Hydrodynamic Properties of Phospholipid Vesicles and of Sucrase Isomaltase-Phospholipid Vesicles*

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The hydrodynamic properties of phosphatidylcholine vesicles prepared by the cholate removal method (Brunner, J., Skrabal, P. and Hauser, H. (1978) Biochim. Biophys. Acta 455, 322-331) were determined by analytical ultracentrifugation and compared with those of sonicated vesicles. The former were homogeneous as characterized by a narrow particle size distribution, but showed some fluctuation in the average particle size from one preparation to another. In contrast, sonicated vesicles showed a wide particle size distribution with their average particle diameter depending on the conditions of the ultrasonic irradiation. The molecular weight of the enzyme complex sucrase-isomaltase, which is a major intrinsic protein of small intestinal brush border membrane, was determined in the analytical ultracentrifuge in the presence of two different detergents: sodium cholate and octyl tetraoxethylene. The values thus obtained were 2.7 $\times$ 10$^6$ and 2.8 $\times$ 10$^6$, respectively. Upon removal of the detergent, the protein aggregated to regular oligomeric structures which were speroidal and had a diameter of 28 $\pm$ 4 nm (i.e. about twice the diameter of the sucrase-isomaltase complex).

A model membrane system was reconstituted from phosphatidylcholine and sucrase-isomaltase by the cholate removal technique (Brunner, J., Hauser, H., and Semenza, G. (1978) J. Biol. Chem. 253, 7538-7546). Papain digestion of this model membrane yielded a water-soluble form of sucrase-isomaltase with a molecular weight of $M_r$ = 2.37 $\pm$ 0.09 $\times$ 10$^6$ indistinguishable from that obtained by papain digestion of brush border membrane vesicles. This finding supports the notion that the mode of lipid-protein interaction in the model membrane system resembles that in the brush border membrane. Reconstituted sucrase-isomaltase-phospholipid vesicles were investigated in the analytical ultracentrifuge. Depending on the protein/lipid ratio, populations with 1 to 6 protein molecules inserted into the lipid bilayer of one vesicle were observed. This shows unambiguously that the monomeric form of the protein is incorporated into the lipid bilayer. However, it was not possible to fit a Poisson distribution to the population of vesicles differing in the number of protein molecules inserted. This was attributed to the presence of some undissociated protein dimers which also interacted with the lipid bilayer.

The enzyme complex sucrase-isomaltase is anchored in the brush border membrane by a small hydrophobic peptide located near the NH$_2$-terminal end of the isomaltase subunit (1). Pure sucrase-isomaltase can readily be assembled into phosphatidylcholine bilayers (2). The resulting model membrane system (sucrase-isomaltase PC vesicles) resembles in many ways the "native" brush border membrane as regards the mode of the sucrase-isomaltase phospholipid interaction (1-3).

Here we characterize, mainly by analytical ultracentrifugation, the reconstituted membrane system. Particularly, the question of stoichiometry and of whether the enzyme is inserted in the phospholipid bilayer as monomers or dimers are important in view of the possible functional role(s) of this membrane-bound enzyme. A previous study (2, 3) of the model membrane system led to some tentative conclusions regarding the protein-lipid stoichiometry. A detailed ultracentrifugal analysis was precluded because the exact value of the molecular weight of sucrase-isomaltase was unknown. Here we present the molecular weight determination of sucrase-isomaltase and the analysis of the protein-lipid stoichiometry based on this molecular weight. The results provide strong support for the tentative interpretation given previously (2, 3).

**EXPERIMENTAL PROCEDURES**

**Materials**

Egg phosphatidylcholine was purchased from Lipid Products (South Nutfield, Surrey, United Kingdom). 1,2-Dimyristoyl-sn-phosphatidylcholine and 1,2-dioleoyl-sn-phosphatidylcholine were synthesized by Mr. R. Berchtold, Biochemisches Labor, Berne, Switzerland. All lipids were chromatographically pure and used without further purification. H$_2$O (99.8%) was obtained from EIR (Wurenlingen, Switzerland). Con A-Sepharose 4B was a Pharmacia product. Cholic acid was recrystallized from acetone/water (4:1, v/v). All other chemicals were analytical reagent grade.

