Kinetic Properties of Glycogen Synthase and Phosphorylase and Structural Aspects of Glycogen in the *db/db* Mouse Liver

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SUMMARY

Kinetic studies were carried out on liver glycogen synthase and phosphorylase isolated from genetically diabetic *db/db* mice. Glycogen synthase *a* and *b* enzymes from diabetic mice had V_{max} values 30% and 20% lower, respectively, than the enzymes from normal mice. Glycogen synthase *b* from diabetic mice also had a 30% lower $I_{0.5}$ for P_i and ATP at physiologic concentrations of UDP-glucose (0.25 mM) compared with the normal enzyme. Kinetic studies of phosphorylase *a* showed that, at low glycogen concentrations (0.25 mg/ml), the V_{max} of the diabetic enzyme was twofold greater than that of the normal enzyme. This was probably related to the diabetic phosphorylase *a* having a lower apparent K_m for glycogen. This enzyme also had a slightly higher $I_{0.5}$ for ATP compared with the enzyme from normal mice.

Structural studies of liver glycogen isolated from these diabetic mice showed differences from normal mouse glycogen. Both the α- and β-amylase limits were lower in the diabetic glycogen, and the average chain lengths, exterior chain lengths, and interior chain lengths calculated from these limits were all shorter in the glycogen from diabetic mice. Although both normal and diabetic glycogen absorbed light maximally at 430 nm when complexed with iodine, the absolute absorbance value was significantly lower for the diabetic glycogen. These data suggest an altered branching pattern of liver glycogen from the diabetic mice and it is suggested that this altered structure may ultimately influence the activities of glycogen-metabolizing enzymes. These results provide further characterization of the *db/db* mouse and show heretofore undescribed changes in phosphorylase *a* kinetics and glycogen structure that occur in diabetes. DIABETES 1986; 35:210–16.

Glycogen synthase and phosphorylase are the rate-limiting enzymes of glycogen synthesis and degradation, respectively. Several studies have shown that these activities in liver are altered in animal models of diabetes.[1–3] A study by Akatsuka et al.[4] showed that there were changes in the kinetic and structural properties of glycogen synthase from streptozocin-induced diabetic rats and suggested that different forms of the enzyme may exist under different physiologic conditions. Bahnak and Gold, however, found no changes in glycogen synthase isolated from alloxan-induced diabetic rats.[5] The disparate results between these two studies may be due to the different chemicals used to induce the diabetes or to other reasons.

The genetically diabetic mouse (C57BL/KsJ-*db/db*) has been used as a model of type II or maturity-onset diabetes. Various aspects of its hepatic glycogen metabolism have been studied. Both glycogen synthase and phosphorylase activities have been shown to be elevated,[1,3] implicating an increased glycogen turnover rate.[1] However, there have been no reports of the kinetic properties of these enzymes isolated from this diabetic mouse. In the present study, we have examined the kinetics of hepatic glycogen synthase (*a* and *b* activities) and phosphorylase *a* from this mouse, in which nonspecific effects of diabetogenic drugs are not a concern. Moreover, because these mice are hyperphagic,[6] we also decided to examine certain structural aspects of their liver glycogen, since alterations in structure have been observed in different fed states.[7] This study provides further characterization of glycogen metabolism in this mouse model of diabetes. To our knowledge, this is the first report of changes in phosphorylase *a* kinetic properties and glycogen structure in a diabetic state.

MATERIALS AND METHODS

Animals. The mice used in this study were 8–10 wk of age and were offspring from a breeding colony of C57BL/KsJ-*db*/+ obtained from Jackson Laboratories (Bar Harbor, Maine). Heterozygote (*db*/+) mice were used as controls. All the mice used in this study were fed ad libitum and kept under a constant 12-h light-12-h dark cycle, the light period extending from 0600 to 1800 h.

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Enzyme isolation. Glycogen synthase and phosphorylase were isolated from frozen mouse livers as described by Haverstick and Gold[8] for adult rat, except that glycogen synthase eluted from the DEAE-cellulose column with 0.1 M NaCl. The phosphorylase, which did not bind to this ion-exchange matrix, was dialyzed against a lower ionic strength buffer (4 mM Tris, pH 7.4, 1 mM EDTA, and 1 mM 2-mercaptoethanol) and applied to a DEAE-cellulose column equilibrated with this buffer. The phosphorylase was then eluted with 0.1 M NaCl. The glycogen synthase isolated in this manner was purified 900-fold with a specific activity of around 900 U/mg protein and had an activity ratio of 0.06 from normal mouse liver and 0.01 from diabetic liver. This preparation was therefore used for the kinetic studies of the inactive glycogen synthase *b*. The phosphorylase obtained was purified 200-fold with a specific activity of about 15,000 U/mg protein.

