Biochem. J. (1990) 267, 697–702 (Printed in Great Britain)

Purification and characterization of the invertase from *Schizosaccharomyces pombe*

A comparative analysis with the invertase from *Saccharomyces cerevisiae*

Sergio MORENO,*‡ Yolanda SANCHEZ* and Luis RODRIGUEZ†

*Departamento de Microbiología, Genética, Medicina Preventiva y Salud Pública, Facultad de Biología, Universidad de Salamanca, 37007 Salamanca, and †Departamento de Microbiología y Biología Celular, Facultad de Farmacia, Universidad de La Laguna, 38071 La Laguna, Tenerife, Spain

Invertase (EC 3.2.1.26) was purified to homogeneity from exponentially growing cells of *Schizosaccharomyces pombe* fully de-repressed for synthesis of the enzyme, and was shown to be a high-molecular-mass glycoprotein that can be dissociated in the presence of 8 M-urea/1% SDS into identical subunits with an apparent molecular mass of 205 kDa. The carbohydrate moiety, accounting for 67% of the total mass, is composed of equimolar amounts of mannose and galactose. There is a small amount of glucosamine, which is probably involved in the linkage to the protein moiety, since the enzyme is sensitive to treatment with endoglycosidase H. The composition of the carbohydrate moiety resembles that found in higher-eukaryotic glycoproteins and differs from glycoproteins found in *Saccharomyces cerevisiae*. The protein portion of each subunit is a polypeptide of molecular mass 60 kDa, very similar to the invertase of *Sacch. cerevisiae*. Both proteins cross-react with antibodies raised against the protein fractions of the other, indicating that the two enzymes are similar.

INTRODUCTION

The fission yeast *Schizosaccharomyces pombe*, like *Saccharomyces cerevisiae*, synthesizes invertase that is secreted into the cell wall. In both yeasts biosynthesis of the enzyme is regulated by catabolite repression (Gascon & Ottolenghi, 1967; Mitchison & Creanor, 1969), and, when exponentially growing cells are transfered to a medium with a low percentage of glucose, enzyme synthesis is de-repressed. *Sacch. cerevisiae* cells contain two forms of the enzyme: one located intracellularly without carbohydrate, which corresponds to the repressed form of invertase, and a second extracellular one, containing nine or ten *N*-glycosidically linked oligosaccharides, which corresponds to the de-repressed form of the enzyme (Neuman & Lampen, 1967; Gascon & Lampen, 1968; Gascon *et al.*, 1968; Tarentino *et al.*, 1974; Huffaker & Robbins, 1983). Both enzymes are synthesized from the same structural gene, and their protein moieties have a molecular mass of 60 kDa (Carlson & Botstein, 1982). In the fission yeast invertase is a high-molecular-mass glycoprotein located outside the plasma membrane (Moreno *et al.*, 1985).

Since study of the *Sacch. cerevisiae* invertase has yielded valuable information on the nature of yeast glycoproteins (Cohen & Ballou, 1981), we have purified and characterized the invertase from *Schizosacch. pombe* both in order to compare it with the similar enzyme in *Sacch. cerevisiae* and to gain information about the composition of *Schizosacch. pombe* glycoproteins.

MATERIALS AND METHODS

Materials

Reagents for gel electrophoresis and Bio-Gel A-5m were obtained from Bio-Rad Laboratories, Watford, Herts., U.K. DEAE-Sephadex A-50 and ampholytes for isoelectric focusing (Pharmalyte 2.5–5) were from Pharmacia–LKB, Uppsala, Sweden. Proteins used as molecular-mass markers, *Sacch. cerevisiae* invertase, 2,3,5-triphenyltetrazolium chloride and *Staphylococcus aureus* proteinase V8 were from Sigma Chemical Co., St. Louis, MO, U.S.A. Endoglycosidase H was from Miles Scientific, Miles Martin, Madrid, Spain. [^{35}S]Methionine and nuclease-treated reticulocyte lysate were from Amersham International, Amersham, Bucks., U.K. Oligo(dT)–cellulose was from Collaborative Research, Bedford, MA, U.S.A. Inactivated *Staph. aureus* cells (IgG Sorb) were from The Enzyme Center, Malden, MA, U.S.A. All other reagents were of analytical grade.

