the membrane-bound protein) times the yield of solubilized protein is plotted as a function of the protein/detergent weight ratio. Fig. 2B demonstrates that the nonionic detergents decyl maltoside and decenyl maltoside are well suited for solubilization of Na^+/K^+-ATPase. With detergent/protein weight ratios of 2.5 for decyl maltoside and 3.0 for decenyl maltoside (Fig. 2), about 50–60% yields of protein were obtained. With a retention of a specific enzyme activity of up to 90% after dialysis of the detergent, transport-active vesicle preparations were obtained with up to 40% enzymatic activity (Fig. 2B).

Octyl glucoside proved to be unsatisfactory for the solubilization of Na^+/K^+-ATPase. With detergent/protein weight ratios of 1–2 about 30% of the original enzyme activity was obtained. Weight ratios above 2 caused a rapid irreversible inactivation of the protein. This finding is in agreement with results from the literature [28,29].

We have also studied the stability of the enzyme when it was stored at 0°C in solubilized form at optimal detergent/protein weight ratio. As shown in Fig. 3, inactivation with decenyl maltoside as detergent is much slower than with decyl maltoside.

Formation of proteoliposomes

In a recent paper [13] we described the formation of large unilamellar liposomes by dialysis of mixed micellar solutions of DOPC, DOPS and the nonionic detergent decyl maltoside. The formation of multilamellar lipid structures, which is a tendency in using nonionic detergents [30], could be prevented by addition of at least 10% by weight of negatively charged lipids (DOPS, DOPG).

Attempts to accommodate this system to the formation of proteoliposomes containing Na^+/K^+-ATPase failed when both the lipid and the protein were solubilized by decyl maltoside. The resulting liposome suspensions revealed up to 100% recovery of the original enzyme activity, but in ion-flux measurements no, or only low, pump activity could be detected. Determination of lipid and protein distribution after density-gradient centrifugation showed that no, or only small, amounts of protein had been associated with lipid. These results could not be improved by the use of mixtures of DOPC, DOPS and DOPE of different weight ratios.

In contrast to these results, proteoliposomes with high pump activities and 40–60% of the original enzyme activity were formed when DOPC was solubilized by octyl glucoside and the protein by decyl maltoside. These solutions were mixed immediately before starting the dialysis. With lipid concentrations of 4 mg/ml and protein/lipid weight ratios of 0.2–0.06, tight proteoliposomes with functionally intact Na^+/K^+-ATPase were obtained.

A successful reconstitution was strongly dependent on the lipid concentration. DOPC concentrations above 5 mg/ml caused a cloudy precipitation of presumably multilamellar material, which was clearly distinguishable from the slightly opalescent appearance of a liposome suspension.

After mixing the lipid and protein solutions, the protein/ decyl-maltoside/ octyl-glucoside weight ratio was 1:5:15. This excess of octyl glucoside would normally lead to irreversible inactivation of the protein within a short time, as was confirmed by solubilization experiments with Na^+/K^+-ATPase and octyl glucoside alone. A protein/octyl-glucoside ratio of 1:9 (w/w) caused a complete and irreversible inactivation of the enzyme when the protein was solubilized as outlined above. A possible explanation for the stabilizing effect of decyl maltoside is that in the mixed micellar solution, octyl glucoside is bound to the lipid, and presumably the exchange of detergent between lipid/octyl glucoside and protein/decyl maltoside micelles is slow enough to prevent severe inactivation. Moreover, decyl maltoside is more hydrophobic than octyl glucoside and is probably not appreciably exchanged because of its stronger binding to the protein. Inactivation of the enzyme