Glycogen synthase *a* was isolated essentially as described above for the *b*-form with the following changes. NaF was excluded from all buffers during the isolation procedure. The glycogen pellet was suspended in 50 mM imidazole (pH 7.6), 45 mM 2-mercaptoethanol, 20 mM $MgCl_2$, 1 mM benzamidine, and 0.1 mM N-α-P-tosyl-L-lysine-chloromethyl ketone, L-1-tosylamido-2-phenylethylchloromethyl ketone, and phenylmethylsulfonyl fluoride, and incubated at 25°C for 90 min. During this incubation, the endogenous glycogen synthase phosphatase(s) converted glycogen synthase *b* to the *a* form. This mixture was then treated as described above for glycogen synthase *b* isolation. The glycogen synthase isolated had a specific activity of approximately 900 U/mg protein and an activity ratio of 0.92 from normal mouse liver and 0.96 from diabetic liver. Purification was about 900-fold in both groups of mice.

Kinetic studies. All of the assays for the kinetic studies were carried out at 30°C. The glycogen synthase assay measured the amount of [^{14}C]glucose transferred from UDP-[^{14}C]glucose to glycogen per minute.[9] The phosphorylase assay measured the amount of [^{14}C]glucose transferred from [^{14}C]glucose-1-P into glycogen per minute.[10] In both assays, the radioactive glycogen was precipitated on Whatman 31ET paper (2 × 2 cm), washed, and counted for radioactivity as described previously.[2] The length of incubation was limited such that no more than 10% of the radioactive substrate was utilized.

Apparent K_m values were obtained by the double-reciprocal plot and confirmed by the Eadie-Hofstee plot. V_{max} values were also obtained from these plots and were expressed per milligram of enzymic protein. Activation and inhibition constants were obtained by Hill plots using the V_{max} calculated from the double-reciprocal plots of the same data. In the studies concerning phosphorylase *a* inhibition by glucose, glucose-6-P, and UDP-glucose, the $I_{0.5}$ were estimated from the plot of percent initial activity versus concentration of inhibitor.

Glycogen structure experiments. Liver glycogen from normal and diabetic mice was isolated by the $HgCl_2$ method,[11] which prevents degradation of the high-molecular-weight glycogen particles often observed when acid or alkali treatments are used.[7,12] The iodine absorption spectrum of glycogen was performed as described by Krisman.[13] The α-amylase and β-amylase limits were determined as described previously[14] and were based on the methods of Kjolberg and Manners,[15] the only exception being that for the determination of the β-amylase limit we used 0.5 mg of β-amylase and the total volume of the final reaction mixture was reduced to 10 ml. The maltose released by these amylase digestions was determined by the method of Nelson.[16] The average chain length ($\overline{CL}$), expressed as glucose residues, was determined as described by Manners and Wright[17] based on the extent of α-amylolysis. The exterior chain length (ECL) was calculated on the basis of the β-amylase limit and the interior chain length (ICL) was calculated from the ECL and $\overline{CL}$.[18]

Fractionation of the purified liver glycogen by sucrose density centrifugation was carried out as described by Konishi and Fuwa:[7] 250 μl of a 1% (wt/vol) glycogen solution was layered on top of a 10–40% sucrose gradient (13 ml) prepared in a cellulose tube. These tubes were centrifuged at 15,000 rpm for 35 min in a swinging bucket rotor (IEC type SB-283). After centrifugation, 0.5-ml fractions were collected from the bottom of the tube and assayed for glycogen by iodine absorption.[13]

Analytic procedures. SDS-polyacrylamide gel electrophoresis was performed by the method of Laemmli[19] using 8% acrylamide gels. Protein was measured by the method of Bradford.[20]

Materials. [^{14}C]glucose-1-P and UDP-[^{14}C]glucose were obtained from New England Nuclear, Boston, Massachusetts. UDP-glucose, glucose-1-P, maltose, type III rabbit liver glycogen, AMP, 2-(N-morpholino) ethane sulfonic acid (Mes), mercuric chloride, type 1-B β-amylase, and type 1-A α-amylase were from Sigma, St. Louis, Missouri. All other chemicals were of reagent grade.

