Effect of detergents on sterol synthesis in a cell-free system of yeast

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Abstract In order to obtain information about the reactivity of enzymes in sterol synthesis of yeast, the effects of some detergents were investigated. Among the detergents tested, Triton X-100 was found to exert a unique action, and its effect on the incorporation of ^{14}C-labeled acetate, mevalonate, farnesyl pyrophosphate, or *S*-adenosyl-L-methionine into squalene, 2,3-oxidosqualene, and sterols in a cell-free system was examined. Triton X-100 showed virtually no effect on the enzyme activities in the reactions from acetyl CoA to farnesyl pyrophosphate, but it had a marked effect on reactions from farnesyl pyrophosphate to ergosterol. Evidence was obtained suggesting that Triton X-100 apparently activated squalene synthetase (EC 2.5.1.21) but inhibited squalene epoxidase (EC 1.14.99.7) and Δ^{24}-sterol methyltransferase (EC 2.1.1.41). The activity of epoxidase was protected from the inhibition by increasing the concentration of cell-free extracts or by the prior addition of lecithin liposomes to the reaction mixture. The inhibition of methyltransferase was partially reversed by treatment with Bio-beads SM-2, but that of epoxidase was not reversed by the treatment.—**Hata, S., T. Nishino, N. Ariga, and H. Katsuki.** Effect of detergents on sterol synthesis in a cell-free system of yeast. *J. Lipid Res.* 1982. **23:** 803–810.

Supplementary key words Triton X-100 • squalene synthetase • squalene epoxidase • Δ^{24}-sterol methyltransferase

The subcellular distribution of enzymes involved in ergosterol synthesis by yeast have been elucidated in the last ten years. Shimizu et al. (1) discovered that 3-hydroxy-3-methylglutaryl CoA reductase (EC 1.1.1.34), the enzyme in the rate-limiting step of the synthesis, was localized in mitochondria of yeast. This was confirmed by other groups of investigators (2, 3). Moreover, Shimizu et al. (4) reported that the reactions from acetyl CoA to mevalonate (MVA), the early stage of the synthesis, proceeded in mitochondria. The reactions from MVA to farnesyl pyrophosphate (FPP), the middle stage of the synthesis, were shown to occur in the cytoplasm (5–7). Although some of the enzymes involved in the reactions from FPP to ergosterol, the late stage of the synthesis, were reported to be located in mitochondria (8, 9), we obtained results indicating that all of the enzymes were located in microsomes (10–13).

In spite of the development of studies on the subcellular distribution of enzymes, the state of the intermediate sterols and the reactivity of the enzymes have not been studied so well. In this connection, it has been pointed out that the water insolubility of substrate and product and the variability of activities of the microsomal-bound enzymes, as a result of differences in membrane structure, make the analysis of the reactions difficult. In addition, it has been reported that the sterol pool present in membranes is heterogeneous. For example, the presence of an endogenous "triterpenoid" pool, which behaved differently as a substrate for the synthesis depending on the rate of synthesis, was reported for rat-liver microsomes (14). Moreover, the presence of at least two pools of cholesterol in rat liver microsomes, one more accessible than the other to cholesterol 7α-hydroxylase, has been reported (15).

In studies of sterol synthesis in vitro, detergents have often been used to solubilize the membrane enzymes or to disperse substrates in the reaction mixtures. Detergents are also known to stimulate or inhibit sterol synthesis, depending on their type and concentration. However, virtually no systematic biochemical study has been reported on the effects of detergents in sterol synthesis in cell-free systems of yeast and mammalian tissues, although the mechanism of detergent action in the solubilization of membrane proteins has been investigated, primarily from a biophysical point of view (16).

In the present study on the effects of detergents on sterol synthesis in cell-free extracts of yeast, we report that Triton X-100 and lysolecithin among the detergents tested exerted a unique action on some microsomal enzymes. Evidence is described suggesting that the mechanism of action of Triton X-100 on the enzymes studied is closely related to the alteration of microsomal structure.

Abbreviations: MVA, mevalonate; FPP, farnesyl pyrophosphate; SAM, *S*-adenosyl-L-methionine.

