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Analysis of Hepatic Glycogen-Associated Proteins

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Abstract

Glycogen particles are associated with a population of proteins that mediate its biological functions, including: management of glucose flux into and out of the glycogen particle, maintenance of glycogen structure and regulation of particle size, number, and cellular location. A survey of the glycogen-associated proteome would be predicted to identify the relative representation of known members of this population, and associations with unexpected proteins that have the potential to mediate other functions of the glycogen particle. We therefore purified glycogen particles from both mouse and rat liver, using different techniques, and analyzed the resulting tryptic peptides by mass spectrometry. We also specifically eluted glycogen-binding proteins from the pellet using malto-oligosaccharides. Comparison of the rat and mouse populations, and analysis of specifically eluted proteins, allow some conclusions to be made about the hepatic glycogen sub-proteome. With the exception of glycogen branching enzyme all glycogen metabolic proteins were detected. Novel associations were identified, including ferritin and starch-binding domain protein 1, a protein that contains both a transmembrane endoplasmic reticulum signal peptide and a carbohydrate-binding module. This study therefore provides insight into the organization of the glycogen proteome, identifies other associated proteins and provides a starting point to explore the dynamic nature and cellular distribution of this metabolically important protein population.

Keywords

Glycogen Particle; Carbohydrate Binding Domains; Glucose Metabolism; Starch Binding Domain Protein 1; Sub-cellular Localization

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

1. INTRODUCTION

The storage of glucose as glycogen is integral to cell biology, and its dysregulation is a feature of many disease states. Glycogen exists in all cells, but in mammals the primary reserves are in muscle and the liver. In muscle, glycogen provides glucose to meet the energetic requirements associated with movement, and is the major destination of insulin-dependent glucose transport [1]. In liver, glycogen incorporates excess glucose from either dietary or gluconeogenic sources and contributes to hepatic glucose output in response to hypoglycemia [1,2].

Glycogen is a branched polymer of glucose. It is initiated by the autoglucosylation of glycogenin and elongated by the activities of glycogen synthase (GS) and glycogen branching enzyme (GBE). It is comprised of α 1,4-glycosidic bonds with branch points occurring at α 1,6-glycosidic linkages [1]. Mature liver glycogen molecules, or beta-particles, have up to 55,000 glucosyl units, are 20 to 30 nm in diameter, and aggregate to form larger alpha-rosettes [3–5]. Glucose is mobilized from glycogen by the concerted action of glycogen phosphorylase (GP) and glycogen debranching enzyme [1]. Proteins involved in glycogen metabolism have been documented to interact with the particle to form a dynamic carbohydrate/protein complex [6]. The glycogen particle therefore is a sub-cellular compartment that has the required composition to maintain the macromolecule and respond to the metabolic and synthetic requirements of the cell.

Because of its central role in carbohydrate metabolism, glycogen is highly regulated. There are three modes of regulation. The catalytic activities of glycogen metabolic enzymes, particularly GP and GS, are controlled by a combination of post-translational modification and allosteric activators [1,7]. Glycogen metabolic proteins translocate in a controlled way between the glycogen particle and other cellular structures [8–10]. Finally, the glycogen particle is spatially and temporally regulated. Microscopic studies reveal association of the particle with specific cellular structures, particularly endoplasmic reticulum membranes and mitochondria, and the particle distribution in the cell responds dynamically to metabolic conditions [4,11].

Defining the hepatic glycogen proteome is therefore a challenge, given the dynamic nature of the protein/carbohydrate complex and cellular structures that would be predicted to specifically co-purify with the particle. To address difficulties in articulating the line between simple contamination and specific association we define the proteome by both broad and stringent criteria. The broadly defined glycogen proteome was based on repeated and consistent association with the glycogen pellet [12] (Table 1.). To increase confidence in specific associations we took two approaches. We analyzed the population of proteins solubilized by the addition of malto-oligosaccharides, which specifically compete for glycogen binding activities releasing these proteins from the insoluble glycogen molecule (Table 2.). Secondly, we contrasted the population with a glycogen-associated protein population isolated from rat liver using a different purification method (Tables 3 and 4).

This strategy resulted in a survey of the mouse and rat hepatic glycogen particle that identified and analyzed a combination of expected associations with glycogen binding proteins as well as unexpected identifications that have the potential to represent meaningful roles in glycogen metabolism and provide a measure of the sub-cellular distribution of glycogen particles. Each of these observations provides the starting point for additional studies on the dynamic nature of the glycogen proteome and its role in glycogen function including regulation of glucose concentrations, cellular distribution and integration in cellular metabolic pathways.

