Acid Phosphatase of Bakers' Yeast: An Enzyme of the External Cell Surface*

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Living bakers' yeast has phosphatase activity with an optimum near *p*H 3.6. The acid phosphatase of living yeast cells practically equals that of yeast homogenates obtained under optimal conditions of cell disintegration. No acid phosphatase is excreted into the medium by living yeast. Hydrolysis of unlabeled β-glycerophosphate by living P^{32}-labeled yeast does not result in appreciable decreases of the radioactivity of the cells. The total acid phosphatase activity of yeast homogenates is found in the supernatant obtained by sedimentation at 96,000 × *g*. These observations suggest that the acid phosphatase of bakers' yeast occurs in a cell compartment near the cell surface, in analogy to the localization of the alkaline phosphatase of P-starved *E. coli* cells (Malamy and Horecker, 1961).

The studies of Schmidt *et al.* (1946, 1949) and of Wiame (1946) on bakers' yeast and those of Rautanen and Mikkulainen (1951) on *Torulopsis utilis* showed that cultivation of these yeasts in phosphate-deficient media strongly increased their capability to accumulate intracellular inorganic polyphosphate after their transfer to phosphate-containing nutrient solutions. Further studies concerning the influence of phosphate deprivation on the regulatory mechanisms of phosphate metabolism revealed striking increases of the activities of certain phosphatases in some phosphate-starved microorganisms. Rautanen and Kaerkkaeinen (1951) discovered strong increases of the activity of an acid phosphatase during incubation of *Torulopsis utilis* in a phosphate-deficient medium. Schmidt et al. (1956) and Liss (1958) found an analogous behavior for a different acid phosphatase in phosphate-starved bakers' yeast. Torriani (1960) reported stimulation of the activity of the alkaline phosphatase of *E. coli* during growth in a medium of low orthophosphate content. Garen and Levinthal (1960) demonstrated that *de novo* synthesis of alkaline phosphatase is the mechanism of its enhanced activity in *E. coli* strains grown under such conditions. Information concerning the intracellular localization of the alkaline phosphatase of *E. coli* was obtained by Malamy and Horecker (1961), who found that all enzymatic activity was released into the medium when the cells were converted to spheroblasts by lysozyme. The authors concluded that the phosphatase was located between cell membrane and cell wall, and that the exclusive occurrence in this space was maintained by compartmentalization of the cells.

Studies concerning the intracellular distribution of the constituents of yeast cells were greatly facilitated when Lammanna and Mallette (1954) developed an efficient technique for their disruption. This technique was applied in the present investigation of the distribution of the acid phosphatase of bakers' yeast. It will be shown that the similarities between the effects of phosphate starvation on three different species of microorganisms are not limited to the enhancement of the activities of certain phosphatases, but that close similarities are also found in the intracellular localization of these enzymes.

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EXPERIMENTAL PROCEDURE

Substrates.—Commercial sodium diphenyl phosphate, which was often found to be contaminated with orthophosphate, was purified by converting it to the crystalline barium salt and by recrystallizing the latter twice by slow addition of ethanol to its aqueous solution. Disodium β-glycerophosphate was purchased and used without further purification.

Yeast.—All experiments were carried out with Fleischmann's Yeast which was procured from the local wholesale dealer on the day of its arrival from the fermentation plant. Commercial samples of bakers' yeast contain only very small amounts of acid phosphatase, presumably because of the high orthophosphate concentrations in the media used for industrial growth of yeast. Enrichment of the phosphatase concentration was achieved by incubating the yeast in the laboratory either in a complete growth medium of low orthophosphate content (medium a) or in a salt-dextrose mixture devoid of any source of phosphorus (medium b).

Medium a ("Low-Phosphate" Medium).—The yeast samples were strongly aerated in solutions containing 1% Difco Neopeptone and 6% dextrose at temperatures ranging between 28° and 31.5°. For some experiments, the yeast was grown from small inocula (100 mg of yeast per liter of medium) for 2 or 3 days. After 36 hours, 5 g of Neopeptone and 60 g of glucose were again added per liter of yeast suspension. For large-scale preparations of acid phosphatase, 1% suspensions of yeast were aerated during periods ranging between 8 and 16 hours. The Neopeptone-dextrose medium contained 20 mg of phosphorus per liter and was free of orthophosphate.

