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Nonspecific Acid Phosphatase from *Schizosaccharomyces pombe.* Purification and Physical Chemical Properties†

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ABSTRACT: Repressible nonspecific acid phosphatase from *Schizosaccharomyces pombe* was purified to apparent homogeneity, as ascertained from ultracentrifugal, electrophoretic, and chromatographic data. The native protein has a molecular weight of 383,000 as determined by sucrose density gradient centrifugation and 381,000 as determined by gel filtration. The native protein can be dissociated in the presence of 8 *M* urea–1% sodium dodecyl sulfate into subunits possessing an approximate molecular weight of 104,000. Neutral sugars account for about 66% of the total molecular weight and contribute to the high solubility and some of the other physical properties of this enzyme. Purified enzyme preparations have a K_m for 4-nitrophenyl phosphate of 0.17 m*M* and a broad substrate specificity, but do not show diesterase activity. Phosphate and sulfate are competitive inhibitors. The enzyme is inactivated at neutral and alkaline pH and at relatively low temperatures. Mannose and galactose were found as the main components of the carbohydrate moiety; glucosamine was present in lower amounts. The amino acid analysis revealed a high content of aspartate, threonine, and serine; no sulfhydryl group could be detected. P_i is released in stoichiometric amount (1 mol per enzyme monomer) on protein digestion.

The regulatory mechanisms of phosphatase formation have not yet been clearly elucidated in eukaryotic microorganisms. The available data on the regulation of the synthesis of the nonspecific acid phosphatase of *Saccharomyces cerevisiae* (Shurr and Yagil, 1971; Toh-e et al., 1973) and nonspecific alkaline phosphatase of *Neurospora crassa* (Lehman et al., 1973; Toh-e and Ishikawa, 1971) suggest a more complex regulatory mechanism with respect to that of the corresponding alkaline phosphatase in *Escherichia coli,* which has been extensively studied by Garen and his colleagues (Garen and Echols, 1962a,b). Our previous work on the nonspecific acid phosphatase (orthophosphoric-monoester phosphohydrolase, EC 3.1.3.2) in *Schizosaccharomyces pombe* has been concerned with preliminary characterization both in intact cells and in cell-free extract and investigations on the factors affecting the enzymatic activity of the cells (Dibenedetto, 1972). Our results suggested that, besides the repression by inorganic phosphate, additional factors influence the levels and changes of phosphatase activity. Evidence was then obtained indicating that unimpeded formation of mannan is a necessary condition for the formation of the acid phosphatase. To investigate further the genetic control of the formation of the acid phosphatase in *S. pombe* it was essential to obtain pure preparations and to investigate the chemical and physical features of the enzyme molecule. The present article describes the purification procedure and the physical and chemical properties of the pure enzyme.

Materials and Methods

Urea was recrystallized from hot methanol, norleucine was obtained from Mann Research Laboratories, and dansyl-Cl[1] was from Calbiochem. Diisopropyl fluorophosphate treated carboxypeptidase A was supplied by Worthington. Twice distilled constant boiling HCl was used for hydrolyses. Bovine serum albumin (from Calbiochem), β-galactosidase (from Sigma), and catalase (from Boehringer) were used without further purification. Phosphorylase was kindly supplied by the laboratory of Dr. E. Fischer and arginine decarboxylase by Dr. E. Boeker. Bio-Gel A-5m, 200–400 mesh, was obtained from Bio-Rad and washed with buffer before using. Dextrans T-150, T-250, and T-500 were purchased from Pharmacia, Fine Chemicals.

Activity Assay. The nonspecific acid phosphatase was assayed as described before (Dibenedetto, 1972) by a modification of the method of Torriani (1960) and by determination of phosphorus using the method of Fiske and Subba-Row (1925) with minor modification. One unit of acid phosphatase activity represents the amount of enzyme catalyzing the release of 1 μmol of 4-nitrophenol/min at 30°,

† From the Department of Genetics, University of Washington, Seattle, Washington 98195. *Received October 9, 1974.* The present experimental work was supported by National Institutes of Health Grant No. 5R01-A100328.