2. MATERIALS and METHODS

Preparation and Trypsinization of Liver Glycogen Particles

Rat and mouse liver glycogen particles were purified as previously described or as detailed in the supplemental section [13]. All glycogen particle preparations originated from single livers were treated by addition of 20 µg TPCK-treated trypsin (Trypsin Gold, Promega, Madison, WI) and incubated at room temperature for 16 h. Peptides were purified by C18 ZipTip™ desalting columns (Millipore, Billerica, MA), eluted into 10 µl 50% methanol, 0.5% acetic acid and 2 µl acetonitrile and the analyzed by LC/MS/MS. All non-organic buffers were passed through a Sep-Pak reversed-phase tC18 solid phase extraction column (Waters Corp., Milford, MA).

Mass Spectrometry

Tryptic preparations of glycogen-associated proteins were analyzed by LC/MS/MS analysis using an ESI Ion-Trap/FTMS hybrid mass spectrometer (LTQ-FT, ThermoElectron, Corp., Waltham, MA). An aliquot of 0.5% to 20% of each sample was injected onto a nano-LC column (75 µm ID × 10 cm, Atlantis C18 RP, 3 µm particle size, Waters, Milford, MA) using a nano-LC system (NanoLC 1D, Eksigent Technologies, Dublin, CA) with a gradient of 9 % to 60% acetonitrile in 0.1% formic acid at 400 nL/min (Supplemental Table 1A). Primary mass spectra were acquired in the FTMS portion of the instrument and MS/MS sequence information was collected in the linear ion trap using collision-induced dissociation (CID). Primary mass spectra were acquired with typically better than 2 ppm mass accuracy; CID fragmentation spectra were acquired with less than 0.3 Da mass error. A total a four mouse and four rat replicate hepatic glycogen preparations, 22 samples in total, were analyzed (Supplemental Table 1).

Data Analysis

Peaklists for database searching were generated for peptide precursor ions (i.e. +1, +2 and/or +3 charge states) and corresponding CID fragmentation data using SEQUEST (BioWorks Browser, revision 3.2, ThermoElectron Corp.) with the default parameters. Resulting DTA files from individual LC/MS/MS runs for each species were concatenated into a single mouse and rat file and analyzed using MASCOT (software version 2.1.03, Matrix Science, Inc., Boston, MA). The mouse and rat datasets had 139,711 and 28,664 queries respectively. Both datasets were searched using the mammalia taxonomy classification within the MSDB database (down loaded 08/06/2007, with 3,239,079 sequences). The broader “mammalia” taxonomy, rather than specifically “rodentia” or “mouse” taxonomy, was employed in order to detect potential contamination from human keratins and porcine trypsin. The following MASCOT search parameters were used in the analysis: tryptic-specific peptides, maximum of 3 missed cleavages, no fixed modifications, variable methionine oxidation, mass tolerances of 5 ppm for precursor ions and 0.3 Da for MS/MS CID fragment ions. A significance threshold of $p < 0.05$ for identified proteins was used. Individually identified peptides with expectation scores below 0.05 were considered significant and included. Any protein or peptide with greater than a 5% chance of false assignment were excluded. In order to be classed as a glycogen-associated protein, two unique peptides, with different primary sequence and expectation scores less than 0.05, were required to be identified from the gene product.

To gain a conservative estimate of the false discovery rate (FDR) a decoy database was developed by the MASCOT software. The FDR was calculated as the number of false positives (FP), determined by searching the decoy database, divided by the number of matches (M) in the target “mammalia” database ($FDR = FP/M$). The decoy database consisted of random sequences, of the same length and average amino acid distribution of

the “mammalia” database, generated for each identified peptide. The same criteria for acceptance was employed for both target and decoy databases. Searches of the mammalian database resulted in identification of 12498 and 2539 unique peptide sequences from the mouse and rat datasets (from 14 and 7 LC/MS/MS runs respectively), which satisfied the Mascot “identity threshold”. The malto-oligosaccharide-treated dataset contained 1054 peptides (from 3 LC/MS/MS runs). The relative size of the datasets was consistent with the number of LC/MS/MS runs. Decoy databases based on these datasets resulted in 1744 and 330 matches, giving an FDR of 13.95% and 13.00% for the mouse and rat datasets respectively. The decoy database for the smaller malto-oligosaccharide eluted preparation resulted in 118 matches and an FDR value of 11.06%. These are high values, but were consistent with the large size of the datasets.

In order to achieve consistent protein assignment, Mascot search results were exported in the comma-delimited CSV format and arrayed in a spreadsheet (Microsoft® Excel® 2004 for Mac Version 11.3.7). Peptides from poorly annotated protein assignments were submitted to the International Protein Index (IPI) mouse or rat database (April 2008) using the PROWL website (<http://prowl.rockefeller.edu>). The Swiss-Prot identifier was obtained, submitted to the UNIPROT database (<http://www.uniprot.org>), and the resulting UNIPROT identifiers referenced.

