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On the Phosphorus Fractions and the Uptake of Phosphate by Low-phosphorus *Torulopsis utilis*

NIILO RAUTANEN and PAULA MIIKKULAINEN

Biochemical Laboratory of the University, Biochemical Institute, Helsinki, Finland

Working with *Torulopsis utilis* yeast we have observed that through starvation in respect to phosphorus the total phosphorus content of yeast cells can be lowered to one seventh or more from the normal content. On the other hand, when this low-phosphorus yeast was again placed in a medium containing phosphate, the total phosphorus of the cells rapidly increased so that they could contain two times more phosphorus than the normal yeast and about ten times more than the low-phosphorus yeast. The question arises how these great changes are possible and which cell fractions are affected by them. Further are these changes in chemical composition followed by changes in the physiological properties.

In the present work the object was to investigate especially the various phosphorus fractions of low-phosphorus *Torulopsis utilis* yeast and the uptake of phosphate by it.

EXPERIMENTAL

Cultivation

All cultivation experiments were performed in Kluyver flask at 29 °C with powerful aeration (400 l/hour). The pH was kept within the range 4.5—5.0. The *normal yeast* was cultivated in a solution containing an excess of phosphate, ammonia nitrogen and glucose. The *low-phosphorus yeast* was cultivated by placing the normal yeast in a medium with normal composition but devoid of phosphate.

Analytical methods

The fractionation of yeast substances for analyses was performed with tricloracetic acid as follows: The first extraction was carried out with 10 per cent tricloracetic acid at room temperature (*cold TCA extract*). This fraction contained acid-soluble phosphorus and nitrogen compounds. The remaining cell mass was treated 15 min. with 5 per cent tricloracetic acid at 90 °C (*hot TCA extract*). In this hydrolysis nucleic acids and some

other phosphorus compounds were completely extracted (*cf. Di Carlo* and *Schulz* [1] and *Wiame* [2]). *The insoluble residue* after extraction with hot tricloracetic acid contained stabile phosphorus and nitrogen compounds, mainly protein.

Total nitrogen was determined by the Kjeldahl micro method. *Phosphate* determination was carried out according to the modification of *Berenblum and Chain* [3], in which the colour extraction with isobutyl alcohol was used. *Total phosphorus* was determined as phosphate after wet combustion with sulfuric acid and hydrogen peroxide. *Nucleic acid* content was determined from the hot tricloracetic acid extract with the Beckman spectrophotometer at 260 mμ. The amount of nucleic acid equivalent to the density readings was read from a standard curve constructed with ribonucleic acid preparation (Ribonucleic acid from yeast B.D.H.). As a relative measure of the amount of soluble nucleotides in the cold tricloracetic acid extract the transmission density at 260 mμ even of this fraction was determined.

RESULTS AND DISCUSSION

The growth of the normal yeast when placed in a medium devoid of phosphate was at the beginning very intensive. Only after some hours the rate of growth gradually diminished without a clear endpoint as the cells were exhausted in respect to phosphorus on account of their multiplication.

The analyses of yeast samples (Table 1) taken during the starvation process showed that dry matter content had a slight tendency to increase. In this experiment the total P of the cells gradually diminished to one seventh of the original normal value. The total N content also decreased to some extent.

Table 1. Dry matter, phosphorus and nitrogen content of the yeast cells during cultivation in the absence of phosphate.

	Cultivation hours			
	0	3	6	9
Dry matter % of fresh matter	17.8	18.0	19.4	21.0
Total P % of dry matter	2.25	1.12	0.52	0.33
» N » » » »	8.78	8.30	8.26	7.80

Table 2 shows the results of the more minute analyses of the different fractions of normal yeast and low-P yeast (starved 9 hours in respect to phosphorus). For the sake of comparison we have also presented corresponding values of low-N yeast (starved 7 hours in respect to nitrogen). The dry matter contents of both the low-P and low-N yeasts were clearly greater than that of the normal yeast. This is mainly due, as can be seen from the N content of the insoluble residue fraction, to the disappearance of the water binding proteins from the cells during the starvation processes (*cf.* even Roine [4]). Accordingly

Table 2. Different fractions of normal yeast, low-phosphorus yeast and low-nitrogen yeast.

