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Purification and Properties of Yeast Invertase*

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ABSTRACT: Although the enzyme, yeast invertase, has been studied extensively in the past, it has never been chemically characterized and there has been a great deal of uncertainty concerning a number of its physical properties. The present studies describe a rapid method for the isolation of highly purified enzyme in good yield. The enzyme is homogeneous by polyacrylamide gel electrophoresis. Sedimentation velocity studies reveal a major component with an $s_{20,w}$ of 10.4 S. Heavier components which are also present in much smaller amounts are enzymatically active and indicate the presence of an association–dissociation equilibrium. The molecular weight of the enzyme is about 270,000, as determined by sedimentation equilibrium measurements. The chemical properties of the enzyme have been examined and the amino acid composition determined. Invertase is shown to be a glycoprotein which contains about 50% carbohydrate (predominantly mannan with a small percentage of glucosamine).

Recently, Sutton and Lampen (1962) and Islam and Lampen (1962) have described the secretion of invertase by yeast protoplasts. To understand this process, it is essential to characterize the invertase of the intact yeast cell (which is primarily localized in the cell wall) to permit eventual comparison of this material with the secreted enzyme and with the invertase present inside the cell membrane.

Yeast invertase was first isolated by Berthelot (1860) by alcohol precipitation. Since that time, this enzyme has been studied by a number of workers. Much of the early work has been reviewed by Neuberg and Roberts (1946). Myrbäck (1960) has summarized the more recent results. A great deal is known about the enzyme from a kinetic point of view particularly from the inhibition studies by Myrbäck and his coworkers. Nevertheless, relatively little is known about the detailed chemistry of the molecule. Considerable controversy exists concerning physical properties as

* From the Institute of Microbiology, Rutgers, The State University, New Brunswick, New Jersey. *Received November 14, 1966.* This investigation was supported by a U. S. Public Health Service grant (AI-04572). Some of the findings were presented at the 50th Annual Meeting of the Federation of American Societies for Experimental Biology, Atlantic City, N. J., April 1966.

well. In particular, a wide range of molecular weight has been reported. The present paper reports the preparation of cell wall invertase with a high degree of homogeneity from a particular strain of yeast as well as a description of some of its chemical and physical properties.

Experimental Procedures

Invertase Assay. The assay used was one similar to that recently described by Messer and Dahlquist (1966). Hydrolysis of the substrate, sucrose, was measured by determining the amount of glucose produced using a coupled reaction involving glucose oxidase, peroxidase, and a chromogen (Keston, 1956; McComb and Yushkok, 1958). Samples of enzyme were incubated at 50° together with substrate and Glucostat Special[1] (a commercial preparation of enzymes and chromogen formulated for glucose estimation obtainable from Worthington Biochemical Corp., Freehold, N. J.). The final concentration of substrate was 4.2% and the reaction volume was 2.5 ml. The pH was maintained at 5.0 with sodium acetate buffer of 0.04 ionic strength. After 15-min incubation, the reaction was stopped by the addition of 2.5 ml of 60% (v/v) sulfuric acid and the red color produced was read in a photometer at 520 mμ. By means of an appropriate factor, the readings were converted to invertase units (micromoles of sucrose hydrolyzed per minute at 30°).

Analytical. Total carbohydrate was measured by the phenol–sulfuric acid method of Dubois *et al.* (1956) and nitrogen by a micro-Kjeldahl procedure (Markham, 1942). Glucosamine was determined by the ninhydrin method during amino acid analysis. Initial identification was made by the method of Elson and Morgan (1933). Absorption at 280 mμ or the procedure of Lowry *et al.* (1951) was used for the estimation of protein. Amino acid analyses were performed with a Technicon AutoAnalyzer (Technicon Chromatography Corp., Chauncy, N. Y.) which utilizes principles developed by Spackman *et al.* (1958) and Piez and Morris (1960). Samples for analysis were hydrolyzed in 6 N HCl at 110° for 20 hr[2] in sealed evacuated tubes (Moore and Stein, 1963). Performic acid oxidation for the determination of half-cystine as cysteic acid was performed as described by Moore (1963). Tryptophan was determined spectrophotometrically by the method of Goodwin and Morton (1946). The sulfhydryl content of the enzyme was measured by the procedure of Ellman (1959) with 5,5′-dithiobis-(2-nitrobenzoic acid) as modified by Schramm (1964) or chemically by determination of *S*-carboxymethylcysteine after reaction with iodoacetic acid under conditions similar to those described by Crestfield *et al.* (1963) but without prior reduction of the enzyme. For tests of inhibition by *p*-hydroxymercuribenzoate and other sulfhydryl reagents the enzyme was incubated in the presence of various levels of the inhibitor in 0.04 M acetate buffer of pH 5.0 at room temperature for 20 min and then diluted and assayed as usual. A modification of the method of Laki *et al.* (1954) was used to estimate amide nitrogen. Polyacrylamide gel electrophoresis was performed according to Ornstein and Davis.[3] In certain instances the sample and spacer gels were eliminated and the sample was added by layering under the buffer in the presence of sucrose, as described by Hjerten *et al.* (1965). Localization of invertase activity in the gels was performed according to Eilers *et al.* (1964) and carbohydrate by the periodic acid–Schiff reaction (Keyser, 1964). Immunodiffusion tests were done by the Ouchterlony method (Kabat and Mayer, 1961) with antisera from rabbits injected intramuscularly with purified enzyme in complete Freunds' adjuvant.

