Purification, characterization and partial amino acid sequence of glycogen synthase from *Saccharomyces cerevisiae*

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Glycogen synthase from *Saccharomyces cerevisiae* was purified to homogeneity. The enzyme showed a subunit molecular mass of 80 kDa. The holoenzyme appears to be a tetramer. Antibodies developed against purified yeast glycogen synthase inactivated the enzyme in yeast extracts and allowed the detection of the protein in Western blots. Amino acid analysis showed that the enzyme is very rich in glutamate and/or glutamine residues. The N-terminal sequence (11 amino acid residues) was determined. In addition, selected tryptic-digest peptides were purified by reverse-phase h.p.l.c. and submitted to gas-phase sequencing. Up to eight sequences (79 amino acid residues) could be aligned with the human muscle enzyme sequence. Levels of identity range between 37 and 100 %, indicating that, although human and yeast glycogen synthases probably share some conserved regions, significant differences in their primary structure should be expected.

INTRODUCTION

Glycogen synthase (EC 2.4.1.11) is the enzyme responsible for the synthesis of glycogen in a wide variety of species, from yeast to mammals. In contrast with mammalian glycogen synthase, the enzyme from yeast has not been extensively characterized. Mainly from the work of Cabib and collaborators, it is known that yeast glycogen synthase exists as an oligomer that contains a single type of subunit [1]. As observed for the mammalian enzyme, glycogen synthase appears in yeast in two forms, I and D, which differ in their sensitivity to glucose 6-phosphate and are interconvertible [2]. Interconversion is, most probably, produced by phosphorylation–dephosphorylation reactions [1,2]. In addition, the yeast enzyme appears to be very sensitive to proteolysis.

Recently our laboratory became interested in the regulation of glycogen synthesis in yeast. Therefore we decided to characterize yeast glycogen synthase in order to obtain structural information to understand its regulation in yeast cells. In the present paper we describe a rapid purification method for yeast glycogen synthase, and our results indicate that, although yeast and mammalian glycogen synthases probably share regions with a high degree of sequence identity, significant differences in their primary structure should be expected.

MATERIALS AND METHODS

Materials

Trypsin [tosylphenylalanylchloromethane- ('TPCK'-)treated], rabbit liver glycogen (type III), phenylmethanesulphonyl fluoride, leupeptin and benzamidine were from Sigma Chemical Co. DEAE-cellulose (DE-32) was from Whatman. Sepharose 4B and Sephacryl S-300 came from Pharmacia. Other reagents were from Merck or Sigma Chemical Co.

α -Amylase was purified from human saliva as described in ref. [3]. Amylase activity was determined at 37 °C with maltotetraose as a substrate with a kit from Roche.

The Sepharose 4B–hexamethylenediamino-3-carboxypropionylglucosamine 6-phosphate affinity system was prepared as follows. Sepharose 4B was activated with CNBr and coupled to

1,6-diaminohexane as described by the manufacturer. The hexamethylenediamino-Sepharose 4B matrix was resuspended in water, succinic anhydride (1 mmol/ml of gel) was added and the reaction was carried out at pH 6.0 for 5 h at 4 °C. Subsequently, glucosamine 6-phosphate was coupled at pH 4.5 in the presence of 1-(dimethylaminopropyl)-3-ethylcarbodi-imide.

Growth of yeast

Saccharomyces cerevisiae ABYS[−] (a mutant lacking four major proteinases [4]) was grown in medium containing glucose (2 %, w/v), peptone (1 %, w/v) and yeast extract (1 %, w/v) at 30 °C. Cells were harvested shortly after the onset of the stationary phase.

Purification of glycogen synthase

All the purification steps were carried out between 0 and 4 °C unless otherwise stated.