	Normal yeast	low-P yeast	low-N yeast
Dry matter % of fresh matter	17.75	20.0	21.5
Total P % of dry matter	2.09	0.53	1.77
» N » » » »	8.95	7.93	4.78
Cold TCA extract			
P % of dry matter	0.80	0.11	0.80
PO_4—P » » » »	0.42	0.05	0.41
N » » » »	1.09	2.10	0.37
Transmission density at 260 mμ	0.118	0.055	0.109
Hot TCA extract			
P % of dry matter	1.12	0.29	0.82
N » » » »	1.46	0.63	0.65
Nucleic acids % of dry matter	7.46	1.88	2.02
Insoluble residue			
P % of dry matter	0.17	0.13	0.15
N » » » »	6.40	5.20	3.76

even the total N content of low-P yeast is lower than that of normal yeast. On the other hand the starvation in respect to nitrogen had a similar effect on the P content of low-N yeast. This proves that certain parts of nitrogen and phosphorus of the cells are so closely connected with each other that the changes in one of these elements cannot fail to influence the other. In both cases this parallel decrease of P and N was due to the disappearance of compounds which contain both of these elements viz. nucleic acids and hence proteins. Accordingly this reciprocal influence of P and N was greatest in the compounds of the hot TCA extract and in those of the insoluble residue. The amount of nucleic acids in low-P and low-N yeasts showed an equal and very considerable decrease, which in low-P yeast is quite parallel with the decrease of P content of the hot TCA extract. In low-N yeast the P content of the hot TCA extract has decreased noticeably less than the nucleic acid content. On the other hand the N content of this fraction of both low-P and low-N yeasts has decreased equally. The low nucleic acid content of low-N *Torulopsis* yeast has already been shown by Virtanen and Miettinen [5]. In the acid-soluble fraction (cold TCA extract) the P content of low-P yeast showed the greatest decrease, which is parallel with the decrease of the free PO_4-P of this fraction. This is very interesting because the P content of this fraction in low-N yeast has not changed from the normal value. Accordingly the N content of this soluble fraction seems to

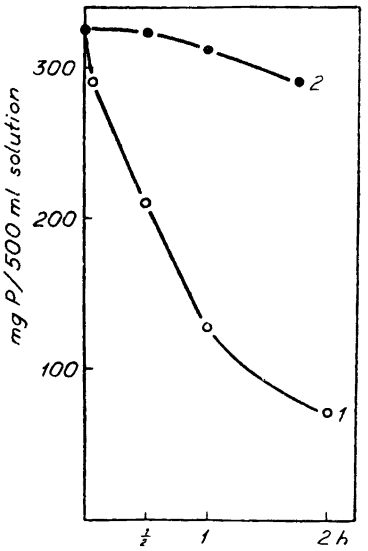

Fig. 1. Decrease of phosphate in culture solution during the uptake of phosphate by low-phosphorus and normal yeasts.

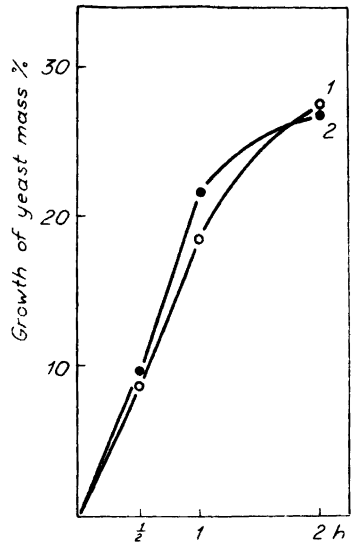

Fig. 2. Growth of low-phosphorus and normal yeasts during the uptake of phosphate.