**Methods**

Brush border membranes were prepared from frozen rabbit small intestine according to (4). Sucrase-isomaltase was isolated from brush border membranes using the detergent Triton X-100 (from Serva, Heidelberg, Federal Republic of Germany) (5). Triton X-100 was removed on Biosetion SM-2 and the resulting protein was extensively dialyzed against 0.1 M NaCl, 0.01 M sodium borate (pH 9.0), 0.02% NaN$_3$ containing 2% (w/v) sodium cholate or against 0.1 M NaCl, 0.02 M sodium phosphate (pH 7.0), 0.02% NaN$_3$ containing 1% (w/v) octyl

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1 The abbreviations used are: PC, phosphatidylcholine; DMPC, 1,2-dimyristoyl-sn-phosphatidylcholine; DOPC, 1,2-dioleoyl-sn-phosphatidylcholine; P-SI, water-soluble fraction of sucrase-isomaltase, released from brush border membrane by digestion with pepsin; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; $T_c$, transition temperature.

2 The term "reconstitution" is used as described (2).

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tetraoxethylene (6). By controlled papain digestion of brush border membrane a water-soluble form of sucrase-isomaltase was released from the membrane (papain-solubilized sucrase-isomaltase, P-SI). Similarly, controlled papain digestion of sucrase-isomaltase PC vesicles, also produced a water-soluble form of sucrase-isomaltase (2).

Phospholipid dispersions in 0.1 M NaCl, 0.01 M Tris HCl (pH 7.3), 0.02% NaN3 were prepared and sonicated as described before (2). Egg PC and DOPC were in ice water while DMPC was sonicated above the temperature transition in a 35 °C water bath. The unsaturated lipids were stored at 4 °C while DMPC was kept at 26 °C (T = 23 °C). The preparation of single-bilayer vesicles without sonication (7) and the assembly of sucrase-isomaltase into egg phosphatidylcholine bilayers using the cholate removal technique have been described before (5). Before the removal of the detergent, the egg PC/cholate molar ratio varied between 0.35-0.5. The resulting model membrane systems are referred to as "cholate" vesicles and sucrase-isomaltase PC vesicles, respectively. Phospholipid and protein concentrations were determined according to Refs. 6 and 9, respectively.

Analytical Ultracentrifugation—Analytical ultracentrifugation was carried out in a Beckman model E instrument equipped with an RTIC temperature control unit using an An-D rotor and a 12-mm double-sector, capillary-type synthetic boundary cell. All experiments were carried out at 20 °C except for DMPC which was run at 26 °C. Unless stated otherwise sedimentation runs were performed at ~50,000 rpm using chlorella optics. Apparent sedimentation coefficients, $s_{app}$, were calculated from linear plots of log $r$ versus time $t$ where $r$ is the distance from the center of rotation to the position of the maximum ordinate of the chlorella pattern. Values of $s_{app}$ were corrected to standard values $s_{exp}$ in water at 20 °C. Diffusion measurements were carried out in the analytical ultracentrifuge at about 6500 rpm. Apparent diffusion coefficients, $D_{app}$, were calculated by the maximum ordinate-area method using the relationship $D_{app} = \left(\frac{2}{3} \pi \times k \right) \left(\frac{A}{Kh_{max}}\right)$ where $k$ is the magnification factor along the radial coordinate, $A$ is the area under the chlorella peak at time $t$ after boundary formation, and $H_{max}$ is the peak height. $D_{app}$ values were corrected to standard conditions in H2O at 20 °C ($D_{h2o}$). The heterogeneity parameter, $p$, was calculated from sedimentation experiments according to Ref. 10:

$$s_{p} \gamma_{t} = \frac{D}{v} + \frac{1}{2} \left(\frac{1}{v} \gamma_{t}\right) \left(\frac{r}{v}\right)$$ (1)

where $r_t$ and $s_p$ are the reduced first and second moment of the gradient curve, respectively, $r_t$ is the distance from the center of rotation to the meniscus, $a$ is the angular velocity and $D$ is the weight average diffusion coefficient.