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MATERIALS AND METHODS

Preparation of cell-free extracts

Saccharomyces cerevisiae (ATCC 12341) was grown semi-anaerobically and adapted aerobically as described previously (17). The harvested cells (5 g, wet weight) were suspended in 5 ml of 0.1 M potassium phosphate buffer (pH 7.4) containing 0.2 mM dithiothreitol and disrupted with a Vibrogen cell mill (Edmund Bühler Co., Weilheim, West Germany) as described previously (18). Unless otherwise indicated, the homogenate was centrifuged at 2,500 *g* for 10 min at 4°C and the resulting supernatant (approximately 40 mg of protein/ml) after appropriate dilution, was used as a cell-free extract for enzyme reactions. When the enzyme preparation was to be treated with Bio-beads SM-2, the beads, which were equilibrated with 0.1 M potassium phosphate buffer (pH 7.4), were added in the indicated amounts to the cell-free extracts. After the mixture was stirred for 3 min at 4°C, the enzyme was separated from the beads by filtration through a glass filter.

Enzyme reaction

Unless otherwise indicated, the reaction mixture contained, in a final volume of 1.5 ml, the following constituents: 150 μmol of potassium phosphate buffer (pH 7.4), 0.1 μmol of dithiothreitol, 3 μmol of $MgSO_4$, 4 μmol of $MnSO_4$, 7.5 μmol of NAD, 1.3 μmol of NADP, 6.3 μmol of D-glucose 6-phosphate, 8.4 μmol of D-fructose 1,6-diphosphate, the cell-free extracts (4 mg of protein), detergent, and substrate as indicated: (3RS)-[2-^{14}C]MVA, 129,000 cpm (11.8 nmol); [1-^{14}C]acetate, 275,000 cpm (2.5 nmol); *S*-adenosyl-L-[methyl-^{14}C]methionine (^{14}C-labeled SAM), 41,900 cpm (0.3 nmol); or ^{14}C-labeled FPP, 25,000 cpm (0.1 nmol). When [^{14}C]acetate was to be used as a substrate, the reaction mixture included, in addition, 1 μmol of CoA. When lecithin or lysolecithin was to be added to the reaction mixture, it was dispersed in the buffer as follows. Twenty-five mg of the lipid was added to 0.5 ml of diethyl ether, and the mixture was added to 1 ml of 0.1 M potassium phosphate buffer (pH 7.4). It was agitated vigorously with a vortex mixer under reduced pressure to evaporate the diethyl ether. When other detergents were to be used, a 10% solution (w/v) of the compound was added to the reaction mixture. Unless otherwise indicated, incubation was carried out with shaking for 1 hr at 30°C. When ^{14}C-labeled SAM was used as a substrate, the reaction was carried out for 2 hr at 30°C.

Analysis of nonsaponifiable lipids

After the reaction was completed, the reaction mixture was saponified and nonsaponifiable lipids were extracted with petroleum ether (19). Saponification was carried out for complete extraction of squalene, 2,3-oxidosqualene, and sterols, although most of the radioactive sterols formed under the conditions used were nonesterified sterols (18). Unless otherwise indicated, the nonsaponifiable lipids were separated by thin-layer chromatography on a precoated silica gel plate (0.25 mm thick, Merck Company, Darmstadt, West Germany) using benzene–methanol 98:2 (v/v) as a developing solvent. The lipids were separated into three fractions: squalene (R_f, 0.65–0.75), 2,3-oxidosqualene (R_f, 0.40–0.55), and sterols (R_f, 0.05–0.25). They were visualized with I_2 vapor, and their radioactivities were monitored with a Packard radiochromatogram scanner (Packard Instrument Co., Downer's Grove, IL). For exact determination of the radioactivity, radioactive areas were scraped from the plate into scintillation vials. Δ^{24}-Sterol methyltransferase was assayed by measurement of radioactivity incorporated into the total nonsaponifiable lipid fraction from ^{14}C-labeled SAM, without further separation. Radioactivity was determined in a Beckman LS-230 liquid scintillation spectrometer (Beckman Instruments Inc., Fullerton, CA), using a toluene scintillator containing 0.4% 2,5-diphenyloxazole.

Determination of protein

Protein concentration was determined by the method of Lowry et al. (20), using crystalline bovine serum albumin as a standard.

Chemicals

(3RS)-[2-^{14}C]Mevalonolactone (5 Ci/mol), ^{14}C-labeled SAM (58 Ci/mol), [1-^{14}C]acetate (50 Ci/mol), and [1-^{14}C]isopentenyl pyrophosphate (57 Ci/mol) were obtained from Radiochemical Centre (Amersham, England). Triton X-100 (specially prepared), Tween 80 (extra pure), Tween 40 (extra pure) and Tween 20 (extra pure) were purchased from Nakarai Chemicals Co. (Kyoto, Japan). L-α-Lecithin (extra pure, from egg yolk) was obtained from Merck Company (Darmstadt, West Germany) and further purified by column chromatography on neutral aluminum oxide. L-α-Lysolecithin (Type I, from egg yolk, approx. 99%) was purchased from Sigma Chemical Co. (St. Louis, MO). Bio-beads SM-2 were the product of Bio-Rad Laboratories (Richmond, CA). [^{14}C]Squalene (0.43 Ci/mol) and ^{14}C-labeled FPP (170 Ci/mol) were prepared enzymatically by incubation of ^{14}C-labeled MVA and [^{14}C]isopentenyl pyrophosphate with cell-free extracts of yeast, respectively (21). All other chemicals were of analytical grade.