3. and 4. RESULTS and DISCUSSION

Glycogen particles from mouse liver were gently isolated through repeated centrifugation steps until a stable protein population was obtained (Supplemental Methods and Supplemental Figure 1). The glycogen particles were trypsinized and purified peptide preparations analyzed by tandem reversed phase liquid chromatography/mass spectrometry (LC/MS/MS). Primary mass spectra (MS) with high mass accuracy (< 5 ppm) and Collision Induced Dissociation (CID; MS/MS) spectra were acquired for each peptide. Data files were compared to the “mammalia” database using the MASCOT software algorithm (<http://www.matrixscience.com>) and PROWL search engine (<http://prowl.rockefeller.edu>). Identified proteins from each dataset are listed functionally in order of likelihood of identification in Tables 1 to 3 with a complete listing of proteins and peptides in the Supplemental section (Supplemental Tables 2 to 5). The consistent protein population that resulted from repeated glycogen pelleting steps represents the broadest boundary of the glycogen-associated proteome. To achieve a more stringent definition, two strategies were employed. The glycogen pellet was resuspended in the presence of malto-oligosaccharides and recentrifuged, the supernatant contained proteins or cellular structures that were specifically solubilized based on their glycogen-binding activity (Table 2). Secondly, another glycogen-associated protein population was obtained from rat liver (Table 3) using a different purification methodology allowing qualitative comparison of the two populations (Table 4). Finally, the sub-cellular localization of proteins from each dataset was analyzed (Figure 1).

Identification of glycogen-associated proteins

The analysis was validated by the consistent and frequent identification of proteins known to maintain and associate with the glycogen particle (Tables 1 and 3) [1,12,14–16]. Four of the glycogen metabolic enzymes were present in all preparations: glycogen phosphorylase (GP; Mowse score = 110281 and 13650 from the mouse and rat glycogen proteomes), glycogen debranching enzyme (GDE; 45937 and 4939), glycogen synthase (GS; 40420 and 3528), and glycogenin-1 (18892 and 1555) [1,12,14]. Known regulatory proteins were also identified, such as the mouse protein phosphatase 1 catalytic subunit (648), the rat G_L liver-specific glycogen targeting subunit (346; regulatory subunit 3B), and mouse laforin (56) [17–19]. Other isoforms of the major enzymes GP and GS were identified, consistent with

the presence of other cell types in the preparations, or possible low-level expression in hepatocytes [20]. Glycogen branching enzyme was present in very low amounts in the mouse dataset (56) and absent in the rat, which was confirmed by immunoblot (Supplemental Figure 2.). This was unexpected, particularly as it is relatively abundant in the glycogen sub-proteome of other cell types (data not shown) [16]. However, lack of glycogen binding has been noted in certain muscle glycogen-protein preparations, which provides support for the findings of this survey [21]. This implies that the glycogen-binding activity of this enzyme may be regulated. In contrast, glycogenin-1 was relatively well represented in both rat and mouse datasets [22]. This is unexpected since glycogenin, the primer of glycogen synthesis, is thought to occur at a low stoichiometry of one molecule per glycogen particle, which in any case would be sterically occluded by the mature glycogen polysaccharide [1,22,23]. The abundance of glycogenin in both datasets implies a presence on the particle surface, potentially through interaction with glycogen synthase [24]. Another possibility is a presence in membranes that co-purify with the particle, potentially through the binding activity of STBD1 [25]. Each scenario raises general questions about glycogen initiation. Both pancreatic and salivary amylase were identified in both hepatic glycogen proteomes [12]. Pancreatic amylase is expressed and secreted in the liver, has high affinity for glycogen and most likely co-purifies with glycogen as an artifact of the purification protocols, although there is a report of occasional ectopic distribution within the hepatocyte [26]. The presence of these proteins in the glycogen sub-proteome provides a reminder that glycogen-association needs to be placed within the context of other known properties about a given protein and is not sufficient in and of itself to conclude a functional role in intracellular glycogen metabolism.

AMP-dependent kinase (AMPK) was recently described as associating with hepatic glycogen particles based on immuno-electron microscopic studies together with several *in vitro* studies [27–30]. However in this study, AMPK was not present in either the mouse or rat hepatic glycogen proteome, a finding supported by immunoblots for AMPK (Supplemental Figure 2) [13]. As has been suggested, it is possible that the conditions of glycogen purification were too stringent to identify all glycogen-associated proteins [28]. To confirm that rat AMPK was not likely to dissociate from glycogen during the sucrose gradient step, we measured AMPK β 1-CBM's affinity for sucrose using fluorescence spectrometry but found no evidence of binding (data not shown). Furthermore, mouse liver glycogen was purified using a non-denaturing method that never strayed from physiological conditions and was time efficient (Supplemental Methods), yet AMPK did not associate with glycogen. Failure of AMPK to appear in the two datasets, along with other known glycogen-targeting proteins, such as protein phosphatase-1 glycogen-targeting subunits, phosphorylase kinase, and even glycogen-branching enzyme is a reflection of binding activity and does not preclude a functional role in glycogen metabolism [12,13,19,31].

