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COMPARATIVE PROPERTIES OF 2,3-OXIDOSQUALENE–LANOSTEROL CYCLASE FROM YEAST AND LIVER

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SUMMARY

In yeast the enzyme 2,3-oxidosqualene–lanosterol cyclase (cyclase) is found in the soluble fraction of cell homogenates. The corresponding enzyme from liver is associated with the microsomes. The soluble yeast cyclase is optimally active in solutions of low ionic strength and stimulated by Triton X-100. By contrast, the detergent-solubilized liver cyclase requires deoxycholate and high ionic strength media for optimal activity. After acetone precipitation yeast cyclase is no longer soluble though it still responds to Triton X-100. Acetone-precipitated liver cyclase is rendered soluble by deoxycholate.

INTRODUCTION

Enzyme activities catalyzing the cyclization of 2,3-oxidosqualene to lanosterol have been demonstrated in cell-free extracts of liver[1-3] and yeast[4]. The oxidosqualene–lanosterol cyclase from detergent-solubilized hog liver microsomes has been partially purified and characterized in this laboratory[1,2]. Examining yeast extracts, we have found the properties of enzyme from this source to differ markedly from those of liver cyclase. Most notably, yeast cyclase is found in the highspeed supernatant of otherwise untreated cell homogenates and at this stage is activated by Triton X-100 and inhibited by deoxycholate. On the other hand, on centrifugation of liver homogenates, most of the cyclase activity is recovered in the microsomal fraction and does not become soluble until treated with deoxycholate. The enzymes from the two sources differ also in their response to media of high ionic strength. High salt concentrations markedly enhance the activity of liver cyclase but inhibit the activity of the yeast enzyme.

MATERIALS AND METHODS

Triton X-100 and sodium deoxycholate were purchased from Rohm and Haas, and Sigma Chemical, respectively. The ^{14}C-labeled and the unlabeled 2,3-oxidosqualene used in these experiments was kindly supplied by Professor E. J. Corey and his associates. For final purification, the labeled epoxide was subjected to thin

layer chromatography on silica gel G plates, with 3% ethyl acetate in hexane as developing solvent. 98% of the product was associated with a peak that migrated with R_F of 0.45. Rechromatography did not remove the small amounts of materials at the origin and at the solvent front. It was, therefore, inferred that the 2% impurity was formed during chromatography. Hog liver microsomal fraction was obtained as previously described[1]. The yeast was "Bakers Dried Active Yeast" obtained from Nutritional Biochemical Corp., Cleveland, Ohio. Four grams of this yeast were suspended in 40 ml of 0.05 M potassium phosphate buffer, pH 7.50. The cell suspension was passed twice through a French press at 22 000 lb/inch^2 and then centrifuged at 5000 × *g* for 10 min. The supernatant was collected carefully so as to exclude the floating fatty layer. The collected supernatant was centrifuged at 105 000 × *g* for 90 min and the high speed supernatant (S_{105}) separated from the particulate fraction. The S_{105} so obtained contained 7 to 10 mg protein per ml. Protein was determined according to LOWRY *et al.*[5].

Assay mixtures for the liver enzyme contained in a total volume of 1.5 ml, [1-^{14}C]2,3-oxidosqualene (73 nmoles, 10 000 counts/min), 150 μmoles potassium phosphate or Tris buffer (pH 7.50). The concentrations of KCl, detergent and enzyme are noted in the figures and tables. Incubations were started by the addition of oxidosqualene dispersed in 0.1 ml of 0.01 M potassium phosphate buffer, pH 7.50, with 0.1 mg Tween 80. Reactions were terminated by the addition of 1.0 ml of 10% KOH in methanol (w/v). After standing at 37° for 30 min, the solutions were extracted with three 3-ml quantities of light petroleum. The extracts were dried with anhydrous Na_2SO_4 and solvent removed under a stream of nitrogen at room temperature.

