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ASCARIS SUUM HEXOKINASE: PURIFICATION AND POSSIBLE FUNCTION IN COMPARTMENTATION OF GLUCOSE 6-PHOSPHATE IN MUSCLE

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Summary

Hexokinase (EC 2.7.1.1) is present in a soluble and a bound form in homogenates of *Ascaris suum* muscle. Cellulose acetate electrophoresis, isoelectric focusing, and ion exchange chromatography confirmed the presence of only one molecular form of hexokinase in this muscle.

A procedure for purifying hexokinase from *Ascaris* muscle has been developed utilizing ion-exchange chromatography, ammonium sulfate fractionation and gel filtration. The enzyme is a monomer with a molecular weight of 100 000 as determined by sodium dodecyl sulfate gel filtration. The Stokes' radius, diffusion coefficient, and frictional ratio have been determined. The apparent Michaelis constants for glucose and ATP are $4.7 \cdot 10^{-3}$ M and $2.2 \cdot 10^{-4}$ M, respectively. *Ascaris* hexokinase also exhibits end-product inhibition by glucose 6-phosphate and ADP. It is postulated that the kinetic parameters of the enzyme are the results of its function, that of generating glucose 6-phosphate primarily for glycogen synthesis.

Introduction

The parasitic roundworm, *Ascaris suum*, resides in an environment which is essentially anaerobic, the small intestine of swine. Therefore, the metabolism of this helminth is anaerobic, involving an exclusive utilization of carbohydrates for energy [1—4] (cf. ref. 5 for further details). The parasite is exposed to glucose only when the host is feeding and the worm synthesizes and stores relatively large quantities of glycogen in its muscle tissues [6,7]. This glycogen is then utilized during periods of "fasting" [7]. The catalytic rates of phosphorylase and phosphoglucomutase in this tissue are each approximately eleven

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times greater than hexokinase (EC 2.7.1.1) [8]. Therefore, it has been postulated that the primary source of glucose-6-*P* for glycolysis is glycogen and not free glucose [6,8]. Thus, the hexokinase(s) from this tissue would be of interest to study because it would appear that this enzyme functions primarily to produce glucose-6-*P* for glycogen synthesis rather than for glycolysis. The present communication provides evidence to suggest that the hexokinase from *Ascaris* has many properties which are similar to mammalian hexokinases, but some of the kinetic parameters are distinctly adapted to meet the needs of the parasite. It is postulated that one of the functions of the subcellular distribution of hexokinase is to ensure the presence of glucose-6-*P* for glycogen synthesis.

Materials and Methods

Materials

The following materials were obtained from Sigma: NADP, NADH, glucose-6-*P* dehydrogenase, lactate dehydrogenase, pyruvate kinase, aldolase, fumarase, malate dehydrogenase, phosphorylase *a*, β-galactosidase, cytochrome *c*, chymotrypsinogen, and Triton X-100. Sephadex G-200 and Blue Dextran 200 were acquired from Pharmacia. Carrier ampholines were obtained from LKB Producter. Reagents for disc gel electrophoresis were purchased from Bio-Rad. Titan III cellulose acetate plates were bought from Helena Labs (Beaumont, Texas). All other chemicals were reagent grade.

Methods

Homogenization. Adult female *Ascaris suum* were obtained at the local slaughterhouse and maintained in warm saline solution. The muscle tissue was dissected free of reproductive and digestive organs and washed in cold buffer. The muscle was homogenized in a Waring blender in three volumes of ice-cold buffer containing 10 mM Tris, pH 7.6, 1 mM EDTA, 20 mM 2-mercaptoethanol and 0.1 M glucose. The tissue was homogenized for 4 periods of 30 s. Between each homogenization period, the blender was placed in an ice bath for 5 min.