RESULTS

Effects of various detergents on sterol synthesis

The effects of some non-ionic detergents and phospholipids on sterol synthesis in cell-free system of *S. cer-*

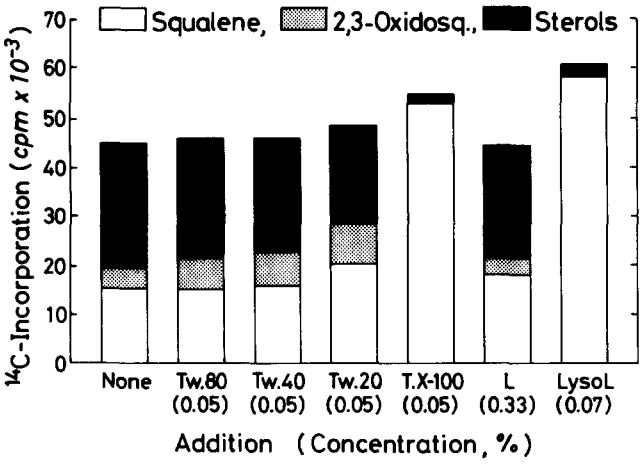

Fig. 1. Effects of detergents and phospholipids on incorporation of ^{14}C from ^{14}C-labeled MVA into squalene, 2,3-oxidosqualene, and sterols. The reaction mixtures contained (3RS)-[2-^{14}C]MVA, cofactors, the cell-free extracts (4 mg of protein), and additions as indicated. Incubation was carried out as described in Materials and Methods. The mixture was saponified and the resulting nonsaponifiable lipids were extracted and separated by thin-layer chromatography into three fractions: squalene (□), 2,3-oxidosqualene (▨), and sterols (■). Each portion was scraped from the plate and its radioactivity was determined. Abbreviations: Tw. 80, Tween 80; Tw. 40, Tween 40; Tw. 20, Tween 20; T. X-100, Triton X-100; L, lecithin; LysoL, lysolecithin.

evisiae were investigated. **Fig. 1** shows the effects of the compounds on incorporation of ^{14}C from ^{14}C-labeled MVA into nonsaponifiable lipids. Most of the radioactivity incorporated was in squalene, 2,3-oxidosqualene, and sterols. Geranylgeraniol, which was reported to be formed in cell-free system of rat brain by Ramsey et al. (22), was not detected by thin-layer chromatography and radio–gas–liquid chromatography in the products of reaction in the presence of these compounds. As shown in Fig. 1, the Tween detergent showed little effect on the composition of lipids. In contrast, Triton X-100 increased, to some degree, ^{14}C incorporation from ^{14}C-labeled MVA into the lipids, which consisted mostly of radioactive squalene, suggesting that it inhibited conversion of squalene to 2,3-oxidosqualene and sterols. Lecithin showed virtually no effect on the composition, whereas lysolecithin showed a similar effect to that of Triton X-100. Throughout the succeeding experiments Triton X-100 was used.

Effect of Triton X-100 on sterol synthesis from various substrates

Table 1 shows the effect of Triton X-100 on incorporation into nonsaponifiable lipids of ^{14}C from various ^{14}C-labeled substrates in the cell-free extracts. Incorporation of ^{14}C into squalene (A) from [^{14}C]acetate, ^{14}C-labeled MVA, or ^{14}C-labeled FPP was increased by the addition of 0.05% Triton X-100, whereas incorporation into 2,3-oxidosqualene (B) and into sterols (C) was decreased. Incorporation of ^{14}C into total nonsaponifiable lipids (A + B + C) was not decreased but rather increased to some degree by the addition of Triton X-100 (Experiments 1–3). These results suggest that the apparent activity of squalene synthetase (EC 2.5.1.21) was stimulated by the detergent and that the activities of enzymes in the steps before FPP synthesis were not inhibited.