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[1] Abbreviation used is: dansyl, 8-dimethylamino-1-naphthalenesulfonate.

and the specific activity was defined as the enzyme units per milligram of protein.

Protein Determination and Absorbancy Index. Protein concentration was determined by the method of Lowry et al. (1951) on impure preparations (specific activity < 100 units/mg) using bovine serum albumin as standard and spectrophotometrically on more highly purified materials, using an absorbancy index $A_{280}^{1\%}$ of 13.2. This value is the average of two independent measurements of protein concentration obtained either by amino acid analysis or refractometrically in the ultracentrifuge assuming for this glycoprotein the refractive index increment of 4.05 fringes/mg reported by Babul and Stellwegen (1969) and calculating the protein's contribution to the refractive index from the percentage of the protein moiety. Values of 13.0 and 13.4, respectively, were obtained and shown to be essentially the same in all buffers used.

Carbohydrate Determination. Total carbohydrate was determined by the orcinol- sulfuric method as modified by Francois et al. (1962) and by the anthrone procedure as modified by Roe (1955). In both cases a calibration curve using mannose as standard was carried out. The carbohydrate content of the protein is given as percentage by total weight or as a ratio of carbohydrate (milligrams) to protein (milligrams). Both methods used gave essentially the same percentage or the same ratio.

Polyacrylamide gel electrophoresis of acid phosphatase was carried out in 5% gels either at neutral pH or at acid pH. At neutral pH the electrophoresis was performed according to the method of Allen and Gockermann (1964) modified as follows: the gels were prepared in 0.01 *M* histidine-HCl buffer at pH 6.5 and the electrophoresis was run in the same buffer for 3 hr at 4°; the sample and the spacer gels were eliminated and the sample (5–50 μg) was added by layering under the buffer in the presence of 20% sucrose. A 1.37% β-alanine–0.05 *M* isobutyric acid buffer at pH 4.5 was used for acid pH both to prepare gels and to run the electrophoresis at 4° for 2 hr. Protein was located by staining for 1 hr with 0.25% Coomassie Brilliant Blue in 50% methanol–8% acetic acid and power destained in 5% methanol–7.5% acetic acid. Phosphatase activity was localized by a diazo coupling method (Dorn, 1965) using a 0.2 *M* acetic acid solution at pH 3, containing 3 mg/ml of disodium α-naphthyl phosphate and 5 mg/ml of Fast Garnet GBC diazonium salt. The detection of carbohydrate was performed by the periodic acid–Schiff reaction (Zacharius and Zell, 1969). Polyacrylamide gel electrophoresis in 0.1% sodium dodecyl sulfate–8 *M* urea was carried out in 7.5 and 10% gels of 8 and 11 cm in length. The gels were prepared in 8 *M* urea–0.1% sodium dodecyl sulfate in 0.1 *M* H_3PO_4–Tris buffer at pH 6.8; the reservoir buffer was the same except for the absence of urea. The samples were pretreated at 100° for 2–3 min in 8 *M* urea–1% sodium dodecyl sulfate; 25 μg was applied for purity checks and 10 μg for molecular weight determinations. Logarithmic plots of relative migration vs. molecular weight were obtained with the following markers: bovine serum albumin monomer (Tanford et al., 1967), catalase (Sund et al., 1967), arginine decarboxylase (Boeker et al., 1969), glycogen phosphorylase (Cohen et al., 1971), and β-galactosidase (Uhlmann et al., 1968).

Molecular Weight by Exclusion Chromatography. A column of Bio-Gel was calibrated for molecular weight determination by gel chromatography with Dextran T-500, Dextran T-250, and Dextran-150 as standards. The column was equilibrated and eluted with 0.2 *M* sodium acetate buffer at pH 4.6 at a flow rate of 26 ml/hr. The fraction's volume was 2.55 ml collected on a drop basis. The acid phosphatase peak was located by monitoring the absorbancy of the effluent at 280 nm. The location of polysaccharide peak was determined by the anthrone reaction. Elution volumes were measured to the maximum height of each peak and were plotted vs. the log of the molecular weight, according to Andrews (1965).

