Adaptation of Yeast to Cadmium VII. Significance of Polyphosphate Metabolism in Cadmium Resistance

by Hakobu NAKAMURA*

Received November 11, 1964

It has been shown that phosphorus-starved yeast cells are susceptible to cadmium¹⁾, and are low in polyphosphate content²⁾. Hence it is interesting to study the relation of polyphosphate to cadmium tolerance of cells.

Materials and Methods

Strains: The parent strain, denoted as *Par*, was *Saccharomyces ellipsoideus* Strain B (Kyoto). A cadmium-resistant strain was obtained by inoculating *Par* in PGY or SG medium which contained 0.4 mM cadmium chloride, followed by repeated sub-culturing in the same medium. Cell population of consistent properties was invariably obtained by this procedure. *Par* and the resistant strain were maintained by alternations of culturing at 30° for 48 hours and storing at 5° for a week, the medium for the resistant strain containing 0.4 mM cadmium. Cells to be used in experiments were harvested directly from 48-hour cultures. For this conditioned culture the normal cadmium-free medium was also used for the resistant strain. The resistant strain thus cultured will be denoted as *Ro*.

Incubation temperature was 30° throughout the present investigation.

Media: The standard media were PGY and SG (cf. Table 1). Micro-elements

Table 1. Composition of media (g/l).

	PGY	SG†	C
Cane sugar	50	50	50
KH ₂ PO ₄	3	3	2
MgCl ₂	—	—	1
MgSO ₄ ·7H ₂ O	1	1	—
K ₂ SO ₄	—	—	1
(NH ₄) ₂ SO ₄	1	2	4
CaCl ₂	0.25	0.25	—
Na-glutamate	1	2	—
Peptone	2	—	—
Yeast extract	2	—	—

† Supplements: Micro-elements (μ g/l): Mn 10, as chloride; Zn 70, Cu 10 and Fe 50, as sulfates; B 10, as borate; Mo 10, as ammonium molybdate. Vitamins (μ g/l): thiamine 200, pyridoxine 200, nicotinamide 200, Ca-pantothenate 200, biotin 2, inositol 10,000.

* Biological Institute, Faculty of Science, Konan University, Kobe, Japan.

and vitamins were omitted from the latter for short period cultures of several hours. Cadmium-containing medium was prepared by sterilizing the nutrient solution and cadmium chloride stock solution (100 mM) separately and mixing them after cooling to room temperature.

Phosphorus starvation of cells: Cells of *Par* and *Ro* were washed twice with one percent sodium chloride solution and once with distilled water, and suspended in C-medium from which phosphate was omitted (cf. Table 1). After incubating for 20 hours, the cells were washed as above for experimental use.

Chemical analyses: Washed cells were divided into three aliquots. One was dried and used for determination of dry weight and total phosphorus content after Allen's method³⁾. The second aliquot was frozen with a mixture of dry ice and ether for 24 hours. The cells thus treated were extracted with 5 per cent trichloroacetic acid (TCA) at 0° for 10 minutes, and washed twice with the same acid solution. The combined extract was used for determination of orthophosphate, acid-soluble organic phosphate and polyphosphate. The residue, after removal of lipid by extracting with a mixture of ethanol and ether (3:1) at 40–45° for an hour, was extracted with 5 per cent TCA at 95° for 5 minutes, and washed twice with the same acid solution. The acid extract and the washings were combined together and used for determination of acid-insoluble polyphosphate.

The third aliquot, after freezing and removal of the lipid (see above), was extracted with water at 0° four times, each for 30 minutes⁴⁾. Polyphosphate thus determined is denoted as water-soluble polyphosphate. The residue was extracted with hot TCA as with the preceding portion, and water-insoluble polyphosphate was determined.

Determination of polyphosphate: The extract by TCA and by water, the latter being acidified with TCA and centrifuged to remove insoluble material, were brought to pH 4.5 with barium acetate solution, and kept overnight in an ice box. The precipitate of barium polyphosphate formed was washed with cold alkaline water, and hydrolyzed with 1 N HCl in boiling water for 30 minutes, to determine the resulting orthophosphate.

It should be noted that only 30 per cent of polyphosphate of the acid-insoluble and the water-insoluble fractions can be digested by the TCA treatment at 95° for 5 minutes⁵⁾.

Determination of orthophosphate and organic phosphate: Orthophosphate was determined by applying the method of Allen³⁾ directly to the water and the acid extracts. Organic phosphate in a fraction was estimated by subtracting the amounts of polyphosphate and orthophosphate from the amount of total acid- or water-soluble phosphorus.

