Biochemical and Biophysical Research Communications **257,** 34–43 (1999)
Article ID bbrc.1999.0396, available online at http://www.idealibrary.com on IDEAL®

Structural and Functional Characterization of the *Drosophila* Glycogen Phosphorylase Gene

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Received February 22, 1999

We identified a P element insertional mutant of the *Drosophila* glycogen phosphorylase (*DGPH*) gene. Glycogen phosphorylase protein concentration and enzyme activity are decreased while glycogen content is increased in flies homozygous for the mutant allele. The *DGPH* gene has been cloned and sequenced; its open reading frame codes for a protein of 844 amino acids with a predicted molecular mass of 97 kDa. Comparison of the conceptual amino acid sequence of the *Drosophila* glycogen phosphorylase with glycogen phosphorylase sequences from other organisms shows a high degree of homology to mammalian enzymes. All the residues of the allosteric effector binding sites, the active site, and the site of phosphorylation are exactly conserved, but some of the residues of the glycogen storage site are not.

Glycogen phosphorylase (1,4 α-D-glucan: orthophosphate α-D glucosyltransferase, E.C.2.4.1.1) is the rate limiting enzyme of glycogen degradation which operates under tight control. Besides its important physiological role, glycogen phosphorylase is also interesting from a theoretical point of view. Rabbit skeletal muscle glycogen phosphorylase was the first example of enzyme regulation by reversible phosphorylation (1). The inactive phosphorylase *b* is phosphorylated and activated by phosphorylase kinase at a single site (Ser-14) and, in turn, phosphorylase *a* is dephosphorylated and inactivated by protein phosphatase 1 (2). The effect of covalent modification is modulated by allosteric effectors (2, 3). Both the fine details of the regulation and the catalytic mechanism of the phosphorylase have been resolved at the atomic level (4, 5). X-ray crystallographic analysis revealed that the enzyme is a homodimer of two identical subunits. In each subunit one molecule of the cofactor pyridoxal 5′-phosphate (PLP) is located at the active center nearby the substrate and competitive inhibitor (glucose) binding sites. In addition, separate binding sites for allosteric activators (AMP and IMP), allosteric inhibitors (caffeine and purine nucleosides), and a specific glycogen storage site were identified at the surface of each diade related monomer. The binding site of another allosteric inhibitor, glucose-6-P, overlaps with that of the nucleotide activators. A complicated network of conformational changes connecting the above mentioned sites within the same and in the symmetry related subunit was detected, and the elementary steps of the catalytic cycle were described (4, 6–10).

Since the publication of the complete amino-acid sequence of the rabbit muscle glycogen phosphorylase (11) a number of phosphorylase cDNA sequences have been reported from various organisms. Complete amino-acid sequences were deduced for the glycogen phosphorylase isozymes from rabbit (12), human (13), rat (13) and cattle (14) muscle; rat (15) and human (16, 17) brain; and rat (18) and human (19) liver. Homologues from lower organisms have been cloned as well; e.g., yeast glycogen phosphorylase (20), phosphorylase 1 (21) and phosphorylase 2 (22) from *Dictiostelium discoideum;* glycogen (23, 24) and maltodextrin phosphorylase (25, 26) from *E. coli,* glycogen phosphorylases from *Bacillus subtilis* (27), *Bacillus stearothermophilus* (28) and *Haemophylus influenzae* (29), type L (30) and type H (31) phosphorylases from potato, phosphorylases from *Vicia faba* (32) and *Ipomea batata* (33). The amino acid sequences of the phosphorylases from different organisms have been compared and

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The nucleotide sequence data reported in the paper can be found in the EMBL GenBank under the Accession Nos. AF073117, AF073178, and AF073179.

Abbreviations used: DGPH, *Drosophila* glycogen phosphorylase; PLP, pyridoxal 5′-phosphate.

0006-291X/99 $30.00

FIG. 1. Molecular map of the region containing the P element insertion in *P79/18*. Sites for endonucleases *Bam*HI (B), *Eco*RI (E), *Hin*dIII (H), *Nco*I (N), *Sal*I (S), *Sph*I (Sp), *Bgl*II (Bg), *Eco*RV (R), *Kpn*I (K), *Pst*I (P), *Sma*I (Sm) and *Xho*I (X) are shown. (A) Intron–exon structure of the DGPH transcription unit. Open arrow indicates the direction of transcription. (B) Restriction map of the region. Site of the P-lacW insertion is designated by a triangle. (C) Genomic fragments used for Northern analysis and to screen the λgt cDNA library. (A), (B) and (C) are drawn on the same scale.

their phylogenetic relationship has been determined (34). It turned out that the structure of the catalytic center including the PLP and glucose binding sites was highly conserved in all examples. On the other hand, the sequences of additional ligand binding sites as well as the structural element connecting the two subunits (tower/gate and cap) were more variable. The characteristic site of covalent modification (Ser-14) was found only in the mammalian phosphorylases; in yeast phosphorylase an unrelated Thr residue is phosphorylated by the cAMP- dependent protein kinase and a yeast-specific phosphorylase kinase, while the phosphorylation site is completely absent from the other homologues. These structural data provide a rational explanation for the diverse substrate specificity and regulation of phosphorylases in different organisms.