Meniscus depletion sedimentation equilibrium experiments were carried out using Rayleigh interference optics. Short columns (~3 mm) of solutions (~0.5 mg/ml) were centrifuged at 1.4-2 times the equilibrium speed for 2-3 h before equilibrating for 12-18 h at 15,000 to 16,000 rpm for PC vesicles, 10,600 rpm for sucrase-isomaltase PC vesicles, and 14,300 rpm for sucrase-isomaltase solutions. Attainment of equilibrium was checked by comparison of photographs taken at 1- to 2-h intervals. For homogeneous solutes the plot of the logarithm of the fringe shift or concentration (in c) versus the square of the radial distance ($r^2$) is linear and the apparent molecular weight was derived from the slope of such a plot according to Ref. 11:

$$M_s = \frac{2RT}{d \ln c} \cdot \frac{d^2}{D_{h2o}}$$ (2)

The molecular weight of sucrase-isomaltase in 1% (w/v) octyl tetraoxethylene was determined at 3 different speeds (4,800, 8,000, and 11,000 rpm) using the photoelectric scanner at 280 nm. The details of the procedure used to determine molecular weights in this detergent are described elsewhere.5 Photographic plates were evaluated in a Nikon microcomparator model 6CT2.

**Partial Specific Volumes ($\rho$)—Densities were measured in a density gradient (DMA 02C from A. Paar K.G., Graz, Austria) at 20 °C. The temperature was maintained at the nominal setting to ±0.001 °C as described before (12). With this kind of temperature control, densities were accurate to ±10^{-3} g/cm^3 and, using these densities, apparent partial specific volumes of phospholipids (at ~1% w/v concentrations) were calculated with a precision of ±0.0003 cm^3/g.

**RESULTS**

The analysis of sucrase-isomaltase PC vesicles in terms of particle weight and protein-lipid stoichiometry requires the knowledge of the particle weight of pure PC vesicles and the molecular weight of sucrase-isomaltase. The results of the

**Table I**

| Sedimenting species | $s_{exp}$ | $D_{h2o}$ | $p^2$ | $M^4$ | $M^4$ |
|---------------------|-----------|-----------|-------|-------|-------|
| Egg PC vesicles (cholate method) | 2.70 ± 0.20* | 1.82 ± 0.10* | 1.10 ± 10^{-14} | (1.95 ± 0.25) × 10^{10} | (2.66 ± 0.12) × 10^{10} |
| Sonicated egg PC | 2.06 ± 0.12 | 1.96 ± 0.04 | 3.50 ± 10^{-14} | (1.44 ± 0.11) × 10^{10} | (1.52 ± 0.10) × 10^{10} |
| Sucrase-isomaltase* | | | | | |
| P-SI | | | | | |
| P-SI | | | | | |

* $p$, heterogeneity parameter as derived from equation 1.

** Sedimentation species calculated from the Svedberg equation $M_s = \frac{s_{exp} \gamma_{t}}{D_{h2o}}$ (1- $\rho_H$). The apparent partial specific volume ($\rho$) of egg PC determined at 10 mg/ml was $\rho = 0.9689 ± 0.0003$ cm^3 g^{-1} (13).

** The particle weight of cholate vesicles determined by sedimentation equilibrium was significantly higher than that derived from sedimentation and diffusion measurements. It was observed that, at rotor speeds below 15,000 rpm, the sedimentation coefficient of these vesicles increased sufficiently to compensate for the difference in molecular weight. A possible explanation of this observation is that the presence of residual cholate makes the vesicle susceptible to changes in shape in the presence of a strong gravitational field.

** $s_{exp}$ and $D_{h2o}$ values are obtained by extrapolation of the linear relationships in Fig. 1 to zero concentration using a least squares analysis.

** The particle weight of sonicated egg PC was significantly lower than that of the cholate vesicles. As reported before (14) the particle weight of sonicated PC was found to depend on the efficiency of the sonoprobe, e.g. the value for egg PC listed in Table 1 was obtained with a new tip. After extensive use of this probe the particle weight of egg PC increased to ~2 × 10^{10} (15). At the same time the sedimentation coefficient ($s_{exp}$) determined at a concentration of 9 mg/ml was 2.5 ± 0.15 S, quite close to the values measured for cholate vesicles at that concentration (Fig. 1a). For comparison the weight of DMPC and DOPC vesicles obtained by sonication with the same, extensively used microtip was determined. The vesicle weight determined by sedimentation equilibrium was 2.33 × 10^{8} (16) and 2.66 × 10^{8} for DMPC and DOPC, respectively. These values were in good agreement with those determined from sedimentation and diffusion measurements which were 2.33 and 2.72 × 10^{8} for DMPC and DOPC, respectively. For the vesicle weight determination the following apparent partial specific volumes were used: $\bar{\rho}$ (DMPC) = 0.9546 ± 0.0003 cm^3 g^{-1} determined at 10 mg/ml and $\bar{\rho}$ (DOPC) = 0.987 cm^3 g^{-1}, which was taken from Ref. 17.