Enzyme assays. Hexokinase activity was measured spectrophotometrically at 30°C by measuring either the rates of glucose-6-*P* or ADP formed by the reaction. To measure glucose-6-*P*, a modification of the method of DiPietro and Weinhouse [9] was utilized. The assay mixture consisted of 50 mM Tris, pH 7.4, 0.75 mM $NADP^+$, 50 mM glucose, 4 mM ATP, 8 mM $MgCl_2$, and 0.5 units of yeast glucose-6-*P* dehydrogenase in a final volume of 1 ml. When D-fructose was used as substrate, 0.5 units of yeast phosphoglucose isomerase was included in the assay mixture. Initial velocities were determined by measuring the reduction of $NADP^+$ at 340 nm.

When glucose 6-phosphate inhibition studies were performed, the reaction was followed by the rate of ADP formation according to the method of Kornberg and Pricer [10]. The assay micture, in a volume of 1 ml contained: 50 mM Tris · Cl, pH 7.5, 100 mM KCl, 8 mM $MgSO_4$, 4 mM ATP, 1 mM phosphoenolpyruvate, 0.36 mM NADH, 50 mM glucose, 0.5 units each of rabbit muscle

lactate dehydrogenase and pyruvate kinase. Initial velocities were determined by the continuous recording of the oxidation of NADH at 340 nm. One unit of hexokinase activity is defined as the amount of enzyme necessary to convert one μmol of substrate per minute at 30° C. Specific activity is defined as units per mg of protein. During kinetic studies, measurements of initial velocities were made by recording the absorbance change from 0—0.1 *A*.

Ion exchange chromatography. 250 ml of DEAE Bio-Gel were washed several times with 0.01 M NaOH and 0.01 M HCl. The gel was then equilibrated with 5 mM Tris, 1 mM EDTA, 20 mM 2-mercaptoethanol, and 0.1 M glucose at the appropriate pH. The gel was packed into a 2.5 cm × 60 cm column under 30 cm of hydrostatic pressure and several volumes of buffer were run through the column at a flow rate of 45 ml · h^{-1} at 5° C.

Gel filtration. Sephadex G-200 (particle size 40—120 μm) was swollen for 72 h in 50 mM phosphate, pH 7.2, 1 mM EDTA, 15 mM 2-mercaptoethanol, and 0.1 M glucose. The suspended Sephadex was then deaerated for 2 h. The gel was packed into a 2.5 cm × 90 cm column under 10 cm of hydrostatic pressure. The column was washed with the respective buffer for 72 h at 4° C until stabilization of bed height and a flow rate of 6 ml · h^{-1} were achieved. Samples were layered on the top of the bed in 20% sucrose (w/v). Void volume (V_o), internal volume (V_i), and elution volumes (V_e) were determined with Blue Dextran, fumaric acid, and standard proteins, respectively. The elution volumes were correlated with molecular weights [11,12] or with Stokes' radii [13].

Cellulose acetate electrophoresis. Cellulose acetate plates (1 inch × 3 inch) were equilibrated in 0.09 ionic strength Tris/citrate buffer, pH 7.0. Samples of 8 μl each were applied to the plates and were subjected to electrophoresis for 30 min (1.5 mA/strip) in a Gelman Rapid Electrophoresis apparatus. The plates were stained for hexokinase activity by the procedure of Murakami and Ishibashi [14]. Densitometry of the electrophoretogram was carried out with the use of a Helena Densitometer.

Polyacrylamide gel electrophoresis. Sodium dodecyl sulfate gel electrophoresis was conducted according to the procedure of Maizel [15] using a 7.5% monomer concentration in 0.1% sodium dodecyl sulfate. Gels were prepared in Tris · Cl buffer, pH 8.8, and electrophoresis was carried out in Tris/glycine buffer, pH 8.8. Protein subunit molecular weight was correlated with mobility by the method of Weber and Osborn [16]. Protein bands were stained with 0.2% Coomassie Brilliant Blue R-250 dissolved in water and methanol (50 : 50, v/v). Just before use, 7 ml of glacial acetic acid was added to the stain. Destaining was performed electrophoretically in methanol/acetic acid/water (50 : 75 : 875, v/v/v).