Glycogen phosphorylase has been purified from *Drosophila melanogaster* (35). The biochemical properties of the inactive *b* and active *a* forms of the enzyme were determined (35, 36) and the regulation by phosphorylation-dephosphorylation in *Drosophila* was demonstrated (37). In the present communication we report the molecular characterization of the gene encoding the glycogen phosphorylase in *Drosophila*. To our knowledge, this is the first report on the molecular genetic dissection of an insect phosphorylase. We also present a comparison of the *Drosophila* glycogen phosphorylase amino acid sequence with that of the published glycogen phosphorylases in order to identify its place in the phylogenetic tree of phosphorylases.

MATERIALS AND METHODS

Drosophila cultures and stocks. Standard fly techniques were carried out as described by Ashburner (38).Genetic symbols used here can be found in Lindsley and Zimm (39). The y^+CyO balancer chromosome carrying a $P(y^+)$ insertion was kindly provided by Dr. Allen Shearn. The mutant *79/18* was isolated previously (40) by making use of the *P-lacW* enhancer detector (denoted from here on as $P(w^+)$). Stock $Dp(2;2)dpp^{s7}$,*Df(2L)dp-38a*,*net ed dp cl/CyO* (denoted as *Df(2L)dp-38a* from here on) was kindly provided by Dr. William Gelbart. Stock *Df(2L)dp-38a* carries a deletion between 22B1-2 and 22F1-2 which uncovers the region of the P element insertion in *P79/18*.

Generating deletions by imprecise excision of the P element. The $P(w^+)$ element in *P79/18* was remobilized to induce imprecise deletions of the inserted transposon. *yw;* $P(w^+)$*/CyO* females carrying the P element insertion were crossed to *SbΔ2-3/TM6* males carrying the *Δ2-3* transposase source (41). Jumpstarter males carrying both the P element and the transposase source were crossed to *yw;* y^+CyO/Sco females and the white-eyed progeny, possibly lacking the P element

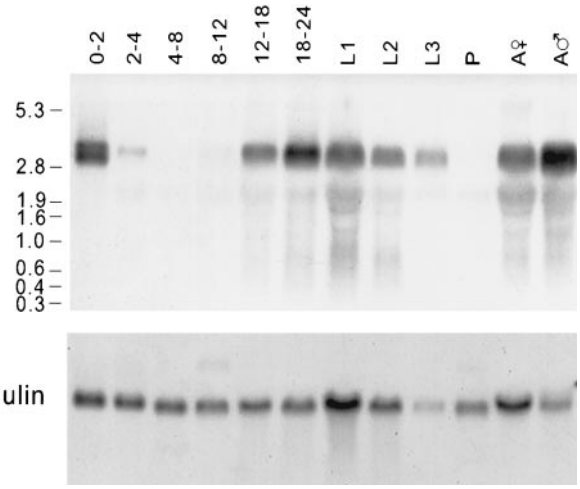

FIG. 2. Developmental Northern analysis of *DGPH* expression. The Northern filter was probed with the g24 DNA fragment (see Fig. 1). To monitor the relative RNA contents of the lanes the same blot was hybridized with a *β*-tubulin probe. Embryonic stages (0–2, 2–4, 4–8, 8–12, 12–18, and 18–24 h), larval stages (L1, L2, and L3), pupal stage (P) and adult females (A♀) and males (A♂) are shown.