Buffers. Buffer A was 50 mM-Tris/HCl buffer, pH 7.4, containing 0.6 M-sucrose, 5 mM-EDTA, 2 mM-EGTA, 1 mM-dithiothreitol, 1 mM-phenylmethanesulphonyl fluoride, 0.25 μ g of leupeptin/ml, 0.1 mM-tosyl-lysylchloromethane ('TLCK') and 0.5 mM-benzamidine. Buffer B was the same as buffer A except that the concentration of sucrose was lowered to 0.25 M and that it contained in addition 2 mM-Na₂SO₄, 10 mM-MgCl₂ and 0.5 mM-glucose 6-phosphate. Buffer C was the same as buffer A except that the concentration of sucrose was lowered to 0.25 M and that tosyl-lysylchloromethane was omitted. Buffer D was 50 mM-imidazole/HCl buffer, pH 7.4, containing 0.25 M-sucrose, 5 mM-EDTA, 2 mM-EGTA, 10 mM-MgCl₂, 1 mM-dithiothreitol and 1 mM-phenylmethanesulphonyl fluoride. Buffer E was 50 mM-Tris/HCl buffer, pH 7.8, containing 25 % (v/v) glycerol, 5 mM-EDTA, 2 mM-EGTA and 1 mM-dithiothreitol.

Extraction. Yeast cells were harvested by centrifugation (7000 g for 10 min) and washed twice with cold distilled water. A 300 g (wet wt.) batch of yeast was resuspended in 600 ml of buffer A. Then 90 ml portions were shaken five times for 1 min with 270 g of glass beads (0.45 mm in diameter) in a pre-cooled Vibrogen cell homogenizer. The temperature was kept below 8 °C. The homogenate was decanted, the glass beads were washed with

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300 ml of buffer A, and the washes and original homogenate were pooled and centrifuged at 13 000 *g* for 30 min at 4 °C. The supernatant was filtered through glass-wool. The filtrate was centrifuged at 117 000 *g* for 90 min at 4 °C. The supernatant was removed and discarded. Pellets were resuspended in 70 ml of buffer B.

Treatment with α -amylase. Human salivary amylase (4000 units) was added and the preparation was incubated at 30 °C for 30 min. After the incubation, the preparation was centrifuged at 170 000 *g* for 30 min at 4 °C and the supernatant recovered.

DEAE-cellulose chromatography. The supernatant was loaded (1 ml/min) on to a 70 ml DEAE-cellulose column equilibrated with buffer C. The column was washed with 100 ml of buffer C, followed by 700 ml of buffer C containing 50 mM-NaCl. Glycogen synthase activity was eluted with buffer C containing 200 mM-NaCl. Fractions containing glycogen synthase activity were pooled and dialysed for 2 h against buffer C containing 10 mM-MgCl₂.

Affinity column chromatography. The preparation was then loaded on to a 4 ml Sepharose 4B-hexamethylenediamino-3-carboxypropionylglucosamine 6-phosphate column equilibrated with buffer D. The column was washed with 10 ml of buffer D including 25 mM-NaCl at 4 °C. The column was then warmed to room temperature, and glycogen synthase was eluted with buffer D containing 25 mM-NaCl and 10 mM-glucose 6-phosphate. Fractions containing glycogen synthase activity were pooled, and rabbit liver glycogen was added to achieve a final concentration of 1 mg/ml. Then the enzyme was precipitated by adding cold ethanol, to 20 % (v/v) final concentration. The pellet was recovered by centrifugation at 18 000 *g* for 15 min at -10 °C, resuspended in 1 ml of buffer E and dialysed against the same buffer.

Determination of protein

Protein concentration was determined by the method of Bradford [5], with BSA as standard.

Electrophoretic analysis

SDS/PAGE was performed in 7 % polyacrylamide slab gels as described by Laemmli [6].

Assay of enzyme activity

The glycogen synthase -glucose 6-phosphate/+glucose 6-phosphate activity ratio was determined by the method of Thomas *et al.* [7]. Glycogen phosphorylase activity was measured by the method described in ref. [8] in the presence of 10 mM-caffeine and 10 mM-EDTA. One unit of enzyme activity is the amount of enzyme that incorporates 1 μ mol of [¹⁴C]glucose/min from UDP-[¹⁴C]glucose (glycogen synthase) or from [¹⁴C]glucose 1-phosphate (glycogen phosphorylase) into glycogen at 30 °C.