Isoelectric focusing. Electrofocusing of *Ascaris* muscle extract was conducted by the method of Fodge et al. [17] utilizing an LKB 8101 electrofocusing column. The column was filled with a sucrose density gradient which contained 1% carrier ampholines (pH 3.5—8), 0.1 M glucose, 10 mM dithiothreitol, and a 25-mg protein sample. Electrofocusing was carried out at 600 V for 72 h. The column was eluted at 60 ml · h^{-1} with a peristaltic pump. Fraction volume was 1 ml and the pH of each fraction was determined immediately at 4° C. All fractions were then assayed for hexokinase activity.

Results

Electrophoretic studies

Initial studies on the hexokinase of *Ascaris* muscle revealed that if the crude homogenate (see Methods) was centrifuged at 105 000 × *g*, approximately 65% of the enzyme activity was present in the pellet and only 35% was in the supernatant solution. Therefore, experiments were carried out to determine if these "particulate" and "soluble" forms represented the same enzyme. Fig. 1A depicts cellulose acetate electrophoresis of the supernatant solution of *Ascaris* muscle after the crude homogenate had been centrifuged at 20 000 × *g*. There are two bands of hexokinase activity, one at the origin and an anodal band. Fig. 1B shows electrophoresis of the same supernatant solution after treatment with Triton X-100, KCl and recentrifugation at 20 000 × *g* (after this step, 97% of the hexokinase activity was recovered in the supernatant solution). There is one anodal band of hexokinase activity which corresponds to the anodal band in Fig. 1A and the band at the origin is not present. This band probably represents enzyme bound to membrane fragments which do not migrate in the electric field. Fig. 1C illustrates the electrophoresis of the supernatant solution from the original 20 000 × *g* pellet which had been rehomogenized, treated with detergent and salt, and then recentrifuged at 20 000 × *g*.

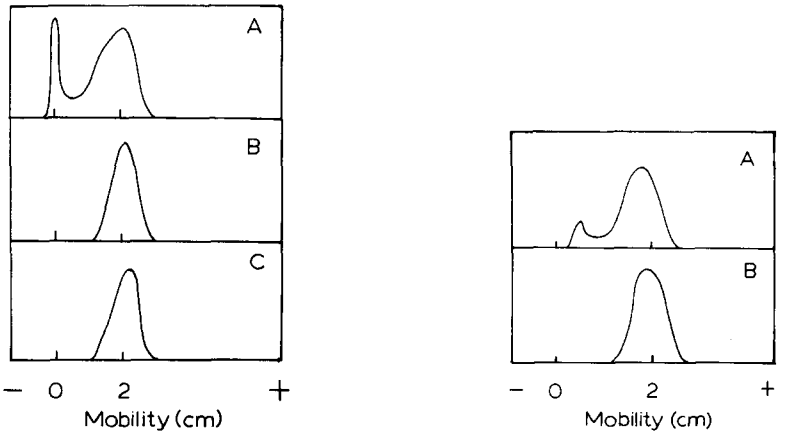


Fig. 1. Densitometer tracings of cellulose acetate electrophoretograms. (A) The crude homogenate of *Ascaris* muscle (See Methods) was centrifuged at 20 000 × *g* and the supernatant solution was subjected to electrophoresis on a 1 inch × 3 inch cellulose acetate plate for 30 min at 1.5 mA per strip. The electrophoresis buffer was Tris/citrate, pH 7.0. Hexokinase activity was visualized as described in Methods. (B) The 20 000 × *g* supernatant (A) was incubated for 1 h with 0.5% Triton X-100, 0.5 M KCl, and recentrifuged at 20 000 × *g*. Electrophoresis of the supernatant solution was carried out as described in (A). (C) The original pellet from the crude homogenate and 20 000 × *g* centrifugation was rehomogenized and then treated with 0.5% Triton X-100, 0.5 M potassium chloride and then recentrifuged at 20 000 × *g*. The supernatant solution was subjected to electrophoresis and activity developed as in (A).

