Cholesterol Metabolism by Purified Cytochrome P-450$_{scc}$ Is Highly Stimulated by Octyl Glucoside and Stearic Acid Exclusively in Large Unilamellar Phospholipid Vesicles†

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Received March 31, 1989; Revised Manuscript Received June 7, 1989

ABSTRACT: Cholesterol side-chain cleavage (CSCC) catalyzed by purified bovine adrenal mitochondrial cytochrome P-450$_{scc}$ is highly dependent on the vesicles that supply cholesterol. Six-fold higher rates are achieved with large unilamellar dioleoylphosphatidylcholine vesicles (diameter 150 nm) prepared by octyl glucoside (OG) dialysis (DOPC–LUV) than with small sonicated vesicles (diameter 30 nm) (DOPC–SUV) (V_{max} = 25 and 4 min^{-1}, respectively. Extensive dialysis that may remove OG decreased V_{max} rates for DOPC–LUV almost to rates seen with DOPC–SUV. These dialyzed DOPC–LUV were, however, very sensitive to addition of OG (EC_{50} = 2.5 μM, 4.3-fold stimulation) while DOPC–SUV were only weakly affected (EC_{50} = 100 μM, 1.6-fold stimulation). This enhancement of CSCC in LUV by OG only occurred when the cholesterol:DOPC exceeded 0.1 and was associated with a 15-fold increase in the K_m for cholesterol. Structural changes in both SUV and LUV at high cholesterol:DOPC ratios (0.1–1) were indicated by decreases in internal volume that were insensitive to OG and did not affect the external diameters. Stearic acid produced a similar stimulation of CSCC in LUV (EC_{50} = 50 μM) and had no effect on SUV. The V_{max} for CSCC, produced by OG activation of DOPC–LUV, is comparable to the highest attained for cytochrome P-450$_{scc}$ (Tween 20/cholesterol). In LUV, a minor proportion of OG (1–5% of cholesterol) is thus sufficient to generate a domain of reactive cholesterol that maintains a near-optimum turnover. This increased CSCC was paralleled by increased binding of cholesterol to P-450$_{scc}$, suggesting that this cholesterol is more readily donated by the membrane to the cytochrome.

Cytochrome P-450 side-chain cleavage (P-450$_{scc}$),[1] located on the matrix side of adrenal cortex mitochondria, catalyzes the conversion of cholesterol to pregnenolone (Churchill et al., 1978; Simpson & Boyd, 1966). The activity of this enzyme limits the extent of steroid production in the adrenal cortex (Stone & Hechter, 1954; Burstein & Gut, 1976). The complete steroid oxygenase system requires two other well-characterized enzymes, namely, adrenodoxin (ADX), an iron–sulfur protein, and adrenodoxin reductase (AR), a flavoprotein (Mitani, 1979). Cholesterol side-chain cleavage (CSCC) activity can be reconstituted in phospholipid vesicles, and the activity is dependent on the phospholipid used in the reconstitution. This modulation of CSCC does not correlate with any physical characteristics of the lipid or the vesicles but for many phospholipids matches the effect of lipid on the K_d for cholesterol binding to cytochrome P-450$_{scc}$ (Lambeth et al., 1980a). Cardiolipin (CL), the most active phospholipid, forms a 1:1 complex with cytochrome P-450$_{scc}$ that has enhanced affinity for cholesterol (Lambeth, 1981). Phospholipid head groups also affect CSCC, probably by modifying the interaction of the cytochrome with the vesicle (Kowluru et al., 1983). Membrane lipids may additionally change the K_d for CSCC by affecting the binding of cholesterol to the membrane. Thus, sphingomyelin has a high affinity for cholesterol and, as a consequence, increases the K_d for cholesterol (Stevens et al., 1986).

The method of generation of unilamellar phospholipid vesicles can greatly affect the size of the vesicle. Thus, dialysis of cholate-solubilized phospholipids or sonication typically generate very small unilamellar vesicles (SUV) (Yamakura et al., 1981), while large unilamellar vesicles (LUV) form when cholate is replaced by a small nonionic detergent, such as octyl glucoside (OG) (Mimms et al. (1981), or when SUV are subjected to repeated freezing and thawing cycles (Pick, 1981). The physical state of cholesterol in phospholipid vesicles is highly dependent on the size of the vesicles which in turn determines the surface curvature (Yeagle et al., 1982). Notably, the preferential distribution of cholesterol to the inner leaflet of egg lecithin SUV has been attributed to the smaller radius of curvature of the inner membrane leaflet (Huang et al., 1974).