Yeast Strains and Culture Conditions. A number of strains of yeast have been examined in these studies as sources of invertase, including a commercial baker's yeast (from Anheuser Busch, Old Bridge, N. J.), *Saccharomyces cerevisiae* strain LK2G12, *Saccharomyces* strain 303-67 isolated by Winge and Roberts (1957) from crosses of *Saccharomyces carlsbergensis*, *Saccharomyces chevalieri*, and *Saccharomyces italicus*, and a mutant (designated FH4C) obtained by ultraviolet irradiation of the 303-67 strain (Symington and Lampen, 1966). Invertase from the mutant strain has been examined in the greatest detail and essentially all the information presented is concerned with this preparation. Strain FH4C was selected for several reasons. The production of invertase by the mutant is not readily repressed by hexose in the medium, and thus it is possible to obtain cells of high specific enzymatic activity at relatively high cell densities. In addition, the invertase is more readily solubilized from this strain by mechanical methods than from a number of other strains which have been tested. Cultures were maintained on Wickerham 1% glucose–agar slants (Wickerham, 1951). For production of invertase, cells were grown in 30 l. in a stainless-steel fermentor, using a yeast extract–peptone medium (0.3% yeast extract and 0.5% Bactopeptone, both from Difco Laboratories, Detroit, Mich.) with an initial pH of 6.8 and with 1% raffinose as carbon source. A 5% inoculum was used. After 19 hr at 30° with moderate aeration, the cells were harvested by centrifugation and stored at −17° until used. Approximately 250 g of cell paste was obtained per run.

Sedimentation Analysis. A Beckman–Spinco Model E ultracentrifuge equipped with a constant temperature device (RTIC unit) and both schlieren and interferometric optics was used. Plates were read with the aid of a Nikon shadowgraph. Molecular weights were

[1] Glucostat Special is used because the regular grade of Glucostat contains enzymes which hydrolyze sucrose, resulting in a high assay blank.

[2] Several runs were performed with samples hydrolyzed for 70 hr to determine correction factors for destruction during hydrolysis. The correction factors obtained for the 20-hr hydrolysis were 0.93 for serine, 0.98 for tyrosine and threonine, and 0.428 for glucosamine.

[3] Print available from Eastman Chemical Co., Rochester, N. Y.

determined by the high-speed equilibrium method of Yphantis (1964). A partial specific volume of 0.683 was used in various calculations. This value was obtained by averaging the value for the protein moiety (calculated from the amino acid composition by the method of Cohn and Edsall (1943) and the value of 0.65 reported for yeast mannan by Korn and Northcote, 1960). For sedimentation velocity studies the lyophilized protein was dissolved in the buffer and dialyzed overnight against the same buffer. Sedimentation in density gradients was performed by procedures similar to those described by Martin and Ames (1961).