** Sucrase-isomaltase was solubilized in 0.1 M NaCl, 0.02 M sodium phosphate (pH 7.0), 0.02% NaN3 containing 1% octyl tetroxyethylene.

** This value represents an upper limit for the molecular weight of sucrase-isomaltase as discussed in the text. Sucrase-isomaltase was solubilized in 0.1 M NaCl, 0.01 M sodium borate (pH 9.0), 0.02% NaN3 containing 2% (w/v) sodium cholate. The borate buffer (pH 9.0) was used to facilitate dissociation of sucrase-isomaltase dimers (cf. Ref. 18).

** Solubilized from brush border membrane vesicles by papain treatment.

** Solubilized from sucrase-isomaltase PC vesicles by papain treatment.
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ultracentrifugal analysis of egg PC vesicles and sucrase-isomaltase are summarized in Table I. The concentration dependence of the sedimentation and diffusion coefficients of sonicated egg PC vesicles and egg PC vesicles produced by the cholate method (7) is shown in Fig. 1. Fig. 1 and Table I document the differences between the two types of vesicle preparations: (a) the average vesicle weight of sonicated egg PC dispersions is usually smaller than that of cholate vesicles; (b) the variation in vesicle weight from one preparation to another is much larger for cholate vesicles as evident from the scatter of the $s_{20,w}$ values (Fig. 1a), but each preparation of cholate vesicles is characterized by a homogeneous particle size distribution. The difference in the homogeneity of the two kinds of vesicles is best illustrated in Fig. 1c. The spreading of the boundary during a sedimentation velocity run was analyzed according to equation 1. The homogeneity parameter ($p$) derived from the linear relationship (Fig. 1c) is significantly smaller for cholate vesicles (Table I). Both linear relationships in Fig. 1c extrapolate to the same intercept on the $s_{20,w}/2tr_{w}$ axis at $r_{w}$, $t \rightarrow 0$. This intercept is the weight average diffusion coefficient and the value thus obtained and corrected to standard conditions is $D_{20,w} = 1.71 \times 10^{-11} \text{ cm}^2 \text{s}^{-1}$ which is about within 10% of the actual diffusion measurements (Table I).

Sucrase-isomaltase was isolated from brush border membranes using Triton X-100. Upon removal of the detergent the protein aggregated to rosette-like structures of relatively uniform size as visualized by electron microscopy of negatively stained samples (data not shown; cf. Ref. 19). Particle size analysis gave an average diameter of about 28 ± 4 nm, which is approximately double the size of papain-treated sucrase-isomaltase (2). The aggregation of sucrose-isomaltase is largely reversible in the sense that most of the aggregates dissociated when 1-2% detergents such as octyl tetroxyethylene or sodium cholate were added. The resultant protein-detergent complexes were analyzed by sedimentation equilibrium. Plotting the data according to equation 2 gave straight lines from the slope of which $M_r (1 - \varepsilon p)$ was obtained; $M_r$

FIG. 1. Concentration dependence of $s_{20,w}$ (a), $D_{20,w}$ (b), and boundary spreading (c) for sonicated egg PC vesicles (x) and egg PC vesicles prepared by the cholate method (O). The solid lines were fitted to the data by least squares analysis and correspond to the following equations: $s_{20,w} = s_{0,w} (1 - k_c c)$ (a) and $D_{20,w} = D_{0,w} (1 - k_p c)$ (b). The $s_{0,w}$ and $D_{0,w}$ values at zero concentration derived from the least squares fit are listed in Table I. For both kinds of vesicles the same value was obtained for $k_c = 0.0036 1/g$. For sonicated egg PC vesicles $k_c = 0.0075 1/g$, while for cholate vesicles there is almost no concentration dependence of $s_{20,w}$. Although $k_c$ is negligible for these vesicles, there is a considerable scatter of the $s_{20,w}$ values. The boundary spreading during sedimentation at 50700 rpm was analyzed according to equation 1 for sonicated egg PC at 17.7 mg/ml (---) and for cholate vesicles at 7.2 mg/ml (----). The lines are least squares fits.