In this paper, we show that the size of the vesicle has a large affect on the rate of CSCC catalyzed by purified cytochrome P-450$_{scc}$. This size selectivity is, however, shown to be dependent on the stimulatory effect of low concentrations of octyl glucoside or certain fatty acids. The characteristics of this stimulation suggest that a small fraction of highly reactive cholesterol is sufficient to maintain maximum CSCC activity.

Materials and Methods

Materials. Lipids were obtained from Serdary Research Laboratories, London, Ontario, Canada; [^{3}H]cholesterol and [^{14}C]inulin were from NEN Research Products DuPont; OG, Sephadex 4B, and NADPH were from Sigma, St. Louis, MO; and Sephadex LH-20 was from Pharmacia, Piscataway, NJ.

† This work was supported by National Institutes of Health Grant DK18585.

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[1] Abbreviations: ADX, adrenodoxin; AR, adrenodoxin reductase; CSCC, cholesterol side-chain cleavage; OG, octyl glucoside; SUV, small unilamellar vesicle(s); LUV, large unilamellar vesicle(s); DOPC, dioleoylphosphatidylcholine; DMPC, dimyristoylphosphatidylcholine; P-450$_{scc}$, P-450 side-chain cleavage.

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Latex beads were obtained from Seragen Diagnostics, Indianapolis, IN.

Enzymes. Cytochrome P-450$_{scc}$ was purified from beef adrenals by using the procedure of Takemori et al. (1975), as modified by Hanukoglu et al. (1981a). This procedure, using adrendoxin–Sepharose column chromatography as the final step, produces purified cytochrome P-450$_{scc}$ with an A_{280}/A_{390} ratio of 1.2. Adrenodoxin and adrenodoxin reductase were also purified from beef adrenal by using the procedure of Orme-Johnson and Beinert (1969) and Hiwatashi et al. (1976), respectively, as modified by Hanukoglu et al. (1981b).

Preparation of Vesicles. For preparation of highly dispersed SUV, a probe sonicator (ultrasonicator, Model 185f) was used. Appropriate concentrations of phospholipids in chloroform and cholesterol (including [^{3}H]cholesterol when required) in ethanol solvents were evaporated under N_2 in 40-mL conical Pyrex tubes and kept under a vacuum overnight. To reduce lipid peroxidation, butylated hydroxytoluene (1:60 molar ratio) was always present during sonication. After complete removal of the solvents, 3 mL of buffer (20 mM Hepes, pH 7.2) was added and vortexed for 2.0 min. The suspension was sonicated to clarity (approximately 15 min) at setting 6 (about 50 W) under a gentle stream of N_2 with the tubes immersed in a circulating water bath at 30 °C. For preparation of the LUV, the method of Mimms et al. (1981) was used. Briefly, phospholipid solution with butylated hydroxytoluene (1:60 molar ratio) to be used was dried to a thin film in a glass tube under a stream of N_2 and then put under vacuum overnight to remove the residual traces of organic solvent. The lipid film was redissolved in 1 mL of 20 mM Hepes buffer and vortexed for 2 min. *n*-Octyl glucoside (OG) was added to the suspension, and the mixture was vortexed to clarity. A molar ratio of 20:1 detergent/lipid was found to be adequate to obtain a clear solution. The solutions were then dialyzed under N_2 against three 1-L changes of buffer for 12 h each (unless otherwise stated). Assuming a half-time for removal of OG of approximately 2 h (Helenius et al., 1977), the proportion of [^{14}C]octyl glucoside remaining in the dialyzed samples of lipid vesicles is about 1:400 (Mimms et al., 1981).

Measurement of Trapped Volume. Trapped volumes were determined by including ^{14}C-labeled inulin in the vesicles and then subjecting the vesicles to gel chromatography to separate the trapped from the untrapped inulin. [^{14}C]Inulin (2×10^6 cpm) was added to the buffer (1 mL) prior to preparation of the vesicles. An aliquot (300 μL) of the vesicles was applied to the Sepharose 4B column, and 1-mL fractions were collected. [^{14}C]Inulin content was determined by using the LKB 1211 mini-beta liquid scintillation counter. Trapped volumes are expressed as microliters of trapped volume per micromole of lipid.

Phospholipid Determination. Phospholipid phosphorus was determined by the method of Ames and Dubin (1960). The ashing was performed according to the method of Hess and Derr (1975). Briefly, samples containing the phospholipid (200 μL) were dried down in glass tubes, and 30 μL of 10 N H_2SO_4 and 90 μL of 60% perchloric acid were added. The tubes were then heated at 190 °C until the samples stopped fuming. Water (0.9 mL) was added, followed by the addition of 2.1 mL of 0.42 M ammonium molybdate·4H_2O in 1 N H_2SO_4/10% ascorbic acid (6:1 mixture). The solutions were then incubated at 45 °C for 30 min, and OD was recorded at 660 nm. A standard curve was constructed by using inorganic phosphorus in the range of 0.1–10 μg.