Enzyme Purification. All steps were carried out at 0–5° unless specified otherwise. In a typical experiment, 169 g of frozen cell paste was thawed and suspended in an equal weight of 0.01 M EDTA, pH 7.0, and passed once through a French pressure cell (American Instrument Co., Silver Spring, Md.) in 40-ml portions. The combined crude extracts were centrifuged at 30,000*g* for 15 min, and the pellet was resuspended in 100 ml of 0.01 M phosphate buffer of pH 6.5 and centrifuged again. To the combined supernatants was added streptomycin sulfate (final concentration of 10 mg/ml) to remove ribosomes and nucleic acid. After 1 hr in an ice bath, the precipitate was removed by centrifugation. The supernatant solution was adjusted to pH 5.0 with 30% acetic acid and stored overnight at 0°. The solution was then heated rapidly over a flame to 50°, maintained at this temperature for 30 min, cooled rapidly, and centrifuged. To the supernatant solution was added an equal volume of 95% ethanol (cooled to −20°) over a period of about 5 min. The temperature was maintained at 0–5°. After 10 min the suspension was centrifuged at 10,000*g* for 10 min. The pellet which contained the activity was dissolved in 0.1 M sodium acetate buffer, pH 5.0. The volume at this stage was about 40 ml. To this solution was added 0.56 g of ammonium sulfate/ml and the solution was stored overnight at 0–5°. The suspension was then centrifuged at 30,000*g* for 30 min and the supernatant, which contained the enzyme, was desalted on a 3.7 × 62 cm column of Sephadex G-25 (Pharmacia, Inc., Piscataway, N. J.), equilibrated with distilled water. For the next step which was chromatography on SE-Sephadex the solution was adjusted to pH 3.65 and a sodium ion concentration of 0.01 M by the addition of 0.5 M sodium citrate buffer of pH 3.65 and sufficient 1 M citric acid to bring the pH to the proper value. This solution was then chromatographed on a 2.5 × 22.5 cm column of SE-Sephadex C-50 (40–120-μ particle size; 2.3 mequiv/g). The starting buffer was sodium citrate of pH 3.65 (0.01 M in Na^+) and fractionation proceeded with a linear gradient of sodium chloride in starting buffer from 0 to 0.3 M. The double-chambered gradient apparatus of Parr (1954) was used with a total of 250 ml in each chamber. Fractions of 10 ml were collected at a flow rate of about 40 ml/hr. The fractions of highest specific activity were pooled and after desalting on Sephadex G-25 were further chromatographed on a 2 × 20.5 cm column of DEAE-Sephadex A-50 (40–120-μ particle size; 3.5 mequiv/g). The starting buffer was sodium phosphate (0.01 M in phosphate) of pH 6.5. The sample was brought to the starting pH and ionic strength with 0.5 M phosphate of pH 6.5. After washing the sample into the column a linear gradient of sodium chloride from 0 to 0.5 M in starting buffer was applied to the column. Each gradient chamber contained 250 ml of solution. After elution of the enzyme, the tubes of highest specific enzymatic activity were pooled, desalted on Sephadex G-25, and lyophilized.

Results

Purification of the Enzyme. A summary of the purification procedure is presented in Table I. The over-all purification is 113-fold with a recovery of about 22% of the starting enzymatic activity. A key step in the purification involves the use of ammonium sulfate which precipitates a great deal of inactive protein while most of the invertase remains soluble. The two final steps are particularly useful in removing large amounts of polysaccharide. Figure 1 illustrates a typical separation on SE-Sephadex. The small amount of enzyme of low specific activity which did not adsorb

TABLE I: Purification of Yeast Invertase from *Saccharomyces* Strain FH4C.

Fraction	Vol. (ml)	Protein (mg)	Invertase (units)	Sp Act. (units/mg of protein)
I, yeast suspension	318	10,400	254,000	24
II, crude juice	350	6,620	212,000	32
III, heat 50°, pH 5.0	335	3,450	208,000	60
IV, ethyl alcohol 50%	40	1,030	157,000	152
V, ammonium sulfate	110	47	103,000	2190
VI, SE- and DEAE-Sephadex	125[a]	21	56,600	2700

[a] Lyophilization of the desalted material yielded 37 mg of enzyme.

to the column was discarded. The fractions of highest specific activity (fractions 29–40) were pooled and chromatographed on DEAE-Sephadex A-50 (shown in Figure 2). The material in the major enzymatically active peak from this run (fractions 74–87) was pooled and, after desalting on Sephadex G-25, was lyophilized. Almost all the data reported were derived from studies on this preparation, or material prepared in a similar fashion and in a similar state of purity.

