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PARTIAL PURIFICATION, CHARACTERIZATION AND LOCALIZATION OF THE MEMBRANE-ASSOCIATED INVERTASE OF YEAST

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Summary

The membrane-associated isozyme of invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26) — precursor of the external glycoprotein invertase (Babczinski, P. and Tanner, W. (1978) Biochim. Biophys. Acta 538, 426—434) — has been purified 60-fold from deoxycholate extracts of derepressed yeast cells. The partially purified enzyme exhibits considerable stability as a salt-free lyophilized powder. Its molecular weight, in this precursor form, has been determined by sodium dodecyl sulphate (SDS) gel electrophoresis to be 180 000 daltons. This correlates well with the presence of only the inner core carbohydrate parts of the external invertase.

The enzyme can be split completely by treatment with endo-β-*N*-acetylglucosaminidase H from *Streptomyces griseus*, demonstrating the presence of a di-*N*-acetylchitobiosyl-asparagine linkage. The proteinaceous split product is still active and has a molecular weight of approx. 120 000. The enzyme cannot be transferred into a supernatant fraction upon osmotic shock treatment of yeast membrane vesicles, indicating that it is strictly membrane-bound.

After separation of yeast membranes on a sucrose density gradient, precursor invertase is predominantly associated with two gradient membrane fractions which most probably represent rough and smooth endoplasmic reticulum.

Introduction

Most proteins which are translocated through cellular membranes, either as membrane or secretory proteins, have been found to be glycosylated [1,2]. It has been shown that the glycosylating enzymes are membrane-bound and that

dolichol derivatives of sugars are intermediate substrates for some of these enzymes (Ref. 3 and references therein). Also, in yeast cells, a number of glycoproteins (partially as glycoenzymes) are either secreted into the cell wall space [4—11] and, in the case of protoplasts, into the medium [12] or are localized in the yeast vacuole [13—15].

Yeast external invertase is a mannoprotein containing 50% mannose and 3% glucosamine by weight [5], both of which are linked to asparagine through *N*-acetylglucosamine [16]. Immunochemical studies have indicated that the carbohydrate structure of *Saccharomyces cerevisiae* invertase mannan is very similar to that of the cell wall mannoprotein [17].

The structure [18] and biosynthesis of yeast mannoproteins (see references in Ref. 19) have been investigated intensively. In the *N*-glycosidically linked carbohydrate part of the molecule, an inner core region of two *N*-acetylglucosamine and approx. 12 to 14 mannose residues can be distinguished from an outer chain of approx. 100 mannoses [18,20,21]. Evidence has been obtained [22,23] that a dolichyl diphosphate oligosaccharide acts as an intermediate in the synthesis of the inner core of yeast mannoproteins.

In a previous publication [19], a membrane-bound precursor for the external invertase in yeast has been described. From its molecular weight and, mainly, from inhibition experiments with tunicamycin it has been assumed that this partially glycosylated invertase isoenzyme most probably consists of the complete protein moiety and of the inner core carbohydrate parts.

In the present study, the precursor invertase was partially purified and characterized. It seems to be located preferentially in membranes of the endoplasmic reticulum. By the use of endo-β-*N*-acetylglucosaminidase H, a direct proof for the presence, in this invertase species, of a carbohydrate moiety connected via a di-*N*-acetylchitobiosyl-asparaginyl linkage has been obtained.

Materials and Methods

Chemicals and instruments. Commercially available analytical grade chemicals were used throughout this study. Calibration proteins for SDS gel electrophoresis and invertase were obtained from Boehringer (Mannheim, F.R.G.), endo-β-*N*-acetylglucosaminidase H from Miles (Maidenhead, Berks, U.K.). β-D-Glucose dehydrogenase (β-D-glucose:NAD(P)$^+$ 1-oxidoreductase, EC 1.1.1.46) and mutarotase (aldolase 1-epimerase, EC 5.1.3.3) were kindly supplied by Dr. H. Lang, Merck (Darmstadt, F.R.G.).

Authentic external invertase was prepared (i) from commercial yeast invertase by an abbreviated version of the method of Neumann and Lampen [5] according to Ref. 24 and (ii) by treating yeast cells with 2-mercaptoethanol according to Ref. 25.

Spectrophotometric determinations were conducted in a Gilford Spectrophotometer 240. Ultrafiltration was performed in an Amicon apparatus (Oosterhout, Holland), and ultracentrifugation runs were carried out in a Spinco L2-65B (Beckman, München, F.R.G.).