Medium b (Phosphate-Free Medium).—Phosphate-starved yeast was prepared by aerating 10-g portions of commercial bakers' yeast after two washings with water in 1 liter of a solution containing 6% dextrose, 0.38% ammonium sulfate, 0.04% potassium chloride, 0.012% magnesium chloride, and 0.02 M potassium citrate buffer of *p*H 5.2 for various time intervals.

Preparation of P^{32}-Labeled Yeast.—P^{32}-labeled yeast cells were obtained by incubating yeast either in medium a or in medium b after addition of 1 mc or 0.1 mc of P^{32}-labeled acid-hydrolyzed phosphate (corresponding to approximately 0.02 or to 0.002 mg P respectively) per liter of each culture.

Processing of Yeast Cells.—The cells were harvested by sedimentation at 2,000 rpm in plastic, 1 liter centrifuge tubes in a Model 2 Centrifuge, International Equipment Company, Boston, at 5°, washed twice with water, and suspended in water or buffer according to the requirements of the various experiments. The

final volume of each suspension was adjusted to 100 ml per liter of yeast culture. For *homogenization*, 40 ml of the suspension was disintegrated in an ice-cooled VirTis Homogenizer Model 45 in the presence of 80 g of *acid-washed* homogenizing glass beads (0.2 mm diameter). After 10 minutes, the motor was stopped for 10 minutes to keep temperature increases within tolerable limits. Six homogenizing periods were sufficient for practically complete disintegration of the cells. The acid treatment of the glass beads was essential for the homogenization of unbuffered cell suspensions because of the considerable amounts of alkali released from the untreated glass beads. After storage of the acid-washed glass beads for a few days, repetition of the acid treatment was necessary. On the other hand, breakage of the acid-washed beads during homogenization did not result in significant increases of the *p*H.

Determination of Phosphorus.—Total, easily hydrolyzable, and inorganic phosphorus were determined according to the method of Fiske and Subba-Row (1925).

Assay of Acid Phosphatase.—Usually, the assays were carried out in 9 ml of incubation mixture containing 0.015 M sodium phenyl phosphate of *p*H 4.0 and 0.05 M sodium acetate buffer of *p*H 4.0. Slight deviations from these conditions in some experiments will be indicated in the Tables. The assay mixtures containing 1 ml of the respective enzyme preparations were mechanically shaken in a water bath of 37.0° for 10 minutes. The reaction was stopped by the addition of 5 ml of 5% trichloroacetic acid. The orthophosphate was determined in the supernatants of the centrifuged suspensions. As shown in Figure 1, the hydrolysis of phenyl phosphate as well as that of β-glycerophosphate closely approached first-order kinetics in cell-free extracts. For the cleavage of phenyl phosphate, this was also the case in enzyme assays with intact cells, whereas the apparent rates of the formation of orthophosphate from glycerophosphate deviated appreciably from first-order kinetics under these conditions, presumably because of utilization of the liberated glycerol and participation of its metabolites in phosphorylation reactions. For this reason, phenyl phosphate was preferentially used as substrate for assay purposes.

Proportionality between concentration of enzyme and rate of hydrolysis and approximation of the latter to maximal velocity was established for the assay conditions in experiments not reported in detail in this paper. For comparative purposes, the figures of phosphatase activities were expressed as activities per ml of yeast culture. In the experiments with large inocula, these values are the activities resulting from the incubation of 10 mg of moist yeast regardless of any weight increase which might have occurred during the incubation.

Determination of Intracellular Phosphorus Fractions of Yeast.—The cells obtained by centrifugation of 10-ml aliquots of the yeast suspensions at various stages of incubation were washed twice with ice-cold water and suspended in water to a total volume of 5 ml. The suspensions of the washed cells were heated for 5 minutes in a boiling water bath. A small portion of the suspension was used for the determination of the total phosphorus. The remaining portion of the suspension was centrifuged. Aliquots of the supernatant were used for determination of inorganic and easily hydrolyzable phosphorus. (Extraction of yeast with hot water was found to result in satisfactory yields of the acid-soluble phosphorus compounds, whereas extraction with acids at low temperature was frequently incomplete.)