Properties of the Purified Enzyme Preparations. Invertase prepared as just described has a specific activity of 2700–3000 μmoles of sucrose hydrolyzed per min per mg of protein at 30°.[4] It is most stable at about pH 5 and can be stored for months in the frozen or lyophilized state. Electrophoresis on cellulose acetate at pH values between 2 and 12 reveals a single protein band which also contains the enzymatic activity. Likewise, only a single band of protein is observed after electrophoresis on polyacrylamide gel. This result is shown in Figure 3. Analyses of several other fractions are included for comparison. It might be noted that a much sharper band is obtained at pH 6.4 where the enzymatic activity is relatively stable than in the standard gel of Ornstein and Davis[3] which is run at pH 9.5. When the gels are stained for enzymatic activity only a single band is obtained coincident with the protein band. Staining for carbohydrate reveals a very faint band of material at the origin in addition to the main carbohydrate band which also is coincident with both protein and enzymatic activity.

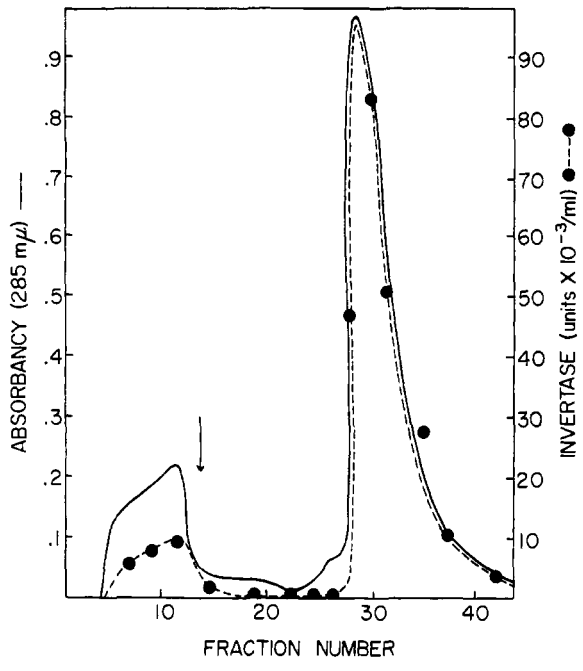

FIGURE 1: Chromatography of yeast invertase on SE-Sephadex. Experimental conditions are described in the text. The arrow marks the point of application of the elution gradient.

The purified enzyme contains about 50% carbohydrate. This is mannan with about 3% glucosamine. No glucose is detectable after acid hydrolysis. The carbohydrate fractionates together with the protein through a wide variety of procedures including adsorption to materials such as bentonite and calcium phosphate gel, column chromatography on SE- and DEAE-Sephadex, electrophoresis on cellulose acetate, polyacrylamide, and starch, and gel filtration through Sephadex, polyacrylamide, or agar. Relatively drastic procedures including gel filtration in the presence of 8 M urea or partitioning between phenol and water were without effect. In addition the enzyme remains soluble in a number of protein precipitants such as saturated ammonium sulfate, picric acid, and trichloroacetic acid. Attempts to remove the carbohydrate by enzymatic digestion with an α-mannosidase from jack bean meal (Li, 1966) resulted in the release of only a few per cent of the carbohydrate as mannose. Oligosaccharides were not detected. Several attempts to isolate an organism by enrichment techniques from soil samples which would produce a mannanase were only partially successful. Only a very limited hydrolysis of mannan was ever obtained.

The ultraviolet absorption spectrum of invertase has a maximum of 280 mμ. The ratio of absorbance at 280:260 mμ was 1.8, indicating a lack of substantial contamination by nucleic acid. The specific extinction coefficient ($E^{1\%}_{280}$) based upon the amount of protein present (calculated as N × 6.27) was found to be 23.0.

Sedimentation velocity experiments (Figure 4) reveal the presence of a major peak whose sedimentation coefficient varies as a function of concentration according to the relation, $s_{20,w} = 10.4(1 - 0.014c)$, where c is in milligrams per milliliter. In addition, there is a minor peak of higher sedimentation coefficient. Analysis of density gradients (Figure 5) shows that both of these components are enzymatically active. The relative proportion of the two components is independent of the initial concentration of the enzyme; also when material from the main peak is analyzed again only a single component is observed. This is in marked contrast to the behavior of the enzyme from the LK2G12 strain which aggregates readily and reversibly under the same conditions. A high-speed sedimentation equilibrium run was performed at an enzyme concentration of 0.25%. A molecular weight calculated from the limiting slope of ln c *vs.* x^2 toward the top of the cell gave a value of 270,000 ± 11,000 daltons. Calculations of a frictional ratio for the molecule gave a value of 1.7. Attempts to measure a molecular weight for the enzyme by gel filtration on columns of Sephadex G-200 were unsuccessful since the enzyme did not penetrate the gel but had an elution volume which was equivalent to the void volume of the column.