Organism and culture. The haploid yeast strain *S. cerevisiae* X 2180 a (supplied by Professor C.E. Ballou, Berkeley, CA, U.S.A.) was cultivated as described [19]. For preparative experiments, 5-l flasks containing 1.5 l of medium were used.

Homogenization of cells. Cells were either broken in a Bio X-press at −25 °C as described [19] or disrupted either in a glass bead homogenizer or in a French pressure cell, both at 4 °C. After removal of whole cells and debris (3000 × *g*, 10 min), membranes were spun down at 48 000 × *g* for 30 min and washed once.

Extraction of invertase. Whole yeast cells were incubated with 1% deoxycholate (w/v, 1 ml/g wet weight) and extracted overnight at room temperature. Membrane preparations were extracted with deoxycholate in the same way (50 µl/mg protein).

Polyacrylamide gel electrophoresis. Separation of invertase species was carried out as described [19], using 5.6% cylindrical gels (pH 7.5; 0.25% SDS), samples contained 0.25% SDS (w/v), and electrophoresis was performed at 4 °C. The previously used staining procedure had to be changed because of the unavailability of glucose oxidase with low enough sucrase contamination. The newly applied method will be published elsewhere in more detail [26]. Gels were rinsed well with distilled water and stained overnight by placing in 0.12 M sodium phosphate buffer (pH 7.6) containing 50 units of 200 U/mg β-glucose dehydrogenase, one unit of 3.9 U/mg mutarotase, 1.3 mM NAD^+, 0.4 mM phenazine methosulphate, 0.6 mM iodonitrotetrazolium chloride, 0.15 M sodium chloride, and 12 mM sucrose. Gels were constantly moved in a swivel shaker. Stained areas were recorded in a gel scanner at 492 nm. The test system was shown to be linear in respect to time and amount of protein applied onto the gel. Cofactors were present in saturating quantities.

Molecular weight estimation. SDS gel electrophoresis was carried out as described above, except that slab gels were used (40 mA) and the electrode buffer contained 0.1% SDS (w/v). Calibration was achieved with RNA polymerase, α-chain (39 000 dalton), β-chain (155 000 dalton), β′-chain (165 000 dalton), bovine serum albumin (68 000 dalton), and trypsin inhibitor from soy bean (21 500 dalton). The calibration samples were pretreated exactly as the invertase samples, i.e. 0.25% SDS was added without heat treatment. Location of invertase activity was performed as in the case of cylindrical gels. Calibration proteins were stained with Coomassie blue.

Estimation of invertase activity. Invertase activity in liquid samples was determined by using basic 3,5-dinitrosalicylate reagent as described [19]. Quantitative determination of relative invertase activity on gels was carried out by measuring the area below the peaks obtained by scanning the gels after activity staining, which has already been shown to be a measure of invertase activity [26]. In order to determine absolute values of invertase activity in distinct gel bands, gels were cut into 2-mm slices which were then assayed in toto for invertase activity with the salicylate reagent. By this means, a correlation can be drawn between the peak area after gel scanning and the actual amount of invertase activity present in a gel band. One unit of invertase is defined as 1 µmol sucrose hydrolyzed per min at 37 °C.

Purification of invertase. Whole derepressed yeast cells (41 g) were extracted overnight with 1% deoxycholate (w/v) at room temperature. After centrifugation at 100 000 × *g*, the deoxycholate extract was dialyzed against 50 mM sodium acetate (pH 5.0), 7 µM phenylmethylsulphonyl fluoride, centrifuged again and concentrated by ultrafiltration over a PM 30 membrane. The resulting extract was passed through a DEAE-Sephadex A-50 column (1.7 × 20

cm) which had been equilibrated with the same buffer. A linear NaCl gradient from 0.1 to 0.7 M, containing 50 mM sodium acetate, pH 5.0, and 7 μM phenylmethylsulphonyl fluoride with a total volume of 600 ml, was applied. Fractions of 1.1 ml were collected at a flow rate of 20 ml/h. Fractions with invertase activity were pooled and concentrated by ultrafiltration; simultaneously the column buffer present in the various samples was diluted with distilled water. For further concentration, a stream of nitrogen was used to aspirate the samples to suitable volumes.