The hepatic glycogen proteome also included novel candidate proteins that have the potential to mediate the sub-cellular localization patterns of glycogen particles or other functions of the glycogen molecule. Starch-binding domain-containing protein 1 (STBD1) contains both a transmembrane endoplasmic reticulum signal peptide (<http://pasub.cs.ualberta.ca:8080/pa/Subcellular>) and a carbohydrate-binding domain (CBM20). We suggest that this is the appropriate structure for a protein mediating association of glycogen particles with endoplasmic membranes [32,33]. While bioinformatic studies have noted the presence of the starch-binding domain on human STBD1, this is the first biochemical evidence of glycogen-binding activity [34,35]. It should be noted that a formal demonstration of glycogen binding for STBD1 is yet to be reported. Laforin, a recently identified glycogen phosphatase, has been demonstrated to associate with the glycogen particle via another CBM20 domain and was also identified in the mouse, but not the rat, hepatic glycogen proteome [36]. This supports the observation that the mouse and rat

datasets are not equivalent due to changes in the gender, age, preparation and species of the originating hepatic material.

Analysis of the stringently purified hepatic glycogen proteins

A basic concern of proteomic analysis is the trade-off between the loss of true and false positives. Co-purification with glycogen particles implies a specific interaction, but may also be due to contaminating “bleed over” or non-specific interaction from abundant proteins. To increase the confidence that identified proteins have a specific interaction with the glycogen molecule the pellet was resuspended in the presence of malto-oligosaccharides, which are chains of 8 to 14 α 1,4-linked glucose monomers that are chemically identical to the unbranched portion of the glycogen macromolecule. These oligosaccharides compete with the glycogen macromolecule for glycogen binding proteins, binding them and preventing immobilization to the glycogen pellet during centrifugation (Supplemental Methods). While this protein population is smaller and less complex, since the dataset results from only three LC/MS/MS runs, many of the features of the broader population are maintained: association of known glycogen metabolic proteins and identification of potentially novel glycogen associated proteins, such as STBD1 and ferritin (Table 2).

Contrasting the glycogen-associated protein populations from mouse and rat livers

A second strategy to increase confidence in defining functional associations with the glycogen is to isolate a related population and identify common proteins. We therefore isolated glycogen particles from female rat livers using differential centrifugation with sucrose gradients instead of repeated glycogen pelleting [13]. The resulting population was analyzed using LC/MS/MS under the identical conditions. As in the mouse glycogen-associated sub-proteome, known glycogen binding proteins were well represented (Table 3). Proteins with novel associations in the mouse proteome, such as Starch Binding Domain Protein 1 (STBD1) and ferritin, were also present. The remainder of the glycogen bound protein population was dramatically different, more than what would be expected from two similar species, regardless of housing conditions and age and gender differences. The greatest difference in these two preparations is the purification protocol, although direct comparison is not possible without a systematic analysis of the two species using the same purification conditions or alternately different purifications using the same initial starting material. The rat glycogen-associated sub-proteome contained many ER-resident proteins. It was particularly rich in ER-resident cytochrome P450 proteins and UDP-glucuronosyltransferases that are involved in hydroxylation and glucuronidation of lipophilic compounds. Glucose-1-phosphate the product of glycogenolysis, is only two metabolic steps removed from UDP-glucuronic acid. The physical proximity of these proteins with glycogen particles validates the concept of ‘glycogen-reticular complexes’ and supports the observation that the glucose required for glucuronidation is derived from glycogen and not gluconeogenesis or glucose transport [37].

Sub-cellular distribution of glycogen associated proteins

Within intact cells glycogen particles are attached to specific structures, particularly endoplasmic membranes, but also mitochondria and cytoskeletal structures [4]. Homogenization disrupts these structures with small fragments co-purifying with the glycogen particle. Glycogen-associated proteins either have primary interactions with the glycogen molecule or have secondary interactions and be part of complexes or cellular structures, particularly membranes, in which only one component has a specific interaction (Table 4). Secondary components of these membranes or other structures provide a potential measure of glycogen sub-cellular distribution within the cell. Based on annotation from the UNIPROT website (<http://uniprot.org>) the sub-cellular localization of each protein in the mouse hepatic glycogen proteome (mouse), the malto-oligosaccharide purified mouse

proteome (MO PROT), and rat hepatic proteome (rat) was collated (Figure 1). While the populations were unequally weighted and cannot be quantitatively compared, certain observations can still be made. Purification using the malto-oligosaccharides resulted in a loss of only two glycogen-associated proteins but all cytoplasmic and nuclear proteins are absent, even though they were previously well represented, indicating that these are most likely non-specific interactions. Mitochondrial, peroxisomal, lysosomal and endoplasmic reticulum proteins are still represented, increasing the possibility glycogen specifically interacts with these cellular structures. There is also evidence of finer sub-organelle control of glycogen distribution. ER-resident peptidylprolyl-isomerase was abundant in the mouse proteome, but was not present in the rat proteome even though the rat dataset was particularly rich in ER-resident proteins (Tables 1 to 3).