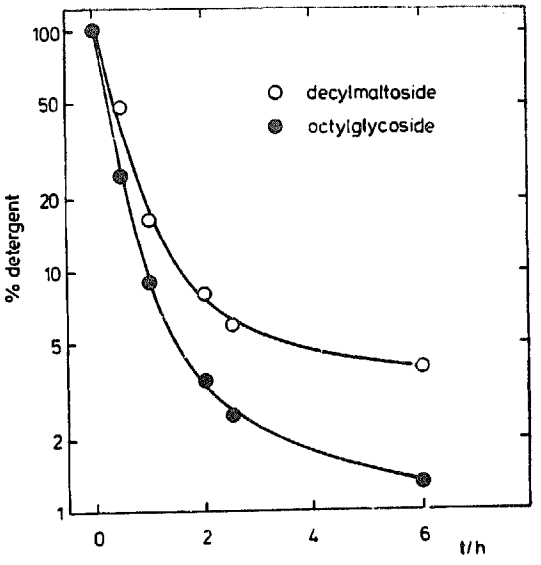

Fig. 4. Time-course of detergent removal during dialysis at 4°C using a Spectra/Pore 2 dialysis tubing. The dialysis was performed with mixed micellar solutions of DOPC/octyl glucoside and Na^+/K^+-ATPase/decyl maltoside in buffer H, which were mixed immediately before starting the dialysis. The relative amounts of detergent in the dialysis medium were determined by HPLC [13]. The remaining fraction of both detergents after 60 h dialysis was about 0.1% of the original amount.

is further reduced by the fast dialysis kinetics. Fig. 4 shows that about 75% of the detergent is removed within 1 h. The observation that the suspension becomes slightly opalescent after 15–20 min indicated that liposomes form early in the dialysis process.

Oxonol experiments

To determine the electrogenic activity of the Na^+/K^+-ATPase, experiments were performed in which the membrane voltage was monitored using the potential-sensitive dye Oxonol VI (Fig. 5). To 1 ml of buffer H was added Oxonol VI to a final concentration of 30 nM and subsequently 1 µl of vesicle preparation (4 µg lipid). After reaching a stable fluorescence signal, ATP was injected to a final concentration of 0.5 mM. The fluorescence increased to a plateau after 80 s. When vanadate, a potent inhibitor of Na^+/K^+-ATPase, was added to the buffer, the signal declined, approaching almost the original fluorescence level prior to ATP addition with a time constant of 44 s. From this time constant the leakage conductance of the membrane can be calculated to be 23 nS/cm² [21]. Using the independently determined fluorescence–voltage calibration [20], the time course of the membrane potential can be obtained.

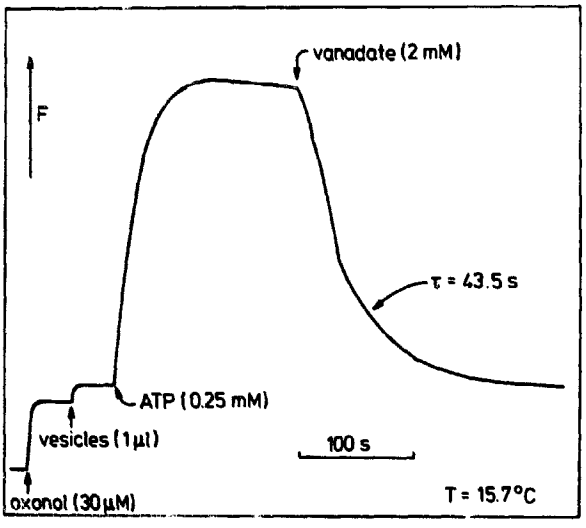

Fig. 5. Time-course of the fluorescence of Oxonol IV. Na^+/K^+-ATPase vesicles were prepared from DOPC/octyl glucoside in buffer H and Na^+/K^+-ATPase/decenyl maltoside in buffer H, containing 140 mM K^+ and 10 mM Na^+. Vesicles were suspended in a buffer containing 140 mM Na^+/10 mM K^+. 30 nM Oxonol VI/1 µl vesicle suspension containing 4.2 µg DOPC/250 µM ATP/2 mM vanadate were successively added. *F* is the fluorescence intensity. The temperature was 15.7°C.