Cholesterol Side-Chain Cleavage Assay. The standard side-chain cleavage assay was carried out by incubation of cytochrome P-450$_{scc}$ (0.4–0.5 μM) and adrenodoxin (7 μM) at 37 °C for 2 min in 20 mM Hepes (pH 7.2) and 100 mM NaCl, with vesicles consisting of 600 μM phospholipid, [^{3}H]cholesterol, and an appropriate amount of cold cholesterol in a total volume of 0.2 mL. Adrenodoxin reductase (0.7 μM) was then added, and the reaction was initiated with 2.5 mM NADPH. After 2 min, the reaction was terminated by addition of 0.2 mL of ice-cold ethanol. Pregnenolone was extracted either with 2 mL of methylene chloride and separated from unreacted cholesterol on LH-20 mini columns (Hanukoglu & Jefcoate, 1980) or with 3 mL of hexane for RIA determination (Abrahams et al., 1973).

Electron Microscopy. For negative staining, vesicle suspensions (1.8 mM ± equimolar cholesterol) were applied to 300-mesh copper grids coated with formvar and washed with 2% ammonium molydate solution and blotted with filter paper. Grids were scanned at low magnification using a JEOL CX100 electron microscope to select regions with good distribution of vesicles and were photographed at 48000× magnification. Distribution of vesicles was determined by measuring the diameters of 100 randomly chosen vesicles by planimetry using the draw program.[2] Micrographs of SUV were enlarged 20 times to minimize errors. The proportion of lipid in vesicles was estimated by using theoretical curves shown in Figure 1. The amount of lipid in each class size was calculated and expressed as a percentage of the total lipid in all vesicle classes.

Spectra. All spectra were performed on an Aminco-Chance DW-2 spectrophotometer in either dual-wavelength or split-beam modes. For studying the removal of cholesterol from the P-450$_{scc}$–cholesterol complex (dissociation), a typical assay contained 20 mM Hepes, 50 mM KCl, and 600 μM phospholipid at 37 °C in a total volume of 1 mL. The assay was started by the addition of 80–100 nM P-450$_{scc}$, and changes in absorption at 420–390 nm were followed in the dual-wavelength mode. Cholesterol transfer to cytochrome P-450$_{scc}$ (association) was measured at equimolar concentrations of phospholipid and cholesterol (600 μM). The reaction was initiated with 80–100 nM cholesterol-depleted cytochrome P-450$_{scc}$ (Hanukoglu et al., 1981a).

Results

Measurements of Vesicle Size. The elution profiles of small and large unilamellar phospholipid vesicles prepared, respectively, by sonication (SUV) and octyl glucoside dialysis (LUV) were compared on a Sepharose 4B column. Latex beads of defined size were used as markers. The order of elution was LUV, 85-nm beads, and SUV followed by 38-nm beads. The positions of elution were consistent with mean sizes for LUV and SUV of approximately 150- and 30-nm diameter, respectively.

The diameter of these unilamellar vesicles is proportional to the aqueous volume trapped within the bilayer (Hope et al., 1986). The internal volumes can be related to external diameter by considering the number of phospholipid molecules in relation to vesicle diameter and internal volume. Theoretical relationships, based on a bilayer thickness of 4.0 nm and an average surface area of 0.7 nm^2 (Huang & Mason, 1978) per phospholipid molecule, are shown in Figure 1. The measured trapped volumes for SUV and LUV without cholesterol (0.3 and 6 μL/μL of phospholipid) are in good agreement with diameters (20 and 200 nm) viewed by electron microscopy (Figure 3). The smaller nonspherical structures in LUV

[2] The draw program was kindly provided by J. Sckulski and R. Kochar of the Department of Neurophysiology, University of Wisconsin—Madison Medical School.

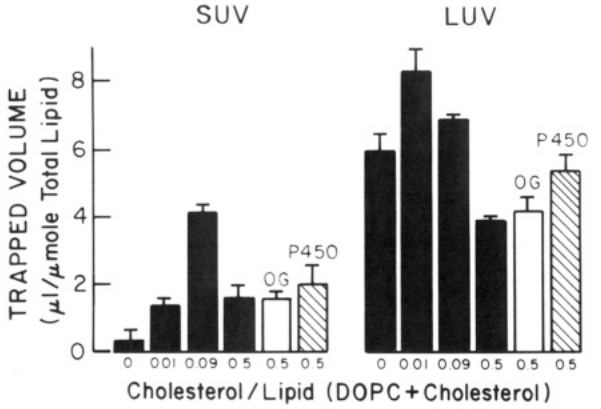

FIGURE 1: Trapped volumes expressed as microliters per micromole of lipid versus cholesterol:lipid (DOPC + cholesterol) ratio for SUV and LUV. Internal volumes were determined by trapping [^{14}C]inulin in the liposomes. An aliquot was chromatographed on a Sepharose 4B column to separate the trapped and untrapped inulin as described under Materials and Methods. Each point represents the mean of these determinations.