RESULTS

Enzyme isolation. The SDS gels of the enzyme preparations used in these studies are shown in Figure 1. Most importantly, the relative Mr of the enzymes from both control and diabetic mice were identical and the preparations were similar in purity. Our glycogen synthase preparations usually consisted of doublets, with the subunits being around Mr 72–76 kDa. The glycogen synthase *a* preparations did not differ from the glycogen synthase *b* samples (results not shown). The phosphorylase preparations consisted of a major band that migrated in the SDS gels identically with the skeletal muscle phosphorylase standards, with a subunit Mr of 97.4 kDa.

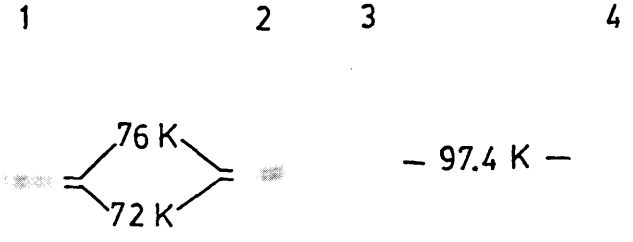

FIGURE 1. SDS-polyacrylamide gel electrophoresis of isolated enzymes used in kinetic studies. Electrophoresis was carried out using 8% acrylamide gels. Lanes 1 and 2: glycogen synthase *b* isolated from normal and diabetic mouse liver, respectively. Lanes 3 and 4: phosphorylase isolated from normal and diabetic mouse liver, respectively.

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TABLE 1
Kinetics of glycogen synthase *a* isolated from normal and diabetic mouse liver

	Normal	Diabetic
V_{max} (nmol/min/mg enzymic protein)	4100, 3800	2900, 2800
K_m UDP-glucose (mM)	0.37, 0.39	0.46, 0.47
$I_{0.5}$ ATP (mM)		
[UDP-glucose] 0.25 mM	0.80	0.85
2.5 mM	4.0	4.0

Assays were carried out at 30°C for 20 min in the presence of 25 mM Tris (pH 7.4), 1% glycogen, 2.5 mM EDTA, and 0.5 mg/ml bovine serum albumin. Final volume in all assay tubes was 0.1 ml. For the K_m (UDP-glucose) determinations, [UDP-glucose] ranged from 0.1 to 4.0 mM. The V_{max} values were calculated from these data. The $I_{0.5}$ (ATP) was determined using [ATP] ranging from 0.2 to 6.0 mM. Values from repeated experiments are separated by commas. Refer to MATERIALS AND METHODS for details on calculations.

Kinetic experiments. The kinetic properties of glycogen synthase *a* isolated from normal and diabetic mice are shown in Table 1. Although there were no changes in substrate affinity (apparent K_m) or inhibition constant for ATP in the diabetic enzyme, the V_{max} was 30% lower than for normal glycogen synthase *a*. This is illustrated further by the double-reciprocal plot shown in Figure 2. Neither enzyme preparation was inhibited by inorganic phosphate but, in fact, both were slightly activated (results not shown). This last observation is in agreement with a previous study.[21]

Glycogen synthase *b* is inactive except in the presence of glucose-6-P.[22] In Table 2, the V_{max} values of the diabetic glycogen synthase *b*, measured at three different concentrations of this activator, are shown to be 20% lower than for the enzyme from normal mouse liver. The apparent K_m values for its substrate, UDP-glucose, were very similar in the two enzymes, as were the activation constants for glucose-6-P. However, the diabetic enzyme did appear to be more sensitive to inhibition by inorganic phosphate and ATP, especially at near-physiologic concentrations (0.25 mM) of UDP-glucose (Figure 3). At this substrate concentration, the $I_{0.5}$ for inorganic phosphate was 0.85 mM for diabetic glycogen synthase *b* compared with 1.25 mM for the normal enzyme. Similarly, the $I_{0.5}$ for ATP was 0.50 and 0.74 mM for the diabetic and normal enzyme, respectively.