Fig. 2. Densitometer tracings of cellulose acetate electrophoretograms. (A) Electrophoresis was performed on a crude enzyme extract that had been treated with 0.5% Triton X-100, 0.5 M KCl, and centrifuged at 20 000 × *g*. Before electrophoresis, the treated extract was dialyzed for 24 h against a buffer containing 1 mM 2-mercaptoethanol. Conditions for electrophoresis are the same as for Fig. 1A. (B) Electrophoresis of the enzyme sample used in Fig. 2A that had been redialyzed against a buffer containing 50 mM 2-mercaptoethanol.

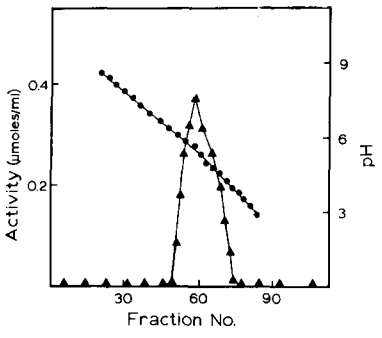


Fig. 3. Isoelectric focusing of *Ascaris* muscle hexokinase. The conditions of the experiment are described in Methods.

There is one anodal band corresponding to the band found in Figs. 1A and 1B. The results of these experiments suggested that hexokinase may be found in the muscle tissue in a soluble form and also present in a bound state in mitochondrial- and plasma membrane-rich fractions (vide infra). However, all forms appear to be electrophoretically identical and do not represent isozymes.

On certain occasions, multiplicity was noted after electrophoresis of extracts of solubilized homogenates. Fig. 2A illustrates electrophoresis of an extract that had been dialyzed 24 h against a buffer with a low concentration of 2-mercaptoethanol (1 mM). Two bands of activity are visible. Fig. 2B is the same sample redialyzed 24 h against the same buffer, but with a much higher concentration (50 mM) of the reducing agent. Only the anodal band is visible. These results suggest that sulfhydryl oxidation is one factor involved in creating pseudoisozymes [18,19,14] of hexokinase in *Ascaris*.

To further corroborate the presence of only one form of hexokinase in *Ascaris* muscle, the solubilized, centrifuged extract was subjected to isoelectric focusing in the presence of 0.1 M glucose and 10 mM dithiothreitol. One peak of hexokinase activity was found with an apparent isoelectric point of 5.9 (Fig. 3).

Purification

In order to further characterize hexokinase from *Ascaris* muscle, the enzyme was purified by the following procedures. All steps, unless otherwise indicated were performed at 4°C. Glucose (0.1 M) was included in all buffers since it appeared to stabilize the hexokinase [20]. *Ascaris suum* muscle (75 g) was homogenized in a buffer containing 10 mM Tris, pH 7.6, 1 mM EDTA, 20 mM 2-mercaptoethanol and 0.1 M glucose. After centrifugation at 20 000 × *g*, the supernatant solution was incubated with 0.5% Triton X-100 and 0.5 M KCl for a period of 1 h. The pellet from the centrifugation was rehomogenized in the same buffer as above, with KCl (0.5 M) added. After homogenization of the pellet, 0.5% Triton X-100 was added and incubated for a period of 1 h. All solutions were then combined and centrifuged for 2 h at 105 000 × *g*. The supernatant solution was then dialyzed overnight against 5 changes of a buffer containing 4 mM Tris, pH 7.8, 1 mM EDTA, 20 mM 2-mercaptoethanol and 0.1 M glucose.