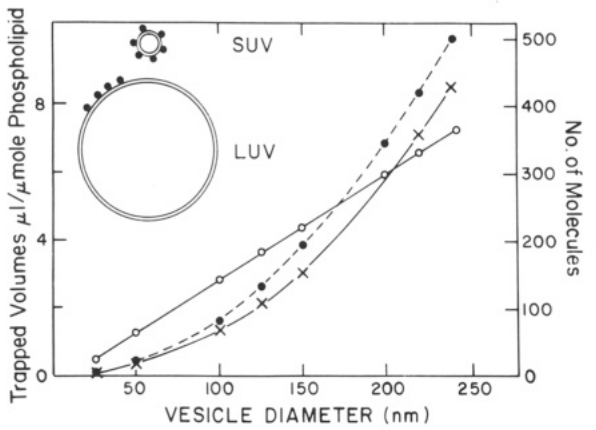

FIGURE 2: Diagrammatic representation of SUV and LUV and the relationship of vesicle diameter with respect to trapped volumes (○), the number of phospholipid molecules [(●) scale $\times 10^{-3}$], and the number of P-450$_{scc}$ molecules (×) (at 0.4–0.5 μM) per vesicle. The values are calculated by assuming a bilayer thickness of 4.0 nm and an average surface area of 0.7 nm^2 per phospholipid molecule (Huang & Mason, 1978). The P-450 molecules (size 30 Å × 60 Å) are shown for comparison at an appropriate scale (Poulos et al., 1985).

micrography probably represent collapsed vesicles. Previously reported internal volumes for SUV are in the range of 0.2–0.5 μL/μmol of phospholipid (Barenholz et al., 1979; Hope et al., 1986), while for LUV prepared by the OG dialysis method, an internal volume of 10 μL/μmol of lipid has been reported (Mimms et al., 1981).

The incorporation of cholesterol (up to 10%) produced a greater than 10-fold increase in the trapped volume of inulin inside SUV, but a smaller increase for LUV (40%) that was maximum at the lowest cholesterol concentration examined (1% of PL) (Figure 2). At equimolar concentrations of cholesterol, the internal volume declined in both vesicles, particularly when expressed as per micromole of total lipid (DOPC + cholesterol). A 3-fold increase in the trapped volumes on inclusion of equimolar cholesterol into SUV has been reported elsewhere (Johnson, 1973). Inclusion of octyl glucoside in vesicles containing equimolar cholesterol (500 μM for SUV, 10 μM for LUV) did not affect the internal volumes. Association of cytochrome P-450$_{scc}$ by the vesicles, however, produced 20% increases.

Electron microscopy of SUV and LUV (Figure 3) corroborated that equimolar cholesterol had little effect on the diameter of LUV but that the mean diameter of SUV increased

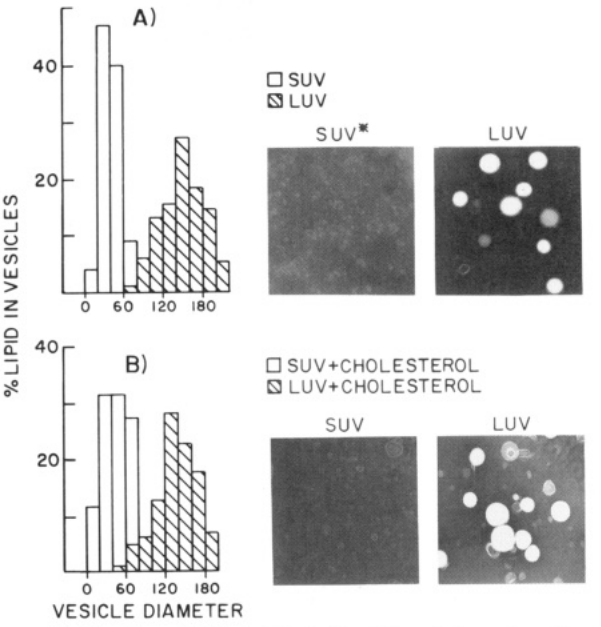

FIGURE 3: Fraction of lipid distributed in different sizes of small and large DOPC vesicles in (A) the presence and (B) the absence of cholesterol. Diameters of 100 randomly chosen vesicles were measured by planimetry from representative fields of the electron micrographs. The proportion of lipid represented in each of the vesicle class is obtained by using the data presented in Figure 1 as described under Materials and Methods. Electron micrographs of these vesicles were taken at a magnification of 19200× (1 cm = 208 nm).