Determination of molecular mass and subunit structure of native glycogen synthase

Purified glycogen synthase (400 μ g in 1 ml) was chromatographed on a Sephacryl S-300 column (1.5 cm $\times$ 90 cm) in 50 mM-Tris/HCl buffer, pH 7.8, containing 5 mM-EDTA, 2 mM-dithiothreitol and 1 M-KCl. Fractions were collected and glycogen synthase activity was measured. Under the same conditions a linear calibration curve was obtained with standard proteins.

Immunochemical techniques

Antisera were raised in rabbits against homogeneous preparations of yeast glycogen synthase. A subcutaneous injection (50 μ g) in Freund's complete adjuvant was given, followed 10 and 20 days later by subcutaneous injection of yeast glycogen synthase (25 μ g) in Freund's incomplete adjuvant. Bleeding was carried out 15 days later. Immunoglobulins were partially purified by precipitation with (NH₄)₂SO₄ (final concentration 45 % saturation at 4 °C) and stored at -20 °C.

Amino acid composition

Glycogen synthase (10 μ g) was reduced and alkylated as described in ref. [9]. Gas-phase hydrolysis was performed *in vacuo* at 110 °C in 6 M-HCl containing 1 % phenol for 24 h. Phenylthiocarbonyl derivatives of the amino acids were identified by h.p.l.c. on a Hypersil ODS (3 μ m particle size) reverse-phase column (0.46 cm $\times$ 25 cm). The solvent A was 55 mM-sodium acetate buffer, pH 5.95, containing 2 % (v/v) acetonitrile and solvent B was acetonitrile. Peaks were monitored at 254 nm and identified and quantified by comparison with known standards.

Determination of N-terminal sequence

SDS/PAGE was performed on 1.5 mm-thick 7 % polyacrylamide gels as described by Laemmli [6]. Gels were pre-run for 15 min before loading of the sample (100 μ g of the glycogen synthase preparation). After electrophoresis, the protein was transferred to Immobilon [poly(vinylidene difluoride)] membranes (Millipore). The blotting was done according to the method of Matsudaira [10]. Blots were stained as described in ref. [11], and the 80 kDa glycogen synthase band was excised and sequenced directly with an Applied Biosystems model 470 A gas-phase sequencer equipped with a model 120 A phenylthiohydantoin analyser. The sequencing was carried out using the manufacturer's recommended programs and procedures.

Production of tryptic peptides and determination of peptide sequences

Glycogen synthase (200 μ g in 300 μ l of 0.4 M-Tris/HCl buffer, pH 8.1, containing 2 mM-EDTA and 6 M-guanidium chloride) was reduced at 37 °C for 2 h in the presence of 5 mM-dithiothreitol and then carboxymethylated by incubation with 90 μ l of 50 mM-iodo[¹⁴C]acetic acid (2200 c.p.m./nmol), pH 8.0, at 37 °C for 1 h. Carboxymethylated glycogen synthase was separated from unused iodoacetic acid by Sepharose G-25 (15 cm $\times$ 1 cm) gel filtration. The protein-containing fractions were evaporated in a Speed-Vac (Savant) and finally dissolved in 0.2 ml of 50 mM-ammonium bicarbonate buffer, pH 8.0. Trypsin was added to a final ratio enzyme/trypsin of 20:1 (w/w) and the digestion was continued for 16 h at 30 °C. The addition of trypsin was repeated twice. The digest was applied to a Brownlee Aqua Pore C₈ reverse-phase h.p.l.c. column. The acetonitrile gradient was 0-100 % (v/v) (100 min) in 0.1 % (v/v) trifluoroacetic acid at a flow rate of 0.8 ml/min. Peaks were monitored at 214 nm. The peptides collected from the column were purified to homogeneity by using a shallower acetonitrile gradient, and their amino acid sequence was determined by automated gas-phase Edman degradation.