FIG. 2. Apparent sedimentation of flotation coefficients measured at 20°C as a function of the solution density $\rho$ of H$_2$O/D$_2$O mixtures. Densities were measured with a density meter (see "Methods"). O, peak 1 (see inset); x, peak 2; \, peak 3. Egg PC concentration, 2-3 mg/ml; sucrase-isomaltase concentration, 0.2 mg/ml. The lines are least squares fits. Inset, typical schlieren pattern from which the plot shown was derived. The lipid/sucrase-isomaltase weight ratio was 12. The photograph was taken after 29 min at 50740 rpm. The schlieren peaks are numbered in the order of increasing $s_{20,w}$ values. Schlieren peak 1 corresponds to protein-free vesicles and peaks 2-5 correspond to sucrose-isomaltase PC vesicles containing 1-4 sucrose-isomaltase molecules, respectively (cf. Table II and text).
and \( \delta_r \) are the weight and the partial specific volume, respectively, of the mixed micelle and \( \rho \) is the solution density. From

\[
M^* (1 - \varepsilon \rho) = M^* [(1 - \varepsilon \rho) + \delta_r (1 - \varepsilon \rho)]
\]

(3)

where the subscripts or superscripts \( c, p, \) and \( d \) refer to complex (mixed micelle), protein, and detergent, respectively, it is seen that the molecular weight of the protein requires the knowledge of \( \varepsilon \rho, \varepsilon \omega, \) and \( \delta_r \), the weight ratio of detergent bound to the protein. Since the product of the partial specific volume of octyl tetroxylethylene and the solvent density is \( \approx 1 \), the contribution of the buoyancy term \( \delta_r (1 - \varepsilon \rho) \) can be neglected (6). Using equation 3 with \( \varepsilon \rho = 0.739 \text{ cm}^3 \cdot \text{g}^{-1} \) (18), a value for the molecular weight of sucrase-iso-maltase of \( (2.8 \pm 0.1) \times 10^5 \) was obtained (Table I). Analysis of the protein-cholate complex yielded \( M^* (1 - \varepsilon \rho) = 6.57 \times 10^5 \); an upper limit of the resulting molecular weight \((2.7 \pm 0.1) \times 10^5\), cf. Table I) can be obtained by assuming that \( \delta_r \) is close to zero.

The molecular weight of the soluble form of sucrase-iso-maltase obtained by papain treatment of brush border membranes (see “Methods” and Refs. 1–3) was determined by sedimentation equilibrium. The value of \((2.37 \pm 0.09) \times 10^5\) thus obtained (Table I) is in good agreement with \(2.35 \times 10^5\) determined by the Archibald method (20) and in reasonable agreement with \(2.21 \times 10^5\) derived from the sedimentation and diffusion coefficients (18) using the Svedberg equation. It is interesting to note that a water-soluble form of sucrase-iso-maltase was also obtained by papain treatment of the reconstituted membrane system. The protein released from the model membrane system gave a molecular weight determined by sedimentation equilibrium which was within the experimental error consistent with that of P-SI liberated from brush border membrane vesicles (Table I). As expected, the sedimentation coefficient of that protein extrapolated to zero concentration \( s_{20,w} \) = 9.9 \pm 0.4 \text{ S} agreed well with the value of \( s_{20,\text{wc}} \) = 9.60 \text{ S} reported for P-SI released from brush border membrane (18).