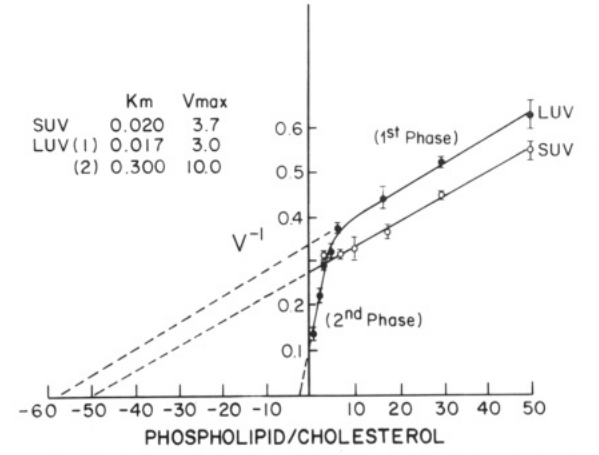

FIGURE 4: Effect of cholesterol concentration on cholesterol side-chain cleavage (CSCC) in SUV and in LUV prepared with only 12 h dialysis of OG. CSCC activity (nanomoles of pregnenolone per nanomole of P-450 per minute) was measured for 2 min in a total assay volume of 0.2 mL containing 20 mM Hepes (pH 7.2), 100 mM NaCl, 2.5 mM NADPH, 7 μM adrenodoxin, 0.7 μM adrenodoxin reductase, and 0.4 μM P-450$_{scc}$. The DOPC concentration was 600 μM, and the proportion of cholesterol was varied (5–600 μM). Each point represents the mean of three determinations.

by approximately 2-fold. Figure 3 also shows the effect of cholesterol on the size distribution of LUV and SUV both with and without cholesterol. The increase in the volume of SUV upon the addition of cholesterol is, however, severalfold too small to account for the large stimulation by cholesterol on the trapped volume of SUV. However, the structures visible in the EM of SUV without cholesterol indicate many small structures that may be miceller. Since such particles may not retain inulin, cholesterol may increase inulin retention by increasing the proportion of lipid in the form of vesicles.

Cholesterol Side-Chain Cleavage. The dependence of the rate of CSCC on the proportion of cholesterol in SUV that contain DOPC is very different from the analogous plot for

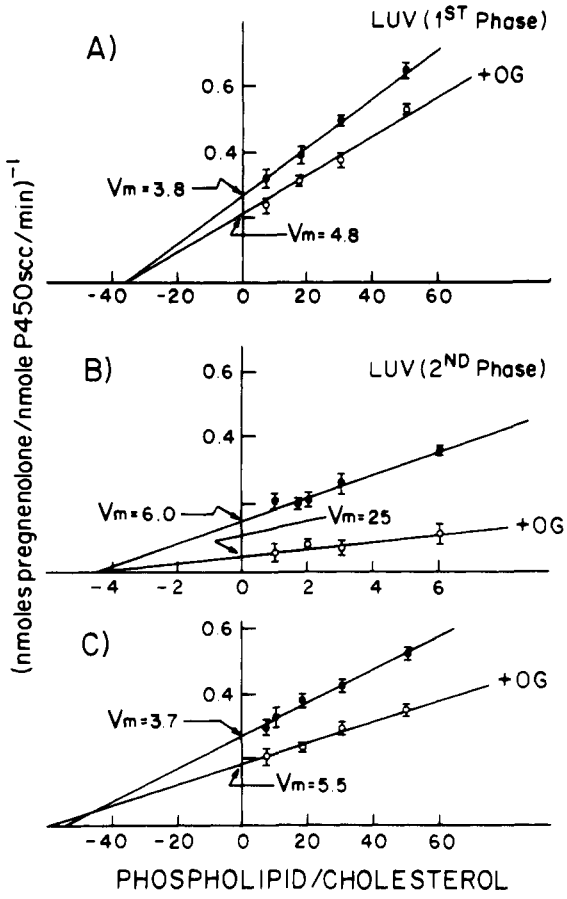

FIGURE 5: Lineweaver–Burk plots of $1/V$ versus DOPC:cholesterol ratio in extensively dialyzed LUV and SUV. 10 and 500 μM OG were added, respectively, to LUV (A and B) and SUV (C). Assay conditions were as in Figure 5. Each data point shows the mean of three determinations.

the corresponding LUV (Figure 4). Since cholesterol is present only in the membrane, the concentration can be defined as the ratio of cholesterol/phospholipid. Lineweaver–Burk plots of $1/V$ versus [DOPC]/[cholesterol] show that activities are similar in SUV and LUV at low cholesterol content (phase 1, ratio <0.1, V_{max} = 3–4 min^{-1}, K_m = 0.02). Thus, there is a sharp transition in LUV for cholesterol ratios above 0.1 to a second type of activity (phase 2) indicated by a 6-fold increase in V_{max} and corresponding to a very high K_m (0.3). By contrast, in DOPC–SUV, the rate of cholesterol metabolism reached a limiting value at a ratio of cholesterol/DOPC of 0.1.

