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Enhanced metabolism of phosphatidylinositol in *Candida tropicalis* in association with filamentous growth caused by ethanol

Yasuhide Uejima, Teiichiro Koga and Teijiro Kamihara

Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan

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Candida tropicalis Pk 233 grows in filamentous form in ethanol-supplemented medium, and *myo*-inositol prevents the ethanol effect [(1979) Biochem. Biophys. Res. Commun. 91, 351–355]. Cells, which were grown with ethanol to the log phase, exhibited an increased rate of phosphatidylinositol turnover as judged by pulse-chase experiments with $^{32}P_i$. Such enhanced metabolism of phosphatidylinositol was not observed in fully developed filamentous cells at the stationary phase or in yeast-like cells which were grown without added ethanol or with ethanol plus *myo*-inositol. The metabolism of other phospholipids was not affected by ethanol.

Fungal dimorphism; Filamentous growth; Phosphatidylinositol turnover; Ethanol; *(Candida tropicalis)*

1. INTRODUCTION

A number of fungi grow in either yeast-like or filamentous form depending on the environmental conditions. Interest in the so-called fungal dimorphism has centred on its being a model as a simple morphogenic system. Despite numerous studies which have been performed from a variety of standpoints [1,2], the biochemical mechanism of dimorphism has been elucidated little.

We have reported that the cells of *Candida tropicalis* Pk 233 grow in filamentous form when cultivated in defined medium containing ethanol (1.5% (v/v) or above), and that the addition of *myo*-inositol (1–5 μg/ml) prevents this ethanol-induced morphological change [3,4]. The effect of *myo*-inositol strongly suggests an important role for membrane phosphoinositides in fungal dimorphism.

It has been subsequently found that the cellular content of PI is extremely low in the ethanol-grown culture at the log phase and increases gradually with cultivation time to a level comparable to that of the control culture grown without added ethanol. This phenomenon would indicate either lowered synthesis or enhanced turnover of PI in cells growing with ethanol.

This paper demonstrates that the enhancement of PI turnover is actually detected in resting cell suspensions which are prepared from growing cells with ethanol.

Correspondence addres: T. Kamihara, Laboratory of Industrial Biochemistry, Department of Industrial Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606, Japan

Abbreviations: PI, phosphatidylinositol; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; CL, cardiolipin; PA, phosphatidic acid

2. EXPERIMENTAL

2.1. *Growth of organism*

C. tropicalis Pk 233 was grown in defined medium with or without the addition of ethanol (2.5% (v/v)) and/or *myo*-inositol (5 μg/ml) as in

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[4]. Cells grown without the supplements, with ethanol, and with ethanol plus *myo*-inositol were designated as control, ethanol, and ethanol + inositol cells, respectively.

2.2. *Incorporation of $^{32}P_i$ into phospholipids*

Cells were harvested at the mid-log and stationary phases (~ 100 mg each, dry wt). After being washed three times with chilled water, cells were suspended in 10 ml of 25 mM Bis-Tris-HCl buffer (pH 6.0) containing 20 mg glucose, 1.25 mg $MgSO_4 \cdot 7H_2O$ and 1.25 mg $CaCl_2$. The suspensions were incubated with $^{32}P_i$ (10 μCi/ml) for 1 h at 30°C. The reaction was terminated by adding trichloroacetic acid at a final concentration of 5%. Extraction of phospholipids (including PA) was carried out by the method of Hanson and Lester [5]. After washing, the trichloroacetic acid precipitate was extracted three times with 3 ml each of a solution of ethanol-water-diethyl ether-pyridine-ammonia water (28%) (15:15:5:1:0.018, v/v) at 60°C for 20 min. To the lipid extract were added 10 ml *n*-propanol, 5 ml of 0.4 M EDTA, 10 ml petroleum ether and 5 ml of 1 M KCl. After shaking, the upper layer was evaporated to dryness. The residue was dissolved in 0.2 ml chloroform and aliquots were analysed for phospholipids by paper chromatography using Whatman SG 81 paper and a solvent system of diisobutylketone-acetic acid-water (40:20:3, v/v) [6]. For analysis of PA, an additional solvent system of diisobutylketone-acetic acid-water (40:25:5, v/v) [7] was employed. Phospholipid spots were located with iodine vapour. The individual phospholipids separated were determined by the method of Skipski and Barclay [8] and radioactivity was measured in toluene containing 0.4% PPO and 0.05% POPOP with an Aloka liquid scintillation counter.

2.3. *Chase of the ^{32}P-labelled phospholipids*

To a portion of the cell suspensions incubated with $^{32}P_i$ for 1 h as above was added 0.5 M sodium potassium phosphate buffer (pH 6.0) at a final concentration of 50 mM, and incubation was continued for a further 2 h to chase the ^{32}P-labelled phospholipids. Analyses of the radioactive phospholipids were carried out after 1 and 2 h incubation in the same way as described above.