Velocity Density Gradient Centrifugation. Linear 10–30% sucrose density gradients (4.8 ml) in 0.2 *M* sodium acetate buffer (pH 4.6) were prepared according to Martin and Ames (1961). Each tube was loaded with 100 μl of either pure enzyme preparation (5 mg/ml) or protein markers (β-galactosidase; catalase; bovine serum albumin; phosphorylase, all at the concentration of 5 mg/ml) and centrifuged for 10 hr, at 56,000 rpm in a Beckman L2-65B ultracentrifuge, using an SW 56 rotor. Fractions of 0.1 ml were collected after tube puncture. The location of protein peaks was performed by monitoring the absorbancy at 280 nm. The molecular weight given is the average of the values obtained with different standards.

Amino Acid Analyses. Nonspecific acid phosphatase was dialyzed exhaustively against distilled water and lyophilized. Duplicate samples of 1 mg of enzyme and 0.1 mol of norleucine, added as an internal standard (Walsh and Brown, 1962), were hydrolyzed at 108° for 24, 48, 72 and 96 hr with 5 ml of 5.7 *N* HCl in sealed evacuated tubes. In drying the hydrolysates, care was taken to avoid raising the temperature and pH, in order to minimize the Maillard reaction (Eastoe, 1972). Analyses were performed on a Beckman Model 120C amino acid analyzer according to Spackmann et al. (1958). The cysteine plus cystine content was determined on duplicate samples after performic acid oxidation of the protein and 22-hr hydrolysis, according to Hirs (1967). Determinations of free sulfhydryl groups were carried out both by titration with 1 m*M* 5,5′-dithiobis(2-nitrobenzoic acid) in 8 *M* urea, according to Ellman (1959), and by selective photooxidation with 1 m*M* crystal violet in 8 *M* urea–0.05 *M* sodium acetate buffer (pH 4.6) for 5 hr, according to Jori et al. (1969). Duplicate tryptophan determinations were made spectrophotometrically, according to Bencze and Schmid (1957).

Carbohydrate Analyses. The chemical composition of carbohydrate was established by gas chromatography of their trimethylsilyl derivatives. Each dried sample (0.7 mg of sugars) was hydrolyzed with 3 ml of 1 *N* methanolic HCl at 80° for 16 hr in sealed tubes, then submitted to acetylation and trimethylsilylation, according to Sweeley and Walker (1964). A 1–6 μl aliquot was injected into a 2 m × 3 mm column of 30% SE-30 at 150° in a 402n Hewlett Packard gas chromatograph equipped with a flame ionization detector and a temperature programmer. Analyses were performed according to Laine et al. (1972), using N_2 as carrier gas. Semiquantitative determinations of the individual components were obtained by cutting out the respective peaks from the chart paper and weighing them. Separate analyses by gas chromatography on a ECNSS-M3 column were also carried out on the reduced and acetylated derivatives of sugars, according to Yang and Hakomori (1971), to check the results of the first method and to identify amino sugars. The quantitative determination of glucosamine was obtained by the ninhydrin method during amino acid analyses.

Phosphate Analysis. Samples of purified acid phosphatase and standard proteins were dialyzed for two successive

Table I: Purification of *S. pombe* Nonspecific Acid Phosphatase.

Step	Total Units (U)	Total Protein (mg)	Sp Act. (U/mg)	Purification Yield (%)
Crude extract (E_1)	87,669	4272	20	100
Concentration freezing–thawing (E_2)	88,515	3150	28	100
Ammonium sulfate precipitation (E_3)	89,945	377	236	100
Eluate from Bio-Gel	69,832	266	412	78

24-hr periods against 2 l. of H_2O, with stirring. Protein digestion and orthophosphate colorimetric determination were carried out according to Shaw (1971). The phosphate content of the samples was calculated from a calibration curve obtained with casein and bovine serum albumin as protein standard and blank, respectively.

Yeast Strain and Cultural Conditions. A wild type strain of fission yeast *Schizosaccharomyces pombe* (wt 972 hr^-) was used in all the experiments. Cells were grown in a low-phosphate minimal medium that provides the best conditions for the maximum derepression of acid phosphatase (Dibenedetto, 1972). Ten liters of a 48-hr culture in low-phosphate medium was added to 90 l. of the same sterile medium in a New Brunswick Scientific Fermacell F-130 fermentor and allowed to grow for 48 hr at 30°, 200 rpm agitation rate and 4 CFM air flowmeter. Approximately 200 g of wet cells was harvested by a large Sharples centrifuge.