The localization data broadly supports the hypothesis that glycogen particles are integrated into different metabolic pathways depending on their sub-cellular location [4–6,38]. While the above data is indirect and not qualitative due to differences in preparation among the respective datasets, a systematic approach using proteomics to analyze glycogen cell localization in different metabolic states is possible if care is taken to conduct a study with a highly defined, amenable cell culture system with equivalent datasets.

4. CONCLUDING REMARKS

We have conducted a proteomic analysis of the hepatic glycogen particle. We identified many proteins previously known to associate with glycogen, including glycogen phosphorylase, glycogen synthase, glycogen debranching enzyme, and glycogenin. Glycogen branching enzyme was underrepresented and glycogenin over represented. Included in the population was ferritin and STBD1, a novel glycogen particle protein that contains an endoplasmic reticulum signal peptide and a carbohydrate-binding module, the primary structure that we predict accounts for the regulated localization and targeting of glycogen particles to endoplasmic membranes [4,34].

This is the first biochemical evidence that STBD1 binds to glycogen, although a formal demonstration is required. In confirmation of physiological and histological studies, we provide mass spectrometric evidence that the glycogen particle is targeted to intracellular loci, supporting the hypothesis that localization of glycogen within the cell is regulated [38]. The methods employed in this study allow analysis of the glycogen proteome in other tissues and in other physiological and metabolic states in amenable cell culture systems. These studies identify proteins with potential regulatory functions in glycogen metabolism, and provide a greater appreciation of the complexity and role of this important protein population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

CBM20 carbohydrate-binding module_family 20

CBM48	carbohydrate-binding module_family 48
DTA	SEQUEST peaklist data file format
emPAI	exponentially modified Protein Abundance Index
FDR	False Discovery Rate
FP	False Positive
FTMS	Fourier transform mass spectrometry
G6P	glucose-6-phosphate
GBE	glycogen branching enzyme
GDE	glycogen debranching enzyme
GP	glycogen phosphorylase
GS	glycogen synthase
MD	maltodextrin
STBD1	starch binding domain protein 1

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Figure 1. Cellular Distribution of Glycogen-Associated Proteins

Proteins from mouse (black bars) and rat (grey bars) hepatic glycogen preparations, which met the standard criteria for designation for glycogen association (Experimental Procedures), were submitted to the UNIPROT database and the cellular distribution obtained and collated. The sub-cellular location of mouse proteins present in the malto-oligosaccharide eluted supernatant are also included (Mouse MO SN, white bar). Abbreviations include: known glycogen associated proteins (g) and proteins located in the cytoplasm (c), mitochondria (m), endoplasmic reticulum and microsomes (er), nucleus (n), lysosomes (ly) and peroxisomes (p). Secreted proteins are not included in the analysis.

The Mouse Hepatic Glycogen Proteome

Mouse hepatic glycogen particles were trypsinized, and peptides analyzed by mass spectrometry (LC/FTMS/MS) and bioinformatic searches of the “mammalia” MSDB database. Mouse proteins identified (name and UniProt identifier, ID), that meet a significance level of less than 0.05, with at least two different unique peptide sequences, are listed functionally in order of likelihood by Mowse score (score) [39]. Other parameters include: the total number of peptides with expectation scores less than 0.05 (total), number of different unique peptide sequences identified corresponding to the specific gene product (#), percent coverage of the protein (%), and the lowest recorded expectation value (E-value) for the protein. Peptides with a mass difference of greater than 3 ppm, compared to the theoretical monoisotopic mass, or with an expectation score greater than 0.05, are excluded from the list of identified peptides for each protein. In the case of proteins with homology to other glycogen-associated proteins, the number of non-redundant unique peptides is indicated in parentheses.