The amino acid composition of the enzyme is shown

[4] Previously reported erroneously as 32,000 μmoles/min (Neumann and Lampen, 1966).

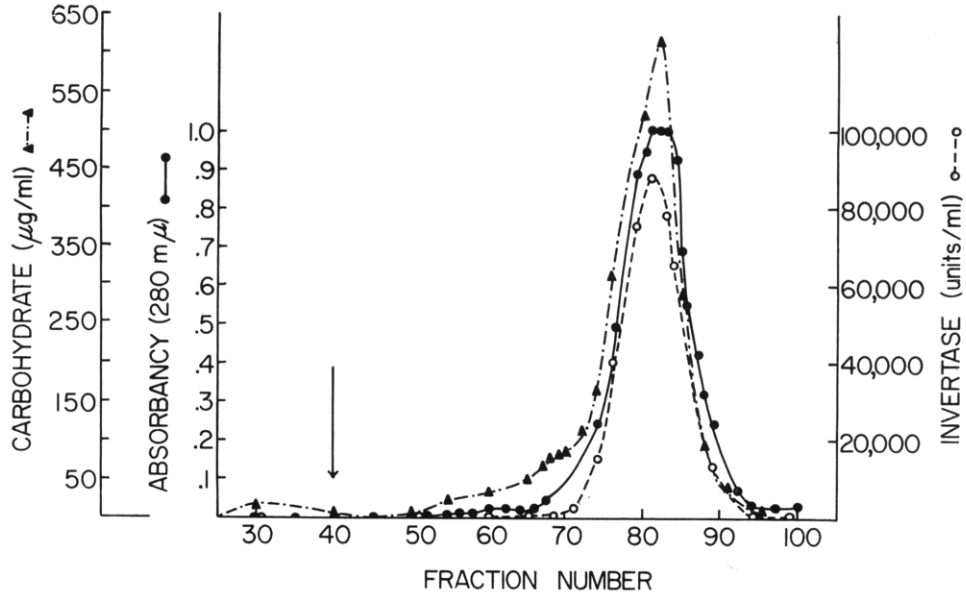

FIGURE 2: Chromatography of yeast invertase on DEAE-Sephadex. Experimental conditions are described in the text. Each fraction contained 10 ml. The arrow marks the point of application of the elution gradient.

in Table II. Very similar values were obtained for invertase from strain LK2G12. A value of 5.2 residues of half-cystine was obtained by measurement as cysteic acid. The sulfhydryl content of the enzyme as measured by spectrophotometric titration with 5,5′-dithiobis(2-nitrobenzoic acid) in the presence of 1% sodium dodecyl sulfate gave a value of 0.80 SH group/mole. The presence of 8 M urea raised this value to two to three SH groups/mole. No reaction was observed in the absence of a denaturing agent. The recovery of car-

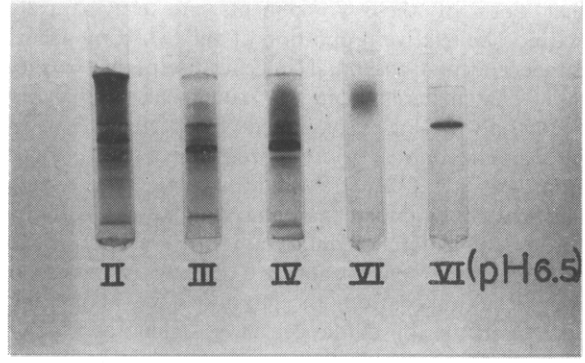

FIGURE 3: Polyacrylamide gel electrophoresis of yeast invertase in various stages of purification. The Roman numerals correspond to the various fractions described in Table I. The gels were run under the conditions described by Ornstein and Davis[3] (running gel is pH 9.5) except for the last gel on the right which was run at pH 6.5. The cathode is toward the top.