RESULTS

Purification of yeast glycogen synthase

We have developed a new method for the purification of yeast glycogen synthase. Briefly, a glycogen pellet is obtained and glycogen synthase released from the glycogen by incubation with α -amylase. After DEAE-cellulose column chromatography,

which removes glycogen phosphorylase, the enzyme is purified by affinity chromatography, with glucosamine 6-phosphate as a ligand. This step is based on the fact that glucose 6-phosphate is a strong allosteric activator of the enzyme. This type of affinity chromatography was first used to purify glycogen synthase from adipose tissue by Miller *et al.* [12]. However, in our case, a hexamethylenediamino-3-carboxypropionyl, instead of a 3-carboxypropionylaminodipropyl, spacer arm is included to avoid steric interactions between the enzyme molecule and the gel matrix. The enzyme is eluted at low salt concentration (25 mM) in the presence of 10 mM-glucose 6-phosphate, and at this step preparations are essentially homogeneous (Fig. 1). Finally, the preparation is concentrated by precipitation with ethanol at low temperature in the presence of added glycogen. The purified enzyme can be stored at -70°C for at least 6 months without significant loss of activity. The whole procedure can be carried out in 48 h, and therefore it is much faster than the protocol described previously [1]. A summary of the purification is provided in Table 1.

SDS/PAGE of the final preparations (Fig. 2) shows a single band corresponding to the glycogen synthase subunit. The calculated molecular mass was 80 kDa, which is somewhat higher than the value described previously [1]. It is worth noting that in our case the source of the enzyme was a strain defective in four

Fig. 1. Affinity chromatography of yeast glycogen synthase
Glycogen synthase preparations were loaded on to a Sepharose 4B-hexamethylenediamino-3-carboxypropionylglucosamine 6-phosphate column and 1 ml fractions were collected. Enzyme activity was eluted with 25 mM-NaCl/10 mM-glucose 6-phosphate (*). No additional glycogen synthase activity was eluted at 100 mM-NaCl/15 mM-glucose 6-phosphate (★). The inset shows SDS/PAGE of fractions eluted from the affinity column. The apparent molecular mass is indicated on the left.

Table 1. Summary of the purification of yeast glycogen synthase

For experimental details see the Materials and methods section.

Fraction	Final volume (ml)	Total activity (units)	Concn. of protein (mg/ml)	Specific activity (units/mg)	Recovery (%)	Purification (fold)
Extract	875	138	15.7	0.010	100	1
Glycogen pellet	80	75	4.17	0.22	54	22
DEAE-cellulose chromatography	50	19.8	0.7	0.56	14.3	56
Affinity chromatography	1.1	17.6	0.7	22.85	12.7	2300

Fig. 2. SDS/PAGE of purified yeast glycogen synthase
A typical preparation of pure yeast glycogen synthase (10 μg) was subjected to SDS/PAGE (7% polyacrylamide) and protein was stained with Coomassie Blue R-250. The mobility of molecular-mass standards is indicated on the left.

major proteinases. When the same procedure was carried out with as starting material baker's yeast, purified preparations contained variable amounts of lower-molecular-mass polypeptides in the range 70–72 kDa, similar to those described in an earlier report [1]. Therefore yeast glycogen synthase may be very sensitive to proteolysis, as is the case for the enzyme from mammalian sources, especially from liver tissue. In this regard, the inclusion in the buffers of several proteinase inhibitors, the use of a strain deficient in proteinases as a starting material and the development of a fast protocol for purification appear as key features in order to obtain a high-molecular-mass enzyme.
Native glycogen synthase shows a molecular mass of 310 kDa, as determined by gel filtration through a Sephacryl S-300 column. Therefore, assuming a molecular mass of 80 kDa for the subunit, the enzyme is probably a tetramer (results not shown).