Table 1

name	ID	score	total	#	%	E-value
glycogen metabolism						
Glycogen phosphorylase, liver form	PYGL_MOUSE	110281	4567	73	79	1.20E-12
Glycogen debranching enzyme*	GDE_HUMAN	45937	1518	31	35	8.30E-12
Glycogen synthase, liver form	GYS2_MOUSE	40420	1686	49	67	4.00E-10
Glycogenin-1	GLYG_MOUSE	18892	643	15	47	7.10E-11
Glycogen phosphorylase, brain form	PYGB_MOUSE	11225	694	28 (18)	43	2.30E-08
Glycogen phosphorylase, muscle form	PYGM_MOUSE	9285	551	17 (4)	26	1.20E-08
Starch-binding domain-containing protein 1	STBD1_MOUSE	4748	119	8	37	6.20E-09
Glycogen synthase, muscle	GYS1_MOUSE	4608	211	13 (11)	25	1.30E-08
Pancreatic alpha-amylase	AMYP_MOUSE	2325	86	19	41	3.20E-07
Salivary and hepatic alpha-amylase	AMY1_MOUSE	1494	43	11 (5)	29	1.10E-04
Protein phosphatase PPI-alpha catalytic subunit	PPIA_MOUSE	648	23	8	30	8.90E-08
Laforin	EPM2A_MOUSE	63	2	2	11	9.20E-04
Glycogen-branching enzyme	GLGB_MOUSE	56	3	3	7	7.30E-03
metabolism						
Carbamoyl-phosphate synthase	CPSM_MOUSE	5637	149	24	22	9.00E-10
3-ketoacyl-CoA thiolase	THIM_MOUSE	3103	65	6	37	5.80E-09
Fructose-bisphosphate aldolase B	ALDOB_MOUSE	1492	31	2	12	3.90E-08
ATP synthase subunit beta	ATPB_MOUSE	1235	35	10	30	4.30E-09
Aldehyde dehydrogenase 2	ALDH2_MOUSE	1121	31	9	23	1.90E-08
Non-specific lipid-transfer protein	NLTP_MOUSE	721	25	4	8	2.00E-06
3-ketoacyl-CoA thiolase A	THIKA_MOUSE	635	15	4	27	6.70E-08

name	ID	score	total	#	%	E-value
ATP synthase subunit alpha	ATPA_MOUSE	518	19	7	20	1.10E-06
Betaine-homocysteine S-methyltransferase 1	BHMT1_MOUSE	470	14	6	22	8.60E-09
Malate dehydrogenase	MDHM_MOUSE	468	7	2	9	1.10E-09
Acyl-coenzyme A oxidase 1	ACOX1_MOUSE	327	9	6	16	9.30E-07
ADP/ATP translocase 1	ADTL_MOUSE	272	7	2	7	2.90E-06
Glyceraldehyde-3-phosphate dehydrogenase	G3P_MOUSE	224	9	4	23	3.00E-06
Peroxisomal bifunctional enzyme	ECHP_MOUSE	194	8	2	3	8.90E-06
Ornithine carbamoyltransferase	OTC_MOUSE	179	6	3	10	1.00E-06
Adenosylhomocysteinase	SAHH_MOUSE	140	4	2	6	6.60E-06
Tripeptidyl-peptidase 1	TPPI_MOUSE	122	3	2	6	5.50E-07
Electron transfer flavoprotein subunit alpha	ETFA_MOUSE	122	4	3	24	8.50E-05
Epoxide hydrolase 2	HYES_MOUSE	109	3	2	4	3.60E-04
Hydroxymethylglutaryl-CoA synthase	HMC52_MOUSE	107	4	2	4	5.70E-04
redox						
Ferritin light chain 1	FRIL1_MOUSE	675	29	8	58	2.40E-08
Glutathione S-transferase P 1	GSTP1_MOUSE	576	11	3	21	6.10E-09
Catalase	CATA_MOUSE	558	19	6	24	2.10E-07
Glutathione S-transferase Mu 1	GSTM1_MOUSE	421	13	5	28	4.30E-06
Ferritin heavy chain	FRIH_MOUSE	134	5	2	17	1.20E-04
RNA						
RNA-binding protein Luc7-like 2	LC7L2_MOUSE	3121	100	5	17	3.10E-09
Splicing factor, arginine/serine-rich 3 ^f	SFRS3_MOUSE	2695	84	5	40	1.30E-06
Splicing factor, arginine/serine-rich 7	SFRS7_MOUSE	1058	29	3	15	9.60E-07
RNA-binding protein 39	RBM39_MOUSE	612	18	4	10	2.40E-09
Heterogeneous nuclear ribonucleoprotein M	HNRPM_MOUSE	455	11	2	3	1.10E-07
Cleavage and polyadenylation specificity factor subunit 6	CPSF6_MOUSE	373	8	3	9	1.10E-09
protein synthesis, metabolism and turnover						
Peptidylprolyl isomerase B	PIIB_MOUSE	8337	260	13	60	5.20E-08
Cathepsin B	CATB_MOUSE	2620	44	3	13	6.70E-11
Cathepsin Z	CATZ_MOUSE	2451	51	3	10	6.90E-08
Translation elongation factor eEF-1 alpha-1 chain	EF1A1_MOUSE	1362	43	6	20	1.20E-08