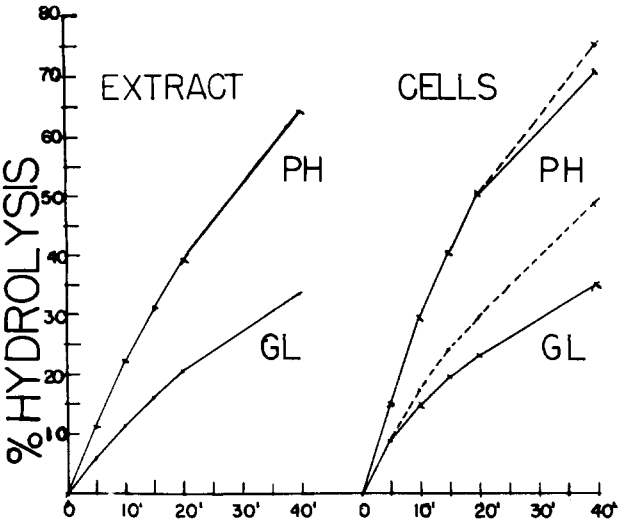

FIG. 1.—Rates of enzymatic hydrolysis of phenyl phosphate (PH) and β-glycerophosphate (GL) at *p*H 4.0 by extracts and intact cells of P-starved bakers' yeast. Unbroken lines: Rates measured experimentally. Broken lines: Rates calculated for first order kinetics from the measured initial rates. Extract: 96,000 × *g* supernatant of yeast homogenate. Cells harvested after a 16-hour period of P-starvation.

RESULTS

1. Influence of pH on the Phosphatase Activity of Living Bakers' Yeast and of Cell-Free Yeast Extracts.—The influence of *p*H on the rates of orthophosphate formation from phenyl phosphate is shown in Table I (third column) for intact yeast cells before and after phosphate starvation and for a cell-free extract of phosphate-starved yeast. Although the increments of orthophosphate during the incubations of the cell-free extract may be considered as a direct measure of phosphatase activity, the interpretation of the figures obtained with the intact yeast cells is more complex. Absorption of orthophosphate formed and its intracellular conversion to polyphosphate and other derivatives, and absorption of substrate and its metabolic utilization, are processes occurring simultaneously with the phosphatic cleavage of the substrate. The *p*H-profiles of these processes might differ from that of the phosphatase involved. Consideration of these possibilities is particularly important in experiments with intact yeast cells because the usual aqueous deproteinizing reagents such as trichloroacetic acid are ineffective for the extraction of intracellular orthophosphate from this organism. Data permitting sufficiently accurate estimates of the extents of orthophosphate absorption, intracellular polyphosphate formation, and substrate absorption are reported in the fourth, fifth, and sixth columns of Table I. Under all *p*H conditions, the absorption of phosphate compounds by the yeast cells was too small to influence significantly the *p*H-profiles of orthophosphate formation (third column). In the neighborhood of the *p*H-optimum, the amounts of the absorbed phosphate compounds were negligible in comparison to those of the liberated orthophosphate, which represent, therefore, an accurate measure of the phosphatase activities of intact yeast cells.

The *p*H-profiles of orthophosphate formation by the two samples of intact yeast cells (third column) are thus predominantly *p*H-profiles of phosphatase activity and may be correlated to the profile obtained for the cleavage of phenyl phosphate by the cell-free extract. The resemblance between the three *p*H-profiles, in

TABLE I

INFLUENCE OF *p*H ON THE PHOSPHATASE ACTIVITIES OF INTACT BAKERS' YEAST AND OF CELL-FREE YEAST EXTRACTS[a]