Characterization of antibodies against yeast glycogen synthase

Antisera against yeast glycogen synthase were obtained as described in the Materials and methods section. Antisera from different batches were tested for their ability to remove glycogen synthase activity from yeast extracts. Extracts were prepared as described for purification of the enzyme, and samples incubated for 2 h at 4°C with different amounts of antiserum. The mixture was centrifuged at 15000 g for 5 min and glycogen synthase

Fig. 3. Binding of antibodies to glycogen synthase from yeast extracts

Extracts from cultures of *S. cerevisiae* (ABYS⁻) were prepared, and to equal portions were added increasing volumes of control serum (○) or anti-(glycogen synthase) serum (●) and the mixtures were incubated for 2 h at 4 °C. Then, samples were centrifuged and glycogen synthase activity was measured in the supernatant (in the presence of 6.6 mM-glucose 6-phosphate). Glycogen phosphorylase activity (△) was also determined in samples incubated with anti-(glycogen synthase) antibodies and is represented as percentage over the controls. The inset shows Western-blot analysis of yeast extracts. Extracts were prepared, and then were subjected to SDS/PAGE (7% polyacrylamide) and the gels were blotted on to nylon membranes. ¹²⁵I-Protein G was used to detect the antigen-antibody reaction. The mobility of molecular-mass standards is indicated on the left.

activity was measured in the supernatant. As shown in Fig. 3, the antibodies were able to remove glycogen synthase activity completely from the extracts. Glycogen phosphorylase activity was not recognized by the antibodies. The antibodies did not cross-react with purified glycogen synthase from rat liver or rabbit muscle.

Glycogen synthase was also detected in yeast extracts by Western-blot techniques. Extracts from *S. cerevisiae* ABYS⁻ cultures were prepared and immediately placed in 1% (w/v) SDS/350 mM-2-mercaptoethanol (final concentrations) and boiled for 5 min. Electrophoresis was performed in 7% polyacrylamide gels. The proteins were blotted on to Immobilon membranes. ¹²⁵I-Protein G was used to detect proteins recognized by the antibody. As shown in Fig. 3 inset, two bands were detected, at 80 kDa and 72 kDa. The 80 kDa band exhibits the same mobility as the purified glycogen synthase subunit. Although precautions against proteolysis were taken during

preparation of the samples, the 72 kDa band was always detected.

N-Terminal sequence analysis of yeast glycogen synthase

Purified yeast glycogen synthase (100 μg) was subjected to SDS/PAGE and the 80 kDa band was transferred electrophoretically to Immobilon membranes. Direct gas-phase sequencing was performed and the sequence obtained is shown in Fig. 4. As can be observed, the N-terminal sequence of yeast glycogen synthase can be aligned with two regions of the human glycogen synthase (residues 14–24 or 28–38). In both cases the level of identity is 45%. Interestingly, yeast glycogen synthase lacks at least 12 amino acid residues that are present in the enzyme obtained from human or rabbit muscle.

Isolation and sequencing of tryptic-digest peptides

Homogeneous glycogen synthase was carboxymethylated and digested with trypsin as described in the Materials and methods section. Tryptic-digest peptides were isolated by reverse-phase h.p.l.c. on a C₈ column (Fig. 5). The peaks numbered in the Figure were collected individually and rechromatographed. Fig. 6 shows the sequence obtained for each peptide.

Tryptic-digest peptides obtained from yeast glycogen synthase could be aligned in most cases (seven out of eight) with specific sequences located throughout the entire coding region of human muscle glycogen synthase (Fig. 6). However, the level of identity varies greatly. Thus peptide no. 6 matches perfectly (eight out of eight residues) with amino acid residues 592–599 of the human muscle enzyme. On the other hand, other peptides show a weaker similarity. For instance, peptide no. 7 shows only a 37% identity with amino acid residues 67–74 of the human muscle enzyme.

Amino acid composition

The amino acid composition of yeast glycogen synthase was determined as described in the Materials and methods section. As shown in Table 2, a noteworthy feature is the high content in glutamic acid/glutamine residues, which account for about 25% of the total expected residues.