The main invertase I and invertase II containing pools were further purified by preparative gel electrophoresis. The gel system was the same as that used for the analytical procedure, except that larger glass tubes (1.4 × 6 cm) were employed. After separation and freezing, slices corresponding to stained areas on parallel gels were cut out and crushed into small pieces. After mixing with new gel solutions, the gel pieces were embedded into a new preparative gel occupying only two thirds of the tube. The residual space was filled with anode buffer and covered with a dialysis membrane which was fastened to the tube by a rubber ring. After a second electrophoretic run, the purified material was recovered from the buffer in the bottom part of the tube by dialysis and lyophilization.

All purification steps were checked qualitatively by analytical gel electrophoresis (glucose dehydrogenase staining) and quantitatively by testing either liquid samples or cut gel slices with salicylate reagent.

Treatment with endoglycosidase H. Purified lyophilized invertase samples were treated at room temperature for 24 h with 0.01 unit of endo-β-*N*-acetylglycosaminidase H from *Streptomyces griseus* in an 0.1 ml reaction volume containing 0.1 M sodium citrate, pH 5.0. Reaction products were analyzed either on cylindrical gels (glucose dehydrogenase staining, see above) or on 5—15% acrylamide SDS slab gels (Coomassie blue staining; [27]).

Density gradient centrifugation. Membranes were produced from derepressed 16 g yeast cells using a glass bead homogenizer. For breakage, cells were taken up (0.6 ml/g) in buffer 1 (0.1 M Tris-HCl, pH 7.2; 50 mM KCl; 10 mM $MgCl_2$; 0.5 M sorbitol) or in buffer 2 (containing 1 mM EDTA instead of $MgCl_2$). For centrifugation, the procedure of Marriott and Tanner [28] was used. The linear sucrose gradients (20—55%, w/w) were prepared in either buffer 1 or 2 (total volume 34 ml). For separation, 3 ml samples (after centrifugation at 3000 × *g* for 10 min) were loaded on top of the gradient and overlayered with 2 ml of buffer 1 or 2, respectively. Gradients were centrifuged for 3.5 h in a SW 27 rotor at 100 000 × *g*. Bands were recovered with a Pasteur pipet, diluted, centrifuged at 100 000 × *g* for 30 min, washed with the respective buffer and extracted with deoxycholate.

Osmotic breakage of membrane vesicles. Derepressed yeast cells (4.4 g wet weight) were taken up in 50 mM sodium acetate, pH 5.0, containing 0.5 M sorbitol and disrupted in a French pressure cell. Cells and debris were removed by centrifugation at 3000 × *g* for 10 min, and a membrane pellet was obtained after centrifugation at 48 000 × *g* for 20 min. This pellet was either osmotically shocked by treatment with distilled water or isotonically treated with starting buffer and was then left standing at room temperature for 1 h.

Other analytical procedures. As a possible marker enzyme for endoplasmic

reticulum membranes, NADPH:cytochrome *c* oxidoreductase was assayed by measuring the reduction of cytochrome *c* at 550 nm [29]. Protein was determined according to the method of Lowry [30].

Results

Purification of invertase I and II

According to decreasing molecular weight, external invertase is designated as invertase I, membrane-associated precursor as invertase II, and internal invertase as invertase III.

Table I summarizes the purification of invertase II from derepressed *S. cerevisiae* X 2180 a cells. Extracts and column fractions have been analyzed by gel electrophoresis (glucose dehydrogenase staining) and quantitated by the reducing sugar test with the salicylate reagent. Deoxycholate treatment yields an extract which produces, besides the invertases, a band on gels in the activity staining procedure which is not dependent on the presence of sucrose in the staining mixture and which runs similar to invertase II. In the salicylate reaction this problem does not arise, therefore quantitation is still possible. This interfering substance is eliminated upon DEAE-Sephadex chromatography.

In the ion-exchange chromatography step, three invertase-active peaks are obtained. Internal invertase can be separated quantitatively (not earlier than 0.2 M NaCl), whereas invertases I and II are eluted as partially overlapping peaks. These two isozymes can be purified to homogeneity (in terms of invertase species) by preparative SDS gel electrophoresis, yielding salt-free lyophilized materials. This purification step was also necessary because large amounts of contaminating protein which were still present in the samples after ion-exchange chromatography and which would have interfered with subsequent experiments (splitting with endoglycosidase H) could be removed. In spite of some contaminating proteins and the low yield in invertases I (not shown) and II (3.3%; largely due to the inability to elute the material from the gel), it was possible to use these materials for further investigations; each respective invertase preparation was free of contamination from other invertase species. The reason for the apparent rise in total activity upon ion-exchange chromatography is unknown.