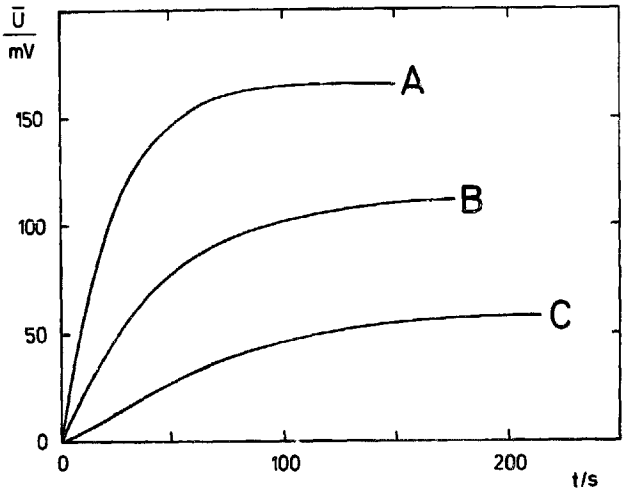

Fig. 6. Average membrane potential, $\bar{U}$, generated by Na^+/K^+-ATPase vesicles prepared from DOPC/octyl glucoside in buffer H and Na^+/K^+-ATPase/decyl maltoside in buffer H. The three preparations differed in protein content: (A), 0.9 mg/ml; (B), 0.5 mg/ml and (C) 0.2 mg/ml. The voltage $\bar{U}$ was obtained from independently determined calibration curves [20] and represents an average of the (heterogeneous) vesicle population. ATP was added at $t = 0$. Buffer conditions were as described in Fig. 5. The temperature was 17.2°C.

In Fig. 6 the electrogenic pump activity of three different vesicle preparations is compared. All three vesicle preparations had the same lipid concentration of 4.2 mg/ml. The protein used came from the same stock solution, solubilized in decyl maltoside, and varied in concentration: (A) 0.9, (B), 0.5, and (C) 0.2 mg/ml. The ratio of decyl maltoside-to-protein was kept constant. The initial increase in membrane potential (5, 2 and 0.7 mV/s, respectively) exhibits a superlinear dependence on protein concentration. The leakage conductance was determined for the different preparations to be 16 nS/cm² (0.9 mg/ml protein), 12.5 nS/cm² (0.5 mg/ml), and 9 nS/cm² (0.2 mg/ml). These values approximately agree with results from vesicle preparations obtained by cholate dialysis [21]. The maximum voltages observed in Fig. 6 are consistent with expected values derived from the initial potential increase and the leak conductance [21]. Reconstituted vesicles prepared from protein solubilized with decyl maltoside and decenyl maltoside showed no significant difference in the oxonol experiments.

^{22}Na influx

Fig. 7 shows a ^{22}Na-influx experiment performed at 25°C with two vesicle preparations containing 0.9 mg/ml protein (A) and 0.5 mg/ml (B). The protein solubilization was carried out with decyl maltoside in buffer H. The uptake of sodium in both experiments followed a monoexponential function of time with a time constant, τ. The comparison of saturation values N^{∞} shows a different pumping capacity of 0.44 μmol Na^+/mg protein (A) and 0.2 μmol/mg (B). Both values are corrected for the contribution of passive ^{22}Na influx (curve C in Fig. 7), which is an indicator for the contribution of leak effects of the membrane to the measured tracer flux. The pumping activity is influenced by two factors: (1) the intravesicular potassium concentration, which reaches very low values during the progress of the experiment, so that the pump has to switch to the slower Na-only mode [37]; and (2) the membrane potential, which reduces the pumping rate with increasing (intravesicular) potentials [21]. From the slope at time zero the initial pumping activity can be calculated by N^{∞}/τ to be 1.6 nmol Na^+/mg protein per s (A) and 315 pmol/mg per s (B) if 50% of the enzyme is oriented inside out. The comparison of both pumping activities shows an even more pronounced superlinear dependence of transport activity on the total amount of protein, as compared with the oxonol experiments. A similar dependence was observed with vesicle preparations obtained by cholate dialysis (unpublished results). The dependence of the pumping capacity on the protein concentration cannot be explained by different vesicle sizes, since the determined radii are indistinguishable. If it is assumed that only enzyme dimers, $\alpha_2\beta_2$, exhibit Na^+/K^+-transport activity [32,33] and that the reconstituted protein is in a monomer–dimer equilibrium, only part of the enzyme contributes to transport, the amount of $\alpha_2\beta_2$ protomers would show superlinear concentration dependence and the observed behaviour could be expected. The comparison of experimentally observed pumping activity of 1.6 nmol Na^+/mg protein per s (Fig. 7, curve A) with the theoretical upper limit of 50 nmol/mg per s (at 25°C) indicates a similar phenomenon. The theoretical value corresponds to the assumption that all protein is present in uniformly oriented units exhibiting an enzymatic activity of