Enzyme purification

Sacch. cerevisiae invertase was obtained from Sigma Chemical Co. and then purified by gel filtration on a Sephacryl S-300 column (1.25 cm × 100 cm) equilibrated in 50 mM-Tris/HCl buffer, pH 7.5. For production of *Schizosacch. pombe* invertase, the wild-type strain *Schizosacch. pombe* 972 h$^-$ was grown in a 14-litre New Brunswick glass fermenter in yeast-extract medium containing 3% (w/v) glucose; 4 litres of medium were grown overnight at 30 °C, with shaking at 200 rev./min and aeration at 10 litres/min, to a density of 2×10^7 cells/ml. Then the culture was diluted with 8 litres of fresh medium containing 0.25% glucose, and incubation was continued for a further 15 h under the same conditions. The cells were harvested and washed twice in ice-cold distilled water and once more with 0.1 M-sodium acetate buffer, pH 4.0. With this procedure it was possible to obtain about 100 g wet wt. of cells in which invertase synthesis was fully de-repressed. Cells were resuspended in 100 ml of 0.1 M-sodium acetate buffer, pH 4.0, and broken by mechanical shaking with an equal volume of acid-washed glass beads (0.45 mm diam.) at 4 °C in a Braun MSK homogenizer. Unless otherwise noted, all further procedures were carried out at 4 °C. The extract was spun first at 5000 *g* for 5 min and then at 40000 *g* for 30 min. The pH of the supernatant of the second centrifugation was adjusted to 4.0 with 1.0 M-acetic acid, and the solution was left overnight in an ice bath and then centrifuged at 40000 *g* for 30 min. Solid $(NH_4)_2SO_4$ was added slowly to the supernatant to 85% satu-

‡ To whom correspondence should be sent, at present address: Microbiology Unit, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

ration (56 g/l). After standing overnight in the cold, precipitated material was removed by centrifugation at 40000 *g* for 30 min. The supernatant, which contained all the enzymic activity, was first dialysed against distilled water and then against 0.1 M-sodium acetate buffer, pH 4.0. Then 6 g of DEAE-Sephadex previously equilibrated in the same buffer was added to the resulting solution. The gel slurry was stirred for 2 h and then packed into a column and washed with 2 litres of buffer containing 10 mM-NaCl, after which the enzyme was eluted with 1 litre of buffer containing 50 mM-NaCl. The enzyme solution was concentrated in an Amicon ultrafiltration cell fitted with an XM-100 membrane and then applied to a DEAE-Sephadex column (2 cm × 15 cm) equilibrated in 0.1 M-sodium acetate buffer, pH 4.0, containing 10 mM-NaCl. The column was washed with 500 ml of this buffer and then eluted with 600 ml of a linear gradient of 10–100 mM-NaCl in the same buffer. Fractions with enzymic activity were pooled, dialysed against 0.1 M-sodium acetate buffer, pH 4.0, concentrated to 4 ml by ultrafiltration and filtered through a Bio-Gel A-5m column (2.5 cm × 175 cm) equilibrated in the same buffer. Fractions with activity were pooled, concentrated and rechromatographed in the same way. SDS/PAGE of this material revealed a single protein band.

The purified invertase was desalted by dialysis against distilled water, freeze-dried and stored at −20 °C; these conditions proved to be adequate for preserving the enzyme over long periods (over 6 months) without any appreciable loss of activity.

Invertase activity

Invertase activity was measured by the method of Goldstein & Lampen (1975) as described previously (Moreno *et al.*, 1985). One unit of invertase is the amount of enzyme that hydrolyses sucrose to yield 1 μmol of glucose/min at 30 °C and pH 4.0.

Protein concentration and amino acid analysis

Protein concentration was measured by the method of Lowry *et al.* (1951), with BSA as standard. Samples for amino acid analysis were hydrolysed in constant-boiling HCl at 110 °C for 24 and 48 h in sealed evacuated tubes. Analyses were performed in a Beckman 119 autoanalyser, fitted with 6 mm (internal diam.) columns, by the procedure described in the Beckman Aminoacid Analysis Manual.