DISCUSSION

We have developed a method for the purification of yeast glycogen synthase. This protocol yields an essentially homogeneous enzyme and is faster than the method described previously [1]. One of the most important problems when purifying glycogen synthase from mammalian tissues is the occurrence of proteolysis, which results in the generation of low-molecular-mass fragments [16,17]. A previous report on purified yeast glycogen synthase [1] showed a molecular mass of 77 kDa for the enzyme subunit, often accompanied by species of lower molecular mass (71 kDa). This molecular mass was lower than that of

Fig. 4. Comparison of the N-terminal sequence of yeast glycogen synthase with those of rabbit muscle and liver and human muscle glycogen synthases

The N-terminal sequence of yeast glycogen synthase can be aligned at two positions (starting at positions 14 and 28), very near the N-termini of human muscle [13], rabbit muscle [14] and rabbit liver [15] glycogen synthases. The numbering system is according to the amino acid sequence of the human enzyme. Alignment was performed using the QGSEARCH program from the PCGENE package (Intelligenetics Inc.).

Fig. 5. H.p.l.c. profile of tryptic-digest peptides from yeast glycogen synthase

The isolated 80 kDa protein was treated with trypsin and submitted to h.p.l.c. on a C_8 reverse-phase column. Numbered peaks yielded easily resolved peptides when rechromatographed as described in the Materials and methods section. These peptides were subsequently used for amino acid sequencing. Peptides no. 4 and no. 5, which were eluted together from the first chromatography (peak 4,5), were well separated from each other when rechromatographed.

Peptide no.			
Human	3	60	D N Y F L
Yeast			I N D F L
			- - -
Human	7	67	P Y T E Q G V R
Yeast			G Y P D Q F F R
			- - -
Human	4	107	L I E G G P
Yeast			L I E G S V
			- - -
Human	1	212	Y L C A G A V D F Y N N L E N
Yeast			Y S N G S N N D D N N N E N
			- - -
Human	8	247	A A A H C A H V F T T V S Q I T A I
Yeast			A A A H S A D V F T T P S Q I T A F
			- - -
Human	5	547	A Y G I Y I L D R
Yeast			H Y - I Y I V D R
			- - -
Human	6	592	L S D L L D W K
Yeast			L S D L L D W K
			- - -

Fig. 6. Comparison between the sequence of human glycogen synthase and those of yeast glycogen synthase tryptic-digest peptides

The amino acid sequences of seven tryptic peptides from yeast glycogen synthase were aligned with the amino acid sequence of human muscle glycogen synthase deduced from a cDNA clone [13]. Identical residues are underlined. The numbering system is according to the amino acid sequence of the human enzyme. The peptide numbers correspond to those indicated in Fig. 5. Tryptic-digest peptide no. 2 does not present a significant similarity to any region of the human sequence. Comparison was performed using the QGSEARCH program from the PCGENE package (Intelligenetics Inc.).

mammalian glycogen synthase. Therefore our main goal was to purify undegraded yeast glycogen synthase for sequence and phosphorylation studies.

Since it is known that several phosphorylation sites occur near the *N*-terminal region in mammalian glycogen synthase and that this region is very sensitive to proteolysis, we considered the

isolation of the complete subunit an important point. For that purpose we selected a yeast strain (ABYS⁻) deficient in several proteinases [4] as a source of the enzyme and developed a fast affinity-chromatography-based procedure to isolate glycogen synthase. SDS/PAGE analysis shows a molecular mass of 80 kDa for the enzyme subunit, which is higher than previously reported

Table 2. Amino acid composition of yeast glycogen synthase

Amino acid residue	Amino acid composition (mol of residue/mol)
Asx	68
Glx	180
Ser	58
Gly	69
His	13
Thr	24
Ala	63
Arg	25
Pro	42
Tyr	27
Val	35
Met	7
Ile	20
Leu	34
Phe	16
Lys	46
Trp	—*
Cys	—*

* Not determined.

values. Data obtained with gel-filtration columns showed that the holoenzyme is eluted as a 310 kDa species, suggesting a tetrameric structure for the yeast glycogen synthase. This observation agrees with the results obtained by Cabib & Huang [1] using equilibrium-sedimentation techniques.

Antibodies developed in rabbit against homogeneous yeast glycogen synthase preparations were able to remove glycogen synthase activity from yeast extracts, whereas they failed to recognize glycogen phosphorylase. The specificity of the antibodies was tested by Western-blot experiments with yeast extracts as a source of protein. In these conditions two bands were detected, at 80 kDa and 72 kDa. Since precautions were taken, it is unlikely that proteolysis would occur during the preparation step. Therefore the possibility that a population of glycogen synthase could exist in the cell as proteolytic products has to be considered. Alternatively, the existence of a protein structurally related to the 80 kDa glycogen synthase subunit must also be taken into consideration.