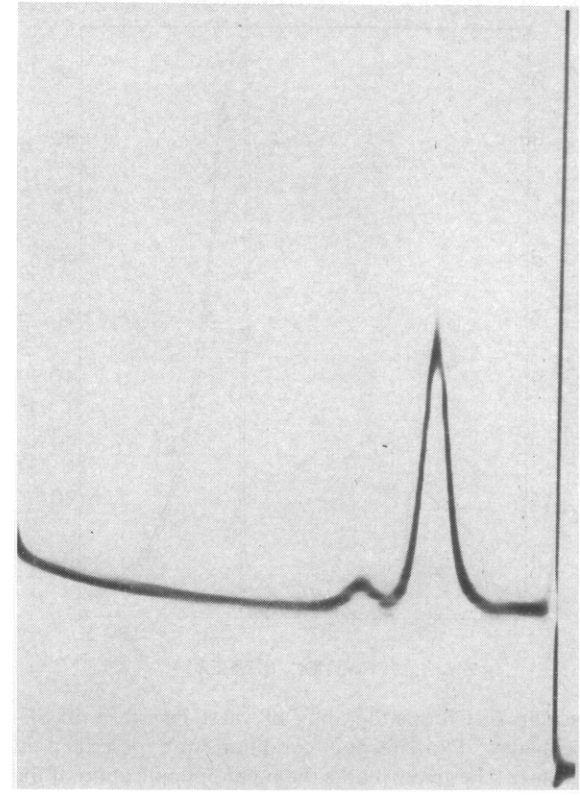

FIGURE 4: Sedimentation velocity schlieren pattern of yeast invertase at 20° approximately 15 min after the ultracentrifuge reached the top speed of 59,780 rpm. The enzyme was in 0.2 M NaCl–0.01 M NaAc buffer of pH 5.4. Sedimentation is from right to left.

TABLE II: Amino Acid Composition of Yeast Invertase (*Saccharomyces* strain FH4C).[a]

Amino Acid	Residues Calcd for Mol Wt of 270,000 (50% carbohydrate)	
	Found	Expressed to Nearest Integer
Glycine	71.3	71
Alanine	68.2	68
Serine	114	114
Threonine	83.6	84
Proline	64.7	65
Valine	68.7	69
Isoleucine[b]	40.2	40
Leucine	83.1	83
Phenylalanine	79.6	80
Tyrosine	64.6	65
Tryptophan	33.2	33
Half-cystine[c]	5.24	5
Methionine	20.6	21
Aspartic acid	178	178
Glutamic acid	115	115
Amide N	150	150
Arginine	27.1	27
Histidine	15.8	16
Lysine	59.3	60
Glucosamine	38.3	38

[a] The amino acids, glucosamine, and ammonia recovered accounted for 96% of the nitrogen of the sample. The values shown represent in most cases the average of four determinations. [b] The value obtained after 70-hr hydrolysis was used. [c] Measured as cysteic acid.

boxymethylcysteine after reaction with iodoacetate in the presence of 8 M urea was 2.80 residues/mole.

p-Mercuribenzoate inhibited the enzyme only at relatively high ratios of inhibitor to enzyme. Thus at an enzyme concentration of 5×10^{-7} M (0.12 mg/ml), 0, 34, and 66% inhibition was obtained at inhibitor concentrations of 10^{-5}, 10^{-4}, and 10^{-3} M, respectively. Other sulfhydryl reagents such as iodoacetamide, iodoacetic acid, and *N*-ethylmaleimide did not inhibit the enzyme.

Immunodiffusion studies with a purified preparation of invertase from *S. cerevisiae* LK2G12 using the Ouchterlony technique are illustrated in Figure 6. Only a single precipitin band was observed when the invertase was allowed to diffuse against the homologous antiserum. A test with the FH4C enzyme against the same antiserum also resulted in the appearance of a single band. Yeast mannan did not produce a detectable precipitate.