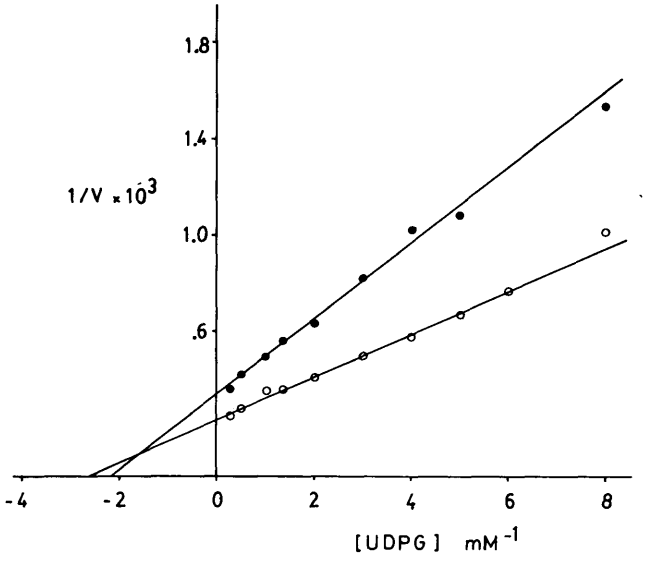

FIGURE 2. Double-reciprocal plot of reaction velocity versus UDPG concentration using the glycogen synthase *a* preparations from normal (○) and diabetic (●) mouse liver. Velocity units and details for the assay are given under MATERIALS AND METHODS and in Table 1.

TABLE 2
Kinetics of glycogen synthase *b* isolated from normal and diabetic mouse liver

	Normal	Diabetic
V_{max} (nmol/min/mg enzymic protein)		
[Glucose-6-P] 0.1 mM	3100	2500
1.0 mM	7100	5600
5.0 mM	5900	4600
K_m UDP-glucose (mM)		
[Glucose-6-P] 0.1 mM	1.58	1.30
1.0 mM	0.30	0.25
5.0 mM	0.21	0.21
$A_{0.5}$ Glucose-6-P (mM)		
[UDP-glucose] 0.25 mM	1.20	1.10
2.50 mM	0.48	0.60
$I_{0.5}$ P_i (mM)		
[UDP-glucose] 0.25 mM	1.25, 1.20	0.85, 0.80
2.50 mM	1.58	1.48
$I_{0.5}$ ATP (mM)		
[UDP-glucose] 0.25 mM	0.74, 0.70	0.50, 0.52
2.50 mM	0.83	0.66

Assays conditions were the same as described in Table 1. For the K_m (UDP-glucose) determinations, [UDP-glucose] ranged between 0.05 and 10.0 mM. The V_{max} values were calculated from these data. The $A_{0.5}$ (glucose-6-P) determinations were made using [glucose-6-P] varying between 0.05 and 5.0 mM. For the inhibition assays, glucose-6-P was present at a concentration of 2 mM. In these assays, [P_i] varied from 0.05 to 6.0 mM while [ATP] were ranged from 0.05 to 4.0 mM. Values from repeated experiments are separated by commas. Refer to MATERIALS AND METHODS for details on calculations.

Phosphorylase *a* kinetics were carried out in the presence of 0.5 mM caffeine, which is a potent inhibitor of phosphorylase *b*.[23] The V_{max} of the diabetic enzyme was twofold greater than that of the normal enzyme at low glycogen concentrations (Table 3), which was probably related to its lower apparent K_m for glycogen observed at two different concentrations of glucose-1-P (Table 3). These differences in apparent K_m for glycogen are illustrated by the double-reciprocal plots (Figure 4, A and B). The enzymes from both groups of mice behaved similarly in the presence of the activator AMP and most of the inhibiting compounds. However, the $I_{0.5}$ for ATP was greater for the diabetic enzyme, and although the difference between the normal and diabetic values was not great, it was a consistent observation in three separate experiments.

Glycogen characterization. Liver glycogen isolated from both types of mice by the $HgCl_2$ method was 93–94% pure as determined by the phenol-sulfuric acid method.[24] The α-amylase limit of normal mouse liver glycogen was 76% (Table 4), which is similar to that observed for rabbit liver glycogen.[25] This value was significantly lower (66%) in diabetic mouse glycogen. The β-amylase limit was also decreased (Table 4). From these values, calculations of average chain length, exterior chain length, and interior chain length were made. All three of these values were lower for the diabetic glycogen (Table 4). Both types of glycogen, when complexed with

