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# On the Nature and Activation of the Phosphatase of *Torulopsis utilis*

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In a previous communication [1] the increase in the phosphatase activity of *Torulopsis utilis* cells during starvation in respect to phosphorus was described. This interesting phenomenon led us to a more detailed study of the nature of the *Torulopsis* phosphatase, as well as of the factors affecting its activation and inhibition.

## EXPERIMENTAL

The cultivation of the yeast mass was carried out in Kluyver flasks at 29° C with aeration (200 l/hour). The pH was kept within the range 4.5—5.0 with sodium hydroxide. The *normal yeast* (yeast with normal phosphorus content) was cultivated in a solution containing 50 g glucose, 3 g ammonium hydrogen phosphate, 4 g ammonium sulfate, 1.5 g potassium sulfate, 1 g magnesium sulfate and 0.5 g calcium chloride per litre of tap water (*cf.* Roine [2]). The *low-phosphorus yeast* (phosphorus-starved yeast) was cultivated by placing washed normal yeast in a medium of the above composition but devoid of phosphate salt. Cultivation was continued until growth ceased owing to the exhaustion of the cells in respect to phosphorus.

In the experiments *lyophilized cells* and *cell-free extracts* of the cells were used. The extracts were obtained by disintegrating a 30 % suspension of fresh cells in distilled water 30 minutes in a Raytheon sonic vibrator at 9 kilocycles. After 10 minutes' centrifugation at 16 000 rpm in a refrigerated centrifuge, the clear supernatant was decanted.

*Phosphatase activity* was measured in veronal buffer, with disodium phenylphosphate as the substrate. The liberated phenol was determined with the Folin-Ciocalteau phenol reagent. When the effect of activators or inhibitors was to be studied, the enzyme preparation was incubated with these substances for 30 min. at 37° C before the substrate was added.

All results are given in Rae-Eastcott units: the amount of phenol in mg liberated per 10 mg of the dry preparation from a 0.01 *M* solution.

## RESULTS AND DISCUSSION

The phosphomonoesterases are classified according to the dependence of their activity on the hydrogen ion concentration as well as on certain divalent metals, of which magnesium is the most important (*cf.* Roche [3]). In a prelimi-

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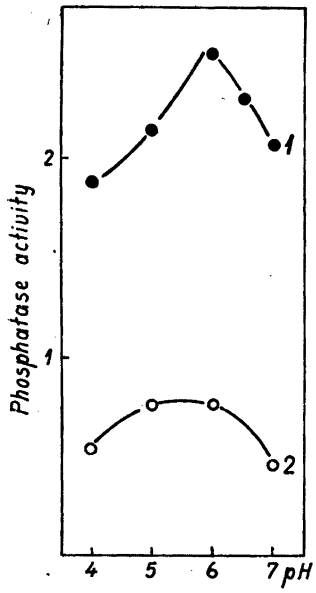


*Fig. 1. Dependence of the phosphatase activity of low-phosphorus cells on hydrogen ion concentration in the presence and in the absence of magnesium.*

*Curve 1: With $10^{-2}$ M $MgCl_2$*
*Curve 2: Without magnesium.*

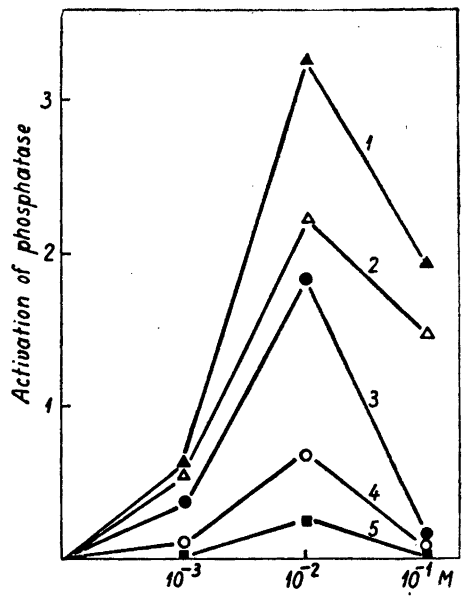


*Fig. 2. Activation of the phosphatase of normal and low-phosphorus cells by manganese, magnesium and calcium at different concentrations.*

*Curve 1: Low-P cells with $MnSO_4$*
*Curve 2: Normal cells with $MnSO_4$*
*Curve 3: Low-P cells with $MgCl_2$*
*Curve 4: Normal cells with $MgCl_2$*
*Curve 5: Low-P cells with $CaCl_2$*

nary test, it was found that magnesium has a stimulatory effect on the *Torulopsis* phosphatase. Owing to the minimal activity of the normal cells, the dependence of the phosphatase activity on the hydrogen ion concentration was studied with the low-phosphorus cells in the presence ($10^{-2}$ *M*) and in the absence of magnesium (Fig. 1).