Mixtures for the yeast enzyme assay contained, in a total volume of 0.5 ml, [1-^{14}C]2,3-oxidosqualene (6 nmoles, 20 000 counts/min), 25 μmoles potassium phosphate buffer, pH 7.5, KCl, detergent, and enzyme as noted in the tables and figure legends. The oxidosqualene was dispersed as described for the rat liver enzyme assays. Incubations were started by the addition of enzyme and stopped by addition of 0.5 ml of acetone. The solutions were extracted immediately with three 3-ml quantities of benzene acetone (3:1, by vol.) and the combined extracts were evaporated to dryness under a stream of nitrogen at room temperature.

The lipid residues recovered from all enzyme assays were applied to pre-coated silica gel (Brinkman) thin-layer chromatographic plates (2 cm × 20 cm) and the plates developed in unsaturated tanks to a height of 10 cm with ethyl acetate benzene (5:95, by vol.). R_F values of 0.9 and 0.5 were consistently obtained for oxidosqualene and lanosterol, respectively. The areas of the thin layer plates corresponding to lanosterol and oxidosqualene were scraped directly into scintillation counting vials and 15 ml of scintillation fluid was added. Samples were counted in a Packard Tri-Carb scintillation spectrometer and counts corrected for quenching by the channel ratio method.

Acetone powders were prepared by adding enzyme solutions dropwise to 60 vol. of acetone at −20°. The precipitated protein was collected by centrifugation and dried under a stream of nitrogen.

Lanosterol formed from the above incubations had the same retention time as an authentic sample of unlabeled lanosterol on gas chromatographic analysis performed with an F·M, Model 8733, instrument. Lanosterol was the sole labeled product obtained with either yeast or liver enzymes.

RESULTS

The distribution of cyclase activity after centrifugation of broken cell preparations of yeast and liver is shown in Table I. Both types of cells were homogenized in buffer without addition of detergent. In the absence of detergent, both the sedimented and supernatant yeast fractions showed relatively low activity. Subsequent addition of Triton X-100, however, stimulated cyclase activity of the soluble yeast fraction 5- to 6-fold. The detergent did not raise the low activity of the particulate yeast enzyme. As already reported[1,2], the bulk of liver cyclase activity resides in the microsomal fraction and becomes soluble (non-sedimentable) on treatment with deoxycholate. The detergent-solubilized liver cyclase can be purified by ammonium sulfate fractionation, gel filtration, and by chromatography on DEAE-cellulose or hydroxylapatite[6].

Although the yeast cyclase remains in solution when subjected to centrifugation at 100 000 × *g* for periods up to 3 h, attempts to purify it either in the presence or absence of Triton X-100 by ammonium sulfate fractionation, gel filtration or

TABLE I

2,3-OXIDOSQUALENE-LANOSTEROL CYCLASE ACTIVITY IN CELL FRACTIONS OF LIVER AND YEAST

Source of enzyme	*Relative activity* (%)
Liver	
Microsomal fraction	28.0
Microsomal fraction + 0.2% deoxycholate	100*
High speed supernatant	3.44
High speed supernatant + 0.1% deoxycholate	7.90
Yeast	
Particulate fraction	17.6
Particulate fraction + 0.2% Triton X-100	17.2
High speed supernatant	18
High speed supernatant + 0.2% Triton X-100	100*

* Under the standard assay conditions, enzyme activity was linear with time. A relative activity of 100 represents a 15% conversion of substrate by liver enzyme and 19–20% conversion of substrate by yeast enzyme.

chromatography on either DEAE-cellulose or hydroxylapatite, all failed resulting in nearly complete loss of enzyme activity. We do not know whether this failure is due to the intrinsic instability of yeast cyclase or whether the enzyme, though soluble by the usual centrifugal criteria, is, in fact, particulate in nature. It seems remarkable at any rate that yeast cyclase can be obtained from homogenates in a non-sedimentable form without the aid of a surface-active agent whereas the corresponding liver enzyme requires detergent for solubilization. The activities of the two enzymes also respond differently when exposed to detergents. The neutral Triton X-100 raises the activity of the soluble yeast cyclase 5- to 7-fold without affecting the low activity of the particulate yeast fraction. The anionic deoxycholate does not raise yeast cyclase activity significantly (Fig. 1A). Conversely, liver enzyme is almost totally dependent on deoxycholate at all stages of purification. The deoxycholate stimulation