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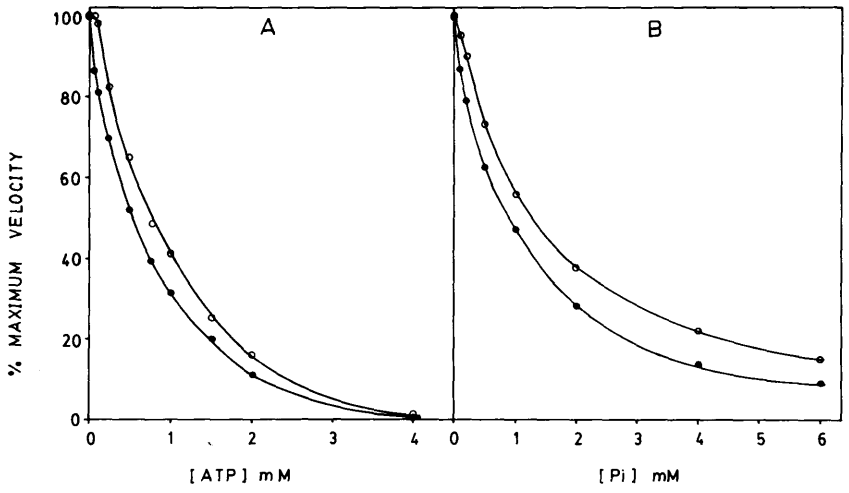

FIGURE 3. Inhibition of glycogen synthase *b* by ATP (A) and Pi (B). Values given are expressed as percent of the activity measured in the absence of inhibitor. Details for the assay are given under MATERIALS AND METHODS and in Table 2. ○, normal mouse liver glycogen synthase *b*; and ●, diabetic liver enzyme.

iodine, absorbed light maximally at a wavelength of 430 nm (Table 4). However, the absolute absorbance value at this wavelength was significantly less in glycogen isolated from diabetic mouse liver, being 0.194 ± 0.004 compared with 0.221 ± 0.005 from normal glycogen (Table 4).

Sucrose density centrifugation of these purified liver glycogens revealed that the bulk of glycogen from both normal and diabetic mice is homogeneous in size, layering in fractions 3–8 (Figure 5). However, glycogen from normal mice appeared to contain larger proportions of heavier glycogen, sedimenting lower in the gradient, whereas diabetic glycogen was more homogeneous and contained less amounts of heavy particles. Similar results were obtained in three separate experiments (data not shown).

DISCUSSION

The genetically diabetic C57BL/KsJ-*db*/*db* mouse has been used as a model of maturity-onset or type II diabetes. Several studies have indicated that the activities of glycogen synthase and phosphorylase, the rate-limiting enzymes of glycogenesis and glycogenolysis, respectively, are altered in this mouse model.[1,3,26] Akatsuka et al.[4] have shown that glycogen synthase isolated from diabetic rats (induced by streptozocin) differs from normal glycogen synthase with respect to kinetic and possibly conformational properties. For the above reasons, we examined the kinetic properties of glycogen synthase *a* and *b* and phosphorylase *a* isolated from diabetic mouse liver.

Values for the subunit molecular weight of mouse liver glycogen synthase have not been previously reported. The enzyme preparations used in this study had a subunit Mr doublet of 72 and 76 kDa (Figure 1). In several different preparations carried out in our laboratory, the enzyme isolated had a subunit Mr ranging from 72 to 80 kDa. The inclusion of proteolytic inhibitors throughout the purification protocol did not alter the apparent subunit molecular weight, in agreement with the results of Bahnak and Gold.[5] These values are similar to those obtained for the rat liver enzyme (77–80 kDa) by McVerry and Kim[27] and Bahnak and Gold,[5] although others have reported values of 80–93 kDa.[28–32] Bahnak and Gold[33] have suggested that these higher-molecular-weight forms may be due to incomplete hydrolysis of glycogen before purification. It is possible that the 72-kDa species (present study) is a proteolytic product, since gly-