Results

Purification Procedure of the Enzyme. Conditions for optimum yield of the enzyme were systematically investigated during the establishment of each purification step. The procedure described below leads to homogeneous material with 70% overall yield. Unless otherwise indicated, all operations were carried out at 4°; centrifugations were performed at 48,000*g* in a Sorvall RC2-B centrifuge.

Preparation of the Crude Extract. Freshly harvested cells (100 g) were suspended 1:1 in 0.2 *M* sodium acetate buffer (pH 4.6) and disrupted at low temperature, using a Braun MSK homogenizer. Equal volumes (30 ml) of yeast suspension and acid-washed glass beads (0.45–0.50 mm in diameter) were shaken together for 4 min at 4000 rpm. Glacial acetic acid (100 μl) was added to the mixture to avoid a raise in pH to alkaline values during the homogenization. The glass beads were removed by simple sedimentation; broken cells and other particulate material were removed by centrifugation. The sediment was washed twice with 150 ml of the buffer used above and the supernatants were collected (crude extract or E_1).

Concentration, Freezing, and Thawing. The crude extract was concentrated by dialysis against polyethylene glycol (20,000) to half its original volume. The resulting solution was frozen overnight, then thawed in a refrigerator, and centrifuged for 30 min; the supernatant was collected (E_2).

Ammonium Sulfate Precipitation. To the solution E_2 solid ammonium sulfate was slowly added (while stirring) to a concentration of 0.6 g/ml and the solution was allowed to stand overnight. The suspension was centrifuged at 27,000*g* for 1 hr. The supernatant, which contained all the enzymatic activity, was dialyzed overnight against 10 l. of

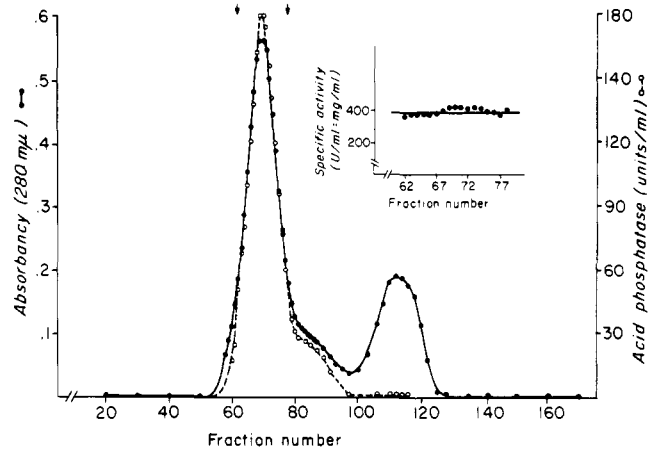

FIGURE 1: Chromatography of yeast acid phosphatase on Bio-Gel. The column was equilibrated with 0.2 *M* sodium acetate buffer (pH 4.6), loaded with 3 ml of E_3, and eluted with the same buffer at a flow rate of 27 ml/hr; 3.2-ml fractions were collected and analyzed for absorbancy at 280 nm, acid phosphatase, and carbohydrate content. The insert reports the specific activity through the peak. The fractions between the bars were pooled for subsequent characterization.

0.2 *M* acetic acid adjusted to pH 3.0 with 0.2 *M* sodium acetate. After dialysis, the enzyme solution was brought to pH 4.2 with 1 *M* sodium acetate, concentrated by dialysis against polyethylene glycol (20,000) and, after centrifugation, the clear supernatant was collected (E_3).

Gel Filtration. The final purification step consisted of a batchwise filtration through a column (2.6 × 70 cm) of Bio-Gel. The elution diagram is presented in Figure 1. No increase in specific activity was observed after recycling of the purified enzyme on Bio-Gel or Sephadex G-200, where the carbohydrate peak and the activity and protein peak coincided and the ratio protein/carbohydrate was constant through the peak.