The schlieren pattern of reconstituted sucrase-iso-maltase PC vesicles which were separated from protein-free egg PC vesicles by adsorption chromatography on Con A-Sepharose 4B. The experiment was carried out at 16.9 °C and at a rotor speed of 10,517 rpm; lipid concentration, 0.4 mg/ml; lipid/protein weight ratio, 11. Interference optics were used and fringe shifts, which are proportional to the concentrations, were plotted as a function of \( r^2 \), \( r \) being the distance from the rotor centre to the point of the fringe shift measurements. Curve x was calculated on the basis of the 1:1 mixture of vesicles containing 1 and 2 sucrase-iso-maltase molecules; curve o was calculated for a 1:1 mixture of vesicles containing 2 and 4 sucrase-iso-maltase molecules (for details of the calculation, see “Appendix”). Only the former calculation gives a satisfactory fit, although the calculated curve deviates from the experiment at low \( r^2 \) values. Decreasing the molecular weight of sucrase-iso-maltase to \( 2.6 \times 10^5 \) and increasing the ratio of (vesicles containing 1 sucrase-iso-maltase/vesicles containing 2 sucrase-iso-maltase) = 5:45 improved the fit. The experimental curve corresponds to a 1:1 mixture of vesicles containing 1 or 2 sucrase-iso-maltase molecules can also be demonstrated by calculating average values for \( [M^* (1 - \varepsilon \rho)] \) according to Ref. 21. The experimental [\( M^* (1 - \varepsilon \rho) \) ] value is 1.28 \times 10^6, in good agreement with the value of 1.31 \times 10^6 calculated for a 1:1 mixture of vesicles containing 1 and 2 sucrase-iso-maltase molecules. It is consistent with the value of 2.18 \times 10^5 calculated for a 1:1 mixture of vesicles containing 2 and 4 sucrase-iso-maltase molecules.

### Table II

| Schlieren peak number | Experimental | Computed | Number of sucrase-iso-maltase molecules/vesicle |
|----------------------|--------------|----------|-----------------------------------------------|
| 1                    | 1.018        | 1.016    | 0                                             |
| 2                    | 1.041        | 1.040    | 1                                             |
| 3                    | 1.064        | 1.061    | 2                                             |
|                      |              |          | 1.079                                         |

*The schlieren peaks (inset, Fig. 2) are numbered in the order of increasing sedimentation coefficients.

The calculation was carried out for 1, 2, or 4 sucrase-iso-maltase molecules of \( M_1 = 2.75 \times 10^5 \) being inserted into the lipid bilayer of egg PC vesicles. It has been shown before (2) that the vesicle size remains unchanged upon incorporation of the protein and hence the average vesicle weight used in the calculation is that of protein-free egg PC vesicles determined by sedimentation equilibrium: \( 2.66 \times 10^5 \) (see Table I). The evidence that the vesicle size remains unchanged when protein is inserted in the lipid bilayer is based on gel filtration (2) and \(^{1}H\) and \(^{31}P\) NMR measurements in the presence of paramagnetic cations and anions. These ions are known to induce perturbations, such as chemical shift changes or line broadening, in the polar group resonances. With sucrase-iso-maltase egg PC vesicles approximately 50% of the total signal intensity of the \(^{1}H\) polar group signals or the \(^{31}P\) signal were shifted in the presence of Pr(NO\(_3\))\(_3\). The ratio of shifted to unshifted signal intensity, which depends on the vesicle size, agreed within experimental error with the ratio measured for protein-free vesicles (2), indicating that the incorporation of sucrase-iso-maltase into the lipid bilayer does not affect the average size of the lipid vesicle.
rough estimate of their relative amounts can be derived from the schlieren peak areas.\(^4\)

The simplest mixture of sucrase-isomaltase lipid vesicles consisting of only 3 peaks was produced when lipid and protein were mixed at weight ratios ≈20. At higher lipid/protein weight ratios the two protein-containing peaks 2 and 3 (inset, Fig. 2) decreased simultaneously and eventually disappeared. When the lipid/protein ratio decreased, an increasing number of peaks was detected in the schlieren pattern, e.g. as a lipid/protein weight ratio of 1.5, 6 clearly discernible peaks in addition to the phospholipid peak were observed (cf. Fig. 1 of Ref. 2). Phospholipid vesicles free of protein could be removed on a Con A-Sepharose 4B column (dimension, 0.6 × 13 cm; flow rate, 3.5 ml/h). Sucrase-isomaltase lipid vesicles were retained on such a column and could be eluted with 50 mM 6-aminohexanoate. The schlieren pattern of reconstituted sucrase-isomaltase egg PC vesicles treated in this way consisted therefore of only 2 peaks of about equal area (data not shown). The sedimentation equilibrium analysis of such a preparation is shown in Fig. 3. A curved line was obtained (○) when the data were plotted according to equation 2. This plot is in reasonable agreement with the one computed (×) on the basis that the preparation consists of equal populations of vesicles containing 1 and 2 sucrase-isomaltase molecules. In contrast, equal populations of vesicles containing 2 and 4 sucrase-isomaltase molecules are clearly inconsistent with the experimental data (Fig. 3).