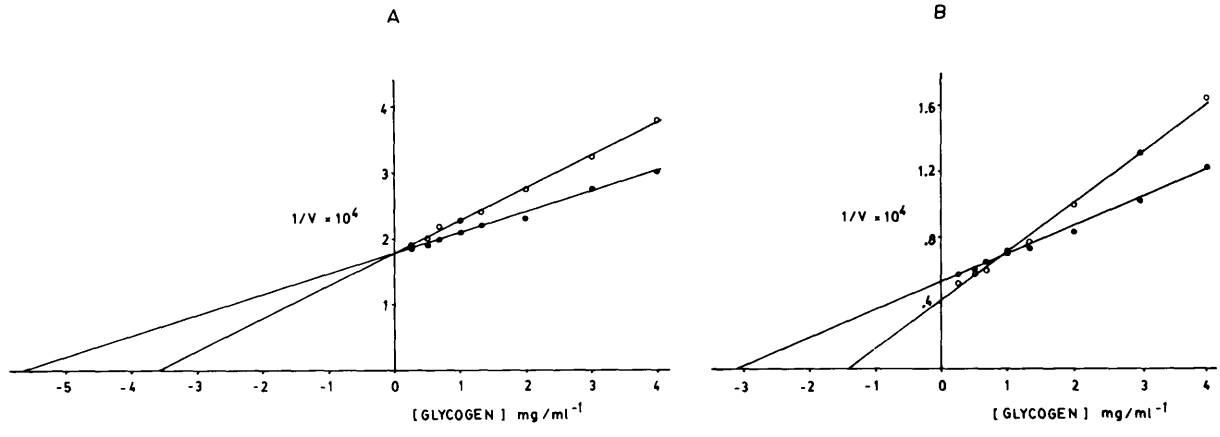

FIGURE 4. Double-reciprocal plots of phosphorylase *a* velocity versus glycogen concentration using enzymes from normal mouse liver (○) and diabetic mouse liver (●). A and B: reaction carried out in the presence of 0.5 and 5.0 mM glucose-1-phosphate, respectively. Velocity units and details for the assay are given under MATERIALS AND METHODS and in Table 3.

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TABLE 3
Kinetics of phosphorylase *a* partially purified from normal and diabetic mouse liver

	Normal	Diabetic
V_{max} (nmol/min/mg enzymic protein)		
[Glycogen] 0.25 mg/ml	7200	15,700
5.0 mg/ml	31,000	23,600
K_m Glucose-1-P (mM)		
[Glycogen] 0.25 mg/ml	2.0	2.3
5.0 mg/ml	4.8	4.8
K_m Glycogen (mg/ml)		
[Glucose-1-P] 0.5 mM	0.29, 0.27	0.18, 0.19
5.0 mM	0.45, 0.69	0.30, 0.32
$A_{0.5}$ AMP (mM)	0.05	0.05
$I_{0.5}$ ATP (mM)	7.7, 7.7, 7.5	8.7, 8.8, 8.6
$I_{0.5}$ UDP-glucose (mM)	~11	~11
$I_{0.5}$ Glucose (mM)	~35	~35
$I_{0.5}$ Glucose-6-P	both showed ~35% inhibition at 15 mM glucose-6-P	

Assays were carried out at 30°C for 20 min in the presence of 33 mM Mes (pH 6.5), 0.5 mM caffeine, 100 mM NaF, and 0.5 mg/ml bovine serum albumin. Final volume in assay tubes was 0.1 ml. For the K_m (glucose-1-P) determinations, [glucose-1-P] was varied from 0.1 to 20.0 mM. V_{max} calculations were made from these data. The K_m (glycogen) experiments were carried out using [glycogen] ranging from 0.1 to 10.0 mg/ml. The $A_{0.5}$ (AMP) determinations were made in the presence of 1 mg/ml glycogen and 2 mM glucose-1-P, with [AMP] ranging from 0.02 to 5.0 mM. For the studies on phosphorylase *a* inhibition by ATP, UDP-glucose, glucose, and glucose-6-P, [glycogen] was 5 mg/ml and [glucose-1-P] was 10 mM. For $I_{0.5}$ (ATP), [ATP] varied from 6.5 to 13.0 mM. For $I_{0.5}$ (UDP-glucose), [UDP-glucose] varied from 2.0 to 15.0 mM. Glucose-6-P inhibition was examined using [glucose-6-P] from 0.5 to 15.0 mM. Glucose inhibition studies utilized [glucose] ranging from 1 to 50 mM. Values from repeated experiments are separated by commas. Refer to MATERIALS AND METHODS for details on calculations.