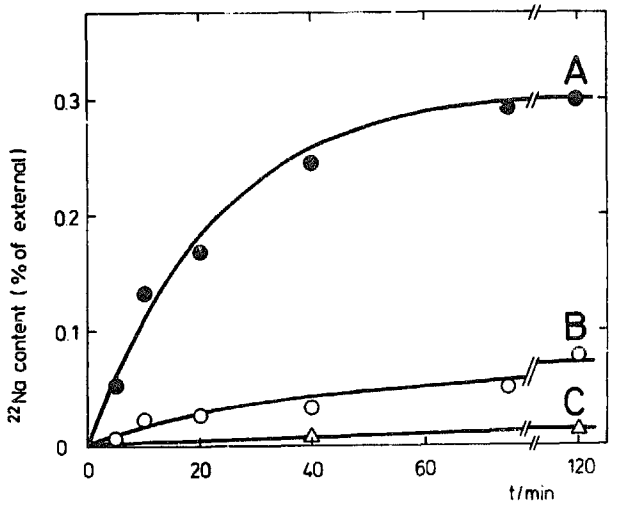

Fig. 7. Na^+ influx generated by Na^+/K^+-ATPase in reconstituted vesicles. At time $t = 0$, 20 mM Mg-ATP were added. The temperature was 25°C. Curve A, vesicle preparation containing 4.2 mg/ml DOPC/0.9 mg/ml protein; curve B, 4.2 mg/ml DOPC/0.5 mg/ml protein; curve C, Na^+ uptake of preparation A and B in the absence of ATP. The ordinate is the ^{22}Na content of the vesicles referred to the total ^{22}Na content of the vesicle suspension (in percent).

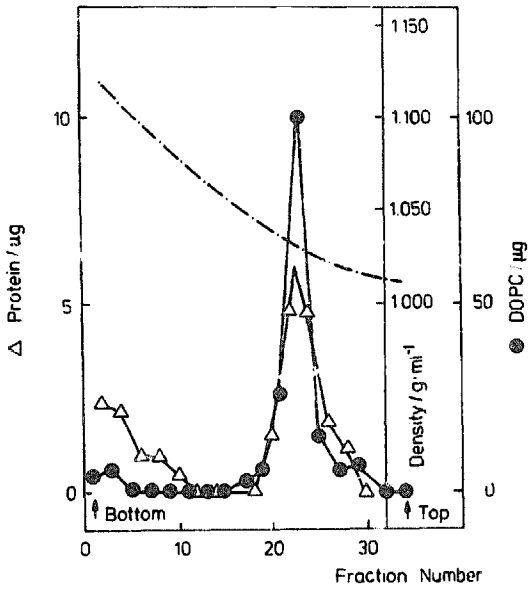

Fig. 8. Fractionation of proteoliposomes on a sucrose-density gradient. 0.1 ml of reconstituted proteoliposomes was applied to 2 ml of a continuous sucrose gradient, 5–20% (w/w). Centrifugation was performed for 4 h in a TLS-55 rotor at 260000 × g and 4°C. Fractions of 60 μl were collected from the bottom of the tube and analyzed for protein and DOPC content. The protein had been solubilized by decenyl maltoside before dialysis.