The pH optimum lies between 5 and 6, probably at about 6.

In addition to magnesium, some other divalent metals appeared to activate this phosphatase. The effects of these on the normal and low-phosphorus cells at different concentrations are illustrated in Fig. 2.

Manganese is the most potent activator. The activation of the almost inactive normal cells by manganese is considerably greater than the activation of the active low-P cells by magnesium. Calcium has a slight but distinct effect on the low-P cells, but no effect on the normal cells. All these cations cause the maximal activation at approximately the same concentration ($10^{-2}$ *M* or a little higher). A negligible effect of zinc can be observed at a lower concentration (0.06 units at $10^{-4}$ *M* $ZnSO_4$) and a similar effect of cobalt at a higher concentration (0.05 units at $10^{-1}$ *M* $CoCl_2$).

*Table 1. Dependence of the phosphatase activity of lyophilized cells and cell-free extracts on their phosphorus contents in the presence and in the absence of magnesium.*

| | Total P % of dry matter | Phosphatase activity No addition | $10^{-2}$ *M* $MgCl_2$ | Activation by Mg |
|---|---|---|---|---|
| *Lyophilized cells* | | | | |
| Normal | 1.97 | 0.09 | 0.81 | 0.72 |
| Low-P | 0.29 | 0.86 | 2.46 | 1.60 |
| *Cell-free extracts* | | | | |
| Normal | 4.03 | 0.05 | 0.78 | 0.73 |
| Low-P | 0.73 | 1.48 | 5.31 | 4.53 |

According to Folley and Kay [4], four main types of isodynamic phosphomonoesterases may be distinguished on the basis of pH activity relationships and specific activation with the divalent metals. In regard to its pH optimum, between 5 and 6, and its activation by magnesium and manganese, the phosphatase of the yeast *Torulopsis* resembles the phosphatase found, for example, in bottom yeast and red blood cells, and belongs to the fourth group.

It was very interesting to compare the properties and the activation of the whole lyophilized cells and of the cell-free extracts of the same origin. Table 1 shows the dependence of the phosphatase activity on the phosphorus content of the preparations and the activation by magnesium at optimal concentration ($10^{-2}$ *M*).

The enormous difference in the phosphorus contents of the whole cells of normal and low-phosphorus yeasts can also be seen in the phosphorus contents of the cell-free extracts of these cells. The phosphatase activity of both the lyophilized cells and the cell-free extract of the normal yeast is very low. The activity of the low-phosphorus cells is about ten-fold compared to that of the normal cells. However, the activity of the cell-free extract from the low-phosphorus yeast is about thirty-fold compared to the extract from the normal yeast. Accordingly, the destruction of the cell structure when preparing the extract with a sonic vibrator is followed by a coactivation of the enzyme of the low-phosphorus cells, but has no effect on the enzyme of the normal cells.

The activation by magnesium is another very interesting point of this experiment. In normal yeast the activation both of the lyophilized cells and of the cell-free extract is the same. The activation of the low-phosphorus preparations is much more marked than the activation of the preparations with normal phosphorus content, the activation of the low-P cells being twice that of the normal cells and the activation of the cell-free extract of the low-P cells about six times that of the extract of the normal cells. Accordingly in low-P preparations the activation of the cell-free extract is much greater than that of the whole cells. It is interesting to note that the activity of the cell-free extract from low-P cells activated by magnesium is more than 100-fold compared with the activity of the non-activated extract from normal cells.

In addition to the activators, the effect of some commonly known inhibitors of phosphatases have been investigated. Owing to the very low activity of the normal yeast, the inhibition can be studied only with low-P preparations. The results of the experiments with lyophilized cells and cell-free extract are given in Fig. 3 and Table 2.

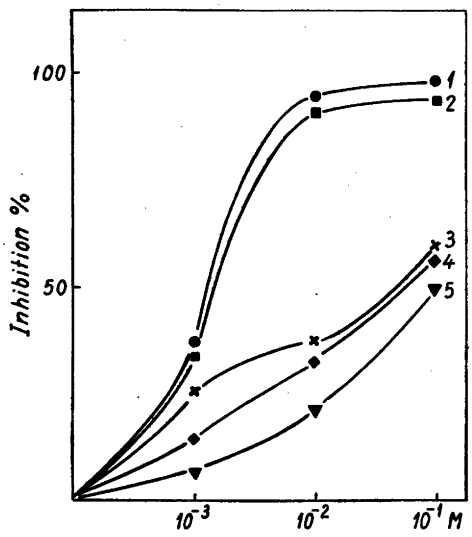


*Fig 3. Effect of inhibitors at different concentrations on the phosphatase activity of low phosphorus cells.*