The fractions with highest and constant specific activity were pooled and concentrated by precipitation with 70% (v/v) of 95% ethanol precooled to −20°. After 5 min the suspension was centrifuged at 18,800*g* for 10 min. The sediment was redissolved in 3 ml of 0.2 *M* sodium acetate buffer (pH 4.6) and dialyzed overnight against the same buffer to eliminate traces of alcohol. The pure enzyme can be stored at 0° for several weeks without appreciable loss of activity.

A typical purification from 100 g of yeast is summarized in Table I. The final specific activity was routinely between 360 and 420 units/mg.

Purity of the Nonspecific Acid Phosphatase Preparations. The final preparations were found to be homogeneous by a number of criteria. The purified enzyme was analyzed by sucrose density gradient sedimentation. As shown in Figure 2, a single protein peak was obtained with constant specific activity.

Disc gel electrophoresis at pH 6.5 yielded a single sharp protein band corresponding to the enzymatic activity and to the carbohydrate band (Figure 3). The same result is obtained at pH 4.5 (Figure 3); under these conditions no protein band could be detected when electrophoresis was carried out with reversed electrodes. It should be noted that at this pH the protein still migrates toward the anode.

Single bands were also obtained when the samples were run in the presence of 8 *M* urea–1% sodium dodecyl sulfate, using several concentrations of polyacrylamide and lengths of gels (Figure 4). The carbohydrate staining showed that,

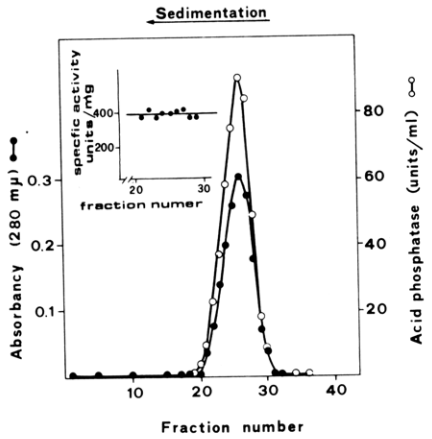

FIGURE 2: Centrifugation of *S. pombe* acid phosphatase in 10–30% sucrose density gradient. Aliquots (100 μl.) were layered on top of a 4.8-ml sucrose gradient in 0.2 *M* acetate (pH 4.6) and centrifuged 6 hr at 56,000 rpm in an SW-56 rotor. Fractions of 100 μl each were collected. The initial concentration of enzyme was 6.4 mg/ml.

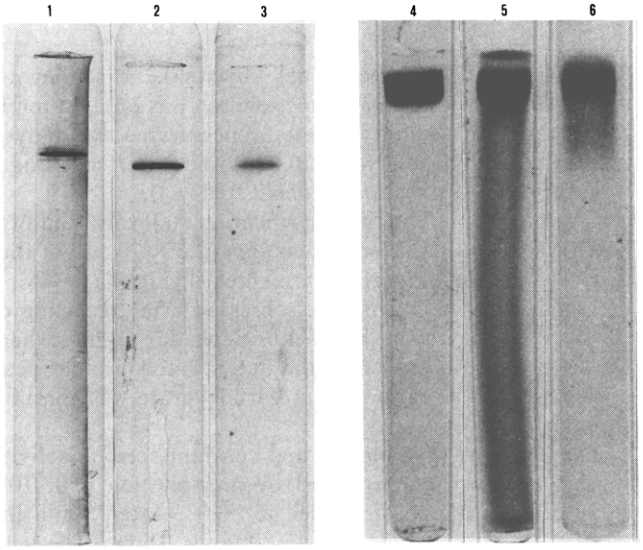

FIGURE 3: Polyacrylamide gel electrophoresis. Staining for proteins (1), for activity (2), and for carbohydrate (3) of gels at pH 6.5. Staining for activity (4), for proteins (5), and for carbohydrate (6) of gels at pH 4.5. The experimental conditions for both electrophoreses are described in the text.