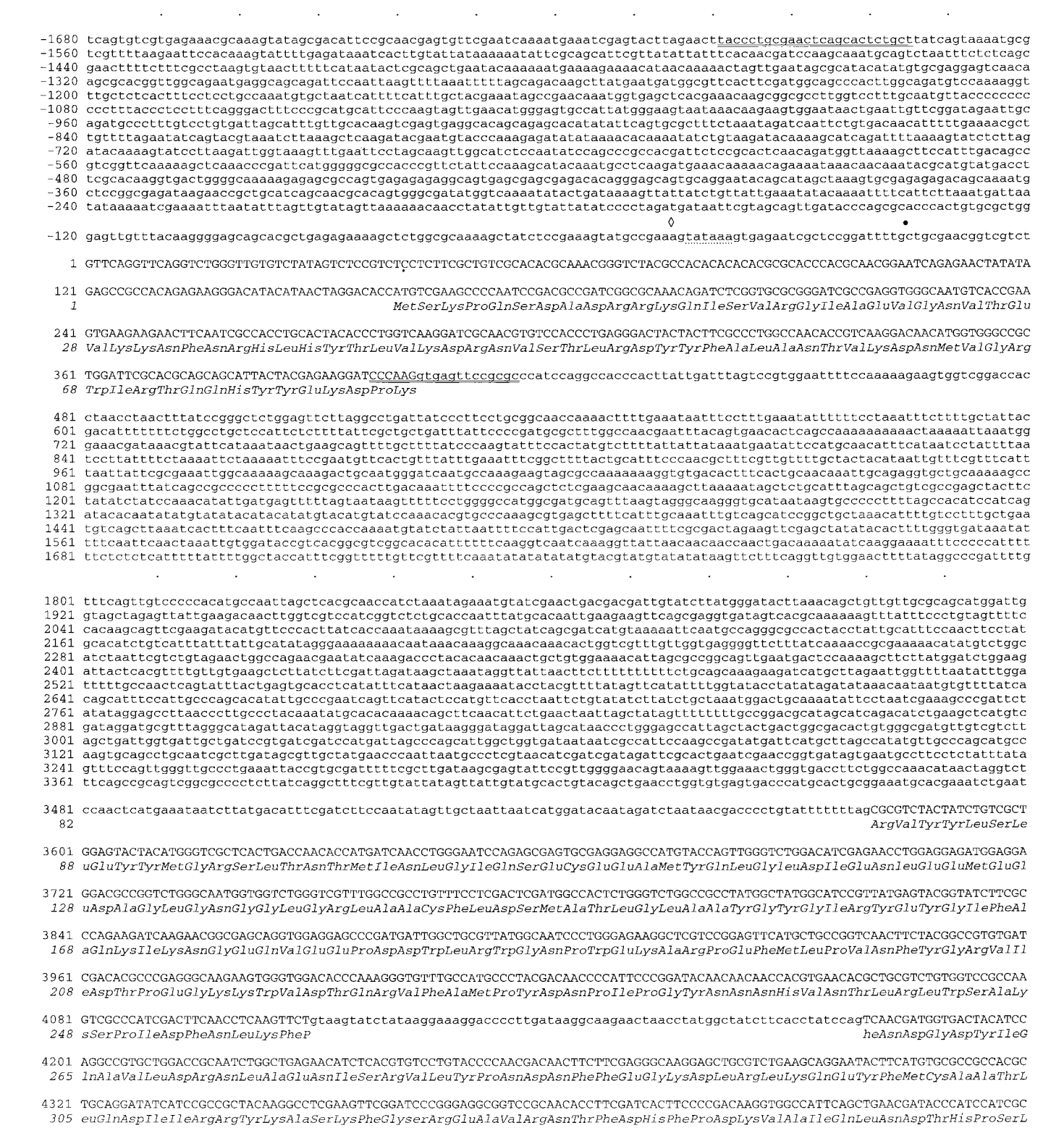

FIG. 3. Sequence of the DGPH gene and predicted amino acid sequence of its product. Intron and untranscribed sequences are shown in lowercase letters, exons are shown in uppercase letters. The first nucleotide of the longest cDNA isolated in the present work is positioned at +1 and the last nucleotides of the two transcribed variants are indicated by an open circle (o) above the nucleotide sequence. The first nucleotide of the longest EST sequence (EST No. GH08049, Accession No. AI108791) corresponding to DGPH in the clot 485 of the Berkeley Drosophila Genome Project is indicated by a closed circle (•) at position −23 above the nucleotide sequence. The insertion of the *P-lacW* transposon in P79/18 at position −52 is indicated by a diamond (◇).

FIG. 3—*Continued*

was selected. Such white eyed males were crossed to *yw; y⁺CyO/Sco* females in one male crosses. Resulting *yw; P(w⁺)/y⁺CyO* males and females were crossed to each other and the lack of Cy^+ flies (flies without the *y⁺CyO* balancer) indicated possible imprecise deletion resulting in lethality.

Nucleic acid procedures. Standard molecular biology techniques were performed as described by Sambrook *et al.* (42), unless otherwise indicated. DNA sequences adjacent to the insertion were isolated by plasmid rescue: genomic DNA from *P79/18/CyO* flies was digested with *Eco*RI, ligated and transformed into XL-1 Blue competent cells. The rescued clones were analyzed by Southern blotting. A genomic fragment near the P element insertion was used as a probe to screen a genomic EMBL3 λ phage library. The phage clones were analyzed by restriction mapping, and a clone overlapping the P element insertion on both sides was chosen for further subcloning. A fragment overlapping (g24) and two adjacent (g18 and g19) to the insertion site (Fig. 1) were subcloned and used to screen a λ gt11 cDNA library, kindly provided by Dr. L. Kauvar, (43) made of 0- to 16-h-old embryos. The inserts of the cDNA clones were subcloned into Bluescript SK^+ vector and sequenced. Dideoxy chain termination method was used with a USB Sequenase Ver. 2.0 kit to sequence cDNA clones and most of the subclones of the genomic λ-phage. To help sequencing, some internal primers were synthesized. In the homozygous strain $DGPH^{Ins31}/DGPH^{Ins31}$, the fragment carrying the imprecise P element excision was PCR amplified with primers E4FD: 5′-TACCCTGCGAACTCAGCACTCTGC-3′and B4UP: 5′-GCGCGGAACTCACCTTGGG-3′ (Fig. 3) using Takara ExTaq DNA polymerase and the PCR product was sequenced. Some regions were sequenced on an ABI 50 DNA sequencer. Sequence analysis was done using the DNA Star software. Oligonucleotides were synthesized on a Pharmacia Gene Assembler by phosphoramidite chemistry.

For a developmental Northern analysis, $polyA^+$ RNA was isolated, electrophoresed and transferred to nitrocellulose filters as described in Török *et al.* (44). $PolyA^+$ RNA was isolated from embryos, larvae and adults, and the RNA blot was probed with fragment g24 which overlaps the P element insertion.

The homozygous nonviable lines derived from the remobilization experiment of *P79/18* were screened by Southern analysis for deletions or remaining P element sequences.

Western blot analysis. Proteins from third instar larval homogenates were separated in 8% SDS gel and transferred to nitrocellulose membrane (42). Following incubation with affinity-purified primary rabbit anti-DGPH antibody (kindly provided by Dr. Andor Udvardy) in a 1:15000 dilution, the filter was washed and incubated in secondary peroxidase-conjugated goat anti-rabbit antibody (Dako) in a

1:200 dilution. ECL Western blot detection reagents (Amersham Life Science) were used to visualize the DGPH protein.