Carbohydrate analysis

Total carbohydrate was measured by the phenol/H_2SO_4 method (Dubois *et al.*, 1956), with mannose as standard. Individual sugars were identified by g.l.c. of their alditol acetate derivates as described by Albersheim *et al.* (1967) in a Varian 2400 chromatograph. Samples for sugar analysis, containing inositol (1 mg/ml) as internal standard, were previously hydrolysed in 2 M-trifluoroacetic acid at 121 °C for 1 h in sealed evacuated tubes. Identification of neutral sugars was performed on a 3% ECNSS-M column at 190 °C and amino sugars were separated on a 3% SP2340 column at 180 °C for 6 min, after which the temperature was increased by 2 °C/min to 300 °C; in both cases injector and detector temperatures were 210 °C and 250 °C respectively, and the carrier gas was He at 60 ml/min.

Electrophoresis

SDS/PAGE was performed by the method of Laemmli (1970); in some experiments, to avoid denaturing conditions for the samples, SDS was omitted from both the gel and the buffers. Linear gradient (5–15%) SDS/PAGE was carried out as described by Hames (1981), and two-dimensional electrophoresis was performed as described by Pollard (1984), except that the treatment of the samples with DNAase and RNAase was omitted.

Proteins were detected either by staining the gels with Coomasie Brilliant Blue R-250 by the procedure of Hames (1981) or by the $AgNO_3$ method of Morrisey (1981). Invertase activity was detected *in situ* in the gels by the method of Gabriel & Wang (1969) as modified by Carlson *et al.* (1981).

Isoelectric focusing was performed on a 3%-polyacrylamide/0.6%-agarose vertical gel slab as described by An der Lan & Chrambach (1981).

Antibody preparation

A 1 mg portion of purified enzyme was denatured by boiling in 0.1% SDS/100 mM-2-mercaptoethanol, and then digested with endoglycosidase H by the procedure of Tarentino *et al.* (1974). Protein fractions were purified by SDS/PAGE, and the 60 kDa bands were cut out and electro-eluted in 25 mM-Tris/glycine buffer, pH 8.4, at 100 V for 2 h. Samples containing 100 μg of pure protein were injected into rabbits every 28 days. After the fourth injection the rabbits were bled out and the serum was checked for the ability to immunoprecipitate the homologous antigen.

Immunodiffusion

A 15 μl sample of serum was tested by two-dimensional diffusion in agarose gels in 150 mM-NaCl/10 mM-sodium phosphate buffer, pH 7.0, by the procedure of Ouchterlony & Nilsson (1978).

RNA preparation, translation *in vitro* of the polyadenylated RNA, and immunoprecipitation and electrophoresis

Total *Sacch. cerevisiae* and *Schizosacch. pombe* RNA was obtained from 100 ml of culture in mid-exponential phase (10^7 cells/ml) as described by Kaufer *et al.* (1985). Polyadenylated RNA was prepared by using oligo(dT)–cellulose as described by Clemens (1984).

To 10 μl of rabbit reticulocyte lysate was added 1 μl of polyadenylated RNA (1 μg/μl) and 1 μl of [^{35}S]methionine (10 μCi/μl, 1150 Ci/mmol) and the mixture was incubated at 30 °C for 1 h. The reaction was stopped by adding 1 μl of 10% (w/v) sodium deoxycholate, and the mixture was diluted to 0.5 ml with 50 mM-Tris/HCl buffer, pH 7.5, containing 1% Triton X-100, 5 mM-EDTA, 50 mM-NaCl, 10 mM-methionine and 0.5 mg of BSA/ml. The immunoprecipitations were carried out in this buffer by adding 20 μl of antiserum and 5 μl of a solution of 200 mM-phenylmethanesulphonyl fluoride in ethanol. After incubation overnight at 4 °C, 50 μl of 10% (w/v) inactivated *Staph. aureus* cells in 50 mM-Tris/HCl buffer, pH 7.4, containing 150 mM-NaCl, 5 mM-EDTA and 0.5% Triton X-100 were added and the mixture was incubated for a further 30 min at 30 °C. Then the suspensions were spun down and the cells were washed with four 1 ml portions of 0.5 M-Tris/HCl buffer, pH 8.2, containing 0.1% SDS, 7.5% sucrose and 1 mg of BSA/ml and once with the same buffer without BSA. Eventually the cells were resuspended in electrophoresis sample buffer, boiled for 5 min and spun down, and the supernatant was loaded on a 10% polyacrylamide gel.

Peptide mapping

Samples containing 2 μg of deglycosylated invertase were treated with 10 ng of proteinase V8 from *Staph. aureus* for 20 min at room temperature in the stacking gel as described by Cleveland *et al.* (1977). The peptides were separated by SDS/PAGE on a 14%-polyacrylamide gel.