Curve 1: Low-P yeast
Curve 2: Normal yeast

be quite independent of the corresponding P content and vice versa. The relative amount of soluble free nucleotides (transmission density at 260 mμ) decreased in low-P yeast to one half of the original, whereas in low-N yeast no remarkable drop of these substances occurred.

The first task when beginning to study the resynthesis of the compounds which had disappeared from the yeast cells during the phosphorus starvation was to follow the uptake of phosphate and the growth of the P-starved cells in a solution containing phosphate, ammonium, and glucose in ample amounts. For the sake of comparison the corresponding processes were followed even in normal yeast. As it can be seen from the curves in Fig. 1 and 2 the very vigorous uptake of phosphorus by low-P yeast begins immediately, and the amount of phosphorus taken up by it during the first few minutes is as great as taken up by the normal yeast in two hours. This enormous consumption lasts, however, only approximately two hours, whereafter it continues at the normal rate. On the other hand the growth curves of low-P and normal yeasts presented in Fig. 2 are practically identical. This proves that only a very small quantity of the consumed phosphorus in low-P yeast is necessary for the growth.

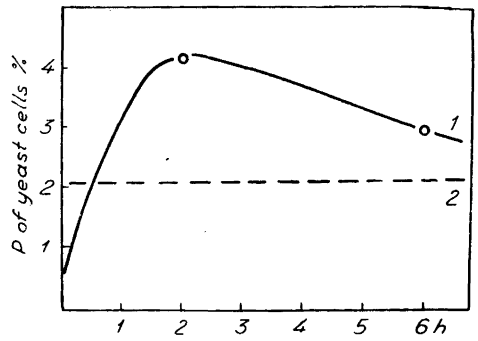

Fig. 3. Total phosphorus content of low-phosphorus yeast during the uptake of phosphate.

Curve 1: Low-P yeast
Curve 2: Level of normal yeast

The fate of the phosphorus taken up by the yeast cells was examined in another experiment of longer duration. As can be seen from Fig. 3 the total P content of the yeast cells increases very rapidly and reaches its temporary very high maximum in 2 hours, after which it begins to fall. The other facts of the analyses of this experiment are given in Table 3. The original values of normal yeast are even presented.

Table 3. Different fractions of low-phosphorus yeast during cultivation in the presence of phosphate, compared with the corresponding fractions of normal yeast.

	Low-P yeast Cultivation hours			Normal yeast
	0	2	6	
Dry matter % of fresh matter	20.0	19.25	19.20	17.75
Total P % of dry matter	0.53	4.19	2.94	2.09
» N » » » »	7.93	7.77	8.62	8.95
Cold TCA extract				
P % of dry matter	0.11	2.06	1.15	0.80
PO_4—P » » » »	0.05	0.65	0.52	0.42
N » » » »	2.10	1.76	1.48	1.09
Transmission density at 260 mμ	0.055	0.118	0.143	0.118
Hot TCA extract				
P % of dry matter	0.29	1.99	1.56	1.12
N » » » »	0.63	1.16	1.42	1.46
Nucleic acids % of dry matter	1.88	5.37	7.19	7.46
Insoluble residue				
P % of dry matter	0.13	0.14	0.23	0.17
N » » » »	5.20	4.80	5.72	6.40

Table 4. Different fractions of normal yeast during cultivation in the presence of great excess of phosphate.

	Cultivation hours		
	0	2	6
Dry matter % of fresh matter	17.8	17.9	19.4
Total P % of dry matter	2.09	2.32	2.14
» N » » » »	8.95	8.65	8.70
Cold TCA extract			
P % of dry matter	0.80	0.83	0.82
PO_4—P » » » »	0.42	0.47	0.41
N » » » »	1.09	1.32	1.28
Transmission density at 260 mμ	0.118	0.123	0.175
Hot TCA extract			
P % of dry matter	1.12	1.08	1.19
N » » » »	1.46	1.43	1.36
Nucleic acids % of dry matter	7.46	7.25	6.83
Insoluble residue			
P % of dry matter	0.17	0.41	0.13
N » » » »	6.40	5.90	6.06

Dry matter content of the starved yeast rises all the time towards the level of the normal yeast. During the very intensive accumulation of phosphorus (at 2 hours) the increase of total P is 8-fold, the increase of acid-soluble P (cold TCA extract) approximately 20-fold which is much more greater than the increase of free PO_4-P of this fraction. The increase of P of the fraction containing *inter alia* nucleic acids (hot TCA extract) is 6-fold. The simultaneous increase of nucleic acids is only 3-fold. Accordingly the accumulation of inorganic orthophosphate and the resynthesis of the nucleic acids shows no parallellism with this accumulation of phosphorus *per se*.