TABLE I

PURIFICATION OF INVERTASE II FROM X 2180 a DEREPRESSED YEAST CELLS

Cells were extracted with 1% deoxycholate. The extract was loaded onto a DEAE-Sephadex column which was developed in a linear NaCl gradient. Invertase II-containing fractions were pooled, deionized, concentrated by ultrafiltration and finally reduced to a small volume using a stream of nitrogen. The sample was further purified on preparative SDS gels. Slices corresponding to parallel stained gels were cut out and the invertase II-containing fraction was recovered by preparative SDS gel electrophoresis. Data represent a typical experiment.

Purification step	Volume (ml)	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Purification (-fold)	Yield (%)
Deoxycholate extract	47	239.7	4.84	0.02	1	100
DEAE-Sephadex A-50 column chromatography	2.3	11.3	7.2	0.64	32	149
Preparative SDS-gel electrophoresis	0.3	0.13	0.16	1.26	63	3.3

Stability of invertase isozymes

Both invertase I and II species (aqueous solutions) lose only very little activity upon storage for 6 days either at room temperature (1 mM NaN_3), in ice or at −20°C. Freezing in 3 M glycerol for 10 days reduces activity to 47%, whereas freezing in 0.25 M glycine causes only approx. 19 or 16% of invertase II or I activity to be lost, respectively.

Regarding the amount of invertase II solubilized from membranes with 1% deoxycholate as 100%, only 52% of this activity is obtained with 0.5% deoxycholate. Higher concentrations seem to reduce enzyme activity. It was also observed that fresh detergent solutions are more active in terms of solubilization than old ones. In both cases, short extraction times (3 h) are not sufficient to solubilize invertase II from yeast membranes appreciably. On the other hand, deoxycholate extracts show loss of invertase activity upon prolonged standing. Enzyme activity seems to be unaffected by 0.25% SDS (during gel electrophoresis).

Revised molecular weight of invertase isozymes

A refined gel electrophoresis system has been developed to separate invertase species and to prepare simultaneously a calibration curve for molecular weight estimations. Using 5.6% acrylamide slab gels, the molecular weight of invertase II was redetermined to lie between 176 000 and 183 000 (this is in contrast to the value obtained earlier [19]). This discrepancy may have arisen due to a different treatment of the calibration proteins, as compared to the invertase samples, before electrophoresis. Invertase I was found to have a molecular weight of 235 000—245 000 daltons, using 4% acrylamide gels. If invertase I was analyzed by slab gel electrophoresis according to Ref. 27, a major protein band appeared in the 120 000 dalton range. The same result was achieved with authentic external invertase (Boehringer) or with 2-mercaptoethanol-released invertase from whole yeast cells. A molecular weight of 135 000 daltons has been reported for internal invertase [31]. In the gel system used here, a molecular weight of 65 000—75 000 daltons has been determined, which indicates that internal invertase, which is not a glycoprotein, might dissociate during SDS gel electrophoresis.

Treatment of invertase II with endo-β-N-acetylglucosaminidase H

Fig. 1 (lower part) shows that invertase II is completely split by the endoglycosidase into one split product which is not identical in size to internal invertase III. Recovery of enzymatic activity is almost quantitative. Out of four experiments, an average molecular weight for the split product has been determined to be approx. 120 000.

The experiment clearly indicates that invertase II is a glycoprotein with a carbohydrate part attached via an asparaginyl-*N*-acetylglucosamine link. Furthermore, carbohydrate obviously can be removed from invertase II without loss in invertase activity, as has been observed for external invertase [32].

To test whether the split product of invertase II is identical to that of invertase I, this enzyme, after purification, has also been treated with endoglycosidase H. Fig. 1 (upper part) shows that this invertase species is also split