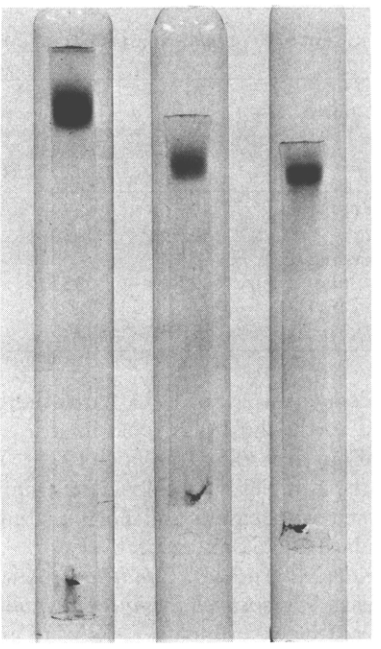

FIGURE 4: Polyacrylamide gel analysis of acid phosphatase in the presence of 8 *M* urea–0.1% sodium dodecyl sulfate: (right) 10% acrylamide, (center) 7.5% acrylamide, and (left) 7.5% acrylamide; length, 11 cm.

also under these conditions, the protein and the carbohydrate bands (not reported in the figure) were superimposed on one another.

Physical and Chemical Properties. The purified enzyme contains 66.5 ± 3% by weight of carbohydrates that fractionate together with the protein through a wide variety of procedures including column chromatography on P-cellulose (not reported here), Sephadex G-200, and Bio-Gel, electrophoresis on polyacrylamide either in the absence or in the presence of 8 *M* urea–0.1% sodium dodecyl sulfate, and sucrose density gradient sedimentation.

The purified enzyme is colorless and the ultraviolet absorbance spectrum has a maximum at 280 nm. The A_{280}/A_{260} ratio is 1.81, indicating a lack of substantial contamination by nucleic acid.

Molecular Weight Determination. The molecular weight of the native enzyme was determined by molecular sieve chromatography and sucrose density gradient sedimentation. By exclusion chromatography the purified phosphatase eluted between the Dextran T-500 and the Dextran T-250, corresponding to a molecular weight of 381,000 ± 17,500. A similar value, 383,400 ± 29,240, is calculated from the relative distances on sucrose density gradient.

Subunit Molecular Weight. Acid phosphatase was subjected to polyacrylamide gel electrophoresis in 8 *M* urea–0.1% sodium dodecyl sulfate using different polyacrylamide gel concentrations. In a 10% polyacrylamide concentration the acid phosphatase displayed a migration, in four determinations, corresponding to a molecular weight of 104,000 when compared to marker proteins. In a 7.5% polyacrylamide gel, migration relative to marker proteins corresponded to a subunit molecular weight of 134,000 ± 2000.

Solubility. The acid phosphatase was quite soluble in a solution of ammonium sulfate; the bulk of the enzyme was not precipitated even when complete saturation with salt was attained. The enzyme is soluble also under acidic conditions, such as 1 *M* acetic acid of 1 *N* HCl, and does not precipitate in 12% trichloroacetic acid.

Enzymatic properties of the pure enzyme such as the pH–activity curve, the substrate specificity, the stability to pH, and inhibitor effects were determined and shown to be very similar to those of the crude preparations (Dibenedetto, 1972).

The K_m value for 4-nitrophenyl phosphate is 0.17 m*M* and the K_i values for competitive inhibitors like phosphate and sulfate are 5×10^{-4} and 1.8×10^{-3} *M*, respectively.

The effect of incubation of the enzyme at temperatures between 35 and 60° was examined: at temperatures higher than 40° the enzyme was rapidly inactivated.

The amino acid composition is reported in Table II. The difference between duplicate samples was less than 3% for each amino acid, except for lysine, glutamic acid, and methionine which differed by about 10%. The destruction of amino acids due to the Maillard reaction was kept in limited extent, as shown by both the total recovery of free amino acids and the recovery of the internal standard after hydrolysis. The high content of aspartic acid, threonine, and serine

Table II: Amino Acid Composition of *S. pombe* Nonspecific Acid Phosphatase.[a]