The amino acid composition shows that the enzyme is very rich in glutamic acid/glutamine residues, which account for about 25 % of the total amino acid residues. In this regard yeast glycogen synthase appears to be quite different from the human muscle enzyme [13] and the bacterial glycogen synthase [18], where the amounts of glutamic acid or glutamine residues are not particularly high.

Several conclusions can be drawn from the comparison of the peptide sequences from yeast glycogen synthase with the complete sequence of the human muscle enzyme derived from a cDNA clone [13]. The *N*-terminal sequence of the yeast enzyme can be aligned with two regions of the human protein (amino acid residues 14–24 and 28–38). In any case, it is clear that the yeast enzyme lacks at least 12 amino acid residues that can be found in the enzymes from human, rabbit and rat muscle [13,14,19], and eight amino acid residues when compared with that from rabbit liver [15]. This finding is particularly interesting since it indicates that serine-7, a phosphorylation site (site 2) that is conserved in the mammalian enzymes, is not present in yeast glycogen synthase. Site 2 is believed to be a major site for the action of cyclic AMP-dependent protein kinase in rabbit and rat muscle and liver tissues. Consequently, if it is assumed that phosphorylation at site 2 is a key feature in the control of

glycogen synthase activity by cyclic AMP-dependent protein kinase in mammalian tissues, it is necessary to conclude that significant differences between mammalian and yeast glycogen synthases should be expected with regard to the control of the enzyme by phosphorylation. In addition, serine-10, a recently identified phosphorylation site in rabbit muscle glycogen synthase [20,21], would be also lacking in the yeast enzyme.

Although the *N*-terminal sequence of yeast glycogen synthase can be aligned with two regions of the enzymes (both of them close to the *N*-terminus) from human, rabbit and rat [13,14,19], we favour the hypothesis that the *N*-terminal yeast sequence corresponds to amino acid residues 28–38 in the sequence of the human muscle enzyme. In this case yeast glycogen synthase *N*-terminus would be 27 amino acid residues shorter than the human or rabbit muscle enzymes. This could account, at least partially, for the lower molecular mass of yeast glycogen synthase compared with the enzyme from mammalian sources. Although the number of identical residues is the same in both regions (five out of 11 residues), changes are more conservative when compared with sequence 28–38 (i.e. glycine for valine, aspartic acid for glutamic acid etc.) In addition, this region is also highly conserved in the enzymes from different mammalian species [19], most probably because it surrounds the putative active site (lysine-39) [22]. Unfortunately, our sequence does not reach far enough to determine whether this lysine residue is conserved in the yeast enzyme. However, it is worth noting that two lysine residues, not present in the mammalian enzyme, are found in the *N*-terminal sequence of the yeast enzyme. Whether or not they are related to catalytic functions is presently unknown.

Tryptic-digest peptide sequences obtained from yeast glycogen synthase can be aligned through the entire coding region of the human muscle enzyme, suggesting that yeast glycogen synthase is much closer to the mammalian enzyme than is bacterial glycogen synthase [18]. However, among the various peptides different degrees of similarity are found. For instance, a 100 % identity is observed for peptide 6 when compared with the sequence of amino acid residues 592–599 of the human muscle enzyme, whereas peptides 7 and 1 can be aligned with only 37 % and 40 % identity, starting at positions 67 and 212 respectively. Yeast glycogen synthase resembles the mammalian enzyme in many features (i.e. sensitivity to glucose 6-phosphate, use of UDP-glucose as a substrate, possible regulation by phosphorylation reactions etc.), but also presents some differences, such as a lower molecular mass. In mammalian tissues glycogen synthase exists as at least two different isoforms, defined as a muscle type and a liver type. Antibodies developed against one of the forms cross-react very poorly or not at all with the other [23,24]. In fact, the known *N*-terminal sequence of the liver enzyme shows a relatively poor level of identity (44 %) when compared with that of the muscle type [15]. In addition, both types show different specificities for several protein kinases. Although some features of the yeast enzyme are closer to those of the muscle type, such as a tetrameric structure and a slightly higher similarity at the *N*-terminal sequence, it is not possible, at present, to decide if yeast glycogen synthase is more closely related to the liver or the muscle enzyme, or unrelated to both forms, although our antibodies against yeast glycogen synthase recognize neither the enzyme from mammalian liver nor that from mammalian muscle. Molecular cloning of yeast glycogen synthase gene would serve to address this and other questions on the subject.