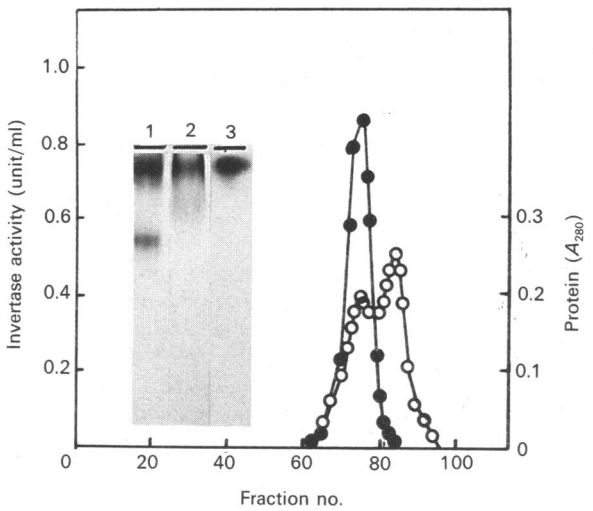

Fig. 1. Chromatography of *Schizosacch. pombe* invertase on Bio-Gel A-5m

Experimental conditions are described in the text. Each fraction contained 5 ml. ○, Protein; ●, invertase activity. The inset shows the electrophoretic analysis (in the absence of SDS) of the material containing the invertase activity, before (lane 1) and after (lanes 2 and 3) filtration through the Bio-Gel column, stained for protein with Coomasie Brilliant Blue (lanes 1 and 2) and for activity (lane 3), under the conditions described in the Materials and methods section.

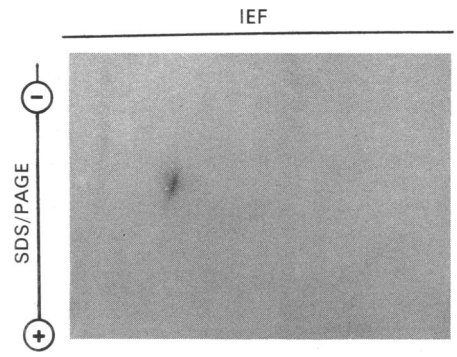

Fig. 2. Two-dimensional electrophoresis of purified *Schizosacch. pombe* invertase

Experimental conditions are described in the Materials and methods section. IEF represents isoelectric focusing (pH 3–10) (first dimension); SDS/PAGE represents polyacrylamide-gel (3–10%) electrophoresis in the presence of 0.1% SDS (second dimension). The gel was silver-stained.

RESULTS AND DISCUSSION

Purification of invertase from *Schizosacch. pombe*

As indicated in the Introduction, the goal of this work was to achieve the purification of invertase from *Schizosacch. pombe* in order to carry out its characterization. The initial approach used was to follow the method described for the purification of the same enzyme from *Sacch. cerevisiae* (Goldstein & Lampen, 1975); however, this method was found to be unsuitable for our purposes, since *Schizosacch. pombe* invertase was not retained by sulphoethyl-Sephadex columns and was eluted with the void volume of Sephadex G-200 columns, two of the purification steps of this method. Accordingly we had to develop a new procedure that would allow purification to apparent homogeneity of the invertase from *Schizosacch. pombe* cells fully de-repressed for the synthesis of the enzyme. This procedure is partly similar to that previously described for the purification of another *Schizosacch. pombe* exocellular glycoprotein enzyme, acid phosphatase (Dibenedetto & Cozzani, 1975), which in itself is an indication that both enzymes must share common physicochemical properties.

The purification procedure described in the Materials and methods section for invertase takes advantage of three of the most relevant properties of this enzyme: (1) its high solubility in concentrated salt solutions, probably due to its high carbohydrate content (see below), (2) its acidic isoelectric point (pI 3.25), which allows binding of the enzyme to anion-exchangers such as DEAE-Sephadex A-50 at low pH values, and (3) its high molecular mass (Moreno *et al.*, 1985; see below), which allows the separation of invertase from its major contaminant, maltase, after the DEAE-Sephadex column by using a Bio-Gel A-5m column (Fig. 1). The purity of the enzyme was assessed by two-dimensional gel electrophoresis; this revealed a single protein (Fig. 2). Table 1 summarizes the relevant purification data.