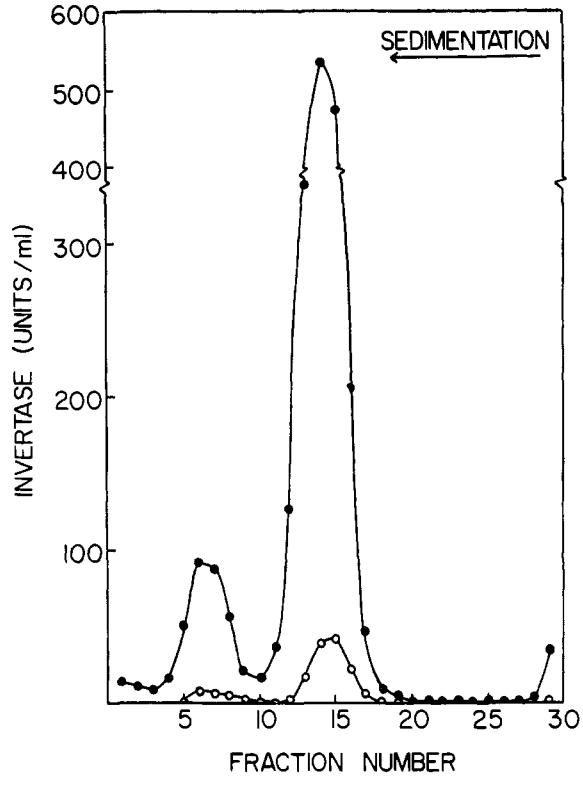

FIGURE 5: Centrifugation of FH4C invertase in a density gradient of 0–20% NaCl in 0.01 M acetate, pH 5.0. Aliquots (100 μl) were layered on top of a 5.3-ml gradient and centrifuged 5 hr at 47,000 rpm fractions of 11 drops each were collected. Initial concentration of enzyme: ●, 100 μg/ml; ○, 10 μg/ml.

Discussion

Many of the earlier attempts to isolate invertase from yeast in pure form have included an initial autolytic step to release the enzyme from the cells. We have used mechanical breakage in order to avoid as much as possible degradation of the enzyme during or prior to isolation. A number of reports in the literature suggest that invertase may exist in more than one form. Thus, Cabib (1952) revealed the presence of two enzymatically active zones after paper chromatography of partially purified yeast extracts. Two electrophoretically distinct enzymatically active components were observed by Yamamoto (1957). Recent observations by Kenkare *et al.* (1964) have shown that proteolytic activity in yeast can cause the conversion of hexokinase into as many as six distinct chromatographic forms. Hoshino and Momose (1966) demonstrated an interconversion of two forms of invertase by extended incubation of the extracts. Thus, until demonstrated otherwise, it seems best to avoid autolytic procedures.

It would be desirable to compare the specific activity of our preparation with that of previous workers. Within the last 10 years, preparations of high specific activity have been described by Yamamoto *et al.*

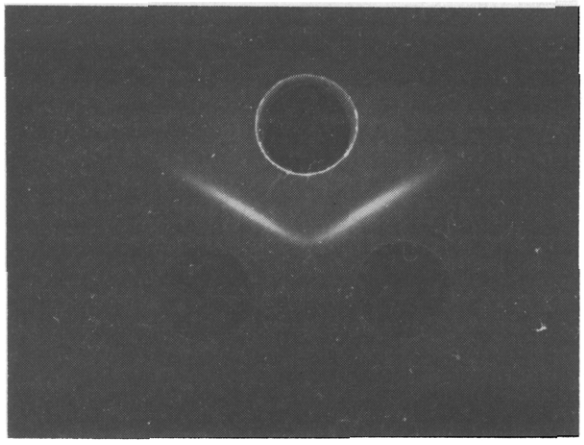

FIGURE 6: Analysis of yeast invertase as an antigen by immunodiffusion. Well at top contained rabbit antiserum to invertase (LK2G12 strain). The well at the bottom right contained the homologous antigen and the well at the left, invertase from the FH4C strain of yeast.

(1957), Anderson (1960), and more recently by Myrbäck and Schilling (1965). Enzymatic activity has generally been expressed in terms of A units (described by Fischer and Kôhtes, 1951). One A unit is the amount of enzyme which catalyzes the formation of 1 mg of reducing sugar from sucrose in 3 min at 20°. Although our assay has been run under different conditions a calculation based upon a Q_{10} of 1.65 (M. F. Islam and J. O. Lampen, 1963, unpublished data) indicates that an A unit is equivalent to 1.53 μmoles of sucrose hydrolyzed/min at 30°. Thus, our preparation would have a specific activity of 11,000–12,000 A units/mg of N. This may be compared with values of 11,000–14,300 for the most active chromatographic fractions of Myrbäck and Schilling. Anderson reported that his preparation had an activity equivalent to 22,000 A units/mg of N at 25° (which when corrected for the temperature difference becomes approximately 18,000). It is not possible to comment further upon the nature of the difference in specific activities of the various preparations without further information upon the properties of these preparations.