Residual OG is probably a major contributor to the high activity in DOPC–LUV. This was strongly suggested by the effect of more prolonged dialysis, which did not change cholesterol SCC at low cholesterol content but almost removed the phase 2 contribution at high cholesterol content. Addition of 10 μM OG to these extensively dialyzed vesicles (600 μM DOPC/60–600 μM cholesterol) restored the phase 2 component that is seen at high cholesterol content while producing only a small increase in cholesterol metabolism at low cholesterol:DOPC ratios. In Figure 5A,B, this action of 10 μM OG on LUV is represented as double-reciprocal plots for, respectively, phase 1 ([cholesterol] <0.1) and phase 2 ([cholesterol] >0.1). OG increases the V_{max} for phase 1 by only 30% with no shift in the K_m for cholesterol (0.02). Without added OG, there is only a small phase 2 contribution that contributes an additional 60% to the maximum CSCC activity in these LUV. Addition of 10 μM OG increases the phase 2 contribution to become 4 times the phase 1 contribution.

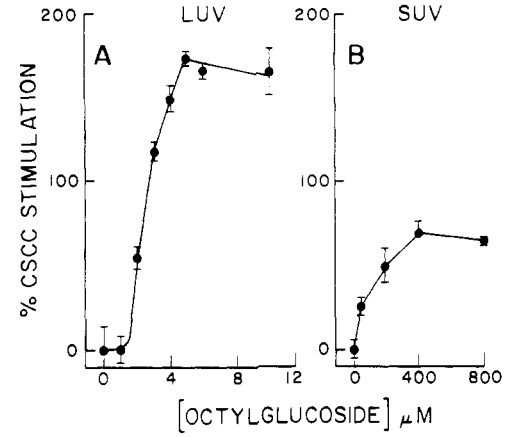

FIGURE 6: CSCC activity as a function of octyl glucoside concentration in LUV (A) and SUV (B). CSCC reaction was carried out as described in Figure 5. Each data point represents the mean of three experimental determinations.

This stimulation represents at least a 10-fold enhancement by OG of the phase 2 contribution. The residual phase 2 contribution even following extensive dialysis probably results from small amounts of residual OG. This residual OG has been measured with [^{14}C]OG to be about 1.5 μM which is consistent with the magnitude of the phase 2 contribution (Mimms et al., 1981).

This action of OG was only seen with LUV. Low concentrations of OG had no effect on CSCC in SUV. High concentrations equivalent to the phospholipid concentration stimulated CSCC by about 50% at low cholesterol concentrations but had no further effect at high cholesterol content (Figure 5C).

The OG stimulation of CSCC in LUV containing equal proportions of cholesterol and DOPC exhibited a sigmoidal dependence on the concentration of OG (Figure 6A). Fifty-fold lower OG concentrations are needed to provide half-maximum stimulation of cholesterol SCC in DOPC–LUV (EC_{50} = 2.5 μM) as compared to DOPC–SUV (EC_{50} = 100 μM) (Figure 6B). Maximum stimulations were produced by, respectively, 5 and 400 μM. The concentration range required for stimulation of LUV by OG represents a ratio of detergent to lipid of only 0.3–1.5% (w/w), while the stimulation of SUV represents nearly equal amounts of detergent and phospholipid (10–70% w/w).

OG also caused a change in the interaction of cholesterol with cytochrome P-450$_{scc}$ that followed the same selectivity described above for CSCC activity. Addition of 10 μM OG increased the proportion of cytochrome P-450$_{scc}$ bound by cholesterol (high-spin state). This increase was unchanged on the addition of adrenodoxin. Without OG, adrenodoxin stimulated cholesterol binding to this same level which represents essentially complete complex formation (Figure 7). There was no effect of OG on the analogous cholesterol complex formation using SUV (data not shown).