*Curve 1: Pyrophosphate*
*Curve 2: Molybdate*
*Curve 3: Fluoride*
*Curve 4: Orthophosphate*
*Curve 5: Oxalate*

The very strong inhibition by pyrophosphate and molybdate is characteristic of this enzyme. Even fluoride, orthophosphate and, to a lesser extent, oxalate cause an inhibition. The phosphorus compounds, which are normal constituents of the cells, reduce the activity of the enzyme in varying degrees (Table 2). Although the values in this table are not in all respects comparable with each other and are not of quantitative importance, they give a qualitative picture of the inhibition.

The phosphorus content of the cell-free extracts can be lowered by removing the freely dialyzable phosphorus compounds by a short period of dialysis. Table 3 shows the effect of 24 hours' dialysis at 4° C against distilled water on the phosphorus and nitrogen contents as well as on the phosphatase activity of the cell-free extracts from normal and low-P cells.

The total phosphorus content of both preparations has decreased very considerably, but the ratio of the P content between normal and low-P preparations has not changed. The total nitrogen content has also decreased, but to a lesser degree. The decrease of the phosphorus contents has, however, no

*Table 2. Inhibitory effect of some phosphorus compounds on the phosphatase activity of the cell-free extract of low-phosphorus cells.*

| | | *Phosphatase activity* |
|---|---|---|
| No addition | | 1.65 |
| Orthophosphate | $10^{-3}$ *M* | 1.65 |
| » | $10^{-2}$ *M* | 1.29 |
| Pyrophosphate | $10^{-2}$ *M* | 0.48 |
| Polymetaphosphate | 0.5 % | 0.51 |
| Ribonucleic acid | » | 1.45 |
| Desoxyribonucleic acid | » | 1.31 |

*Table 3. Effect of dialysis on the phosphorus and nitrogen contents and on the phosphatase activity cell-free extracts of normal and low-phosphorus cells.*

| Cell-free extract | Total phosphorus % of dry matter | | Total nitrogen % of dry matter | | Phosphatase activity | |
|---|---|---|---|---|---|---|
| | before dialysis | after dialysis | before dialysis | after dialysis | before dialysis | after dialysis |
| of normal cells | 4.03 | 1.33 | 2.43 | 1.69 | 0.15 | 0.05 |
| » low-P » | 0.75 | 0.19 | 2.23 | 1.65 | 1.65 | 1.48 |

stimulatory effect on the phosphatase activity. On the contrary, the enzyme activity has in both cases clearly decreased. This decrease can be explained by the fact that the metal present in the enzyme or some other dissociable and dialyzable activators of the enzyme are partly removed, or that the protein is slightly inactivated.

Owing to the limitation of our knowledge concerning the nature of the influence of the factors affecting the phosphatase activity in general, it is very difficult to decide the real cause of the activation of this phosphatase during starvation of the cells in respect to phosphorus. The first supposition would be that the inorganic and organic phosphorus constituents of the cells are the natural inhibitors of phosphatase. The low activity of phosphatase in normal cells would partly be due to the high content of these compounds in the cells and to the formation of combinations of these anions with the metal naturally present in the enzyme. The activation of the enzyme during the starvation in respect to phosphorus would at least to some extent be caused by the considerable disappearance of the phosphorus constituents of the cells. The decrease in different phosphorus fractions of the cells during the starvation has been shown by Rautanen and Miikkulainen [5]. Accordingly the starvation is a very radical process and it would be possible to suppose that it is followed by an anomalous liberation of some active groups of the enzyme protein. Although the phosphorus-starved yeast is an artefact, the characteristics of the phosphatase of this yeast are in all respects comparable with the preparations of normal phosphorus content. Accordingly no change in the fundamental nature of the enzyme seems to have occurred during starvation.

## SUMMARY

The properties of the phosphomonoesterase of *Torulopsis utilis* have been investigated with lyophilized cells and cell-free extracts obtained by sonic disintegration of the fresh cells. The pH optimum lies between 5 and 6, probably at about 6. The enzyme is activated especially by manganese and magnesium. It is also activated by starvation of the cells in respect to phosphorus, and a cell-free extract of very high activity has been obtained from the phosphorus-starved cells. The effect of inhibitors has been studied with the active phosphorus-starved yeast preparations. The enzyme is strongly inhibited by pyrophosphate and molybdate and, to a lesser extent, by other inorganic and organic phosphorus compounds.

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