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REFERENCES

1. Cabib, E. & Huang, K.-P. (1974) *J. Biol. Chem.* **249**, 3851-3857
2. Cabib, E. & Rothman-Denes, L. B. (1971) *Biochemistry* **10**, 1236-1242
3. Bernfeld, P. (1955) *Methods Enzymol.* **1**, 149-158
4. Wolf, D. H. (1982) *Trends Biochem. Sci.* **7**, 35-37
5. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248-254
6. Laemmli, U. K. (1970) *Nature (London)* **227**, 680-685
7. Thomas, J. A., Schlender, K. K. & Lerner, J. (1968) *Anal. Biochem.* **25**, 486-499
8. Gilboe, D. P., Larson, K. L. & Nuttall, F. Q. (1972) *Anal. Biochem.* **47**, 20-27
9. Hawke, D. H., Yuang, P. M., Blacher, R. W., Wilson, K. J. & Hunkapiller, M. W. (1987) *Abstr. Symp. Protein Soc. 1st, San Diego*, abstr. 1051
10. Matsudaira, P. (1987) *J. Biol. Chem.* **262**, 10035-10038
11. Pluskal, M. G., Przekop, M. B., Kavonian, M. R., Vecoli, C. & Hicks, D. A. (1986) *Biotechniques* **4**, 272-282
12. Miller, R. E., Miller, E. A., Fredholm, B., Yellin, J. B., Eichner, R. D., Mayer, S. E. & Steinberg, D. (1975) *Biochemistry* **14**, 2481-2488
13. Browner, M. F., Nakano, K., Bang, A. G. & Fletterick, R. (1989) *Proc. Natl. Acad. Sci. U.S.A.* **86**, 1443-1447
14. Cohen, P., Parker, P. J. & Woodgett, J. R. (1985) in *Molecular Basis of Insulin Action* (Czech, M.P., ed.), pp. 213-233, Plenum Press, New York
15. Wang, Y., Bell, A. W., Hermondsen, M. A. & Roach, P. J. (1986) *J. Biol. Chem.* **261**, 16909-16913
16. Jett, M. F. & Soderling, T. R. (1979) *J. Biol. Chem.* **254**, 6379-6745
17. Camici, M., DePaoli-Roach, A. & Roach, P. J. (1984) *J. Biol. Chem.* **259**, 3429-3434
18. Kumar, A., Larsen, C. E. & Preiss, J. (1986) *J. Biol. Chem.* **261**, 16256-16259
19. Jaspers, S. R., Rulfs, J., Johnson, G., Mole, J. E. & Miller, T. B., Jr. (1989) *Arch. Biochem. Biophys.* **268**, 630-636
20. Poulter, L., Ang, S.-G., Gibson, B. W., Williams, D. H., Holmes, C. F. B., Caudwell, F. B., Pitcher, J. & Cohen, P. (1988) *Eur. J. Biochem.* **175**, 497-510
21. Flotow, H. & Roach, P. J. (1989) *J. Biol. Chem.* **264**, 9126-9128
22. Mahrenholz, A. M., Wang, Y. & Roach, P. J. (1988) *J. Biol. Chem.* **263**, 10561-10567
23. Kaslow, H. R. & Lesikar, D. D. (1984) *FEBS Lett.* **172**, 294-298
24. Kaslow, H. R., Lesikar, D. D., Antwie, D. & Tan, A. W. H. (1985) *J. Biol. Chem.* **260**, 9953-9956

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