Molecular mass and subunit composition of invertase

The molecular mass of the native purified invertase was determined by two different techniques: gel filtration in Bio-Gel A-5m and non-denaturing PAGE.

In the first method, samples containing 7 units of invertase were gel-filtered through a Bio-Gel A-5m column (1.25 cm × 100 cm) equilibrated with 0.1 M-Tris/HCl buffer, pH 7.5, containing 0.2 M-NaCl. An apparent molecular mass of 1200 kDa was extrapolated from a calibration curve obtained by gel-filtering, under the same conditions, samples of thyroglobulin (670 kDa), ferritin (440 kDa) and catalase (232 kDa). The elution volume of the invertase was not modified when a higher salt concentration (3 M-NaCl or 5 M-LiCl) or a detergent (1% Cetrimide) was included both in the sample and in the elution buffer (results not shown).

In the second method, samples of pure invertase containing 10 μg of protein were electrophoresed on gel rods (6 mm × 10 cm) prepared with different concentrations of polyacrylamide, from

Table 1. Purification of *Schizosacch. pombe* invertase

For experimental details see the Materials and methods section.

Step	Total activity (units)	Total protein (mg)	Specific activity (units/mg)
Crude extract, pH 4.0	196000	7630	26
$(NH_4)_2SO_4$ fractionation	164000	526	313
DEAE-Sephadex batch adsorption	74820	146	512
DEAE-Sephadex column chromatography	43720	36	1210
Bio-Gel A-5m column chromatography	7940	5	1590

2.5 to 10%, under non-denaturing conditions along with the following molecular-mass markers: urease tetramer (480 kDa), urease dimer (240 kDa), BSA dimer (132 kDa), BSA monomer (66 kDa) and ovalbumin (45 kDa). A molecular mass for invertase of 1070 kDa was calculated by the procedure outlined in the Sigma Technical Bulletin no. MKR-137 (results not shown).

Subunit molecular mass was estimated by SDS/PAGE in 5%-polyacrylamide gel slabs in the presence of 8 M-urea both in the gel and in the sample buffer. A single smeared wide band was observed which was due to the glycoprotein nature of the enzyme, with a mean molecular mass of 205 kDa (results not shown). This suggests that the native enzyme could be constituted of five or six subunits of identical size.

Carbohydrate composition

The glycoprotein nature of invertase and the presence of mannose and galactose in its carbohydrate moiety were inferred in a previous work (Moreno *et al.*, 1985). In the present study the percentage of carbohydrate was determined as 67% of the total mass of the protein. The individual sugars were identified by g.l.c. as described in the Materials and methods section. Most of the carbohydrate was found to be mannose and galactose in a ratio of about 1:1. The enzyme was also shown to contain glucosamine, but in amounts too small to be quantified by this procedure. Glucosamine is probably involved in the linkage of the carbohydrate and the protein moieties of the enzyme molecule, through an *N*-glycosidic linkage, since the carbohydrate could be separated from the protein portion by treatment with endoglycosidase H under denaturing conditions (see the Materials and methods section). Fig. 3 shows that after this treatment the protein moved as a single and sharp band with a molecular mass close to 60 kDa. This value is in good agreement with the molecular mass calculated for invertase subunits, considering that their protein content is 33% of the total weight.

The sensitivity of invertase to endoglycosidase H is lost when the treatment is performed under non-denaturing conditions; this may mean that the oligosaccharide present in the precursor of invertase undergoes some kind of post-translational processing during secretion to the cell wall, similar to that of animal cell glycoproteins (Hubbard & Ivatt, 1981; Dunphy & Rothman, 1985). Although we have been unable to detect the presence of other components of glycoproteins characteristic of higher eukaryotes, such as fucose or sialic acids, the presence of galactose in the sugar moiety of invertase and of acid phosphatase (Dibenedetto & Cozzani, 1975) may indicate that protein glycosylation in fission yeast takes place through a mechanism more similar to that found in animal cells, generating complex-type or hybrid-type oligosaccharides in glycoproteins, than to that found in *Sacch. cerevisiae*, which generates glycoproteins with high-mannose-type asparagine-linked oligosaccharides (Kornfeld & Kornfeld, 1985). It would be interesting to express in *Schizosacch. pombe* higher-eukaryotic glycoproteins to check whether they are processed correctly and produce the right glycosylation pattern.