DEAE-Bio gel chromatography

The dialyzed solution was centrifuged, the precipitate was discarded, and 420 ml of the extract was applied to a 2.5 cm × 50 cm column of DEAE-Bio Gel. The column was washed with buffer until the eluant was essentially protein-free. The hexokinase was eluted by the application of a linear salt gradient. The reservoir chamber contained 1 l of buffer plus 0.5 M KCl and the mixing chamber contained 1 l of buffer. Hexokinase eluted as a single peak (KCl = 0.26 M) which supports previous data showing only one form of hexokinase in *Ascaris* muscle tissue. Fractions containing maximum hexokinase activity were pooled and dialyzed against a buffer containing 50 mM phosphate, pH 7.4, 1 mM EDTA, 10 mM 2-mercaptoethanol, and 0.1 M glucose.

Ammonium sulfate fractionation

The dialyzed fraction was brought up to 0.10 saturation with solid ammonium sulfate and slowly stirred for 30 min. This fraction was centrifuged at 10 000 × *g* for 20 min and the pellet was discarded. Solid ammonium sulfate was added to the supernatant solution to bring the salt concentration to 0.40. This fraction was centrifuged and the supernatant solution was discarded. The pellet containing the hexokinase activity was dissolved in a minimum amount of the phosphate buffer and dialyzed for 12 h. The dialyzed fraction was brought to 0.40 saturation of ammonium sulfate by the slow addition of ammonium sulfate-saturated buffer (pH 7.4). The extract was centrifuged at 16 000 × *g* for 20 min and the supernatant fraction was discarded. The resulting pellet was dissolved in 5 ml of the phosphate buffer and assayed for hexokinase activity. Buffer containing a saturated solution of ammonium sulfate was added to the redissolved pellet fraction to bring the ammonium sulfate saturation to 0.25. The above steps were then repeated. This procedure was repeated several times, each time decreasing ammonium sulfate saturation by increments of 0.03, until a saturation of 0.10 was reached. Fractions between 0.12—0.21 ammonium sulfate saturation had the highest specific activity and were pooled and dialyzed against 0.70 ammonium sulfate to precipitate the protein. The dialysate was centrifuged at 16 000 × *g* for 20 min and the supernatant solution was discarded.

Sephadex G-200 chromatography

The resulting pellet was redissolved in 2 ml of buffer and dialyzed against 50 mM phosphate, pH 7.2, 1 mM EDTA, 14 mM 2-mercaptoethanol, and 0.1 M glucose. The sample was applied to a 2.5 cm × 90 cm Sephadex G-200 column which had been equilibrated in the above buffer. 2 ml fractions were collected and assayed for hexokinase activity. Fractions containing maximum hexokinase activity were pooled and the enzyme was precipitated in saturated ammonium sulfate. The precipitated protein was spun down and redissolved in 1 ml of the appropriate buffer. The protein sample was again applied to the Sephadex G-200 column used in the preceding step. 1-ml fractions were collected. Fractions having maximum hexokinase activity were pooled and then precipitated in 0.70 ammonium sulfate for stabilization. A summary of the purification procedure is presented in Table I. The above procedure resulted in a 146-fold purification of hexokinase over the crude extract. As the protein is purified it

TABLE I

PURIFICATION OF HEXOKINASE FROM *ASCARIS SUUM*

Fraction	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (overall)	% Yield (overall)
Crude homogenate	395	4120	177	0.041	—	(100)
Post dialysis	420	3521	162	0.046	1.1	92.0
DEAE Bio-gel	230	222	120	0.540	13.0	68.0
Ammonium sulfate I	15	101	83	0.810	20.0	47.0
Ammonium sulfate II	5	41	46	1.100	27.0	26.0
Sephadex G-200 I	1	11	32	2.900	70.0	18.0
Sephadex G-200 II	1	1	6	6.000	146.0	3.3