Material	*p*H	Increase of P_i		Increase of (P_i + 10-min. P) in Cells	Total P in Medium
		In Medium	In Cells		
		(mg P_i per 10 mg yeast[b])			(mg P in 9 ml[b])
Fresh yeast	4.0	0.032	0.0017	—	—
	5.6	0.010	0.0023	—	—
	6.2	0.004	0.0018	—	—
	7.0	0.002	0.0007	—	—
P-starved yeast (16 hr.)	4.0	0.70	—	0.0043	10.4
	5.6	0.45	—	0.0055	10.2
	6.8	0.20	—	0.0066	10.1
Supernatant of homogenate of P-starved yeast	2.2[c]	0.006[c]	—	—	10.4
	3.6	0.390	—	—	10.4
	4.0	0.375	—	—	10.4
	5.6	0.200	—	—	10.4
	6.8	0.150	—	—	10.4

[a] *Assay:* 1 ml yeast suspension or yeast extract of suitable dilution, 5 ml buffer mixture (0.1 M HCl-KCl mixture, 0.1 M sodium acetate buffer or 0.05 M sodium barbital-HCl buffer respectively) and 3 ml 0.112 M solution of sodium phenyl phosphate were incubated for 10 minutes at 36.5°. *p*H measured in substrate-buffer mixtures at 36.5°. The experiment with "fresh" and "P-starved" intact yeast was carried out with the same batch of yeast; the "Supernatant" was prepared from a different batch. [b] All P-values except those of the last column were calculated for aliquots corresponding to 10 mg yeast prior to P-starvation (see Experimental Procedure). [c] Irreversible enzyme inactivation was largely responsible for the low activity at *p*H 2.2.

TABLE II

YIELDS OF ACID PHOSPHATASE OF BAKERS' YEAST OBTAINED BY EXTRACTION AND FRACTIONAL SEDIMENTATION AT DIFFERENT *p*H VALUES

Extraction Liquid		Homogenate *p*H	Phosphatase Activity (mg P_i formed during 10 min. by 10 mg yeast[a])				Condition of Yeast
				Homogenate	Supernatant		
Composition	*p*H		Cells		800 × *g*	96,000 × *g*	
0.1 M Acetate buffer[b]	4.0	4.8	0.56	0.56	0.54	0.52	P-starved (16 hr.)
0.1 M Acetate buffer[b]	5.6	5.9	0.54	0.49	0.47	0.46	P-starved (16 hr.)
Water[b]	6.3	6.4	0.55	0.49	0.47	0.46	P-starved (16 hr.)
Water[c]	9.5[d]	7.5	0.55	0.10	0.10	0.07	P-starved (16 hr.)
0.1 M Acetate buffer[b]	4.0	Not measured	0.025	—	—	0.022	Before P-starvation

[a] See note *b* to Table I. [b] Acid-washed glass beads were used. [c] Acid treatment of glass beads omitted. [d] *p*H of water measured after shaking with the glass beads, but prior to homogenization of yeast.

particular with regard to the characteristic optimum in the region around *p*H 4, is so close that it is justifiable to attribute the hydrolysis of the substrate by the intact yeast cells to the action of the acid phosphatase found in the cell-free extract.

2. Activities of Acid Phosphatase in Intact Cells and in Subcellular Fractions of Bakers' Yeast.

A. INFLUENCE OF *p*H AND TEMPERATURE ON THE STABILITY OF CELL-FREE PREPARATIONS OF ACID YEAST PHOSPHATASE.—The evaluation of comparative data concerning phosphatase activity in living cells and cell-free extracts required information on the stability of this enzyme under the conditions used for cell disintegration. The lability of acid yeast phosphatase at weakly alkaline reaction had been observed by Schaeffner and Krumey (1938). Figures 2 and 3 show time curves of inactivation of acid yeast phosphatase at 1° and at 36.5° for various *p*H values. The enzyme was found to be stable between *p*H 3 and 5, but very labile in solutions of higher acidity or alkalinity. Although the inactivation was considerably retarded by lowering the temperature (Fig. 2), it was found that solutions of acid yeast phosphatase lost almost all their activity when stored for 24 hours at 5° and at *p*H values of 7 or 8. A comparison between the *p*H-activity profiles of Table I and the inactivation curves of Figure 3 suggests a protective influence of the substrate in the weakly alkaline region.