Comparison of the protein fractions of the *Schizosacch. pombe* and *Sacch. cerevisiae* invertases

After treatment with endoglycosidase H, both *Schizosacch. pombe* and *Sacch. cerevisiae* invertase gave rise to a polypeptide of similar molecular mass (Fig. 3). In order to establish the extent of the sequence similarity between the two proteins, we carried out a comparative analysis of both polypeptides.

Purified enzymes were deglycosylated by treatment with endoglycosidase H. The protein fractions were purified by electrophoresis and electro-elution, as indicated in the Materials and methods section.

Comparison of the amino acid compositions of the two enzymes (Table 2) revealed that they were similar except for the lysine contents.

In order to investigate whether the two proteins shared common epitopes in the molecule, polyclonal antibodies were raised against both of them. The sera were tested by two-

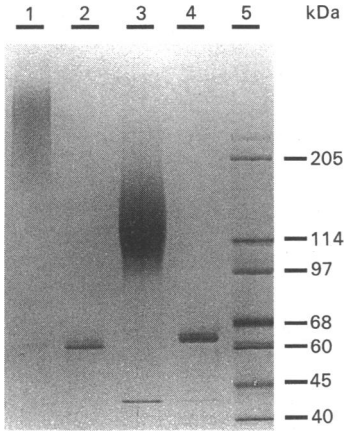

Fig. 3. Effect of deglycosylation on the electrophoretic behaviour of the *Schizosacch. pombe* and *Sacch. cerevisiae* invertases

Samples of purified invertases from *Schizosacch. pombe* (lanes 1 and 2) and *Sacch. cerevisiae* (lanes 3 and 4) were electrophoresed on an SDS/linear-gradient polyacrylamide (5–12%) gel slab before (lanes 1 and 3) and after (lanes 2 and 4) treatment with endoglycosidase H. Experimental conditions are described in the text; the gel was stained with Coomassie Brilliant Blue. At the right-hand side of the gel are indicated the molecular masses (kDa) of the markers (lane 5), from top to bottom: myosin, β-galactosidase, phosphorylase *b*, catalase, ovalbumin and glyceraldehyde-3-phosphate dehydrogenase.

Table 2. Comparison of the percentage amino acid compositions of *Schizosacch. pombe* and *Sacch. cerevisiae* invertases

All values are averages from duplicate determinations after hydrolysis for 24 and 48 h, as described in the Materials and methods section. Abbreviations: N.D., not determined.

Amino acid	Amino acid composition (%)	
	Schizosacch. pombe invertase	*Sacch. cerevisiae* invertase
Asx	13.1	11.8
Thr	7.8	7.1
Ser	8.8	8.1
Pro	5.8	6.0
Glx	9.4	9.6
Gly	7.8	10.8
Ala	7.2	7.8
Val	6.0	5.2
Cys	N.D.	N.D.
Met	N.D.	N.D.
Ile	5.4	5.5
Leu	7.6	6.4
Tyr	5.6	3.8
Phe	4.4	5.8
Lys	2.0	6.9
Hys	1.9	2.4
Trp	N.D.	N.D.
Arg	2.6	2.6

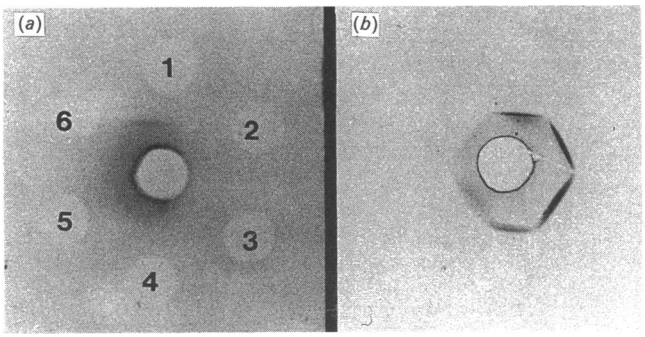

Fig. 4. Cross-reaction between (*a*) *Schizosacch. pombe* invertase and serum raised against invertase from *Sacch. cerevisiae* and (*b*) *Sacch. cerevisiae* invertase and serum raised against invertase from *Schizosacch. pombe*

Key: 1 = (1:1), 2 = (1:2), 3 = (1:4) etc. represent dilutions of the antigen.