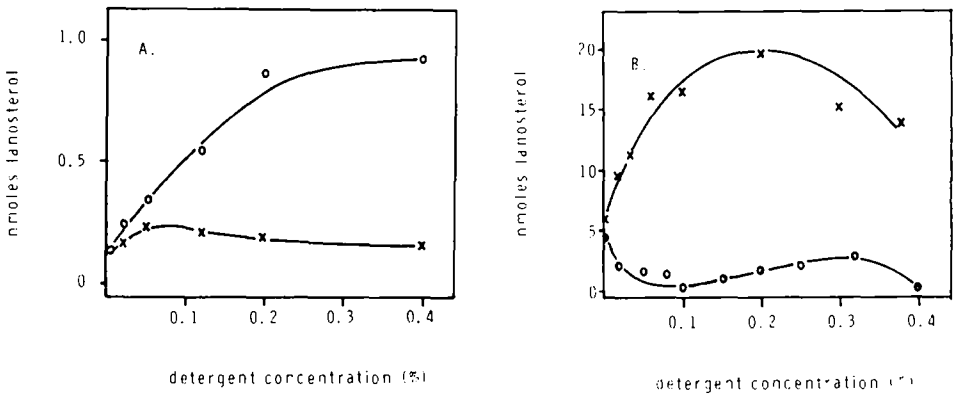

Fig. 1. A. Response of soluble yeast fraction to detergents. Incubation mixtures contained 4 mg supernatant protein, 25 μmoles potassium phosphate buffer, pH 7.6, and 6 nmoles of 2,3-oxidosqualene in a total volume of 0.5 ml. Incubations at 37° for 30 min. ×—×, sodium deoxycholate; ○—○, Triton X-100. B. Response of liver microsomal cyclase to detergents. Incubation mixtures contained 4.2 mg of microsomal protein, 600 μmoles of KCl, 150 μmoles of Tris–HCl buffer, pH 7.5, and 73 nmoles of 2,3-oxidosqualene in a total volume of 1.5 ml. Incubations at 37 for 15 min. ×—×, sodium deoxycholate; ○—○, Triton X-100.

is exhibited in a rather narrow concentration range with an optimal effect at 0.1–0.2% (Fig. 1B).

The cyclase activity of solubilized liver enzyme was previously shown to depend not only on deoxycholate or related anionic detergents, but also on the ionic

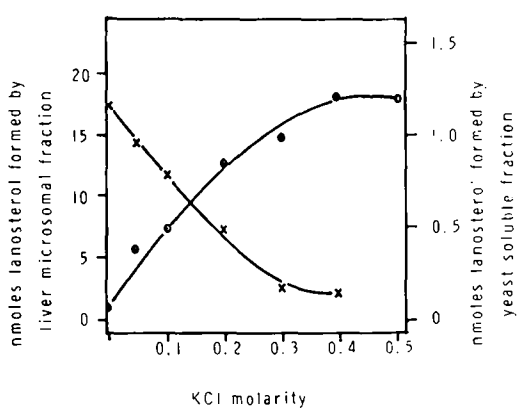

Fig. 2. Effect of ionic strength on liver and yeast cyclase. For the experiments with liver cyclase, assay mixtures contained 1.5 mg of "detergent-dependent" enzyme[1], 73 nmoles of 2,3-oxidosqualene, 15 μmoles of potassium phosphate buffer, pH 7.5, 1.5 mg sodium deoxycholate and KCl as indicated in a total volume of 1.5 ml. Incubations at 37° for 15 min. Yeast cyclase was assayed in solutions containing 4.0 mg supernatant protein, 6 nmoles 2,3-oxidosqualene, 25 μmoles of phosphate buffer, pH 7.6, 1.0 mg of Triton X-100 and KCl as shown in a total volume of 0.5 ml. Incubations at 37° for 15 min. ×—×, yeast cyclase; ○—○, liver cyclase.