DMPC behaves similarly to DOPC with respect to OG stimulation but gives lower basal CSCC rates. We next examined the possibility that OG could be replaced by a natural detergent molecule and consequently tested a series of C18 fatty acids in DMPC vesicles. These acids can also stimulate CSCC in LUV but not SUV (Figure 8A). The saturated stearic acid is more effective (EC_{50} ~ 30 μM) than the unsaturated analogues but is somewhat less effective than OG and the stimulation is dose dependent (Figure 8B). Higher concentrations of stearic acid (<10 mol %) lead to changes in the physical characteristics of LUV. The nearly opaque

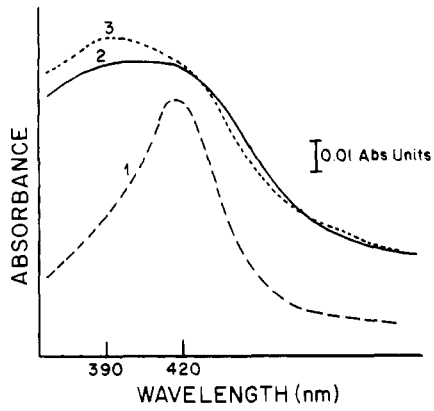

FIGURE 7: Effect of 10 μM OG on cholesterol–P-450$_{scc}$ complex formation in LUV. The figure shows absolute spectra of P-450$_{scc}$ (0.1 μM) in cholesterol-free LUV (1), cholesterol-containing (600 μM) LUV (1:1 cholesterol/DOPC) (2), and the same cholesterol-containing LUV with 10 μM OG added (3). The spectra were conducted at 37 °C in 20 mM Hepes (pH 7.3) containing 50 mM KCl. (Spectrum 1 is offset by 0.02 absorbance unit for clarity.)

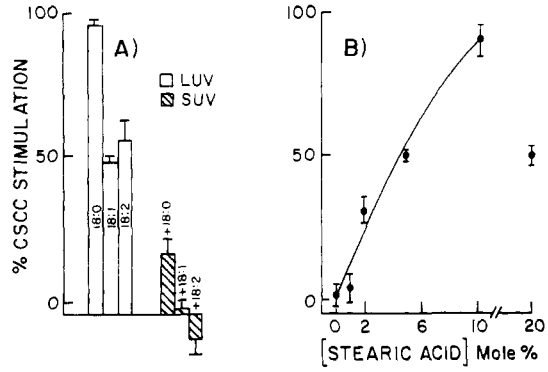

FIGURE 8: Effect of C18 free fatty acids on the rate of CSCC reaction. (A) Vesicles were prepared with 10% (w/w) fatty acids in DMPC vesicles (SUV and LUV, 600 μM); otherwise, assay conditions were as in Figure 5. (B) The activity of CSCC in LUV is represented as a function of stearic acid concentration. Each data point is the mean of three experimental determinations.

liposome suspension becomes increasingly clear as the stearic acid content is raised above this limit. This is accompanied by a decrease in the stimulatory effects of the fatty acid.

Discussion

The rate-limiting transfer of cholesterol to cytochrome P-450$_{scc}$ in adrenal mitochondria involves both transfer of cholesterol between outer and inner membranes and transfer within the inner membrane to P-450$_{scc}$. Several previous papers have shown that the phospholipid composition of the membrane greatly affects the rate of CSCC catalyzed by purified cytochrome P-450$_{scc}$ (Kowluru et al., 1983; Lambeth, 1981; Seybert et al., 1979; Lambeth et al., 1980a; Hsu et al., 1985; Igarashi & Kimura, 1986).

Data presented here show that an exceptionally high rate of cholesterol SCC with purified cytochrome P-450$_{scc}$ can be obtained using large unilamellar vesicles (150–200-nm diameter, LUV) prepared by OG dialysis. This turnover rate (25–30 min^{-1}) is comparable to the highest rate that is seen with this cytochrome when the reaction is carried out with cholesterol and cytochrome bound by the nonionic detergent Tween 20 (Takikawa et al., 1978; Hanukoglu et al., 1981a). Small unilamellar vesicles (20–30-nm diameter; SUV) exhibit rates that are typically about 5-fold lower. The high rates of cholesterol SCC in LUV result from a potent stimulation of the reaction by residual OG. Thus, high activities are lost by exhaustive dialysis and are restored by low concentrations of OG (5-fold stimulation, EC_{50} = 2.5 μM). Only a small stimulation at very high concentrations of detergent occurs in SUV, suggesting that the tight curvature of these vesicles may prevent the stimulation.