Assay of glycogen phosphorylase activity. Phosphorylase activity was determined in the direction of glycogen synthesis as described earlier (35) with the exception that 20 mM NaF was also added to the extraction buffer in order to inhibit phosphorylase phosphatase activity. Assays were carried out in triplicates and in two different dilutions of the extracts in the presence and in the absence of 1 mM AMP. The amount of total phosphorylase was calculated on the basis of the assumptions that the specific activity of phosphorylase *b* was 1.9 times less than that of phosphorylase *a* and phosphorylase *a* was activated by AMP 1.1-fold (36).

Assay of glycogen concentration. Glycogen was assayed by the iodine (I_2-KI) staining in the presence of saturating $CaCl_2$ in a 10% TCA extract using rabbit liver glycogen as standard (45).

RESULTS

Isolation of P element insertions in the Drosophila glycogen phosphorylase (DGPH) gene. The stock carrying the P element insertion in the *Drosophila* glycogen phosphorylase gene was generated in a large scale P element insertional mutagenesis and designated as *79/18* (40). It was isolated as a potential tumour suppressor mutation displaying lethal overgrowth of the brain and the imaginal discs, but later analysis revealed that the overgrowth phenotype and the lethality was the result of a second-site mutation which had no relevance to the P element insertion. The second-site mutation was removed via genetic recombination and the resulting stock was named *P79/18*.

The P element insertion in *P79/18* maps to chromosome 2 at 22B. *P79/18* homozygous animals are fully viable with no phenotypic defect visible. Stock *Df(2L)dp-38a/CyO* carries a deletion which uncovers the region of P element insertion in *P79/18*. *P79/18* proved to be fully viable over the above deletion and no visible phenotypic defects were detectable either. To generate loss of function mutations in the *DGPH* gene, we remobilized the P element insert of *P79/18* and established 100 mobilized lines that had lost their eye color due to the excision of all or part of the P element. These lines were analyzed by Southern blotting (data not shown) to identify imprecise excisions that could disrupt the *Drosophila* glycogen phosphorylase gene. Out of the 100 excision lines none proved to contain deletions in the *DGPH* gene. In one of the excision lines ($DGPH^{Ins31}$) a 209-bp sequence of the 5′ end of the P element was retained but no part of the genomic sequence was deleted, as revealed by sequencing of the allele. $DGPH^{Ins31}$ in combination with *Df(2L)dp-38a/CyO* displays weak viability with only 39% of the $DGPH^{Ins31}$/*Df(2L)dp-38a* animals surviving to adulthood.

Cloning of the Drosophila glycogen phosphorylase gene. To identify the gene responsible for the mutant phenotype, the genomic sequence flanking the P element insertion was cloned via the plasmid rescue method. Screening a *Drosophila* genomic DNA library with a genomic fragment adjacent to the P element insertion yielded a phage clone which contained the insertion site and flanking sequences on both sides (Fig. 1). Northern blot analysis of wild type polyA$^+$ RNA performed with fragment g24 overlapping the P-element insertion site revealed the presence of two relatively abundant transcripts of 2.9 and 3.2 kb in 0-2 hours old embryos (Fig. 2) and the 3.2-kb transcript in the further stages of development. Fragment g24 was also used to screen an embryonic cDNA library and three cDNAs were isolated. Determination of the nucleotide sequence of the genomic DNA and the cDNAs showed that both classes of transcripts belong to the same transcription unit with identical 5′ ends but exhibiting different lengths in their 3′ ends, as indicated in Fig. 1A. The 3.2-kb transcripts were found to contain a 3′ extension of 278 nucleotides by comparison to the 2.9 kb transcripts (Fig. 3). Alignment of the sequences of the three cDNAs with the genomic DNA sequence revealed four exons of 402 (or larger), 529, 1607, and 601 nucleotides separated by three introns of 3178, 70 and 84 nucleotides, respectively. The last exon of the 3.2 kb class of cDNAs is 278 nucleotides longer. The cDNA sequence displays an open reading frame of 844 codons initiated by an ATG present in the first exon 160 nucleotides downstream from the 5′ end of the longest cDNA. This open reading frame encodes a protein with a predicted molecular mass of 97,010 Daltons and is preceded by a sequence which conforms to the *Drosophila* translation start consensus sequence ANN (C/A) A (A/C) (A/C) ATGN (46). A canonical polyA addition site AATAAA is located 30 or 21 nucleotides from the start of the polyA tract in the 2.9 and 3.2 kb classes of transcripts, respectively. The P element insert in 79/18 is inserted 51 nucleotides upstream from the 5′ end of the cDNA or 29 nucleotides upstream from the beginning of the longest EST sequence GH08049 (accession number AI108791) available in the Berkeley Drosophila Genome Project. Search in databases revealed a high degree of homology between the cDNA nucleotide sequence and sequences encoding glycogen phosphorylases of various organisms.

In situ hybridization of fragment g24 to salivary gland chromosomes and to a Southern blot of wild type genomic DNA (data not shown) indicates that there is only one glycogen phosphorylase gene in *Drosophila*.