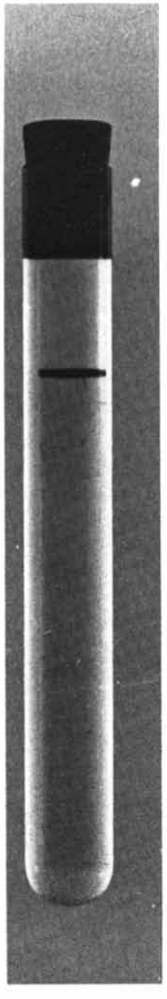
becomes increasingly labile and is extremely unstable in the absence of glucose. Dialysis to remove the glucose results in a total loss of activity. However, if the enzyme is stored in 70% ammonium sulfate and 0.1 M glucose at 4°C, only 10% of the activity is lost within a month.

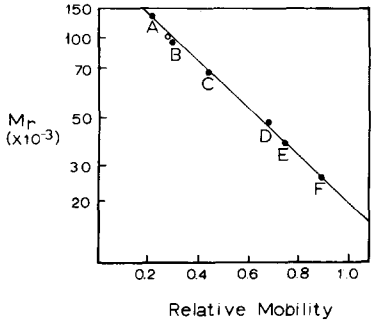
Homogeneity studies and molecular weight

Homogeneity of the enzyme preparation was determined by disc gel electrophoresis in the presence of sodium dodecyl sulfate. As shown in Fig. 4, only one band of protein was detected. The data of Fig. 4 were also used to determine the subunit molecular weight of *Ascaris* hexokinase. Fig. 5 illustrates that the subunit molecular weight is approximately 100 000. Since most mammalian hexokinases are monomers with molecular weights of approximately 100 000 [21,22], it was felt that the ascarid enzyme might also be a monomer. Fig. 6 shows the results of quantitative gel filtration of *Ascaris* hexokinase on Sephadex G-200. These results gave a molecular weight of 97 000. Therefore, we can conclude that the enzyme is a monomer. The results obtained from the gel filtration were also used to calculate other physical parameters of the enzyme. The Stokes' radius of *Ascaris* hexokinase is 37.5 Å (Fig. 7), the diffusion coefficient ($D_{20,w}$) is $5.7 \cdot 10^{-7}$ cm^2 · sec^{-1} and the frictional ratio (f/f_0) was calculated to be 1.3.

Kinetic properties of ascaris hexokinase

Because the purified ascarid hexokinase was unstable in the absence of glucose, all the kinetic studies were carried out on partially purified enzyme (specific activity = 1 unit/mg). Table II lists the apparent kinetic constants for *Ascaris* hexokinase. The apparent K_m for ATP is essentially the same as the reported values for types I, II, and III hexokinase for various rat tissues [23] and type IV hexokinase (glucokinase) from rat liver [24]. However, the apparent K_m for glucose is considerably higher than the "low K_m" hexokinases and is more reminiscent of rat liver glucokinase ($11.2 \cdot 10^{-3}$ M [24]). *Ascaris* hexokinase was also able to catalyze the phosphorylation of fructose; however, the apparent K_m was high ($8.3 \cdot 10^{-2}$ M) and it is doubtful whether it is important as a substrate for *Ascaris* hexokinase.





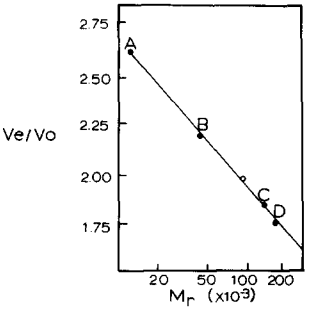


Fig. 4. Sodium dodecyl sulfate disc gel electrophoresis of *Ascaris* muscle hexokinase. A 0.02-mg sample of protein was subjected to electrophoresis. Experimental details are described in the text.