Most of the invertase preparations described in the past have had varying amounts of carbohydrate associated with them. Sumner and O'Kane (1947) clearly indicated that they thought the enzyme was a polysaccharide–protein compound. Our results confirm this idea. We have been able to isolate glycopeptides by gel filtration after extensive proteolytic digestion of the enzyme. These fractions contain predominantly the amino acids serine, threonine, and aspartic acid as well as glucosamine and mannan (Neumann and Lampen, 1966). In view of this it is difficult to explain the ability of Fischer and Kôhtes (1951) to adsorb the enzyme onto bentonite without adsorbing the polysaccharide, as well as the reports of earlier workers such as Salkowski (1900) and Willstaetter and Schneider (1924; *cf.* Neuberg and Roberts, 1946) indicating a separation of enzyme and polysaccharide. One possibility may lie in the initial method of extracting the enzyme from the cells. It seems not at all unlikely that under the proper conditions polysaccharide originally covalently linked to the enzyme may be split from the protein moiety by an autolytic enzyme present in the yeast cell. Our method of preparation utilizing mechanical breakage of cells would help to avoid autolytic breakdown and tend to result in isolation of the intact protein–polysaccharide compound. The picture is somewhat confused by the fact that invertase is associated with a large amount of loosely bound carbohydrate in the early stages of purification, which is subsequently removed. In addition, the enzyme as isolated appears to consist of a mixture of molecules with slightly varying amounts of carbohydrate (note the slight displacement of the polysaccharide peak with respect to the protein peak in Figure 2). Indeed, it should be emphasized that invertase can probably be isolated with widely varying amounts of carbohydrate and that the procedure we have used favors the isolation of a particular fraction of invertase with a limited range of carbohydrate content.

The molecular weight of our preparation (135,000 for the protein moiety) is in reasonably good agreement with the value of 123,000 obtained by Pollard *et al.* (1952) using ionizing radiations as a probe if one assumes that what is in fact being measured by this procedure is the protein portion of the complex. Anderson (1960) reported a molecular weight of 116,000 for a preparation with a nitrogen content of 11.02%. This would be a molecular weight of 80,000 for the protein moiety assuming a N content of 16%. Moelwyn-Hughes (1933) suggested a molecular weight of 50,000 based upon diffusion measurements. According to Dieu (1946), a preparation which he examined contained an enzymatically active component with a molecular weight of 3500 which was accompanied by an inactive material with a molecular weight of 100,000. Thus, there is a considerable range in the magnitude of the proposed molecular weight of yeast invertase. In the present case the molecular weight proposed is near the top of the range, but it is not impossible that our preparation may actually represent an aggregate of several smaller active subunits. However, there is no concrete evidence to suggest this.

Metzenberg (1964) has recently demonstrated the presence of active subunits for the invertase of *Neurospora*. In this connection it might be noted that Hoshino and Momose (1966) reported a sedimentation coefficient for their invertase preparation of 4.2 S, a value considerably lower than the 10.4 S reported in this paper. Work is currently in progress in an attempt to resolve some of these questions.

The failure of the enzyme to penetrate Sephadex G-200 was somewhat surprising initially. This behavior may be related to carbohydrate content and the somewhat higher frictional ratio as compared to most globu-

lar proteins. Whitaker (1963) observed that ovomucoid, a protein with a molecular weight of 27,000 and a carbohydrate content of 27%, behaved as a protein with a molecular weight of 38,000–45,000 by gel filtration on Sephadex.

The possible presence of sulfhydryl groups in the enzyme was of considerable interest in view of the suggestion by Myrbäck and Willstaedt (1958) that iodine may inactivate invertase by oxidation of sulfhydryl groups. The analytical results presented in this paper indicate the presence of three SH groups/mole which are accessible to iodoacetate in 8 M urea. In sodium dodecyl sulfate only one of these groups is able to react with 5,5′-dithiobis(2-nitrobenzoic acid). The failure of a number of sulfhydryl reagents to inhibit the enzyme suggests that the sulfhydryl groups do not participate directly in the catalytic process. The remaining two residues of half-cystine may exist as a disulfide bridge or as sulfhydryl groups which are inaccessible under the conditions used.

Acknowledgment

The authors are grateful to L. McDaniel and E. Bailey for assistance in the production of yeast on a large scale, Miss M. Cora-Figueroa for help with immunization techniques, and Mrs. Y. Kwon and Mrs. L. Schnable for technical assistance.

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