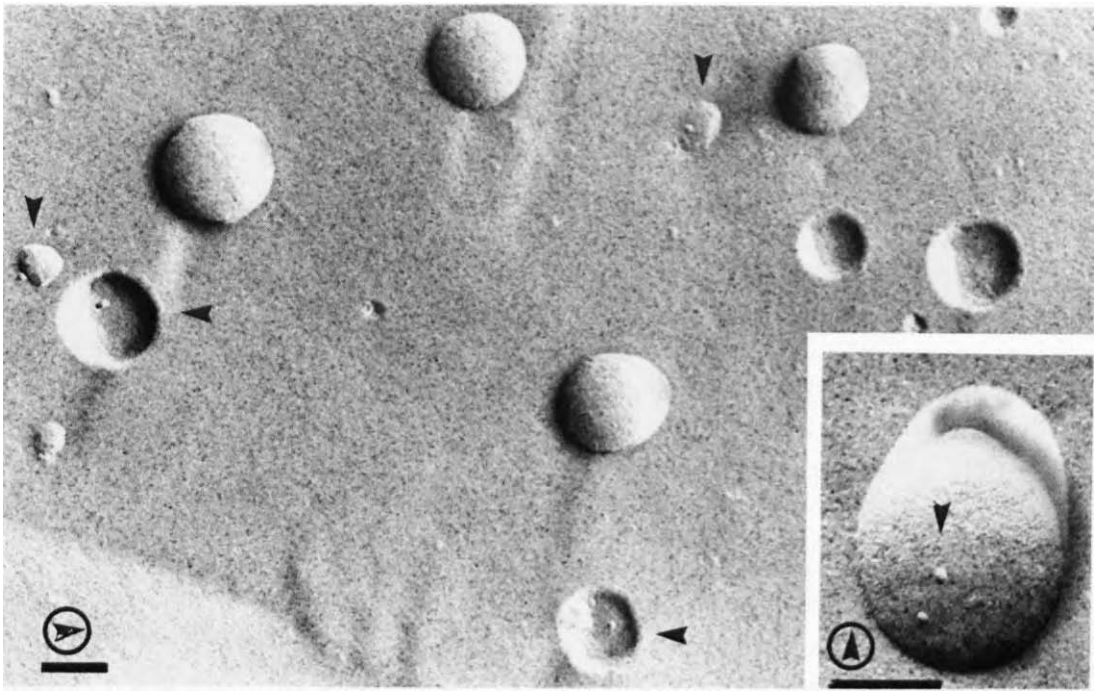

Fig. 9. Freeze-fractured liposomes (see Materials and Methods) show intramembranous particles on both convex (vertical arrowheads) and concave (horizontal arrowheads) fracture faces. The inset shows in detail a convex fracture face with two intramembranous particles at higher magnification. Direction of Pt/C shadowing is indicated by encircled arrowheads. Bars represent 100 nm.

375 μmol P_i/mg protein per h at 37°C and moving three Na^+ per ATP hydrolysed. The discrepancy of a factor of 30 cannot be accounted for by an asymmetry insertion of the reconstituted protein alone. Since ATP hydrolysing activity was observed by $\alpha\beta$ units [33], the discrepancy between enzymatic activity and transport would be understandable assuming $\alpha_2\beta_2$ units are in transport-active form.