According to the very exhaustive work of Wiame [2] concerning the phosphorus uptake by low-P baker's yeast, the accumulation of phosphorus is mainly due to the formation of soluble and insoluble inorganic metaphosphates in the yeast cells. The results of our work are in this respect in good agreement with the facts reported by Wiame, and we can conclude that even in *Torulopsis* occurs an abundant accumulation of soluble and insoluble metaphosphates. In this work, however, no particular attention has been paid to their more minute chemical determination and characterization. The further synthesis of nucleic acids takes place after the accumulation period at the

expense of the metaphosphates. As a consequence of this nucleic acid synthesis even the N content of the hot TCA extract rises continually.

The amount of the soluble nucleotides (transmission density at 260 mμ of the cold TCA extract) have reached their normal level in 2 hours and have later even exceeded this average. The total N content shows hardly any increase during the first hours. This is mainly due to the fact that depending on experimental conditions the powerful resynthesis of the proteins starts not until 2—3 hours atferwards.

The most striking property of the P-starved yeast is its ability to accumulate phosphorus from the phosphate containing medium in a much greater degree than is needed for the synthesis of the organic phosphorus compounds in the cells. The question arises whether this ability is characteristic only of low-P yeast and could not even the normal yeast be able to increase its phosphorus content when placed in a medium of very heavy phosphate concentration. For this reason normal yeast was suspended in a culture solution containing three times as much phosphate as was used in its cultivation. Samples for the analyses of the yeast cells were taken at intervals of 2 hours. The results given in Table 4 show that no increase of the percentage content of total P or of its fractions occurred. The fact that no free PO_4-P was accumulated in the cells deserves special attention. Neither were the analyzed N fractions changed. Only the amount of the soluble nucleotides (transmission density at 260 mμ of the cold TCA extract) have a clear tendency to increase over the normal value. Accordingly the normal yeast cannot take an excess of phosphorus, and its P content is in wide limits independent of the in excess available phosphate in its environment.

SUMMARY

It has been shown that the starvation of *Torulopsis utilis* yeast in respect to phosphorus is followed by considerable decrease in various phosphorus fractions of the cells. Even the nitrogen content of such cells has clearly lowered owing to the disappearance of nitrogen compounds containing also phosphorus, *inter alia* nucleic acids.

The very considerable and rapid increase of the phosphorus content of phosphorus-starved yeast when placed in a medium containing phosphate is caused by the accumulation of phosphorus in the cells in much higher degree than needed for the growth. It is surprising that in consequence of this accumulation the total phosphorus content of the yeast cells exceeds even twice that of the normal yeast, which has been cultivated in the same phosphate

concentration. The resynthesis of the disappeared nucleic acids proceeds at a relatively slow rate at the expense of this accumulated phosphorus.

When cultivating yeast of normal phosphorus content in the presence of a high excess of phosphate no increase of the phosphorus content of the cells occurred.

REFERENCES

1. Di Carlo, F. J., and Schultz, A. S. *Arch. Biochem.* **17** (1948) 296.
2. Wiame, J. M. *J. Biol. Chem.* **178** (1949) 919.
3. Berenblum, I., and Chain, E. *Biochem. J.* **32** (1938) 295.
4. Roine, P. *Ann. Acad. Sci. Fennicae* Ser. A. II. Chem. No. 26 (1947) 1.
5. Virtanen, A. I., and Miettinen, J. K. *Acta Chem. Scand.* **3** (1949) 1437.

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