Functional analysis of the DGPH gene. To determine the expression and the abundance of the *DGPH* transcript, fragment g24 was used to probe a developmental Northern blot of wild type polyA$^+$ RNA. The probe revealed two major transcripts of 3.2 and 2.9 kb and several minor ones (Fig. 2). The size of the two major transcripts is in accordance with the size of the two cDNAs composed from sequencing data. The *DGPH* messages are abundant in preblastoderm embryos (0–2 h) but there is a sudden drop in the amount of the 3.2-kb transcript and the 2.9-kb transcript disappears during blastoderm formation and gastrulation

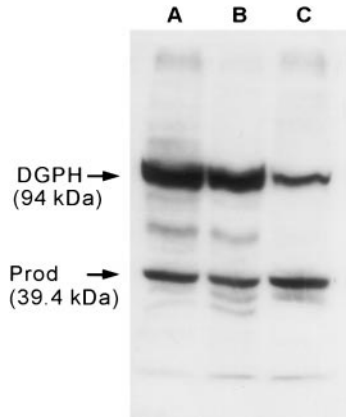

FIG. 4. Western blot of DGPH protein expression in (A) wild type, (B) *P79/18* homozygous and (C) $DGPH^{Ins31}$ homozygous L3 larvae. To monitor the amount of protein loaded in the different lanes, the same blot was treated with anti-Prod antibody.

TABLE 1

Calculated Glycogen Phosphorylase Activities of Wild-Type and DGPH Mutant Adults and Larvae

Stock	Stage	Specific activity (U/mg) ± SD	
Oregon-R (wild type)	A	0.402 ± 0.059	(n = 8)*
	L3	0.233 ± 0.027	(n = 3)
P79/18/P79/18	A	0.374 ± 0.085	(n = 8)
	L3	0.191 ± 0.057	(n = 3)
$DGPH^{Ins31}$/+	A	0.308 ± 0.052	(n = 8)
	L3	0.154 ± 0.009	(n = 3)
$DGPH^{Ins31}/DGPH^{Ins31}$	A	0.069 ± 0.018	(n = 8)
	L3	0.017 ± 0.004	(n = 3)

Note. Glycogen phosphorylase activity was measured as described under Experimental. (n)* is the number of independent extractions. In each extract the activities were measured in triplicates and in two different dilutions. A, adults; L3, third-instar larvae.

(2–4 h). The 3.2-kb transcript also disappears almost completely during germ band extension and at the beginning of germ band shortening (4–8 h) followed by its progressive increase during the later embryonic stages. The amount of the 3.2 kb transcript remains more-or-less constant all through the larval life but decreases suddenly in the pupal stage. Again, the level of the 3.2 kb transcript increases in the adult stage and becomes abundant, especially in males.

The *Drosophila* glycogen phosphorylase protein was visualized using anti-DGPH antibody (Fig. 4). As a control for the amount of proteins loaded in the different lanes, anti-Prod antibody (47) was used. The anti-DGPH antibody detected a major protein of 94 kDa and several smaller ones which are most probably degradation products. While the amount of *DGPH* protein in *P79/18* homozygotes seems to be unaffected, it is radically reduced in $DGPH^{Ins31}$ homozygotes. To examine whether this decrease in the level of the protein correlates with the enzyme activity, we measured the glycogen phosphorylase activity both in third instar larvae and in adults in *P79/18* and $DGPH^{Ins31}$ homozygotes (Table 1). As expected, enzyme activity is greatly reduced in $DGPH^{Ins31}$ homozygous larvae and adults compared to that of wild type animals (thirteen-fold and six-fold, respectively). The increased difference in the enzyme activity between the larvae and the adults of $DGPH^{Ins31}$ homozygotes can be explained by the fact that the lethal phase of the mutation is in the third larval instar. If we suppose that the low *DGPH* level is responsible for the lethality then those mutants which survive to adulthood are expected to have a higher enzyme level than those dying as larvae, and the overall enzyme activity in a larval sample should be lower than that in the adults. There is no significant reduction in the glycogen phosphorylase enzyme activity of *P79/18* homozygotes.

Glycogen content of third instar larvae of both *P79/18* and $DGPH^{Ins31}$ homozygotes was also measured. As expected, $DGPH^{Ins31}$ third instar larvae, which are seriously deficient for glycogen phosphorylase enzyme activity, have a higher glycogen content, while the glycogen content of *P79/18* homozygotes is the same as that of wild type third instar larvae (Table 2).

Comparison of the DGPH protein with glycogen phosphorylases from other organisms. Alignment of the *DGPH* conceptual amino acid sequence with that of other known mammalian sequences is displayed in Fig. 5. The active site, the pyridoxal phosphate binding site, the purine nucleoside inhibitor site, the phosphorylation and AMP binding sites and the glucose-6-p binding site are all highly conserved in the *Drosophila* enzyme; they are identical to those of the rabbit muscle glycogen phosphorylase. Changes of amino acids only occur in the glycogen storage site, all at non-conservative positions.

Phylogenetic relationships of the *Drosophila* glycogen phosphorylase to 21 phosphorylases from other organisms is presented in Fig. 6. The phylogenetic tree indicates that *DGPH* is more closely related to mammalian phosphorylases than to phosphorylases from the other species tested.

TABLE 2

Glycogen Content of Wild-Type and DGPH Mutant Third-Instar Larvae

Stocks	μg glycogen/mg body wt ± SD (n)*
Oregon-R	5.95 ± 0.41 (11)
P79/18/P79/18	3.79 ± 0.40 (10)
$DGPH^{Ins31}$/+	4.91 ± 0.70 (10)
$DGPH^{Ins31}/DGPH^{Ins31}$	8.11 ± 1.20 (13)

Note. Glycogen content of third-instar larvae was measured as described under Experimental. (n)* is the number of independent extractions.