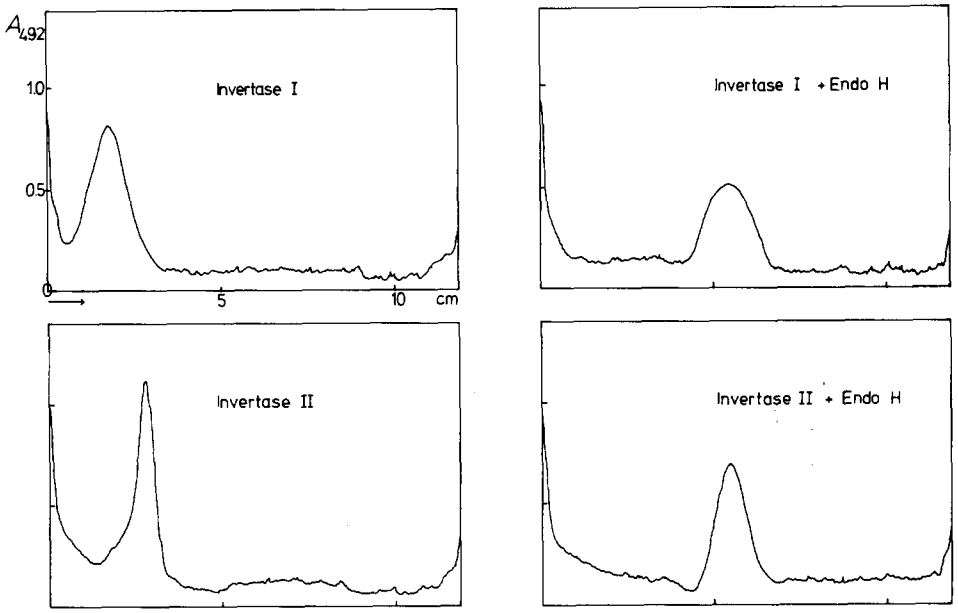


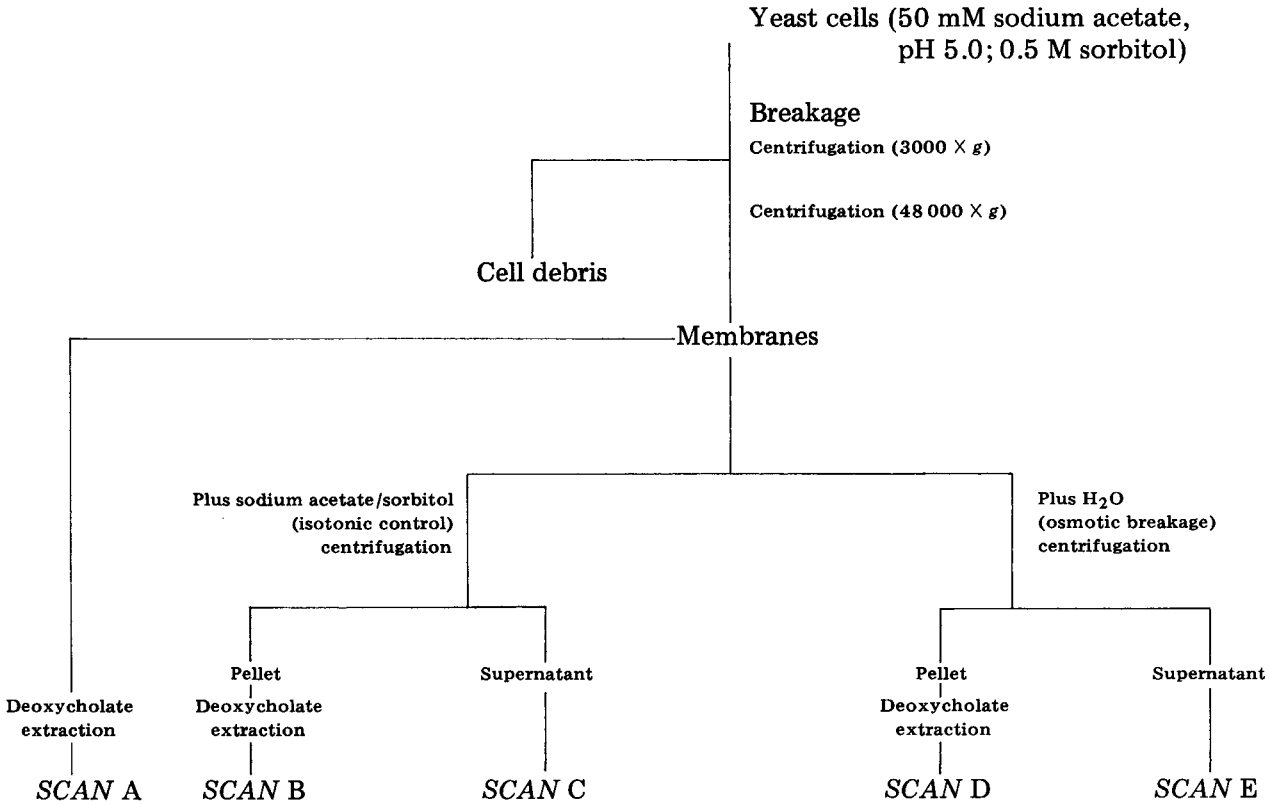
Fig. 1. Treatment of invertase I and II with endo-β-*N*-acetylglucosaminidase H. Separation of products on SDS gels. Purified invertase I and II were treated with citrate buffer (left, control), or with 0.01 unit of the endoglycosidase H in citrate buffer (right) for 24 h, and analyzed for invertase activity on SDS gels. Data represent a typical experiment (four repetitions).

completely, yielding a split product of apparent identical size. The same result was achieved with authentic external invertase prepared from commercially available material (not shown).