The ratios of (B + C)/total nonsaponifiable lipids and C/(B + C) in the incubations with and without Triton X-100 can be regarded as roughly reflecting the relative activities of squalene epoxidase (EC 1.14.99.7) and 2,3-oxidosqualene-lanosterol cyclase (EC 5.4.99.7) under both conditions, respectively. Comparison of the value of (B + C)/total nonsaponifiable lipids in the presence

TABLE 1. Effect of Triton X-100 on incorporation of ^{14}C from ^{14}C-labeled substrates into squalene, 2,3-oxidosqualene and sterols

Experiment	Substrate	Triton 0.05%	^{14}C Incorporation[a]				Ratio	
			A	B	C	A + B + C	B + C / A + B + C	C / B + C
			cpm × 10^{-3}					
1	[^{14}C]Acetate	−	8.8	0.5	2.5	11.8	0.26	0.82
		+	12.5	trace	0.1	12.6	0	
2	[^{14}C]MVA	−	15.0	4.3	26.3	45.6	0.67	0.86
		+	54.1	0.1	1.1	55.3	0.02	0.94
3	[^{14}C]FPP	−	5.0	2.1	11.2	18.3	0.73	0.84
		+	19.6	0.5	4.3	24.4	0.20	0.91
4	[^{14}C]SAM[b]	−			13.6	13.6		
		+			1.2	1.2		

[a] Incorporation of ^{14}C from each substrate into squalene (A), 2,3-oxidosqualene (B), and sterols (C).
[b] Endogenous C_{27} sterols present in the cell-free extracts acted as an acceptor for the methyl group of [^{14}C]SAM.
The reaction mixture contained radioactive substrate, cofactors, the cell-free extracts (4 mg of protein), and 0.05% of Triton X-100 as indicated. In Experiment 1, 1 μmol of CoA was included in the reaction mixture in addition. Incubation was carried out for 1 hr at 30°C in Experiments 1, 2, and 3, and for 2 hr at 30°C in Experiment 4. After the reaction, the mixture was saponified and nonsaponifiable lipids were extracted and analyzed as described in Materials and Methods.

of Triton X-100 with the value in the absence of Triton X-100 suggests that squalene epoxidase activity was markedly inhibited by 0.05% Triton X-100. This was confirmed by the experiment in which NADPH, FAD, and [^{14}C]squalene (dispersed with the aid of 0.1% Tween 80, final concentration) were incubated with the ultracentrifugal precipitate of the cell-free extracts as an enzyme in the presence and absence of Triton X-100. The activity of epoxidase was inhibited about 70 and 85% by 0.1 and 0.3% Triton X-100, respectively.

In contrast to the epoxidase, the activity of cyclase seemed to be scarcely affected by 0.05% Triton X-100. In the present experiment, however, the effect of Triton X-100 on the activity of enzyme was not obvious, since the amount of de novo synthesized [^{14}C]2,3-oxidosqualene as a substrate was too small.

Table 1 also shows the effect of Triton X-100 on the activity of Δ^{24}-sterol methyltransferase (EC 2.1.1.41) (Experiment 4). This enzyme activity was inhibited by 0.05% Triton X-100.

From the above results, it was concluded that Triton X-100 exerted a marked effect on the reactions in the late stage of sterol synthesis in the cell-free extracts of yeast.

Table 2 shows the effect of Triton X-100 on sterol synthesis at various concentrations of the cell-free extracts (Experiment 1). Triton X-100 increased incorporation of ^{14}C from ^{14}C-labeled MVA into nonsaponifiable lipids, suggesting an apparent stimulation of squalene synthetase activity in a wide range of concentrations of the cell-free extracts. In contrast, the activity of squalene epoxidase, which is reflected by the ratio (B + C)/total nonsaponifiable lipids, was inhibited by Triton X-100 when low concentrations of the cell-free extracts were used as an enzyme source. The enzyme activity could be protected from the inhibition by using high concentrations of the cell-free extracts or by adding lecithin liposomes before the addition of Triton X-100. It was presumed from these results that the protection from the Triton inhibition by high concentrations of the cell-free extracts was due to high concentrations of phospholipids in the extracts.