The same reconstitution as described for egg PC (2) was applied to assemble sucrase-isomaltase into bilayers of DMPC except that the procedure was carried out at temperatures well above the transition temperature \(T_c = 23\) °C of DMPC. Working with DMPC at 37 °C, it can be shown that sucrase-isomaltase is readily assembled into sucrase-isomaltase DMPC single-bilayer vesicles with properties similar to sucrase-isomaltase egg PC vesicles. However, lowering the temperature close to the \(T_c\) led to the aggregation and/or fusion of sucrase-isomaltase lipid vesicles and consequently to losses of material; this was not eluted from the Sephadex column and sometimes even caused clogging of the column.

**Discussion**

A prerequisite for an understanding of the ultracentrifugal behavior of reconstituted, complex lipoprotein systems is the knowledge of the behavior of the pure components. One important property of egg PC vesicles produced by the cholate method is that the average size and hence weight remain unchanged (within experimental error) when one or a few peptide chains are inserted in the lipid bilayer. This was shown by several methods such as gel filtration, tracer inclusion, and NMR techniques (cf. Ref. 2 and the legend to Table II). Each preparation of cholate vesicles is characterized by a narrow particle size distribution, i.e. the preparation is homogeneous compared to sonicated vesicles which show a broad particle size distribution (7, 13). However, with cholate vesicles a large scatter of the average particle size was observed from one preparation to another. This variation does not primarily depend on column parameters, such as column size or flow rate, but it was found that varying the weight ratio PC/cholate could account for the variation in the average particle size.\(^5\)

The solubility properties of sucrase-isomaltase are typical for an integral, amphipathic membrane protein. The solubility of sucrase-isomaltase in aqueous solvents is only possible in the presence of detergents. Upon removal of the detergent from the protein-detergent complex, sucrase-isomaltase, like other integral membrane proteins (22), aggregates to regular, oligomeric structures, which in this case are spheroidal. This aggregation is by and large reversible and most of the protein aggregates dissociated at the presence of 2% sodium cholate or 1% octyl tetraoxyethylene. The values for the molecular weight of sucrase-isomaltase determined in these detergents were 2.7 and 2.8 \(\times 10^6\), respectively (cf. Table I). Although the former value obtained in the presence of cholate represents an upper limit, the two determinations are in good agreement, considering the experimental error. The molecular weight is significantly larger than that of the water-soluble form, P-SI, obtained by papain digestion of brush border membrane vesicles or reconstituted sucrase-isomaltase PC vesicles. Papain treatment apparently removes 10-15% of the total protein mass, including the hydrophobic peptide chain responsible for the amphipathic character of sucrase-isomaltase (1-3) (cf. Table I). It is important to note that identical values are obtained for the molecular weight and sedimentation behavior of P-SI produced by papain digestion of brush border membranes and reconstituted sucrase-isomaltase PC vesicles. This result is consistent with evidence presented before (1) which suggest that the way sucrase-isomaltase is inserted in artificial bilayers closely resembles the in vivo situation.

The molecular weights of sucrase-isomaltase and P-SI determined in the analytical ultracentrifuge (Table I) may now be compared to those derived from SDS-PAGE using various marker proteins. The values obtained for the apparent molecular weight of the two subunits sucrase and isomaltase were 1.4 and 1.6 \(\times 10^6\), respectively, giving a total molecular weight of approximately 3.0 \(\times 10^6\). In contrast, the two subunits of P-SI were determined to be 1.4 \(\times 10^6\) each, indicating that papain treatment released a 2 \(\times 10^5\) peptide segment from the isomaltase subunit (1). The higher \(M_r\) values determined by SDS-PAGE are very likely to be due to the fact that both sucrase-isomaltase subunits are glycoproteins. It is well known that the binding of SDS to glycoproteins is anomalous so that the molecular weights of glycoproteins derived from SDS-PAGE are usually too high.