It has been reported [24] that external invertase is split into several products which, on the SDS gel electrophoresis system used, migrated as a family of bands with molecular weights of 60 000 plus *n* times 3000 dalton. With purified invertase I an essentially identical result was obtained (not shown). The same is true for authentic external as well as thiol-released invertase. All the split products could also be stained for carbohydrate content.

Invertase release from membrane vesicles by osmotic shock

To distinguish between membrane-bound and merely entrapped or adsorbed invertase material, the experiment shown in Fig. 2 was performed. It clearly demonstrates that invertase II cannot be transferred into the supernatant fraction upon osmotic shock treatment of vesicles. On the other hand, 16% of the total membrane protein (not shown) and approximately 20% of the invertase I activity can be brought into solution by a single osmotic shock (both values are corrected with the control figures, the amount of invertase I activity in supernatant plus pellet is taken as 100%). Approx. 80% of the invertase III activity is soluble after the hypotonic treatment. However, in the control experiment almost the same amount can be obtained in the soluble fraction just by isotonic washing with buffer. Therefore, this invertase species seems to be merely adsorbed to the vesicles.


Yeast cells (50 mM sodium acetate, pH 5.0; 0.5 M sorbitol)
Breakage
Centrifugation (3000 × g)
Centrifugation (48 000 × g)
Cell debris
Membranes
Plus sodium acetate/sorbitol (isotonic control) centrifugation
Plus H_2O (osmotic breakage) centrifugation
Deoxycholate extraction
Pellet
Deoxycholate extraction
Supernatant
Pellet
Deoxycholate extraction
Supernatant
SCAN A
SCAN B
SCAN C
SCAN D
SCAN E