Fig. 5. Determination of subunit molecular weight of *Ascaris* hexokinase by sodium dodecyl sulfate disc gel electrophoresis. *Ascaris* hexokinase (0.03 mg) and standard proteins were prepared in 1% sodium dodecyl sulfate and subjected to electrophoresis in separate and/or single gels. Gels were subjected to electrophoresis for 3 h at 5 mA per gel. The mobility of each protein was plotted against the log of its molecular weight. Letters designate standard proteins: (A), β-galactosidase, (B), phosphorylase *a*, (C), bovine serum albumin, (D), fumarase, (E), aldolase, and (F), chymotrypsinogen. (o), Hexokinase.

Fig. 6. Determination of molecular weight of *Ascaris* muscle hexokinase by gel filtration. A 2.5 cm × 50 cm Sephadex G-200 column was calibrated with (A), cytochrome *c*, (B), ovalbumin, (C), aldolase, and (D), fumarase. (o), Hexokinase.

Like mammalian hexokinase, *Ascaris* muscle hexokinase shows end product inhibition with ADP and glucose-6-*P*. The apparent K_i values for ADP with respect to glucose and ATP (Table II) are similar to the values reported for the "low K_m" hexokinases from rat tissues [23]. On the other hand, glucose-6-*P* is

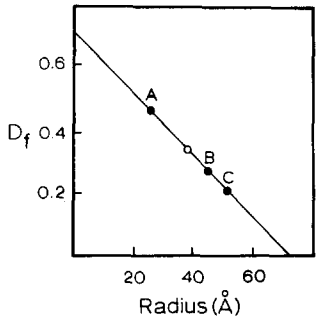


Fig. 7. Determination of Stokes' radius for *Ascaris* hexokinase by Sephadex G-200 column chromatography. The column was calibrated with (A), ovalbumin, (B), aldolase, and (C), fumarase. (o), Hexokinase. $D_f = V_e - V_o/V_i$, the ratio of the elution volume (V_e) minus the void volume (V_o) to the internal volume (V_i).

not as potent an inhibitor of *Ascaris* hexokinase as it is for mammalian hexokinases. The apparent K_i values are at least 1 order of magnitude higher [23]. The types of end-product inhibition are also similar to mammalian "low K_m" hexokinases. It should be noted that the reciprocal plots of varied glucose in the presence of several fixed concentrations of ADP or glucose-6-*P* were almost uncompetitive. Therefore, it is assumed that the ascarid hexokinase has a random kinetic mechanism [25,26].

Discussion

The results of the present investigation suggest that the hexokinase of *Ascaris suum* muscle is a monomer with a molecular weight of approx. 100 000. Although the initial studies on the enzyme revealed electrophoretic multiplicity, these forms were shown to be artifacts due to sulfhydryl oxidation and binding to subcellular organelles. There appears to be only one form of hexokinase in this tissue.

Like many of the mammalian hexokinases [27—32], the enzyme from the aschelminth probably exhibits a subcellular distribution which includes the cytosol, mitochondria- and membrane-rich fractions. The muscle cells of *Ascaris suum* are very unique in that they are essentially devoid of sarcoplasmic reticulum with the exception of the very minute dyads [33]. Therefore, it

TABLE II

APPARENT KINETIC PARAMETERS OF *ASCARIS SUUM* HEXOKINASE

Apparent kinetic constant		Value (M)	Type of inhibition
K_m	(ATP)	$2.2 \cdot 10^{-4}$	—
K_m	(Glucose)	$4.7 \cdot 10^{-3}$	—
K_m	(Fructose)	$8.3 \cdot 10^{-2}$	—
K_i	(ADP vs. ATP)	$6.6 \cdot 10^{-4}$	Non-competitive
K_i	(ADP vs. glucose)	$5.4 \cdot 10^{-3}$	Non-competitive
K_i	(Glc-6-*P* vs. ATP)	$2.5 \cdot 10^{-4}$	Competitive
K_i	(Glc-6-*P* vs. glucose)	$3.4 \cdot 10^{-3}$	Non-competitive

is probable that a portion of the particulate hexokinase is attached to the plasma membranes.