CSCC in LUV prepared by OG dialysis exhibits two types of activity (phases 1 and 2) that are dependent on the OG concentration. Phase 2 activity appears as an elevation of activity at high cholesterol content (ratio >0.1) and is characterized by an exceptionally high K_m for cholesterol (0.25 M). In extensively dialyzed vesicles where OG levels have fallen to 1:400 or less of the DOPC concentration (<1.5 μM), phase 2 contributes very little additional activity (30% above phase 1 extrapolation). OG, however, increases the rate of phase 2 CSCC by 10-fold with little effect on phase 1 metabolism. The phase 2 contribution following dialysis is fully consistent with the residual content of OG. SUV show no evidence of phase 2 behavior and may even show a decrease of plateau of activity at these high cholesterol levels.

It seems probable that phase 1 and phase 2 reactions reflect different types of cholesterol disposition within LUV. Heterogeneity in the distribution of cholesterol in phospholipid vesicles has been observed by other workers (Huang et al., 1974; Yeagle et al., 1982; Schroeder, 1981; Nemecz & Schroeder, 1988). Certainly, different pools of cholesterol within the lipid bilayer have been reported by many investigators (Blöj & Zilversmit, 1982; Pozansky & Czckanski, 1982). The slower phase 1 mechanism may involve cholesterol that is typically fully inserted into the vesicular bilayer between the fatty acid chains of DOPC. This mechanism then operates similarly in LUV and SUV and is relatively insensitive to OG. The fast phase 2 mechanism may reflect a second membrane configuration for cholesterol that occurs in the presence of high cholesterol when potentiated by OG. Cardiolipin (CL) increases the affinity of P-450$_{scc}$ for cholesterol through the formation of 1:1 complex with the cytochrome and enhances the rate of CSCC activity (Lambeth, 1981). OG, on the other hand, acts on the membrane, thereby releasing cholesterol for complex formation. The stimulatory effects of OG and cardiolipin are additive. In DMPC–LUV, CL (10%) and OG (1%) produce a 3–4-fold increase in the CSCC activity. However, a synergistic effect (~10-fold increase) is observed when both OG and CL are present together (Dhariwal et al., unpublished experiments), therefore suggesting that OG and CL are acting by different mechanisms.

Recent work has documented effects of OG on the disposition of cholesterol in phospholipid vesicles but only at very high OG concentrations. Octyl glucoside monomers incorporate into the phospholipid bilayers until above 18 mM OG concentrations, where lamellar to micellar transitions begin to occur (Paternostre et al., 1988). The presence of OG (0–15 mM) in the bilayers is thought to produce a small "fluidizing" effect as indicated by changes in the NMR and fluorescence anisotropy parameters (Jackson et al., 1982). No physical change in the membrane is observable in LUV at 10 μM levels of detergent, but a proportion of this fluidizing domain that is below detection limits remains possible. In the system under study, the optimal OG effect is realized at a very large excess of cholesterol over OG (50 to 1), suggesting that only a minor proportion of total vesicular cholesterol is needed in this active form to sustain the maximum rate of CSCC. This optimal effect of OG occurs at a 10-fold excess of OG over P-450$_{scc}$. The sigmoidal dose response curve for OG suggests a cooperative interaction with the vesicle in which one molecule facilitates the insertion of a second molecule. Patches of fluidized membrane formed by 10 OG molecules could be

sufficient for insertion of each P-450$_{scc}$ into the membrane with a subsequent rapid release of adjacent cholesterol for metabolism.

Optical spectra indicate that the increase of K_m for cholesterol in the phase 2 reaction does not reflect a comparable decrease in binding affinity. Thus, cytochrome P-450$_{scc}$ under reaction conditions prior to the addition of NADPH (with excess ADX) is fully bound by cholesterol. This saturation of binding in the presence of ADX is seen even in the higher range of phase 1 cholesterol concentrations. However, when the affinity for cholesterol is decreased by omission of ADX, OG significantly enhances cholesterol binding to P-450$_{scc}$ at phase 2 concentrations. Previous kinetic studies have indicated an unexplained similarity between the K_m for CSCC and the K_d for cholesterol that is apparently maintained in these experiments (Honukoglu, 1980). Consequently, it is probable that cholesterol binding to P-450$_{scc}$ is rate limiting and is greatly enhanced under phase 2 conditions.

During ACTH stimulation of adrenal cells, free fatty acids are generated at the same time as cholesterol (Boyd & Trezeciak, 1973). Here we have shown that free fatty acids may contribute to the maintenance of cholesterol transfer to cytochrome P-450$_{scc}$. The effect of long-chain fatty acids appears strictly analogous to that of OG in that LUV are selectively stimulated. The greater effectiveness of saturated stearic acid relative to unsaturated analogues may relate to the ability of unsaturated chains to greatly decrease the ordering effects of cholesterol (Kusumi et al., 1986). This may reflect different packing of cholesterol in saturated and unsaturated lipid bilayers. The possible effects of fatty acids on mitochondrial cholesterol transfer remains to be elucidated.

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