B. YIELDS OF ACID PHOSPHATASE OBTAINED BY HOMOGENIZATION OF BAKERS' YEAST AT DIFFERENT *p*H VALUES.—Representative figures of the phosphatase activities recovered in homogenates of bakers' yeast and in the fractions obtained by their sedimentation are given in Table II. When the disintegration of the cells was carried out between *p*H 4.0 and 5.0, the activities recovered in the homogenates practically equalled those found in the living yeast cells. Smaller yields were obtained by homogenization between *p*H 5.0 and 6.5. Homogenization at neutral or weakly alkaline reaction resulted in large losses of enzyme activity. These surprisingly strong *p*H effects on the yields of acid phosphatase in the homogenates, in particular the almost complete loss of enzyme activity after homogenization of the yeast at neutral reaction, are explained by the stability properties of acid yeast phosphatase (see Fig. 2 and 3). It is thus plausible to assume that the amounts of acid phosphatase which were found in the homogenates obtained within the *p*H

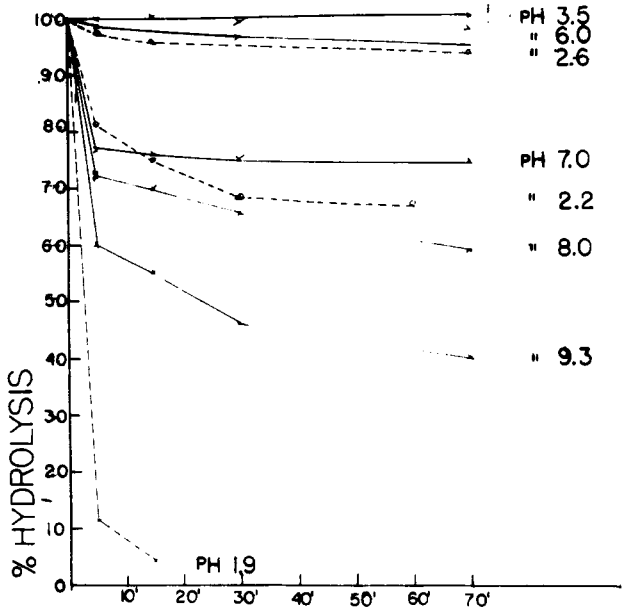

FIG. 2.—Rates of inactivation of acid phosphatase of bakers' yeast at +1° at various *p*H values. Enzyme preparation: 96,000 × *g* supernatant of homogenate of bakers' yeast obtained after 16 hours of P-starvation. 1 ml portions of the dialyzed supernatant were mixed respectively with 4 ml of 0.05 M KCl-HCl and glycine-HCl buffers in the *p*H range below 3.5, of 0.1 M sodium acetate buffers in the *p*H range between 4.0 and 5.5, and of 0.05 M Tris-maleate buffers and of 0.05 M sodium barbital solutions above *p*H 5.5. The mixtures were shaken in an ice bath in a cold room during the various time intervals indicated. (The *p*H values indicated were measured in the enzyme buffer mixture at approximately +3°.) *Assay*. 1 ml of the respective enzyme buffer mixtures was incubated after the various inactivation periods with 9 ml of prewarmed substrate buffer mixture (containing 8 ml of 0.1 M sodium acetate buffer of *p*H 4.0) as described in the section on Experimental Procedure. Substrate: 0.017 M β-glycerophosphate (final concentration in assay mixture).

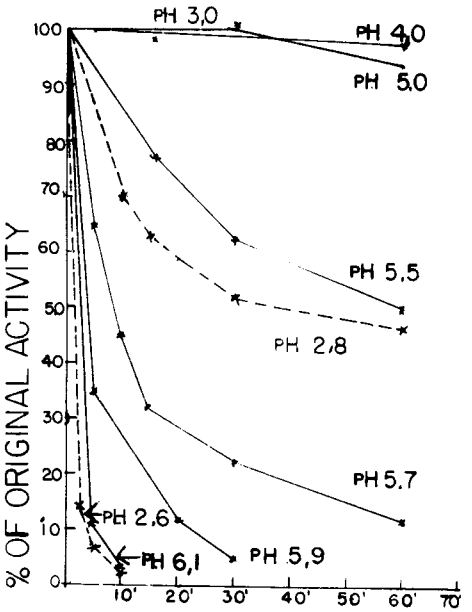

FIG. 3.—Rates of inactivation of acid phosphatase of bakers' yeast at 36.5° at various *p*H values. The experimental details were similar to those described in the legend to Figure 2. The incubations were carried out in a water bath of 36.5°. The *p*H values indicated were measured in the enzyme buffer mixtures at approximately 36°.