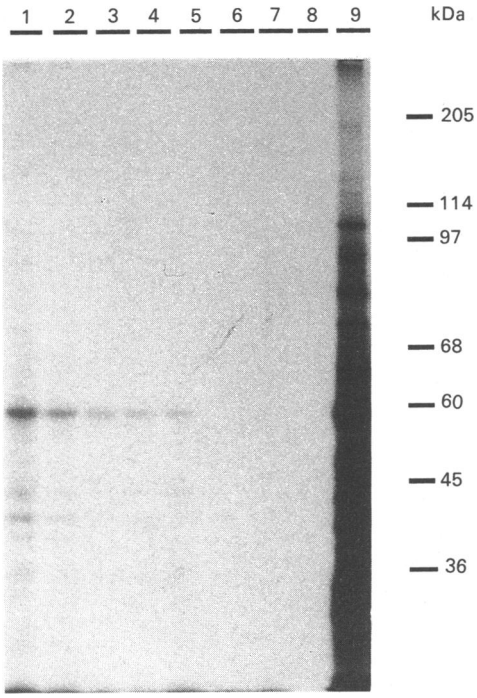

Fig. 5. SDS/PAGE (10% polyacrylamide) of the peptides synthesized *in vitro* from *Schizosacch. pombe* polyadenylated RNA

The translation products were immunoprecipitated with 20 μl of serum raised against invertase from *Sacch. cerevisiae*, in the presence of 0 μl (lane 1), 1 μl (lane 2), 2 μl (lane 3), 3 μl (lane 4) or 5 μl (lane 5) of purified deglycosylated *Sacch. cerevisiae* invertase (1 mg/ml). Lanes 8 and 9 are the total products of the reaction in the absence and in the presence of mRNA respectively. The gel was dried and exposed to a Kodak XAR5 film for autoradiography.

dimensional immunodiffusion in agarose gels; in both cases immunoprecipitation bands were seen when the two invertases were used as antigen (results not shown). More interestingly, when the sera were tested for the ability to immunoprecipitate the heterologous antigen, immunoprecipitation bands on an immunodiffusion gel were seen (Fig. 4), indicating that each protein cross-reacts with antibodies raised against either of them.

At this point, it was important to show that the two proteins

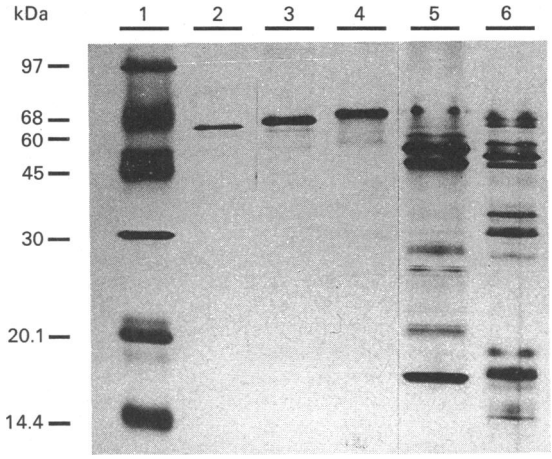

Fig. 6. SDS/PAGE (14% polyacrylamide) of the invertases from *Schizosacch. pombe* (lanes 3 and 5) and *Sacch. cerevisiae* (lanes 4 and 6) before (lanes 3 and 4) and after (lanes 5 and 6) treatment with *Staph. aureus* proteinase V8

Lanes 1 and 2 are molecular-mass markers. The gel was silver-stained.

cross-react because they share common epitopes within the protein fractions and not because of residual carbohydrate that could remain after the treatment with endoglycosidase H. To avoid this possibility, *Schizosacch. pombe* total RNA was prepared from cells grown under conditions in which synthesis of invertase was de-repressed. The RNA was translated *in vitro* in a rabbit reticulocyte lysate, in the presence of [^{35}S]methionine. The products of the reaction were immunoprecipitated with antibodies raised against invertase from *Sacch. cerevisiae*. A protein of 60 kDa was immunoprecipitated (Fig. 5, lane 1). This band could be competed out when purified *Sacch. cerevisiae* invertase was added to the immunoprecipitation reaction (Fig. 5, lanes 2–5). A similar result was obtained when RNA from *Sacch. cerevisiae* was translated *in vitro* and the polypeptide mixture was immunoprecipitated with antibodies raised against invertase from *Schizosacch. pombe* (results not shown).