The ultracentrifugal analysis of sucrase-isomaltase PC vesicles clearly shows that the monomeric form of sucrase-isomaltase is incorporated into PC bilayers. If this assembly were strictly random, a Poisson distribution of monomers into vesicles at any given concentration would be expected. It is evident from the inset of Fig. 2 that this is not the case, an observation holding for the entire range of protein/lipid weight ratios tested. Rather, the population of vesicles containing even numbers of sucrase-isomaltase molecules appear to be larger than predicted from the Poisson distribution.\(^6\) This is likely to be due, at least in part, to the presence of a fraction of sucrase-isomaltase in the dimeric form, a hypothesis supported by sedimentation analyses of sucrase-isomaltase in the presence of 1-2% sodium cholate (not shown). It is also consistent with the observation that P-SI dimerizes at the ionic strength and pH used in our reconstitution experiments (18). The data presented clearly show, however, that dimerization is not a prerequisite for vesicle assembly and that a substantial fraction incorporates as monomers.

It was reported previously (2) that the assembly of sucrase-\(6\)

As pointed out, the refractive index increment of vesicles differing in the number of sucrase-isomaltase molecules/vesicle is unknown and fractional populations estimated from the schlieren peak areas are approximate. However, it is clear that the error thus introduced cannot account for the deviation from the Poisson distribution.
Hydrodynamic Properties of Phospholipid Vesicles

Isomaltase into egg PC bilayers leads to an asymmetric membrane with almost the total protein mass together with the two active centers facing the external medium. It was suggested that the reason for this asymmetric insertion is that sucrase-isomaltase is too large to be accommodated in the vesicle cavity. The peptide segment by which the isomaltase subunit is anchored in the lipid bilayer has a molecular weight of only \( \sim 6500 \), amounting to \( \sim 2\% \) of the total protein mass. Consequently, the protein mass to be accommodated in the vesicle cavity is greater than \( 2.5 \times 10^6 \). Test experiments with various soluble marker proteins have shown (2) that proteins of mass greater than \( 2.5 \times 10^6 \) are practically excluded from the vesicle cavity. This shows that the protein size alone cannot account for the asymmetric insertion although specific effects governing the insertion of the protein cannot be ruled out at the present stage.

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APPENDIX

Rearranging and integrating equation 2 gives:

\[
c_i(r) = \exp(\alpha r^2 + C_i)
\]

(4)

where \( c_i(r) \) is the fringe shift of component \( i \) at position \( r \),

\[
\alpha_i = \frac{M_i}{2RT} \left( 1 - \frac{v_i}{v} \right) \omega^2
\]

(5)

and \( C_i \) is an integration constant.

The total mass of component \( i \) in the sector-shaped cell (such as a meniscus; \( d \), thickness) is

\[
m_i = \frac{q d}{2a} \int_{r_m}^{r_b} c_i(r) r \, dr
\]

(6)

where \( r_m \) and \( r_b \) are the radial distances to the meniscus and bottom of the cell, respectively. Substituting \( c_i(r) \) by equation 4 and integrating equation 6 together with the fact that \( c_i(r_m) \to 0 \) under the conditions of meniscus depletion give:

\[
m_i = \frac{q d}{2a} \exp(\alpha r^2 + C_i)
\]

(7)

\( m_i \) may be expressed as a fraction of the total mass \( m_t \) so that \( m_i = f_i m_t \); an approximate value for \( f_i \) may be derived from the area under the schlieren peak of component \( i \). A separation by gel filtration on Sepharose 4B or Con-A-Sepharose 4B of sucrase-isomaltase PC vesicles differing in the number of sucrase-isomaltase molecules/vesicle was not possible, and, hence, the refractive index increment of different sucrase-isomaltase PC vesicles could not be measured. The area under the schlieren peak gives therefore only an approximate estimate of \( m_i \). With this approximation and using equation 7, the integration constant \( C_i \) can be calculated. Equation 4 is then used to calculate \( \ln c_i(r) \) as a function of \( r^2 \) for component \( i \). The total fringe shift \( c_t \) is the sum of all components:

\[
c_t = \sum C_i
\]

(8)

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\footnote{M. Spiess, unpublished data.}