range of stability of the enzyme were identical with the total amounts of acid phosphatase of the yeast cells prior to their disintegration. This leads to the conclusion that the hydrolysis rate of extracellular phenyl phosphate during incubation with intact yeast cells corresponds to the activity of the total amount of intracellular acid phosphatase.

The phosphatase activity of all homogenized preparations was exclusively and practically completely found in the supernatant obtained after centrifugation at 96,000 × *g*.

3. Phosphatase Activity of Living Bakers' Yeast at Different Time Intervals of Phosphate Starvation.—Figure 4 shows the phosphatase activities and the contents of protein nitrogen (including the nucleic acid nitrogen) at different time intervals of incubation in a phosphate-free nutrient medium. The values for both cell constituents increased without an apparent lag period, and the relative increase of the phosphatase activities during this period was more than ten times as large as that of the protein nitrogen.

4. Behavior of P^{32}-Labeled Intact Cells of Bakers' Yeast During Short Incubation with Nonlabeled β-Glycerophosphate.—When samples of P^{32}-labeled yeast corresponding to 30 mg dry weight were incubated at 36.5° for 10 minutes with nonlabeled β-glycerophosphate, no measurable decreases of the radioactivities of any of the intracellular phosphorus fractions were observed (Table III), although the amounts of orthophosphate liberated from the substrate exceeded those of the intracellular orthophosphate by 30 times. In the control samples without added substrate, amounts of radioactivity which averaged 0.2% of the total radioactivity of the cells were excreted into the medium. The samples incubated with glycerophosphate showed excretion values of radioactivity which were identical with those of the controls. In the latter, the excretion proceeded at a practically constant rate for 2 hours and continued afterwards at gradually diminishing rates. Very similar results were obtained when the amounts of added labeled phosphate were only 10% of those used in the experiment of Table III. This renders it unlikely that the somewhat surprising release of phosphorus compounds from phosphate-starved yeast indicated radiation damage of the cells. Preliminary experiments suggested a stimulation of the rates of excretion of phosphorus compounds by the acidity of the medium. It should be pointed out, however, that *p*H 4.0 of the nutrient medium is within the physiologic range for the growth of bakers yeast.

5. Phosphatase Activity of Bakers' Yeast Grown in a Medium of High Orthophosphate Content.—Rautanen and Kaerkkaeinen (1951) observed that the activity of the acid phosphatase of *Torulopsis utilis* which had been grown in presence of high concentrations of orthophosphate were much smaller than those of phosphate-starved cultures. The data of Table IV demonstrate an analogous behavior for bakers' yeast. The Neopeptone (Difco), which was the only source of phosphorus and nitrogen in the basal medium, contained 0.2% phosphorus (all organically bound), corresponding to a 0.66 mM concentration of organic phosphorus in the medium. The phosphatase activity of the yeast cultured in this medium was more than ten times higher than that of bakers' yeast which had been grown in basal medium supplemented with 0.1 M potassium phosphate. On the other hand, the weight increases of the yeast were the same in both media.

The last column of Table IV shows that the addition of orthophosphate to the medium resulted in a large