environment. Raising the ionic strength in a medium containing 0.1% deoxycholate from 0.1 to 0.4 M (KCl) increases enzyme activity approximately 6-fold. The opposite effect is observed with yeast cyclase. This enzyme, in the presence of optimal concentrations of Triton X-100, suffers a sharp decline of activity with rising ionic strength of the assay medium (Fig. 2). In the case of the liver enzyme, the ionic strength effect is producible by a variety of salts[1] and therefore nonspecific. The specific activities for the yeast and the liver enzymes are 0.02 nmole/mg protein per min and 0.75 nmole/mg protein per min, respectively. Thus, the solubilized liver enzyme is about 37 times more active than the crude yeast preparation.

Many particulate enzymes or enzymes of particulate origin are adversely affected by treatment with acetone. When deoxycholate-solubilized liver cyclase is precipitated with acetone, enzyme activity is lost, but can be restored fully and resolubilized by addition of deoxycholate to the buffer-suspended acetone powder (Table II). Presumably, acetone removes the detergent which is essential both for the activity of the liver enzyme and for keeping it in solution. Yeast cyclase precipitated from the 100 000 × *g* supernatant by acetone is still active and stimulated by Triton X-100 but is rendered insoluble by this organic solvent treatment (Table II). The

TABLE II

CYCLASE ACTIVITY OF ACETONE-TREATED HOMOGENATES

The acetone powders were thoroughly dispersed in the indicated buffer with a Potter–Elvehjem homogenizer to give a final concentration of 7 mg protein per ml. The protein suspensions were centrifuged at 105000 × *g* for 90 min. Portions of the resulting supernatants and resuspended precipitates were assayed.

Fraction	*Homogenization buffer*	*Total activity** (%)
Liver		
High speed supernatant	0.033 M potassium phosphate buffer (pH 7.5) containing 1% deoxycholate	69
Precipitate		17
Yeast		
High speed supernatant	0.5 M potassium phosphate buffer (pH 7.5) containing 0.2% Triton X-100	4.8
Precipitate		61.3

* Relative to whole particle suspension in the presence of optimal detergent concentration.

acetone-soluble material obtained by this procedure neither solubilizes nor stimulates the precipitated yeast cyclase activity.

The cyclases from liver and yeast have also been exposed to various lytic enzymes. Proteases (pronase, trypsin, chymotrypsin) failed to solubilize or inactivate the microsomal liver cyclase. Moreover, after solubilization by deoxycholate, liver cyclase proved remarkably resistant to the proteolytic enzymes, both with respect to catalytic activity and to behavior on Sephadex G-200 gel filtration. These digestions also failed to alter the dependence of liver cyclase activity on detergent and high ionic strength[6].

An attempt to resolubilize the acetone-precipitated yeast cyclase by digesting the powder with proteolytic enzymes was unsuccessful and led to a 50% loss of enzyme activity. Phospholipases were similarly ineffective resolubilizing agents. Moreover, they did not reduce yeast cyclase activity.

DISCUSSION

The findings reported here illustrate the marked differences in properties between functionally identical enzymes of different origin. Liver cyclase as isolated from homogenates is clearly a microsomal enzyme and obtainable in a particle-free soluble form only with the aid of detergents. All the evidence so far suggests that liver cyclase is not a lipoprotein nor does it seem to require a specific lipid for activity (F. W. SWEAT, unpublished). Nevertheless, the solubilized cyclase retains many of the properties characteristic of particle-bound enzymes.

Yeast cyclase, as initially obtained by centrifugation of cell homogenates, appears to be a soluble enzyme which is dissociable from the particulate cell elements without detergent. However, the fact that this enzyme cannot be handled by procedures ordinarily applicable to soluble enzymes and that acetone precipitation leads to an insoluble preparation suggests that yeast cyclase also may be a particulate enzyme. Conceivably, the yeast cell contains molecules with detergent properties which facilitate the solubilization of the enzyme and keep it soluble during the initial extraction procedure.

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