2.4. *Chemicals*

$^{32}P_i$ (carrier-free) was purchased from NEN Research Products. Phospholipids were from Sigma. Other chemicals were of analytical reagent grade.

3. RESULTS

Table 1 (A) lists the specific activities of phospholipids from control, ethanol, and ethanol + inositol cells after 1 h incubation of these cells with $^{32}P_i$. Incorporation of $^{32}P_i$ into PI was greatly in-

Table 1

Turnover of phospholipids in cells of *Candida tropicalis* Pk 233

(A) Incorporation of $^{32}P_i$ into phospholipids

Cells	Specific activity (cpm/μmol $\times 10^{-5}$)					
	PI	PC	PE	PS	CL	PA
Mid-log phase						
Control	5.80	1.34	2.65	3.62	1.15	1.01
Ethanol	49.6	2.00	2.02	2.54	1.28	7.47
Ethanol + inositol	8.44	2.48	2.63	2.66	2.25	2.20
Stationary phase						
Control	5.26	1.24	5.94	7.47	2.66	2.37
Ethanol	7.99	1.10	7.35	5.47	2.90	2.35
Ethanol + inositol	6.08	1.70	7.74	2.99	2.70	2.69

(B) Chase of ^{32}P-labelled PI and PA in log-phase ethanol cells

Time for chase (h)	Specific activity (cpm/μmol $\times 10^{-5}$)	
	PI	PA
0	49.6	7.47
1	24.8	4.16
2	12.1	2.70

Incorporation of $^{32}P_i$ into phospholipids (A) was carried out at 30°C and pH 6.0 for 1 h in resting suspensions of cells grown as indicated. For chase of labelled PI and PA (B), $^{32}P_i$-treated suspensions were incubated for a further 2 h after adding excess unlabelled P_i. Samples from the 1 h incubation for $^{32}P_i$ incorporation and from the 1 and 2 h incubation for chase were analysed for specific activities of individual phospholipids as described in section 2

creased in ethanol cells grown to the mid-log phase. However, the increased specific activity of PI was reduced to the control level at the stationary phase where cells had a fully developed filamentous form. Ethanol + inositol cells had the same level of $^{32}P_i$ incorporation into PI as control cells at both growth phases. No appreciable changes were detected in $^{32}P_i$ incorporation into the other phospholipids except PA whose specific activity was significantly higher at the mid-log phase in ethanol cells than in the other cells. The increases in specific activities of PI and PA suggest enhancement of their turnover. This was confirmed by chase of the labelled PI and PA (table 1, B). The addition of unlabelled P_i in excess to suspensions of the log-phase ethanol cells, which had been incubated for 1 h with $^{32}P_i$, caused a significant reduction in specific activities of PI and PA: these values decreased approx. 50% per h. No appreciable changes in cellular levels of PI and PA were detected during incubation for chase. It is therefore conceivable that PI turnover, probably via PA, would be enhanced specifically in cells growing with ethanol.

4. DISCUSSION

This work has demonstrated the enhanced metabolism of PI and PA in cells of *C. tropicalis* Pk 233 growing exponentially in ethanol-supplemented culture. The ethanol-induced enhancement of PI and PA metabolism was indicated to be that of PI turnover via PA. This suggests the existence in microbial morphogenesis of a mechanism similar to that of the well-known signal transduction through inositol phospholipid turnover in a variety of cells of higher organism [9,10]. However, the response to ethanol was associated with cell proliferation and hence needed a longer period than those to agonists in mammalian cells. Moreover, the PI response was measured in resting cells suspended in ethanol-free buffer. Therefore, if the enhancement of PI turnover were caused by ethanol, the effect might have been retained in the cells after its removal.

Yeast cells are known to contain polyphosphoinositides [11,12] as higher eukaryotic cells. It is therefore very likely that, as in the case of higher eukaryotes [10], PI 4,5-bisphosphate rather than PI is the main target in inositol phospholipid turnover which is suggested here to be stimulated in association with filamentous growth. It has recently been found in *Saccharomyces cerevisiae* that inositol phospholipid metabolism or turnover is enhanced prior to the initiation of ergosterol-induced growth of cells [13] and in association with glucose-induced stimulation of cells which should lead to the transition from the G_0/G_1 phase of the cell cycle [14]. The present paper is the first to suggest the participation of turnover in morphogenesis. These findings should promote investigations on the regulatory mechanism of inositol phospholipid turnover with the advantages of using microorganisms, particularly the ease of applying genetic approaches.

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