Variation of sterol synthesis with increasing concentrations of Triton X-100

Variation of the activity of ergosterol synthesis using ^{14}C-labeled MVA or ^{14}C-labeled FPP as a substrate with increasing concentrations of Triton X-100 was examined (**Fig. 2**). A slight increase in ^{14}C incorporation into nonsaponifiable lipids (□) from ^{14}C-labeled MVA by the addition of Triton X-100 was observed in a wide range of concentrations from 0.01 to 0.5%, showing an optimum at 0.03–0.2% (Fig. 2A). In contrast, incorporation of ^{14}C from ^{14}C-labeled MVA into 2,3-oxidosqualene and sterols (△) was markedly decreased by the detergent at concentrations more than 0.04%. The concentration required for half-maximum inhibition was about 0.03% (Fig. 2A, inset). This again indicates a marked inhibition of squalene epoxidase activity by Triton X-100. Similar experiments with ^{14}C-labeled FPP showed that ^{14}C incorporation into nonsaponifiable lipids (□) was slightly increased by the addition of Triton X-100 in a range of concentrations from 0.02 to 0.2% (Fig. 2B). In contrast, incorporation of ^{14}C into 2,3-oxidosqualene and sterols (△) was decreased by the detergent at concentrations more than 0.04%. The curves for incorporation of ^{14}C into sterols (○) and into 2,3-oxidosqualene and sterols (△) almost overlapped each other, suggesting that the

TABLE 2. Effect of Triton X-100 on incorporation of ^{14}C from ^{14}C-labeled MVA into squalene, 2,3-oxidosqualene, and sterols under various conditions

Experiment	Enzyme Source	Triton 0.05%	Lecithin 0.67%	^{14}C Incorporation[a]				Ratio	
				A	B	C	A + B + C	B + C / A + B + C	C / B + C
	mg of protein			*cpm* × 10^{-3}					
1	3	−	−	24.6	4.6	18.6	47.8	0.48	0.80
	3	+	−	58.1	trace	trace	58.1	0	
	6	−	−	22.7	5.0	16.5	44.2	0.49	0.77
	6	+	−	45.1	5.8	7.7	58.6	0.23	0.57
	30	−	−	28.3	3.7	12.0	44.0	0.35	0.76
	30	+	−	34.0	4.5	14.4	52.9	0.36	0.76
2	4	−	−	13.8	4.7	11.5	30.0	0.54	0.71
	4	+	−	41.7	2.3	2.1	46.1	0.10	0.48
	4	−	+	20.3	7.3	10.3	37.9	0.46	0.59
	4	+	+	21.7	7.5	13.7	42.9	0.49	0.65

[a] Incorporation of ^{14}C from (3R)-[^{14}C]MVA into squalene (A), 2,3-oxidosqualene (B), and sterols (C).

The reaction mixture contained (3RS)-[2-^{14}C]MVA, cofactors, indicated concentrations of the cell-free extracts, and 0.05% Triton X-100 and 0.67% lecithin where indicated. Other experimental conditions were the same as described in Materials and Methods.

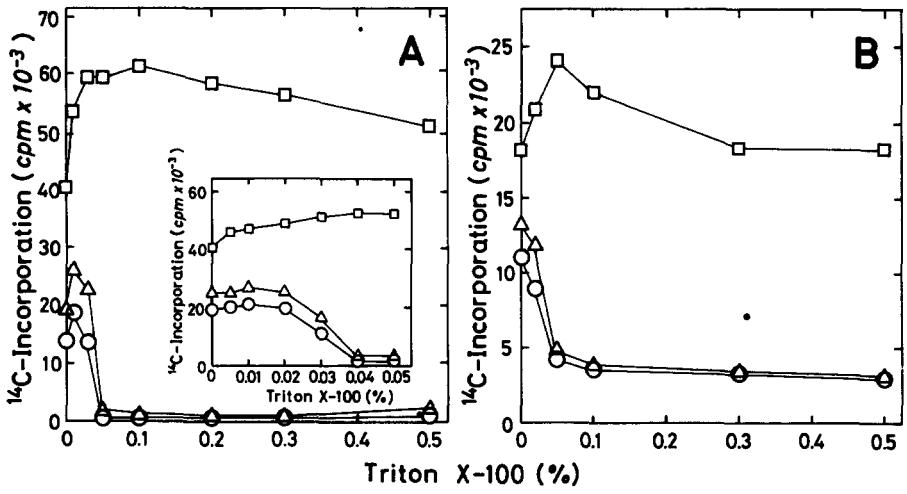

Fig. 2. Variations of incorporation of ^{14}C from ^{14}C-labeled substrates into squalene, 2,3-oxidosqualene, and sterols with increasing concentrations of Triton X-100. The incubations were carried out using (3RS)-[2-^{14}C]MVA (A) or [^{14}C]FPP (B) at different concentrations of Triton X-100 as described in Materials and Methods for determination of ^{14}C incorporation into squalene plus 2,3-oxidosqualene plus sterols (□), 2,3-oxidosqualene plus sterols (△), and sterols (○). The amount of enzyme was 4 mg of protein. The inset in A shows the result of a separate similar experiment in which lower concentrations of Triton X-100 were used.

activity of cyclase was at least not inhibited by Triton X-100.