TABLE 4
Molecular structure and properties of liver glycogen isolated from normal and diabetic mice by the $HgCl_2$ method[11]

	Normal	Diabetic
Purity (%)	94	93
α-amylase limit	76 ± 3	66 ± 1*
β-amylase limit	14 ± 1	8 ± 0.4†
Average chain length	14 ± 1	11 ± 0.3
Exterior chain length	4.4 ± 0.2	3.4 ± 0.1†
Interior chain length	8.4 ± 1.0	6.2 ± 0.3
Iodine complex λ_{max} (nm)	430	430
$E^{1\,cm}_{max}$ at λ_{max}	0.221 ± 0.005	0.194 ± 0.004*

Values shown (except for purity and λ_{max}) are the mean ± SEM of four samples. Statistical analysis was performed by Student's *t*-test. See MATERIALS AND METHODS for details on assay procedures and calculations.
*$P < 0.05$ and †$P < 0.025$.

cogen synthase is susceptible to proteolysis.[34,35] The proteolytic products of the rabbit liver enzyme have been shown to have kinetic properties different from those of the unproteolyzed enzyme.[34] However, because the subunit patterns on SDS-polyacrylamide gels are similar for the normal and diabetic enzymes, the differences in kinetic properties observed between these two enzyme preparations are not due to the presence, absence, or differing amounts of the lower-molecular-weight species in the samples.

Both forms of glycogen synthase from diabetic mice had 20–30% lower V_{max} values compared with normal glycogen synthase, but the substrate affinities (apparent K_m for UDP-glucose) were very similar (Tables 1 and 2). The only other difference observed was in glycogen synthase *b*, in which the $I_{0.5}$ for ATP and Pi at 0.25mM UDP-glucose was lower for the diabetic enzyme. These results indicate that only minor differences occur in this enzyme as a result of the diabetic state. This was further established by the fact that the glycogen synthase subunits from normal and diabetic samples migrated identically on SDS-polyacrylamide gels (Figure 1) and the enzymes had similar pH profiles (results not shown). These results differ with those of Akatsuka et al.[4] and may reflect the different type of diabetes studied. In the latter study, a streptozocin-induced diabetic rat model was used, resembling juvenile-onset diabetes, whereas in the present study a genetic mouse model of maturity-onset diabetes was employed. Bahnak and Gold,[5] using alloxan-induced diabetic rats, observed no changes in the kinetic and structural properties of glycogen synthase. The reason for the different results in the studies by Akatsuka et al.[4] and Bahnak and Gold[5] may be due to the use of different drugs for the induction of diabetes. For obvious reasons, this type of problem does not arise when using a genetically derived model of diabetes such as the one used in the present study.

There have been no previous reports on the kinetics of liver phosphorylase in diabetic animals. In the present study, the most striking change in the phosphorylase *a* kinetics is the

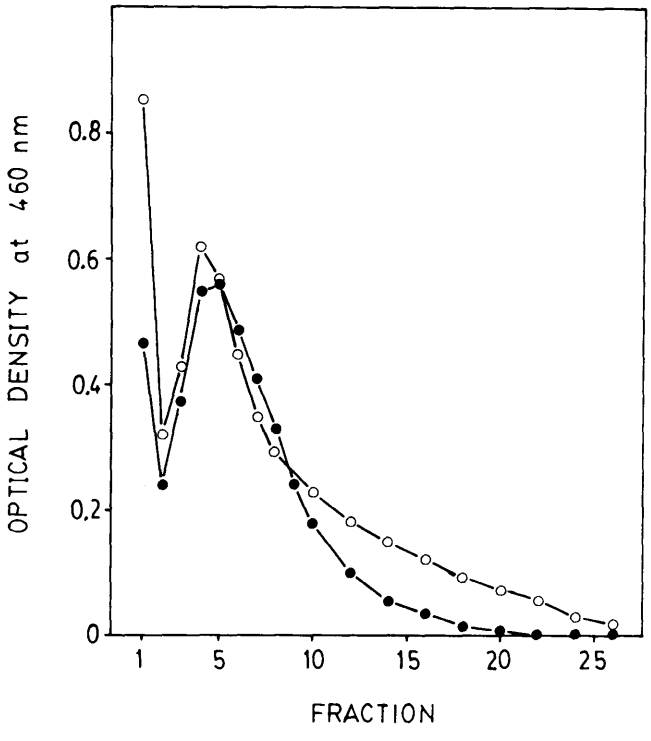

FIGURE 5. Fractionation of purified liver glycogen from normal (○) and diabetic (●) mice. Glycogen solutions (1%) were layered on top of a 10–40% (wt/vol) sucrose gradient in a cellulose tube. Details on centrifugation and glycogen measurement in the fractions are described in MATERIALS AND METHODS. Fraction 1 represents the top fraction in the tube.