While the precise physiological function of the subcellular distribution of hexokinase is not known, in mammalian systems, several hypotheses have been advanced to account for it. These include: regulation of hexokinase activity [34]; positioning the enzyme next to the source of ATP in the mitochondria [35,36]; significance in insulin action and sugar transport [37,36]; and compartmentation for nuclear glycolysis [25]. It is possible that yet another function may be ascribed to the bound hexokinase. The muscle tissue of *Ascaris* is sporadically supplied with glucose and stores large quantities of glycogen (15% of fresh weight, [6]). This glycogen is utilized rapidly during periods of starvation by a complete complement of glycolytic enzymes [7,8]. Thus, it is possible that one function of the plasma membrane-bound hexokinase is to effectively "sequester" the production of glucose-6-*P* [38] to an area which would ensure that a portion of the product of hexokinase would be utilized for glycogen synthesis and not glycolysis. In *Ascaris* muscle, the storage site for glycogen (belly of the muscle, [7]) is continuous with, but spatially separated from the contractile elements which presumably contain the highest concentrations of glycolytic enzymes [39]. Therefore, it is reasonable to assume that a portion of the hexokinase would be localized within an area of glycogen synthesis and storage and not necessarily within the contractile elements. In the case of *Ascaris*, this area would include the plasma membrane surrounding the belly and arm portions of the muscle cell. Therefore, as glucose enters the cell from the perienteric fluid, it could be phosphorylated, and, depending upon the requirements of the cell at the particular time, would either be shunted toward glycogen or glycolysis. The relatively high K_i values for glucose-6-*P* would argue for this interpretation. If the K_i values for glucose-6-*P* were similar to those of mammalian types I, II and III hexokinases [23], then this would essentially prevent glycogen synthesis. From the anatomical considerations [7], it is possible that most of the glucose might enter the muscle cell of *Ascaris* via the belly region, where it could be phosphorylated by membrane-associated hexokinase, incorporated into glycogen and move into the contractile region as the need arises [7]. Therefore, a physical separation may exist between hexokinase and the rest of the glycolytic enzymes. Further, it is also conceivable that hexokinase may be associated with the plasma membrane surrounding the contractile elements (T-tubules, [7]). Presumably, the function of this hexokinase would be to produce glucose-6-*P* for glycolytic flux.

It is possible that the same type of separation could explain the distribution of hexokinase in mammalian muscles. Sigel and Pette [40] have demonstrated histochemically that most of the hexokinase in rabbit muscle is associated with the mitochondria and sarcolemma. In rat muscle, biochemical studies have shown that type II or muscle hexokinase is found in the sarcoplasmic reticulum and in a soluble form with less enzyme affiliated with mitochondrial fractions [41]. Glycogen and the glycogen metabolizing enzymes are closely associated with the sarcoplasmic reticulum [42], whereas several of the glycolytic enzymes are located primarily in the I band of the contractile elements [39,40]. Therefore, glucose, upon entering the muscle cell, could be phosphorylated by hexokinase in the region of the sarcoplasmic reticulum and

be shunted either to glycolysis or glycogen [38]. It is of interest to note here that muscle hexokinase (type II) appears to respond more slowly to inhibition by glucose-6-*P* than does the type I enzyme [43]. Although Kosow and Rose [44] have suggested that this phenomenon functions in controlling glycolytic rates, it is possible that it serves this role as well as allowing glycogen synthesis to occur under conditions in which glycolysis is slowing down. Alternatively, this may be a function only of the sarcoplasmic reticulum-bound hexokinase.

Previous studies from this laboratory [45] have suggested that certain of the enzymes of parasitic helminths are very similar, structurally and functionally, to their counterparts in mammalian tissues. Hexokinase from *Ascaris suum* muscle now appears to fit into this category. The subtle differences that do exist may be the result of the altered metabolism of the worm.

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