name	ID	score	total	#	%	E-value
60 kDa heat shock protein	CH60_MOUSE	541	14	6	24	3.50E-08
40S ribosomal protein S18	RS18_MOUSE	418	12	3	20	1.80E-07
40S ribosomal protein S27a	RS27A_MOUSE	227	8	2	17	1.80E-05
Stress-70 protein	GRP75_MOUSE	175	4	2	4	7.30E-07
structural proteins						
Tubulin beta-2C chain	TBB2C_MOUSE	536	18	4	15	3.50E-06
Tubulin alpha-4A chain	TBA4A_MOUSE	302	6	3	11	1.30E-08

* The UniProt listing for GDE-MOUSE is incomplete. Therefore the closet match in the database was GDE_HUMAN.

† Insufficient data was obtained to conclusively match the identified peptides with a unique gene product.

Table 2

Specifically Eluted Mouse Glycogen-Associated Proteins

Mouse glycogen Proteins were further purified by solubilization with 50 mg/ml malto-oligosaccharides, as described in the Experimental Procedures section. Mouse proteins identified (name and UniProt identifier, ID), that meet a significance level of less than 0.05, with at least two different unique peptide sequences, are listed functionally in order of likelihood by Mowse score (score) [39]. Other parameters include: the total number of peptides with expectation scores less than 0.05 (total), number of different unique peptide sequences identified corresponding to the specific gene product (#), percent coverage of the protein (%), and the lowest recorded expectation value (E-value) for the protein. Peptides with a mass difference of greater than 3 ppm, compared to the theoretical monoisotopic mass, or with an expectation score greater than 0.05, are excluded from the list of identified peptides for each protein. In the case of proteins with homology to other glycogen-associated proteins, the number of non-redundant unique peptides is indicated in parentheses.

name	ID	score	total	#	%	E-value
Glycogen debranching enzyme*	GDE_RAT	8384	275	37	27.7	9.20E-10
Glycogen phosphorylase, liver form	PYGL_MOUSE	7619	299	44	55.1	2.30E-10
Glycogen synthase 2	Q8VCB3_MOUSE	4496	153	26	39.2	2.30E-08
Starch-binding domain-containing protein 1	STBD1_MOUSE	1059	18	3	12.1	1.10E-07
Glycogen phosphorylase, brain form	PYGB_MOUSE	967	64	11 (4)	14.1	1.30E-05
Glycogen phosphorylase, muscle form	PYGM_MOUSE	964	59	8 (2)	11.9	1.10E-07
Cathepsin Z	CATZ_MOUSE	575	12	2	7.2	4.80E-07
Glycogenin-1	GLYG_MOUSE	546	17	3	11.1	1.30E-06
Peptidylprolyl isomerase B	PPIB_MOUSE	286	9	2	10.6	1.10E-04
Protein phosphatase 1-alpha catalytic subunit	PPIA_MOUSE	248	8	7	21.8	6.40E-07
Cathepsin B	CATB_MOUSE	235	5	2	9.1	9.10E-07
Ferritin light chain 1	FRIL1_MOUSE	220	6	6	41	1.10E-07
Carbamoyl-phosphate synthase	CPSM_MOUSE	196	5	3	2.3	2.90E-06
Salivary and hepatic alpha-amylase	AMY1_MOUSE	146	3	2	5.3	4.80E-06
Ferritin heavy chain	FRIH_MOUSE	95	3	2	13.2	2.10E-03
Catalase	CATA_MOUSE	77	2	2	5.7	5.10E-03
Hemoglobin subunit beta-2	HBB2_MOUSE	66	2	2	14.6	1.60E-03

*The UniProt listing for GDE-MOUSE is incomplete. Therefore the closet match in the database was GDE_HUMAN.

The Rat Hepatic Glycogen Proteome

Proteins isolated from rat hepatic glycogen particles were trypsinized, purified, and analyzed by mass spectrometry (LC/FTMS/MS) and bioinformatic searches of the “mammalia” MSDB database. Rat proteins identified (name and UniProt identifier, ID), that meet a significance level of less than 0.05, with at least two different unique peptide sequences identified, are listed functionally in order of likelihood by Mowse score (score) [39]. Other parameters include: the total number of peptides with expectation scores less than 0.05 (total), number of different unique peptide sequences identified that correspond to that specific gene product (#), percent coverage of the protein (%), and the lowest recorded expectation value (E-value) for the protein. Peptides with a mass difference of greater than 3 ppm, compared to the theoretical monoisotopic mass, or with an expectation score greater than 0.05, are not included in the list of identified peptides for each protein. In the case of proteins with homology to other glycogen-associated proteins, the number of non-redundant unique peptides is indicated in parentheses.