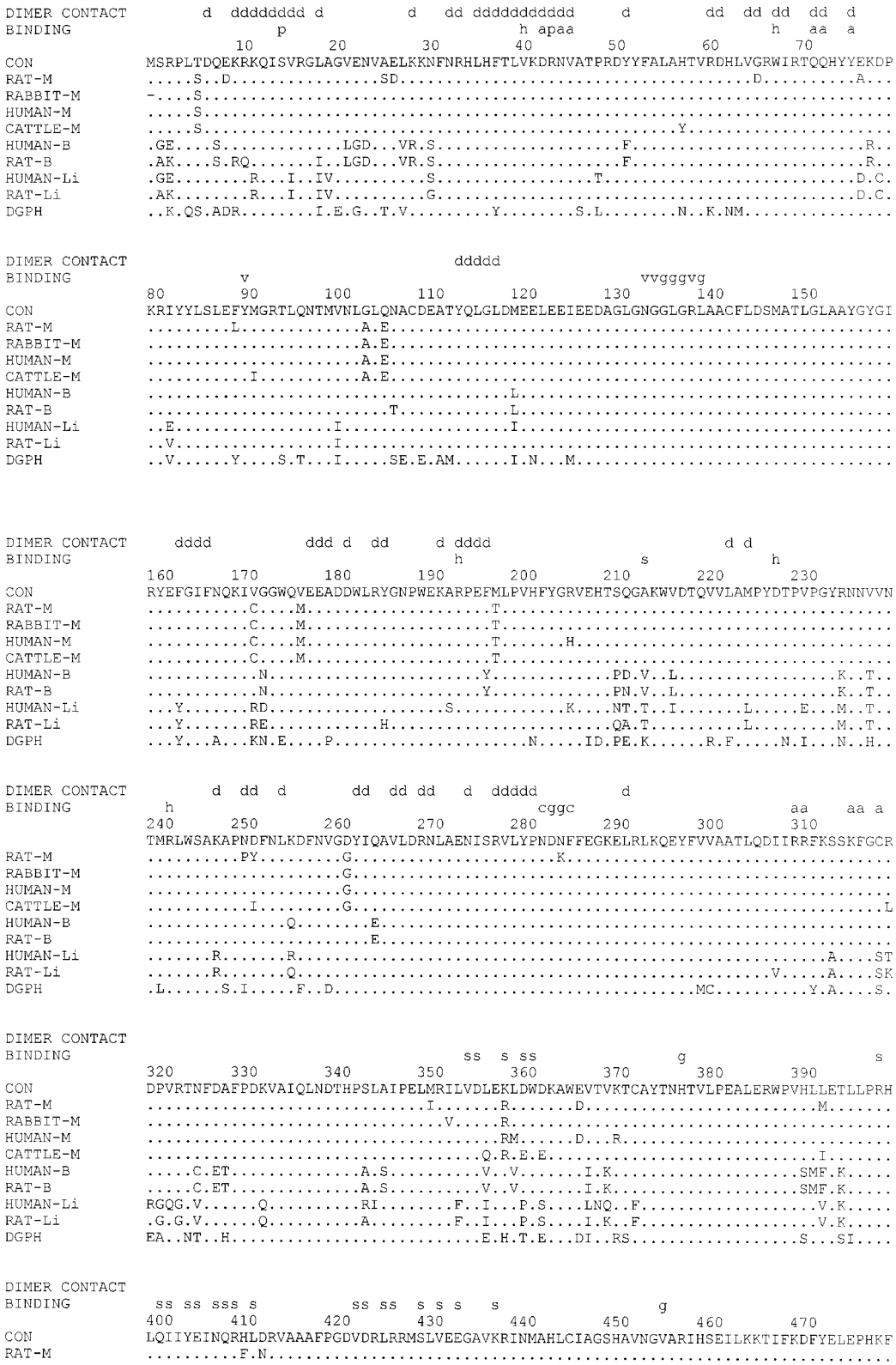

FIG. 5. Multiple alignment of the DGPH protein with the available mammalian glycogen phosphorylases. Alignment was done using the DNA Star software with the identity weight table of the Clustal method. Dot indicates amino acid identity. M stands for muscle, B for brain and Li for liver type glycogen phosphorylases. Residues forming contacts with the other subunit are indicated with *d* in the Dimer Contact row. Different ligand binding residues are indicated in the Binding row: *p* Ser-14 phosphorylation site; *h* glucose-6-P binding site; *a* AMP binding site; *v* pyridoxal phosphate binding site; *g* active site; *s* glycogen storage site; and *c* purine nucleoside inhibitor site. The consensus sequence, indicated as *con*, was generated by the DNA Star MegAlign program. Accession numbers of the aligned sequences are the same as in Fig. 6.