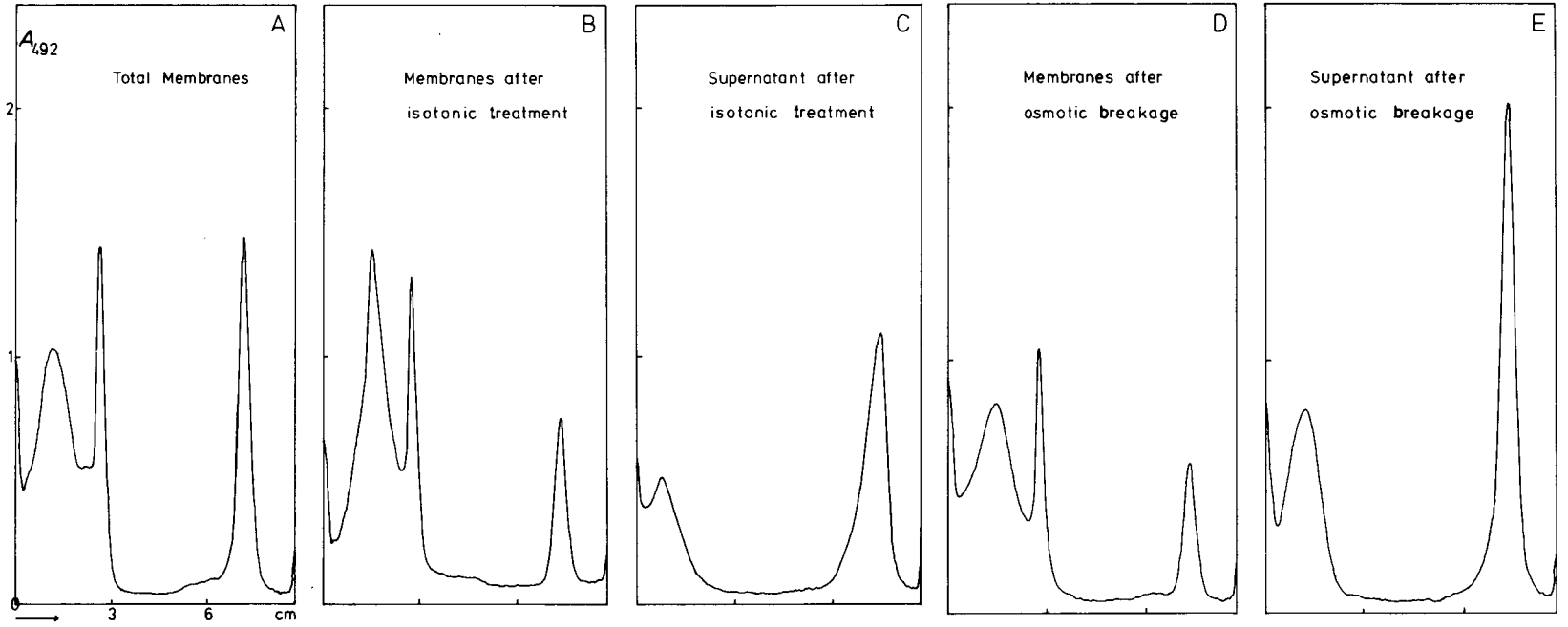


Fig. 2. Osmotic breakage of yeast membrane vesicles. Membranes were prepared from derepressed yeast cells in 0.5 M sorbitol-containing buffer, and either treated with distilled water or with sorbitol-containing buffer (control). After incubation, the respective supernatants and pellets (after deoxycholate extraction) were analyzed for invertase activity on gels (SCANS A—E). The experiment was repeated twice.

TABLE II

NADPH: CYTOCHROME *c* OXIDOREDUCTASE ACTIVITY IN SUCROSE DENSITY GRADIENT BANDS

Membranes were prepared from derepressed yeast cells, either in Mg^{2+}-containing or Mg^{2+}-free buffer, and fractionated by ultracentrifugation on a linear sucrose gradient. Separated bands were analyzed for the endoplasmic reticulum marker enzyme. Data are mean values of two experiments. Total protein of the whole gradient is taken as 100%. One unit is defined as initial rate of $\Delta E_{550} = 1.0$/min at 22°C (27 μM cytochrome *c*).

Fraction	Minus Mg^{2+}			Plus Mg^{2+}		
	Protein distribution (%)	Specific activity (U/mg)	Total activity (U)	Protein distribution (%)	Specific activity (U/mg)	Total activity (U)
3	9	0.13	0.65	5	0.2	0.84
4	4	0.06	0.13	7	0.23	1.38

Subcellular localization of invertase II

Following the procedure of Marriott and Tanner [28], membranes from glass-bead-broken yeast cells were separated on a sucrose density gradient. Five more or less well separated fractions could be obtained both in the absence and presence of Mg^{2+}. The different distribution of protein between fractions 3 and 4, depending on Mg^{2+} (Table II), is in agreement with the known stabilizing effect of Mg^{2+} on the attachment of ribosomes to rough endoplasmic reticulum [33].

Further evidence for the assumption that fractions 3 and 4 contain smooth and rough endoplasmic reticulum comes from studies with NADPH:cytochrome *c* oxidoreductase, which may be used as a marker enzyme for endoplasmic reticulum in yeast [34]. In the presence of Mg^{2+}, the specific as well as the total activity in fraction 4 predominates over that in fraction 3. The reverse is true for the situation where Mg^{2+} has been omitted (Table II).

The relative specific activity of invertase II (expressed as percent of the sum of invertase I, II, and III activities) in deoxycholate extracts from whole mem-

TABLE III

DISTRIBUTION OF INVERTASE ISOZYME ACTIVITIES IN SUCROSE DENSITY GRADIENT BANDS

Membranes were prepared from derepressed yeast cells, either in Mg^{2+}-containing or Mg^{2+}-free buffer, and fractionated by ultracentrifugation on a linear sucrose gradient. Separated bands were diluted, recentrifuged, extracted with deoxycholate and analyzed for invertase activity on SDS gels. Data are mean values out of two experiments. Units · 10^{-1}. Activity of all three isozymes is taken as 100%.

Invertase isozyme	Membranes				Fractions 3 and 4			
	Minus Mg^{2+}		Plus Mg^{2+}		Minus Mg^{2+}		Plus Mg^{2+}	
	Activity	%	Activity	%	Activity	%	Activity	%
I	9.8	74	15.0	67	1.2	61	1.6	59
II	2.0	15	3.5	15	0.6	31	0.7	26
III	1.5	11	4.0	18	0.16	8	0.4	15

branes has been compared to the values of extracts from fractions 3 plus 4 (Table III). An approx. 2-fold enrichment of invertase II is observed in fractions 3 and 4, regardless of whether Mg^{2+} was included in the membrane preparation or not. Beyond that, fractions 3 and 4 contain more than 70% of the total invertase II activity (not shown). Neither invertase I nor invertase III follow these patterns.