Finally, peptide mapping of both proteins was carried out with the use of *Staph. aureus* proteinase V8. As shown in Fig. 6, the peptides generated were not identical, suggesting that the sequence similarity between the two enzymes might be limited to small regions of the proteins that are antigenically similar.

To summarize, we have purified the *Schizosacch. pombe* invertase and shown that it is a high-molecular-mass glycoprotein composed of several identical subunits. The protein portion is very similar to that of the invertase from *Sacch. cerevisiae*, but the carbohydrate is different, resembling that of glycoproteins from higher eukaryotes.

We thank Chris Norbury and Paul Nurse for their comments on the manuscript. This work was supported in part by Grant 0780/84 from the Comisión Asesora de Investigación Científica y Técnica and by predoctoral fellowships (to S.M. and Y.S.) from the Fondo de Investigaciones Sanitarias de la Seguridad Social.

REFERENCES

Albersheim, P., Nevins, D. J. & English, P. D. (1967) Carbohydr. Res. **5**, 340–345

An der Lan, B. & Chrambach, A. (1981) in Gel Electrophoresis of Proteins: A Practical Approach (Hames, B. D. & Rickwood, D., eds.), pp. 157–187, IRL Press, London and Washington

Carlson, M. & Botstein, D. (1982) Cell **28**, 145–154

Carlson, M., Osmond, B. C. & Botstein, D. (1981) Genetics **98**, 25–40

Clemens, M. J. (1984) in Transcription and Translation: A Practical Approach (Hames, B. D. & Higgins, S. J., eds.), pp. 211–230, IRL Press, Oxford
Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) J. Biol. Chem. **252**, 1102–1106
Cohen, R. E. & Ballou, C. E. (1981) Encycl. Plant Physiol. New Ser. **13B**, 441–458
Dibenedetto, G. & Cozzani, I. (1975) Biochemistry **14**, 2847–2852
Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. & Smith, F. (1956) Anal. Chem. **28**, 350–356
Dunphy, W. G. & Rothman, J. E. (1985) Cell **42**, 13–21
Gabriel, O. & Wang, S. F. (1969) Anal. Biochem. **27**, 545–554
Gascon, S. & Ottolenghi, P. (1967) C. R. Trav. Lab. Carlsberg. **36**, 85–93
Gascon, S. & Lampen, J. O. (1968) J. Biol. Chem. **243**, 1567–1572
Gascon, S., Neuman, N. P. & Lampen, J. O. (1968) J. Biol. Chem. **243**, 1573–1577
Goldstein, A. & Lampen, J. O. (1975) Methods Enzymol. **42**, 505–511
Hames, B. D. (1981) in Gel Electrophoresis of Proteins: A Practical Approach (Hames, B. D. & Rickwood, D., eds.), pp. 1–91, IRL Press, London and Washington
Hubbard, S. C. & Ivatt, R. J. (1981) Annu. Rev. Biochem. **50**, 555–583
Huffaker, T. C. & Robbins, P. W. (1983) Proc. Natl. Acad. Sci. U.S.A. **80**, 7466–7470
Kaufer, N. F., Simanis, V. & Nurse, P. (1985) Nature (London) **318**, 78–80
Kornfeld, R. & Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631–664
Laemmli, U. K. (1970) Nature (London) **227**, 680–685
Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) J. Biol. Chem. **193**, 265–275
Mitchison, J. M. & Creanor, J. (1969) J. Cell Sci **5**, 373–391
Moreno, S., Ruiz, T., Sanchez, Y., Villanueva, J. R. & Rodriguez, L. (1985) Arch. Microbiol. **142**, 370–374
Morrisey, J. H. (1981) Anal. Biochem. **117**, 307–310
Neuman, N. P. & Lampen, J. O. (1967) Biochemistry **6**, 468–475
Ouchterlony, Ö. & Nilsson, C. A. (1978) Handb. Exp. Immunol. 3rd Ed. **1**, 19.1–19.44
Pollard, J. W. (1984) in Methods in Molecular Biology, vol. 1: Proteins (Walker, J. M., ed.), pp. 81–96, Humana Press, Clifton
Tarentino, A. L., Plummer, T. H. & Maley, F. (1974) J. Biol. Chem. **249**, 818–824

Received 19 September 1989/13 November 1989; accepted 24 November 1989