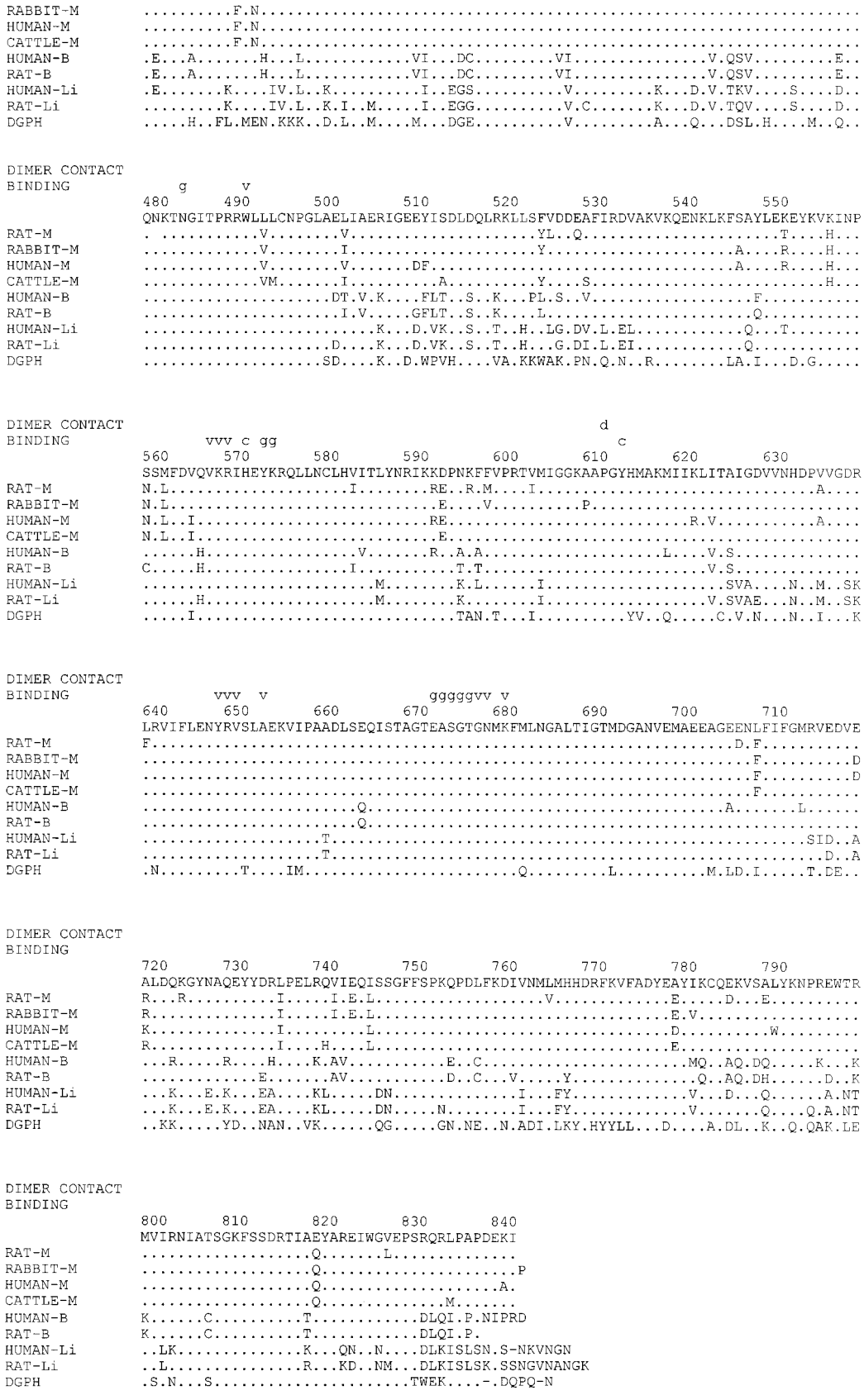

FIG. 5—*Continued*

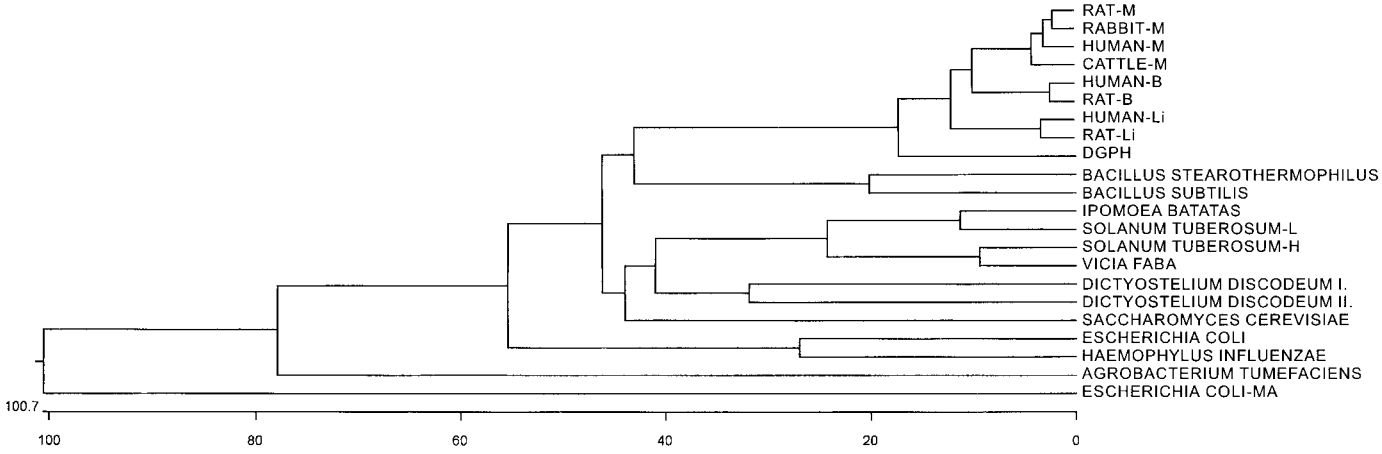

FIG. 6. Phylogeny of glycogen phosphorylases based on their complete amino acid sequences. Multiple alignment of complete protein sequences was done by the Clustal Method with PAM 250 value to obtain the phylogenetic tree using DNAStar software. The abscissa shows the number of amino acid substitution events. M indicates muscle type, Li liver type and B brain type glycogen phosphorylases, MA stands for maltodextrin phosphorylase. Accession numbers of the glycogen phosphorylase sequences are the following: Rat-M P09812; Rabbit-M P00489; Human-M B23093; Cattle-M S82859; Human-B P11216; Rat-B P53534; Human-Li P06737; Rat-Li P09811; *Drosophila* glycogen phosphorylase AF073178, AF073179; *Bacillus stearotermophilus* D87026; *Bacillus subtilis* P39123; *Ipomea batata* L25626; *Solanum tuberosum*-L P04045; *Solanum tuberosum*-H M69038; *Vicia faba* Z36880; *Dictiostelium discoideum* I X62142; *Dictiostelium discoideum* II P34114; Yeast P06738; *Escherichia coli* P13031; *Escherichia coli*-MA V00304; *Haemophylus influenzae* P45180; *Agrobacterium tumefaciens* AF033856.

DISCUSSION

In this paper, we describe the *Drosophila* glycogen phosphorylase gene and the effect of its decreased activity. The P element insertion in *P79/18* did not result in a significant decrease of either the amount of protein or the enzyme activity. The glycogen content of third instar larvae did not increase either. However, $DGPH^{Ins31}$ homozygotes display a marked reduction in both the amount of protein and the enzyme activity and consequently have an increased glycogen content.

The *DGPH* gene has been found to encode a protein of 844 amino acids with a predicted molecular mass of 97,000 Da. This prediction is in correlation with the estimated molecular mass of the purified protein (35) and that of the protein detected by the anti-phosphorylase antibody in a crude Drosophila extract (Fig. 4). The DGPH protein shows a high degree of homology to mammalian glycogen phosphorylases. The homology to the rabbit muscle glycogen phosphorylase begins close to the N terminus, at aa 3, and extends nearly to the C terminus, till aa 840. The rabbit and the *Drosophila* glycogen phosphorylase amino acid sequences can be aligned without insertions or deletions; *DGPH* is only longer due to the two amino acids added at the C terminus. All the residues comprising the active site, the pyridoxal phosphate binding site, the purine nucleoside inhibitor site, the phosphorylation and AMP binding sites and the glucose-6-P binding site in *DGPH* are identical to those of the rabbit muscle glycogen phosphorylase. Changes of