Table 3

name	ID	score	total	#	%	E-value
glycogen metabolism						
Glycogen phosphorylase, liver form	PYGL_RAT	13650	574	54	55	7.60E-13
Glycogen debranching enzyme*	GDE_HUMAN	4939	198	28	18	9.90E-11
Glycogen synthase, liver form	GYS2_RAT	3528	145	31	38	4.70E-08
Glycogen phosphorylase, brain form	PYGB_RAT	2685	166	13 (8)	17	9.50E-09
Glycogenin-1	GLYG_RAT	1555	62	7	18	7.70E-07
Starch-binding domain-containing protein 1	STBD1_RAT	1015	36	11	36	2.60E-06
Pancreatic alpha-amylase	AMYP_RAT	903	26	14	37	1.70E-08
Protein phosphatase 1 regulatory subunit 3B	PPR3B_RAT	409	10	3	11	8.70E-09
steroid metabolism and xenobiotic response						
UDP-glucuronosyltransferase 2B2	UD2B2_RAT	269	12	5	12	3.90E-05
Cytochrome P450 2C7	CP2C7_RAT	194	7	5	14	6.90E-06
Cytochrome P450 2C23	CP2CN_RAT	185	6	2	6	3.80E-07
Cytochrome P450 2D26	CP2DQ_RAT	172	15	9	19	6.00E-05
Cytochrome P450 2D10	CP2DA_RAT	168	11	7 (2)	14	1.10E-04
Epoxide hydrolase 1	HYEP_RAT	132	6	3	6	7.70E-04
Cytochrome b5	CYB5_RAT	94	5	3	33	3.30E-03
Cytochrome P450 2C12, female-specific	CP2CC_RAT	63	6	4	11	1.60E-03
metabolism						
Apolipoprotein A-I	APOA1_RAT	553	17	6	28	1.70E-07
Apolipoprotein E	APOE_RAT	499	13	5	18	9.70E-08
Long-chain-fatty-acid-CoA ligase 1	ACSL1_RAT	479	15	6	13	4.10E-08

name	ID	score	total	#	%	E-value
Retinol dehydrogenase 3	RDH3_RAT	250	9	5	17	1.80E-05
NADH-cytochrome b5 reductase 3	NB5R3_RAT	210	5	2	11	9.80E-07
Bile acyl-CoA synthetase	S27A5_RAT	133	2	2	5	3.10E-08
redox						
Ferritin light chain 1	FRIL1_RAT	784	20	6	49	4.80E-09
Ferritin heavy chain	FRIH_RAT	180	6	4	23	2.80E-05
protein synthesis, maintenance and turnover						
78 kDa glucose-regulated protein	GRP78_RAT	107	3	3	7	2.50E-05
40S ribosomal protein S27a	RS27A_RAT	104	4	3	22	3.20E-04
Protein disulfide-isomerase A3	PDIA3_RAT	84	2	2	4	3.90E-06
Calreticulin	CALR_RAT	53	2	2	5	3.70E-03

*The UniProt listing for GDE-RAT is incomplete, therefore the parameters indicated correspond to the closet match in the database, GDE_HUMAN.

Table 4
Common Proteins in the Mouse and Rat Glycogen-Associated Sub-Proteome

Glycogen preparations were isolated from both mouse and rat liver as described in the methods. Proteins common to the two sub-proteomes are listed. Mouse proteins also present in the stringently purified malto-oligosaccharide supernatant are indicated in bold.

name	mouse	rat
Glycogen phosphorylase, liver form	PYGL_MOUSE	PYGL_RAT
Glycogen debranching enzyme*	GDE_HUMAN	GDE_HUMAN
Glycogen synthase, liver form	GYS2_MOUSE	GYS2_RAT
Glycogenin-1	GLYG_MOUSE	GLYG_RAT
Glycogen phosphorylase, brain form	PYGB_MOUSE	PYGB_RAT
Starch-binding domain-containing protein 1	STBD1_MOUSE	STBD1_RAT
Pancreatic alpha-amylase	AMYP_MOUSE	AMYP_RAT
Ferritin light chain 1	FRIL1_MOUSE	FRIL1_RAT
Apolipoprotein A-I	APOA1_MOUSE	APOA1_RAT
Long-chain-fatty-acid--CoA ligase 1	ACSL1_MOUSE	ACSL1_RAT
Ferritin heavy chain	FRIH_MOUSE	FRIH_RAT
Cytochrome P450 2D10	CP2DA_MOUSE	CP2DA_RAT
40S ribosomal protein S27a	RS27A_MOUSE	RS27A_RAT
Cytochrome b5	CYB5_MOUSE	CYB5_RAT