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twofold increase in V_{max} at low glycogen concentrations (Table 3). This increase is probably related to the lower apparent K_m for glycogen exhibited by the diabetic enzyme (Table 3). It is not clear why this increased V_{max} is not present at the higher glycogen concentration of 5 mg/ml. This phenomenon is clearly shown in Figure 4, A and B, where at low glycogen concentrations the velocity of the reaction catalyzed by the diabetic enzyme is higher than that of the control enzyme, but this difference is diminished as glycogen levels are increased. Obviously, glycogen is more rate limiting for the reaction catalyzed by the control enzyme, but the presence of high glycogen levels overcomes this limitation. A consistent observation with the enzyme isolated from diabetic liver was that it appeared slightly less sensitive to ATP inhibition. The basis for these altered kinetic properties is not clear. Akatsuka et al.[4] have suggested, with regard to the glycogen synthase molecule, that different forms of an enzyme may be expressed depending on the physiologic state of the animal. However, the diabetic phosphorylase *a* migrated on SDS-polyacrylamide gels identical to the normal enzyme (Figure 1) and antibodies raised against normal mouse liver phosphorylase interacted equally well with the diabetic enzyme (Roesler and Khandelwal, unpublished observations). This implies that if a different form of the enzyme is present in the diabetic state, the molecular changes are subtle.

The kinetics of phosphorylase *b*, the inactive form, were not examined in the present study because of the presence of significant proportions of the *a*-form in all of our preparations. Although rapid conversion (i.e., dephosphorylation) occurred with glycogen synthase, such that >90% was in the *b*-form, phosphorylase dephosphorylation using endogenous phosphatases never proceeded beyond 50% completion. Moreover, incubation with the purified catalytic subunit of phosphorylase phosphatase[36,37] resulted in little conversion. Because there is no specific inhibitor known for phosphorylase *a*, this activity interfered with measurements of phosphorylase *b* activity and made any kinetic studies impossible.

Alterations in glycogen structural properties have been detected in hepatomas[38] and in different fed states.[7] Because the mice in the present study are hyperphagic[6] and have alterations in the enzyme activities that control glycogen synthesis and breakdown,[1-3] alterations in glycogen structure might be expected. The results of the present study indicate that differences are present. Diabetic liver glycogen appeared to be more homogeneous compared with normal glycogen (Figure 5). This glycogen also absorbed less iodine and had shorter exterior and interior chains than normal glycogen (Table 4), which together suggest alterations in the branching pattern of the diabetic glycogen. The cause of this altered glycogen structure may be associated with the observed alterations in glycogen synthase and phosphorylase and the increased glycogen turnover in these mice.[1] It is also likely that changes in the branching and debranching enzymes of glycogen metabolism[39-41] are involved in these glycogen structure changes, since they influence the degree of branching. Whether changes in these activities occur in diabetes awaits further characterization of these poorly understood enzymes.

It is apparent from this study that mouse liver glycogen differs from the rabbit and rat liver polymer. Although all three types of glycogen sediment similarly in sucrose gradients and have similar α-amylase limits, the β-amylase limit of rabbit and rat glycogen is typically 30–50%[7,42] compared with 14% for mouse glycogen (present study). Since β-amylase hydrolyzes outer chains and stops at the first branch point it encounters, this implies that the exterior chains of mouse glycogen are much shorter than in the rat and rabbit glycogen molecule. Our β-amylase digestion method was verified by using rabbit liver glycogen type III (Sigma) and glycogen isolated from rabbit liver by the $HgCl_2$ method. In these cases, the β-amylase limits were 51% and 30%, respectively (results not shown).

It has been recently reported[43] that glycogen synthase bound to the glycogen particle behaves differently than the free enzyme with respect to the degree of phosphorylation and sites of phosphorylation by several protein kinases. This suggests a role for glycogen in determining the activity of this enzyme. One could speculate that the altered glycogen structure in the diabetic mouse might also influence glycogen synthase activity, possibly by blocking certain phosphorylation sites or allowing new sites to be recognized.[43] A role for glycogen particle regulation of phosphorylase activity could also be possible. Answers to these questions will have to await further examination of the intimate relationship that glycogen shares with these enzymes.

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