Fig. 3 shows variation of Δ^{24}-sterol methyltransferase activity with increasing concentrations of Triton X-100. This enzyme activity was markedly inhibited by Triton X-100 at concentrations higher than 0.04% as was observed with the epoxidase activity. The concentration necessary for half-maximum inhibition was approximately 0.01%, indicating a higher sensitivity of this enzyme to Triton X-100 than that of the epoxidase.

As mentioned above, the effect of Triton X-100 on the activities of sterol synthesis in its late stage is considerably complicated. It can be summarized as follows. The activity of squalene synthetase is apparently stimulated whereas activities of squalene epoxidase and Δ^{24}-sterol methyltransferase are inhibited by Triton X-100, although the concentrations necessary for the inhibition are different from one another.

Restoration of enzyme activity from the Triton inhibition by treatment with Bio-beads SM-2

In order to examine whether or not the enzyme activities which were inhibited by Triton X-100 can be restored, the detergent was removed from the reaction mixture by treatment with Bio-beads SM-2 and the enzyme activities were determined. **Table 3** shows the result of an experiment with Δ^{24}-sterol methyltransferase. The Bio-beads treatment did not affect the enzyme activity itself. The severe inhibition of the enzyme was partially reversed (nearly 40%) by the treatment with Bio-beads. This suggests that the methyltransferase had not been inactivated or denatured. A similar experiment was carried out with squalene epoxidase using ^{14}C-labeled MVA as a substrate. In the absence of Triton X-100, the radioactivity incorporated into 2,3-oxidosqualene and sterols from ^{14}C-labeled MVA was 20,500 cpm; in the presence of 0.05% Triton X-100, it was 1,900 cpm. When the Triton-inhibited enzyme was treated with Bio-beads, the radioactivity was 1,400 cpm. These results indicated that the epoxidase activity was not restored from the Triton inhibition, although this did not necessarily indicate denaturation of the enzyme protein by Triton X-100. This will be discussed later.

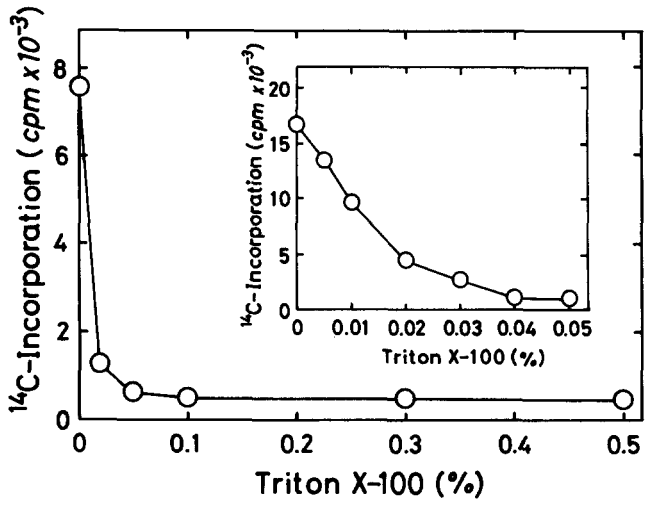

Fig. 3. Variation of Δ^{24}-sterol methyltransferase activity with increasing concentrations of Triton X-100. Incorporation of ^{14}C from ^{14}C-labeled SAM into sterol fraction was determined. The amount of enzyme was 4 mg of protein. The inset represents the result of a separate similar experiment in which lower concentrations of Triton X-100 and the same enzyme preparation as that used in the experiment shown in the inset of Fig. 2A were used.

TABLE 3. Restoration of the Triton-inhibited Δ^{24}-sterol methyltransferase activity by treatment with Bio-beads SM-2

Experiment	Treatment of Enzyme: Triton 0.08%	Treatment of Enzyme: Bio-beads	^{14}C Incorporation from [^{14}C]SAM into Sterols
			cpm × 10^{-3}
1	−	−	10.9
2	−	+	10.9
3	+	−	0.9
4	+	+	4.3

The cell-free extracts (15 ml, 60 mg of protein) were divided into two portions. Triton X-100 (0.08% in final concentration) was added to one portion (7.5 ml in Experiments 3 and 4). An aliquot (5 ml) was withdrawn from each portion and treated with Bio-beads SM-2 (1 g, wet weight) as described in Materials and Methods (Experiments 2 and 4). No treatment was carried out on another aliquot from each portion (Experiments 1 and 3). Incubation was carried out with the above four enzyme preparations (4 mg of protein) for the assay of Δ^{24}-sterol methyltransferase.