Results

1. *Recovery of resistance and accumulation of polyphosphate in phosphorus-starved cells by replenishment:* When cells of phosphorus-starved *Par* and *Ro* were pour-plated with SG agar media containing 0.4 mM and 0.8 mM cadmium, the viable ratios were about 30 and 40 per cent, respectively. When the starved cells were inoculated into C-medium (cf. Table 1) and pour-plated in the cadmium-containing SG agar media after various periods of pre-incubation, the viability in the presence of cadmium rose with the replenishment, as shown in Fig. 1. The rate of resistance recovery as observed in this way did not significantly differ with the concentration of KH_2PO_4 in C-medium between 0.5 and 4.0 mg/ml.

Fig. 1. Recovery of cadmium resistance of phosphorus-starved cells by replenishing incubation. KH_2PO_4 was supplied at 0 time and cells sampled after indicated time of pre-incubation were pour-plated in 0.8 mM cadmium agar medium for *Ro* (Curve A) and in 0.4 mM cadmium agar medium for *Par* (Curve B).

Fig. 2. Synthesis of polyphosphates in the phosphorus-starved cells by replenishment. KH_2PO_4 was added at 0 time. Experimental conditions were the same as in Fig. 1.

Key to curves: —, *Par*; - - - - , *Ro*; ○, acid-soluble poly-P; △, acid-insoluble poly-P; ▲, total acid-soluble P; ×, inorganic P.

A previous experiment showed that in phosphorus-starved cells, orthophosphate, acid-soluble polyphosphate and acid-insoluble polyphosphate decreased remarkably, but the level of total nitrogen, desoxyribonucleic acid, ribonucleic acid and nucleotides remained unaffected²⁾. Fig. 2 shows that on pre-incubation in the phosphorus-containing medium acid-soluble polyphosphates increase with the recovery of resistance in both *Par* and *Ro*, whereas orthophosphate does not.

2. *Polyphosphate as a binder of cadmium in the cell*: It was previously reported that in *Par* and *Ro* cells incubated in cadmium-containing PGY medium, their cadmium content increased in the early stage of growth to reach a maximum at the accelerated phase of growth. The maximal cadmium content thus reached was higher in *Par* than in *Ro*⁶⁾, the cadmium content being parallel with the total phosphorus content of cells¹⁾. Unpublished data also showed that an appropriate concentration of cadmium in the culture medium accelerated the accumulation of acid-soluble polyphosphate in cells during the early growth phase.

Accordingly, a test was conducted to see whether polyphosphates play a role as a cadmium binder in cells. SG media containing and not containing 0.5 mM cadmium were inoculated with *Par* and *Ro* cells and incubated for 5 hours. And the water-

Table 2. Water-soluble and -insoluble polyphosphates in *Par* and *Ro* cells. SG-media containing and not containing 0.5 mM cadmium chloride were inoculated with two-day-old cells, and incubated for 5 hours. Figures in parentheses represent the amount of each phosphorus fraction in percentage of the total phosphorus of the cells.

	Phosphorus content of cells ($\mu\text{g P/mg dry wt.}$)			
	<i>Par</i>		<i>Ro</i>	
	Without Cd	With Cd	Without Cd	With Cd
Total water-soluble P	16.1 $\pm$ 0.01 (50.0)	14.9 $\pm$ 0.01 (72.1)	16.9 $\pm$ 0.01 (60.2)	15.0 $\pm$ 0.01 (58.4)
Inorganic P	5.09 $\pm$ 0.12 (16.9)	4.39 $\pm$ 0.13 (21.5)	4.09 $\pm$ 0.04 (14.6)	3.75 $\pm$ 0.01 (14.5)
Water-soluble poly-P	9.25 $\pm$ 0.48 (30.8)	9.53 $\pm$ 0.08 (45.8)	12.3 $\pm$ 0.40 (44.4)	10.1 $\pm$ 0.30 (39.1)
Water-insoluble poly-P	0.37 $\pm$ 0.09 (1.2)	0.23 $\pm$ 0.06 (1.1)	0.25 $\pm$ 0.09 (8.8)	0.13 $\pm$ 0.04 (5.1)

soluble and -insoluble polyphosphates in the harvested cells were determined. The results were as shown in Table 2. A greater part of polyphosphate in the cells was water-soluble, especially in *Par* cells. The amount of water-insoluble polyphosphate was smaller in *Ro* than in *Par*, and larger in cells from cadmium-free cultures than in cells cultured in the presence of cadmium. Hence, if polyphosphate is water-insoluble when bound to cadmium, as in model experiment, polyphosphate does not seem to play a role of detoxicating cadmium in the cells.

3. Status of phosphate in parent and resistant strains: The two strains *Par* and *Ro* were compared with respect to the content of various forms of phosphorus. They were cultured in SG medium without cadmium addition and harvested in the stationary phase of growth. The analytical results are represented in Fig. 3. There is a great difference between *Par* and *Ro* in the extractive patterns of cellular polyphosphates with TCA and water.