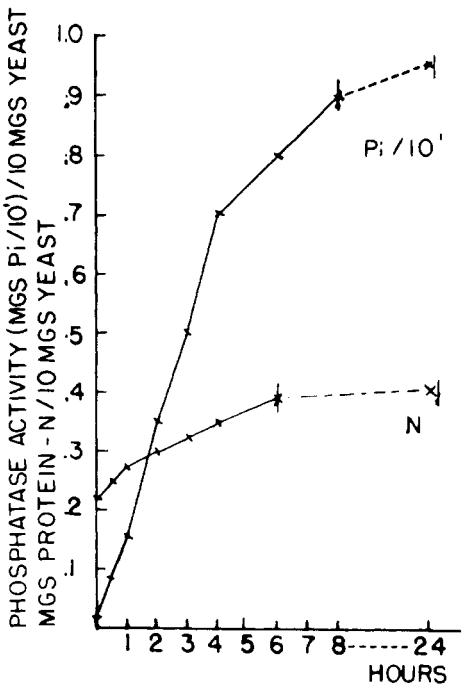

FIG. 4. Protein nitrogen content and acid phosphatase activity of bakers' yeast after various intervals of phosphate starvation. All values are calculated for the indicated weight units of moist yeast before incubation in the phosphate-free medium. Substrate of assay: 0.015 M phenyl phosphate (final concentration).

increase in the intracellular concentration of orthophosphate. It remains an open question whether the influence of the orthophosphate concentration of the medium on the phosphatase activity of the yeast cells represents an effect on surface structures or on cytoplasmic constituents of the yeast cells.

6. Effect of Magnesium and Manganese Ions on Acid Phosphatase of Bakers' Yeast.—The initial rates of hydrolysis of β-glycerophosphate by acid phosphatase of bakers' yeast decreased by about 5% in presence of 0.01 M magnesium chloride or manganous chloride and by 25% in presence of 0.01 M manganous sulfate. This behavior contrasts with the strong activation of the acid phosphatase of *T. utilis* by magnesium and manganese ions (Rautanen and Kylae-Siurola, 1954).

Discussion

Intracellular Localization of Acid Phosphatase of Bakers' Yeast.—The similarity between the *p*H-profiles of the phosphatase activities of intact cells of bakers' yeast and of the acid phosphatase of cell-free yeast preparations suggests that the presence of this enzyme near the cell surface is responsible for the phosphatase activity of intact yeast cells. It should be noted that the characteristic *p*H-profile of the action of this enzyme represents a *p*H-activity curve only between *p*H 2.8 and 5.2. On both sides beyond this range, the over-all *p*H-profile results from the combined effects of the hydrogen ion concentration on the activity and on the stability of the enzyme. This observation, however, does not diminish the value of the *p*H-profile in the neighborhood of the *p*H optimum as a means for the identification of the acid phosphatase of bakers' yeast.

The close quantitative agreement between the acid phosphatase activities of intact yeast cells and comparable quantities of cell-free yeast preparations suggests that the total amount of the acid phosphatase of bakers' yeast occurs near the surface of the cells. Other possible explanations of this quantitative observation are not consistent with the experimental results. No acid phosphatase was detected in the nutrient medium used for the preparation of phosphate-starved yeast (medium b), although the enzyme was found to be stable at this acidity. The possibility of intracellular cleavage of the substrate after its absorption and of the subsequent release of the orthophosphate into the

TABLE III

RADIOACTIVITY OF P^{32}-LABELED, P-STARVED YEAST CELLS BEFORE AND AFTER INCUBATION WITH NON-LABELED β-GLYCEROPHOSPHATE

20 g bakers' yeast was aerated for 16 hours at 31.5° in 2 liters of medium b (see Experimental Procedure) supplemented with 2 mc of P^{32}-labeled orthophosphate (0.017 mg phosphorus) as exclusive source of phosphorus. The harvested cells were washed with water of 5° and suspended in cold water to yield a total volume of 200 ml. 1 ml of the suspension was incubated for 10 minutes at 36° at *p*H 4.0 with 6 ml of a 0.027 M solution of non-labeled sodium β-glycerophosphate in 0.1 M sodium acetate buffer. A control sample without glycerophosphate was incubated under comparable conditions. The incubated samples were centrifuged, the sedimented cells were extracted with 5 ml water in a water bath of 100° for 5 minutes, and the cooled suspensions were centrifuged. The two supernatants and the final washed sediments were analyzed for radioactivity. The supernatants were also analyzed for orthophosphate. The figures represent the values for the total respective fractions of the incubated samples.