Alteration of membrane structure by Triton X-100

Some experiments were carried out to obtain information about the location of the enzyme that was affected by Triton X-100. The cell-free extracts were ultracentrifuged at 100,000 *g* for 60 min in the presence and absence of Triton X-100. The resulting precipitate and supernatant were separated from one another and were treated with Bio-beads in order to eliminate the detergent. Then each fraction was assayed for the activity of Δ^{24}-sterol methyltransferase (**Table 4**). Higher activities (both specific and total activity) of the enzyme were seen in the precipitate than in the supernatant fraction in the experiment without Triton X-100 (Experiments 1 and 2). Some significant enzyme activity found in the supernatant fraction (Experiment 2) seems to have been due to small particles that remained in this fraction. In the experiment with Triton X-100 (Experiments 3 and 4), on the contrary, much higher activities (specific and total) were found in the supernate than in the precipitate. This indicates that the methyltransferase was translocated from the precipitate to the supernatant fraction by the action of Triton X-100. It is presumed that not only the enzyme but also the endogenous sterols were translocated to the supernatant, since sterols had not been added exogenously as an acceptor for the methyl group of ^{14}C-labeled SAM. The smaller value of combined activities in Experiments 3 and 4 than that of combined activities in Experiments 1 and 2 seems to have been due to the insufficient restoration of the enzyme activity from the Triton inhibition by the Bio-beads treatment (see Table 3).

A similar phenomenon was observed in the experiment with ^{14}C-labeled MVA as a substrate. The cell-free extracts were incubated with ^{14}C-labeled MVA in the presence and absence of Triton X-100 and then ultracentrifuged to obtain the precipitate, supernatant, and floating lipid fractions. Each fraction was saponified and radioactivity in nonsaponifiable lipids was measured. When the reaction mixture was incubated in the absence of Triton X-100, most of the total radioactivity was in the precipitate and less than 10% of the total radioactivity was in the supernatant fraction. In contrast, when the mixture was incubated in the presence of 0.05% Triton X-100, approximately 40% of the total radioactivity was found in the supernatant and floating lipid fractions. The results of protein analyses also showed that approximately 30% of protein in the precipitate was translocated to the supernatant fraction through the action of 0.05% Triton X-100. These results indicate that the structure of microsomal membrane was altered by Triton X-100 to some extent.

DISCUSSION

In an attempt to obtain information about the activity of enzymes in yeast involved in sterol synthesis, we investigated the effects of various detergents on enzyme activities from the early to the late stages of synthesis. Among the detergents tested, Tweens and lecithin scarcely affected the activities. This seems to be the reason why they are often used for the dispersion of water-insoluble substrate in a reaction mixture. In contrast,

TABLE 4. Translocation of Δ^{24}-sterol methyltransferase to the 100,000 *g* supernatant by treatment with Triton X-100

Experiment	Treatment of Enzyme: Triton 0.05%	Treatment of Enzyme: 100,000 *g* Fraction	Treatment of Enzyme: Bio-beads	Enzyme Activity: Sp. Act.	Enzyme Activity: Total act.
				cpm/hr per mg of prot.	*cpm/hr*
1	−	ppt	+	3710	10900
2	−	sup	+	1190	9670
3	+	ppt	+	280	620
4	+	sup	+	1530	15200

The cell-free extracts (15 ml, 40 mg of protein) were divided into two portions. Triton X-100 (0.05% in final concentration) was added to one portion (7.5 ml in Experiments 3 and 4). An aliquot (5 ml) was withdrawn from each portion and was centrifuged at 100,000 *g* for 60 min. The resulting precipitate, after separation from the supernatant, was suspended in 2 ml of 0.1 M potassium phosphate buffer (pH 7.4) using a Teflon homogenizer. From each of the four samples, an aliquot (2 ml in Experiments 1 and 3, and 4.5 ml in Experiments 2 and 4) was withdrawn and treated with Bio-beads SM-2 (1 g, wet weight). Final protein concentrations in Experiments 1, 2, 3, and 4 were 1.5, 1.8, 1.1, and 2.2 mg/ml, respectively. The reaction was carried out using 1 ml each of the above preparations and ^{14}C-labeled SAM as a substrate. The endogenous C_{27} sterols present in the enzyme preparation acted as an acceptor for the methyl group of ^{14}C-labeled SAM.