amino acids only occur in the glycogen storage site, all at non-conservative positions. Here, Ala214, Glu406, Gln409, Arg410, and Leu412 are changed to Lys, His, Phe, Leu, and Met, respectively, compared to the rabbit muscle glycogen phosphorylase (Fig. 5). Obviously, these replacements at the glycogen storage site do not affect the kinetics of glycogen utilization at the active site, since the K_M glycogen is nearly the same with both the rabbit and *Drosophila* phosphorylases (35). Residues that form close contact with residues in the other subunit are also highly conserved in the *DGPH;* 89% of the dimer contact residues are identical to those of the muscle enzyme.

The Ser-14 residue, the site of covalent phosphorylation is well conserved in the *Drosophila* glycogen phosphorylase which reflects that the enzyme is more closely related to the mammalian glycogen phosphorylases than to the enzymes of lower species described before. Except for *DGPH,* Ser-14 has only been conserved in mammalian glycogen phosphorylases (34). The presence of the conserved Ser-14 and its environment is in agreement with the observation that the enzyme can be phosphorylated *in vitro* and *in vivo* (37).

DGPH shows the same high degree of homology both to the rabbit muscle and the human liver glycogen phosphorylases (72.4 and 72.7%, respectively) at the amino acid level. Newgard *et al.* (19) have shown that there is a distinct difference in codon usage pattern between the rabbit muscle and the human liver glycogen phosphorylases in particular, and in liver and muscle coding sequences in general. The difference in codon usage can be ascribed to the third codon position,

where 60% of the nucleotides are either deoxyguanosine or deoxycytidine residues in the liver glycogen phosphorylase compared with 85.8% in the muscle enzyme. The *Drosophila* glycogen phosphorylase gene displays a codon usage similar to that of the rabbit muscle glycogen phosphorylase gene with 83% G + C at the third codon position. Thus, we can conclude that though *DGPH* shows the same level of homology to the rabbit muscle and the human liver glycogen phosphorylases at the amino acid level it is phylogenetically more closely related to the rabbit muscle glycogen phosphorylase if we consider codon usage.

ACKNOWLEDGMENTS

We thank Dr. Andor Udvardy for providing the anti-DGPH antibody. We are grateful to Dr. Sándor Bottka for oligonucleotide primers and to Dr. Géza Ádám and Dr. Tibor Török for helpful comments on the manuscript. This work was supported by OTKA Grants 16693, 17575, andF016856. The technical assistance of Szilvia Adányi and Mrs. Margit Bíró is acknowledged.

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