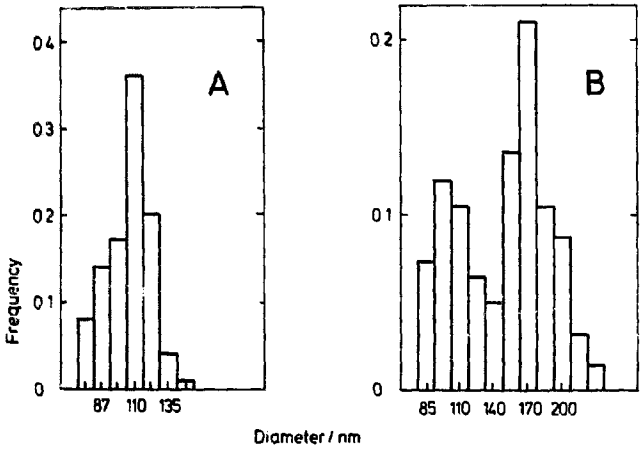

Fig. 10. Size distribution of reconstituted vesicles, as determined from electron micrographs of freeze-fractured preparations of proteoliposomes. About 1000 profile diameters were measured, and the size distribution was determined using the Wicksell transformation [31]. The reconstitution had been performed by dialysis of mixed micellar solutions of (A), DOPC/octyl glucoside and enzyme/decenyl maltoside and (B), DOPC/octyl glucoside and enzyme/decyl maltoside.

Density-gradient centrifugation

The recombination of protein and DOPC was studied by density-gradient centrifugation. Fig. 8 shows the protein/DOPC distribution in a 5–20% (w/w) sucrose gradient. About 90% of the lipid and 50% of the protein migrated together as a sharp opalescent band at a buoyant density of 1.03. The protein/lipid weight ratio of this fraction was about 0.1. A minor fraction with about 20% of the employed protein and a protein/lipid ratio of 0.5 was found at higher densities of 1.10–1.15.

Electron microscopy: size, size distribution and protein reconstitution

From electron micrographs of freeze-fractured liposomes (Fig. 9) about 1000 profile diameters were measured, and the size distribution of the liposomes was determined using the Wicksell

transformation [31]. Fig. 10 shows that the size of the vesicles is affected by the chemical structure of the maltoside detergent. With the unsaturated decenyl maltoside in the mixed micellar solution, a fairly homogeneous size distribution with a maximum at 110 nm was obtained. The saturated decyl maltoside revealed a broader size distribution with a maximum at 170 nm. The occurrence of a second liposome population with smaller diameters (Fig. 10B) was also observed with pure DOPC/cholate dialysis liposomes when the Wicksell transformation was used for the determination of the size distribution of freeze-fractured liposomes (unpublished results). The property of an unsaturated maltoside to produce smaller vesicles than the corresponding saturated compound was also observed with 2,4-decadienyl maltoside [13].

Protein-containing vesicles exhibit intramembrane particles as presented in Fig. 9. These particles appear with equal probability on concave and convex fracture faces. No corresponding pits were recognized, as opposed to Na^+/K^+-ATPase in native membranes [29]. From the apparent frequency distribution, an extrapolation to the total membrane area of the vesicles indicates that the majority of liposomes should contain one or more particles. The apparent size of these particles (8–10 nm) is influenced by the replication procedure, probably increased (as compared to the actual size) by some aggregation of shadowing material. The value observed, however, is in agreement with observations in previous investigations of vesicle preparations with reconstituted Na^+/K^+-ATPase [34–36] and with Na^+/K^+-ATPase in native membranes [29].

Conclusions

Decyl and Δ^4-*cis*-decenyl maltoside prove to be suitable detergents for the solubilization of Na^+/K^+-ATPase from rabbit kidney. Removal of detergent by dialysis from mixed micellar suspensions containing synthetic DOPC/octyl glucoside and Na^+/K^+-ATPase/decyl or decenyl maltoside results in the formation of large unilamellar liposomes with reconstituted, transport-active enzyme.

The molecular structures of the two maltoside detergents evidently influence the inactivation kinetics of the solubilized enzyme and the diameter of the produced proteoliposomes (110 and 170 nm, respectively). At optimum detergent/protein weight ratios the inactivation of the enzyme was slower and the liposome diameters smaller (110 nm) with the unsaturated decenyl maltoside (Figs. 3 and 10).

The different inactivation kinetics of two maltoside detergents had no effect on the transport activity of the resulting proteoliposomes when the dialysis was started immediately after solubilization of the enzyme. A comparison of oxonol experiments with liposomes prepared from decyl maltoside and from decenyl maltoside showed no significant difference in the electrogenic activity of the reconstituted Na^+/K^+-ATPase.

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