Triton X-100 and lysolecithin showed a marked effect on the activities of some enzymes on microsomes. Nes, Patterson, and Bean (23) found that Triton X-100 inhibited micelial growth of *Phytophthora cactorum*, a fungus unable to synthesize sterols, while Tween 80 did not. This observation is compatible with our present data.

Although Triton X-100 scarcely affected enzyme activities in the early and middle stages of the synthesis, it exerted a marked effect on the activities in the late stage (Table 1). As far as is known, no systematic study of the effects of detergents on the sterol synthesis seems to have been made except the one by Ramsey et al. (22). They investigated the effects of detergents and phospholipase on incorporation of ^{14}C from ^{14}C-labeled MVA into sterols in cell-free extracts of rat brain to find the method for stimulation of sterol synthesis. Their results differed from ours; they found that the detergents decreased incorporation of ^{14}C into squalene but increased incorporation into free sterols and geranylgeraniol.

In animals, the presence of a sterol carrier protein that stimulates the enzyme reactions in the late stage of sterol synthesis is established (24). A similar study was preliminarily reported with yeast (25). However, details of this study have not been reported, and it seems that the presence of sterol carrier protein in yeast is not necessarily established. However, if such a protein does exist in yeast, the effect of detergent on the activities of enzymes of ergosterol synthesis in the late stage might be explained in terms of the presence of protein. In spite of an effort to demonstrate the presence of a sterol carrier protein, we could obtain no supporting evidence. In the present study, we suggest that the effect of detergent presented is explainable in terms of the alteration of membrane structure.

The present study showed that squalene synthetase activity was apparently activated by Triton X-100.[3] In contrast, the activities of squalene epoxidase and Δ^{24}-sterol methyltransferase were apparently "inactivated" with increasing concentrations of the detergent and, accordingly, with increasing alteration of microsomal membrane (Figs. 2 and 3). However, the activity of methyltransferase was not "inactivated" itself but was translocated from the microsomal fraction to the ultracentrifugal supernatant by the treatment with Triton X-100 (Table 4). The disappearance of "inactivation" of the epoxidase activity by the increase of concentration of the cell-free extracts or by the prior addition of lecithin liposomes seems to be due to the protection against the alteration of the membrane by the decrease in the effective concentration of Triton X-100. It is worth noting that the sensitivities of the above two enzymes to Triton X-100 were different. The activity of epoxidase was scarcely affected by Triton X-100 at concentrations lower than 0.02%, while that of methyltransferase was so sensitive to the detergent as to be inhibited at concentrations less than its critical micellar concentration (about 0.01%) (16) (Figs. 2 and 3). The two enzymes showed different behavior in the restoration experiment (from the Triton inhibition) by the Bio-beads treatment. The methyltransferase, which is thought to be a simple protein, restored a considerable activity by the treatment (Table 3), while no recovery was obtained with the activity of squalene epoxidase system, which consists of several components. The loss of activities of these enzymes occurred even by the treatment with low concentrations of Triton X-100 that scarcely denatured protein. It is possible that the epoxidase system that had been altered by Triton X-100 could not restore its original conformation even when the detergent was removed from the reaction mixture.

The present study revealed that the epoxidase activity of *S. cerevisiae* was inhibited by Triton X-100. This is in contrast to the results with rat liver microsomes, in which the activity was stimulated more than 10 times by 0.3% Triton X-100 (26). This discrepancy seems to be due to the difference in the location of the enzyme on membrane or in the nature of the enzyme itself. A similar observation was reported by Shechter, Sweat, and Bloch (27) on 2,3-oxidosqualene-lanosterol cyclase. In their experiment using exogenously added [^{14}C]2,3-oxidosqualene as a substrate, they revealed that the activity of the yeast enzyme was stimulated by considerably high concentrations, from 0.2 to 0.4%, of Triton X-100 while that of the hog liver enzyme was not. In the present experiment, however, the effect of detergent on the cyclase activity was not obvious.

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[3] After the manuscript was submitted for publication, a review was published (Poulter, C. D., and H. C. Rilling. 1981. Conversion of farnesyl pyrophosphate to squalene. *In* Biosynthesis of Isoprenoid Compounds. J. W. Porter and S. L. Spurgeon, editors. John Wiley and Sons, New York. **1:** 413–441.). The authors described the activation of squalene synthetase by organic solvents or detergents, citing a Ph.D. thesis (Carlson, J. P. 1971. Thesis, University of Utah, Salt Lake City, UT).

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