Discussion

According to Ref. 5 yeast external invertase contains approx. 50% of its molecular weight of 270 000 in the form of mannose, in addition to 3% of *N*-acetylglucosamine residues in the core region. Our previously published molecular weight of approx. 240 000 [19] could be affirmed in the present study using a refined gel electrophoresis system. This value is also confirmed by experiments using *S*-carboxymethylated invertase [24]. The latter publication provides some evidence that the enzyme is composed of two subunits, each of 120 000 dalton which is also indicated by the experiments reported here using a gel electrophoresis system exhibiting dissociating conditions.

Upon treatment with endoglycosidase H, invertase I yields the same (carbohydrate positive) split product as authentic external invertase. These products migrate in the gel system used for activity staining as a single diffuse band at approx. 120 000 dalton. This gel system does not bring about dissociation into subunits; the split products probably run as dimers as do invertases I and II as well.

Interestingly, the precursor invertase II of this paper shows the same behaviour upon treatment with endoglycosidase H. Using the refined gel system, an average molecular weight for untreated precursor invertase II of approx. 180 000 dalton was determined, which correlates well with a calculated molecular weight for a molecule which consists per subunit of the complete protein moeity (60 000 dalton; [24]) and of the inner core carbohydrate parts (9 times 3000 dalton [21]). Under non-dissociating conditions, this molecule should migrate at approx. 174 000 dalton and, after treatment with endoglycosidase H, near 120 000 dalton.

Split products due to endoglycosidase H treatment of external invertase have been described; they migrate on SDS gels between 60 000 and 70 000 dalton, each differing by 3000 dalton [24,32]. Due to their carbohydrate content, the presence of one or more inner core carbohydrates parts could be assumed. Invertase I, as well as authentic external invertase (Boehringer), yield those split products upon electrophoresis under dissociating conditions (not shown). In the case of invertase II, it was not possible to resolve the split products in the same way. Nevertheless, from the experiment in Fig. 1 (lower part) it might be suggested that the precursor invertase already contains those carbohydrate parts which cannot be removed by endoglycosidase H, as is the case with invertase I.

Upon electrophoresis in gels having a low SDS concentration, invertase I, II and, most probably, also the split products caused by degradation with endoglycosidase H migrate as dimers, whereas internal invertase III dissociates.

Common to the former three enzyme species is their carbohydrate content. Under drastic SDS conditions and treatment with 2-mercaptoethanol [27], all the carbohydrate-containing isozymes dissociate during electrophoresis. Obviously carbohydrate provides some means of preventing dissociation into subunits. As invertase I and II are extracellular and membrane-bound (ultimately extracellular as well), respectively, it is tempting to speculate that this might have some physiological relevance. External invertase is loosely entrapped within the periplasmic space of the yeast cell wall. Its release [25] and reduction in size by thiol treatment [35] have been described.

Precursor invertase is exclusively membrane-bound as is shown in Fig. 2. The observation that after hypotonic treatment internal invertase seems to be still membrane-bound may be due to incomplete treatment; successive osmotic breakages might have shifted the amount of invertase III (and I?) into the supernatant fraction.

The invertase-active membranes have been characterized somewhat by sucrose density gradient centrifugation of the yeast microsomal preparation. Fractions 3 and 4 (Table II) seem to represent membranes of smooth and rough endoplasmic reticulum, where precursor invertase II exhibits a 2-fold enrichment as compared to whole membrane extracts. In the light of biosynthesis of proteins destined for secretion, this result agrees with the proposed pathway elucidated for pancreatic exocrine cells [36] and oviduct cells [37]. Proteins are synthesized vectorially into the cisternae of the endoplasmic reticulum with concomitant core glycosylation. During transportation through endoplasmic reticulum membranes and the Golgi apparatus glycosylation is completed. Budding vesicles carry the proteins to the plasmalemma where final secretion occurs. Although contrary results have been published [38], the described model should hold also for the synthesis of external invertase in yeast. In this connection, it is of interest that core glycosylation reactions have been found to be located preferentially in yeast endoplasmic reticulum membranes [28].

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