Residues	g/100 g of protein	mol per 89.960 g (66% of Carbohydrate)
Lys	2.12 ± 0.08	5.1
His	2.34 ± 0.01	5.2
NH_3	1.90 ± 0.13	34.2
Arg	3.18 ± 0.03	6.2
Asp	13.82 ± 0.17	36.7
Thr	7.51 ± 0.05	22.7
Ser	9.35 ± 0.05	32.8
Glu	8.02 ± 0.46	19.0
Pro	4.45 ± 0.05	14.0
Gly	3.19 ± 0.01	17.1
Ala	4.74 ± 0.06	20.2
Val	5.66 ± 0.02	17.5
Met	1.17 ± 0.08	2.7
Ile	3.91 ± 0.10	10.6
Leu	6.87 ± 0.08	18.6
Tyr	8.28 ± 0.01	15.5
Phe	8.66 ± 0.05	18.0
Half-Cys	1.80	3.6
Trp	4.00 ± 0.01	6.6
Glc	5.34 ± 0.06	10.1

[a] All values except those for half-cystine and tryptophan (see Materials and Methods) are averages computed from duplicate determinations following 24, 48, 72, and 96 hr of hydrolysis. Ammonia, threonine, serine, and glucosamine were determined by extrapolation to zero time of hydrolysis; valine and isoleucine values are for 96 hr of hydrolysis.

residues in this protein is noteworthy.

Sulfhydryl Groups Titration. Following performic acid oxidation, 3.6 residues of cysteic acid were found per 30,000 g of protein (Table II). All were apparently derived from the oxidation of cystinyl residues, since the determination of free sulfhydryl groups by titration with 5,5′-dithiobis(2-nitrobenzoic acid) in 8 *M* urea, using a protein concentration of 2 mg/ml, failed to detect any accessible sulfhydryl group.

Furthermore, to avoid possible spontaneous oxidation of free sulfhydryl groups at alkaline pH, a protein sample from the enzyme stock kept at acid pH was submitted to photooxidation in the presence of crystal violet and 8 *M* urea at pH 4.6. By this method all cysteine groups are selectively converted to cysteic acid, whereas cystine is unaffected. Only negligible amounts of cysteic acid were detected by amino acid analysis following photooxidation, thus confirming the absence of free thiol groups in this protein.

Carbohydrate Composition. Identification of the neutral sugars, accounting for 66% of the total weight of the glycoprotein, was obtained by gas chromatography of their trimethylsilyl derivates and comparison of their retention times with those of standards. The enzyme was found to contain mannose and galactose; the ratio between hexatrimethylsilylmannitol and hexatrimethylsilylgalactitol was about 1:1. The same chemical composition was obtained by gas chromatographic analyses on the alditol derivates, although the ratio between the two sugars was different. The different conditions of hydrolysis and losses during the procedure can explain this discrepancy between the two methods. The enzyme contains also glucosamine; the amount of this amino sugar, obtained by amino acid analyses, is 1.65% by total weight, corresponding to 10 residues per protein monomer.

Phosphate Content. Purified acid phosphates from *S. pombe* contains tightly bound phosphate. As shown in Figure 5, the amount of phosphate released after protein digestion is very close to 1 mol/enzyme monomer.

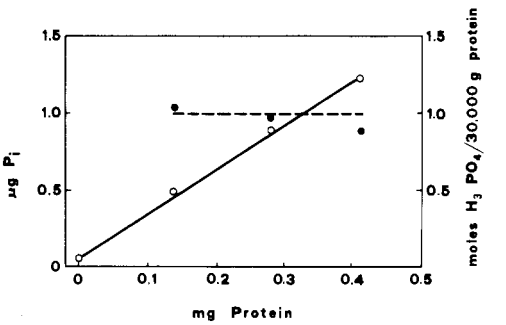

FIGURE 5: Phosphate content of acid phosphatase from *S. pombe*. Samples of purified enzyme, containing the protein amount indicated in the figure, were dialyzed and digested as reported in the text. From the amount of P_i released (○) by each sample, the stoichiometric ratios of P_i:protein monomer molecular weight (●) were calculated.