Fig. 3. Comparison of *Par* and *Ro* at the stationary growth phase for cellular polyphosphates soluble and insoluble in water and cold 5 per cent TCA. Both the strains were cultured in SG medium without cadmium addition for 48 hours.

Discussion

The recovery of cadmium resistance of phosphorus-starved cells by replenishment

appears to be related with the polyphosphate synthesis. The cadmium content of both *Par* and resistant cells during growth in the cadmium-medium is in parallel with the total phosphorus content¹¹.

Unpublished data show that viability of *Par* cells containing cadmium is remarkably raised by treatment of the cells with polyphosphate solution, and that cadmium-polyphosphate complex is water-insoluble. Rothstein⁷ has also suggested that cellular polyphosphate plays an important part as a binder of cations. However, most of the polyphosphate was in a water-extractable form both in the cadmium-containing cells and in the cadmium-free cells, of both strains (Table 2). Hence it appears unlikely that polyphosphate plays a principal role of a fixing agent in the cadmium detoxication in yeast cells.

It is of interest that there is a great difference between *Par* and *Ro* in the extraction patterns of cellular polyphosphates with TCA and water (Fig. 3). Current researches^{4, 5, 8-11} have shown that there is a definite difference in metabolic pattern between TCA-soluble and -insoluble polyphosphates. The difference between *Par* and *Ro* then may be reflecting the differential significance of the two groups of cellular polyphosphates in the cadmium resistance.

Previous publications^{2, 12} have shown that ribonucleic acid and protein metabolism plays an important part in the cadmium resistance of yeast. On the other hand, Mudd *et al.*^{5, 8} and Sall *et al.*⁹, using *Mycobacterium* and *Corynebacterium*, showed that ribonucleic acid-phosphorus is mainly supplied from polyphosphate in cells. Hence, it is suggested that polyphosphate metabolism may play a role in the cadmium resistance, indirectly, through ribonucleic acid and protein metabolism.

Summary

Experiments were made to investigate the roles of cellular polyphosphates in the cadmium resistance of yeast cells.

1. Recovery of cadmium resistance of phosphorus-starved cells by replenishment correlates with accumulation in cells of both acid-soluble and acid-insoluble polyphosphates.

2. Since most part of polyphosphate in cells containing cadmium is in a water-soluble form, and since cadmium-polyphosphate forms a water-insoluble complex, polyphosphate does not seem to be the main cadmium binder in the cells.

3. Contents of polyphosphates insoluble in water and trichloroacetic acid were higher in the resistant cells than in the sensitive cells, even when they were cultured without cadmium.

I wish to express my thanks to Prof. Joji Ashida of Kyoto University for his helpful advice and criticism.

References

- 1) Nakamura, H., Mem. Konan Univ., Sci. Series **5**: 89 (1961).
- 2) ———, *ibid.* **5**: 99 (1961).
- 3) Allen, R. T. L., Biochem. J. **34**: 858 (1940).
- 4) Kuroiwa, Y., J. Ferm. Tech. **34**: 84 (1956).
- 5) Mudd, S., Yoshida, A., and Koike, M., J. Bacteriol. **75**: 224 (1958).
- 6) Nakamura, H., and Ashida, J., Mem. Coll. Sci., Univ. Kyoto, B **26**: 3 (1959).
- 7) Rothstein, A., Membrane phenomena, p 229 (Discussions of the Faraday Society, No. 21, 1956).
- 8) Mudd, S., Takeya, K., Henderson, H. J., J. Bacteriol. **72**: 767 (1956).
- 9) Sall, T., Mudd, S., and Davis, J. C., Arch.

Biochem. Biophys. **60**: 131 (1956). 10) Kornberg, S. R., J. Biol. Chem. **218**: 23 (1956). 11) Yoshida, A., J. Biochem. **42**: 381 (1955). 12) Nakamura, H., Mem. Konan Univ., Sci. Series **6**: 19 (1962).

摘 要

中 村 運: 酵母のカドミウム適応 VII. カドミウム耐性におけるポリリン酸の意義

Saccharomyces ellipsoideus のある株を用い、カドミウム耐性におけるポリリン酸代謝の役割について調べた。細胞をリン飢餓の状態におくと、カドミウム感受性が著しく高まるが、これに無機リン酸を与えると、徐々に、カドミウム耐性が回復する。このとき、酸可溶性および酸不溶性のポリリン酸の細胞内蓄積が平行する。一方、細胞をカドミウム培地に植えると、細胞のカドミウム含量は、誘導期中に、急速に高まり、加速期に最高値に達する¹⁾。この時期の細胞のポリリン酸はほとんど全部水溶性であるから、カドミウムがポリリン酸と結合して無毒化されているとは考えられない。しかし、耐性菌の間には、ポリリン酸の細胞内での存在様式が大きく異なっているから、ポリリン酸代謝がカドミウム耐性になんらかの意義をもっていると想像される。(甲南大学理学部生物学教室)