Incubation Mixture	Medium (mg P_i)	Medium (c/min × 10^{-6})	Boiled Extracts of Cells (mg P_i)	Boiled Extracts of Cells (c/min × 10^{-6})	Residue from Boiled Extract (c/min × 10^{-6})
No glycerophosphate	0	0.032	0.07	1.9	3.9
Glycerophosphate added	2.1	0.032	0.17	1.9	3.9

TABLE IV

EFFECT OF THE CONCENTRATION OF ORTHOPHOSPHATE ON THE ACTIVITY OF ACID PHOSPHATASE OF GROWING BAKERS' YEAST

200 mg portions of bakers' yeast were grown respectively in Neopeptone medium (see Experimental Procedure) without and with addition of 0.1 M KH_2PO_4. Cells harvested after 46 hours. Yield of each culture 7.5 g dried cells (corresponding to 25 g moist yeast). Substrate of assay: 0.017 M sodium β-glycerophosphate.

Condition of Yeast	Homogenate (*p*H)	Phosphatase Activity (mg P_i formed during 10 min. by 10 mg yeast[a]) Supernatant of Homogenate: Cells	Supernatant of Homogenate: 800 × *g*	Supernatant of Homogenate: 96,000 × *g*	Intracellular P_i/10mg Yeast[a]
No orthophosphate added to medium	4.8	0.600	0.575	0.545	0.003
0.1 M orthophosphate added to medium	4.8	0.058	0.045	0.045	0.024

[a] Moist yeast (mg dry weight × 3.3). The moisture content of pressed yeast is 70%.

medium was ruled out by the observation that the radioactivity of P^{32}-labeled yeast did not decrease after incubation of the intact cells with unlabeled glycerophosphate under the assay conditions for acid yeast phosphatase.

In view of the exclusive occurrence of acid yeast phosphatase as freely soluble enzyme in yeast homogenates, its postulated localization near the cell surface is probably maintained by compartmentalization of the cells rather than by association of acid phosphatase with particulate surface constituents. An analogous explanation has been proposed by Malamy and Horecker (1961) for the intracellular localization of the freely soluble alkaline phosphatase formed by certain strains of *E. coli* during growth in low-phosphate media.

Localization of the acid phosphatase at the surface of the yeast cells was found to be characteristic for yeast before and after phosphate starvation. Under both conditions, the activities of the intact cells were quantitatively identical with those of comparative portions of the respective 96,000 $\times$ *g* supernatants of their homogenates. This excludes the possibility that the increased phosphatase activity of intact, phosphate-starved yeast cells might have been caused by a transport of phosphatase molecules from the interior of the cells to their surface as a biological response to phosphate deficiency of the nutrient medium.

The exclusive occurrence of acid yeast phosphatase in the soluble fraction of yeast homogenates is surprising in view of the fact that extraction of this enzyme from the cell debris of ethyl acetate autolysates required enzymatic digestion by extracts of germinating barley (Albers and Albers, 1935), and that autolysis of yeast was reported as essential for the extraction of the phosphatase from dried yeast (Schaeffner and Krumey, 1938). It is possible that the solubilization of the enzyme during homogenization even at ice bath temperature might not be caused exclusively by mechanical liberation, but that an enzymatic process might also be involved. Similar questions have been raised concerning the solubilization of yeast invertase as consequence of the formation of protoplasts from *S. cerevisiae* (Sutton and Lampen, 1962). Myrbäck (1957) reported that, in toluene autolysates of this organism, invertase was tightly bound to structural cell constituents.

So far, only in *E. coli* has *de novo* enzyme synthesis been demonstrated as the mechanism for the enhancement of phosphatase activity during incubation in media of low phosphate concentrations (Garen and Levinthal, 1959). For *S. cerevisiae* and *T. utilis*, direct evidence showing *de novo* enzyme formation by preparative isolation of pure phosphatase is still outstanding. The observations reported in this study on intact cells and cell-free extracts of *S. cerevisiae*, however, strongly suggest reversal of repression of phosphatase biosynthesis as the explanation for the striking increase of phosphatase activity during the incubation of this organism in phosphate-free media. The similarity of the repressor effect of orthophosphate on the profoundly different phosphatase proteins of *E. coli* and *S. cerevisiae* presents an interesting question for further studies.

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