Discussion

Three phosphomonoesterases with distinct properties and locations were demonstrated in yeast (Suomalainen et al., 1960; Tonino and Stein-Parvé, 1963; Chattaway et al., 1971). Consistent data from different laboratories indicate that the bulk of the enzyme with optimum pH around 4 is located outside whereas the enzymes with optimum activity around 6 and 9 are entirely located within the cell membrane (McLellan and Lampen, 1963; Schmidt et al., 1963; Tonino and Stein-Parvé, 1963). In addition, the pH 4 and 9 phosphatases show completely different properties with respect to the inhibition by phosphate, temperature inactivation, cation depletion, and Pronase digestion (Shurr and Yagil, 1971). We have now purified a nonspecific acid phosphatase of *S. pombe* to apparent homegeneity. The purification procedure took advantage of the high solubility of this large glycoprotein in concentrated salt solutions and at acidic pH. The carbohydrate, accounting for about 66% of the total weight, undoubtedly contributes to its solubility. Dextrans have been used as standards for the determination of the molecular weight of the native protein in consideration of the high carbohydrate content. Indeed, the exclusion limits of Sephadex were reported to be lower for polysaccharides than for globular proteins (Andrews, 1965) and we presumed that the purified enzyme could behave during gel filtration more as polysaccharide than as globular protein. In fact, the result obtained by this method, as reported in the case of acid phosphatase from *Candida albicans* by Odds and Hierholzer (1973), is in fairly good agreement with those obtained by velocity sucrose gradient sedimentation and by analytical ultracentrifuge experiments (to be reported separately). The enzyme dissociates in 8 *M* urea–1% sodium dodecyl sulfate in subunits of apparent identical molecular weight of 104,000 in 10% gel acrylamide concentration and 134,000 in 7.5% gels. The increased estimates of molecular weight of 104,000 in 10% gel acrylamide concentration and 134,000 in 7.5% gels. The increased estimates of molecular weight with decreased acrylamide concentration are caused by a decreased binding offum dodecyl sulfate per gram of glycoprotein as compared with standard prosodium dodecyl sulfate gel electrophoresis, and thus a higher apparent molecular weight. By increasing the acrylamide concentration, molecular sieving predominates on the charge as factor determining the separation, and the anomalously high apparent molecular weight decreases, approaching values close to the real size (Segrest and Jackson,

1972; Russ and Polakova, 1973). Because of this behavior, the smaller value of 104,000 should be regarded as an approximation to the real size of the molecule.

The failure to separate the protein from the carbohydrate in strong denaturing agents indicates that covalent bonds are involved. Although the chemical nature of these bonds was not examined, the presence of glucosamine and the contents of aspartic acid, serine, and threonine exceeding the average distribution in proteins might be an indication that a glucosaminyl-asparagine bond could be involved in the protein-carbohydrate linkage, as in the case of yeast invertase (Neumann and Lampen, 1969). In fact, it is known that the sequence near the asparagine involved in a glucosaminyl-asparagine bond is Asn-X-Thr/Ser (Spiro, 1970). If we assume that glucosamine is the linkage point, the presence of ten residues of amino sugar per phosphatase subunit would set an upper limit to the number of carbohydrate chains.

A phosporylated form of bacterial and mammalian alkaline phosphatase was obtained on incubation of the enzyme with low concentrations of P_i (Schwartz and Lipmann, 1961; Fernley, 1971). The phosphoserine resulting from this reaction was shown to play an important role in the reaction mechanism. However, attempts to repeat the experiment with prostatic acid phosphatase were unsuccessful (Hollander, 1971). Therefore, the presence of tightly bound phosphate in *S. pombe* acid phosphatase was not predictable, and its nature, origin, and possible functional role need a complete elucidation.

The general kinetic properties of acid phosphatase from *S. pombe* are qualitatively similar to those of the same enzyme from other yeasts, in particular *S. cerevisiae* (Boer, 1969) and *Candida utilis* (Odds and Hierholzer, 1973). The enzyme from *S. pombe* displays the lowest pH optimum, and the specific activity is about five- to sixfold higher than that reported for purified preparations of acid phosphatase from *S. cerevisiae*. This difference in the specific activity of the same enzyme from closely related yeast species could indicate either different regulation mechanisms or the existence of multiple enzyme forms with different catalytic efficiencies.

Acknowledgments

The authors are extremely grateful to Dr. H. Roman, in whose laboratory a large part of this work has been performed, for his interest and encouragement. We also wish to thank Dr. E. H. Fischer for helpful discussions and for critically reading the manuscript.

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