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Characterization of Key Glycolytic and Oxidative Enzymes in *Steinernema carpocapsae*

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Abstract: The enzyme activities of isocitrate dehydrogenase (ICDH, NADP-specific), lactate dehydrogenase (LDH), malate dehydrogenase (MDH), phosphoenolpyruvate carboxykinase (PEPCK), phosphofructokinase (PFK), pyruvate kinase (PK), and fructose-1,6-bisphosphatase (FBPase) were studied in the third-stage juveniles of *Steinernema carpocapsae*. Reaction requirements, pH optima, substrate and cofactor kinetic constants were similar to those reported previously from other parasitic helminths with the exception of LDH, which was unstable and could not be characterized for specific activity and kinetic constants. The respective pH optima were 7.5 for ICDH, 8.8 for MDH, 6.5 for PEPCK, 7.3 for PFK, 7.2 for PK, and 7.5 for FBPase. The specific activities for ICDH, MDH, PEPCK, PFK, PK, and FBPase at pH 7.5 were 4.8, 1,300, 22, 25, 35, and 6.8 (nmoles substrate · min^{-1} · mg $protein^{-1}$), respectively. In summary, the infective juveniles of *S. carpocapsae* display the metabolism typical of a facultative aerobe.

Key words: aerobic metabolism, anaerobic metabolism, biochemistry, carbon dioxide fixation, entomopathogenic nematode, enzyme, facultative anaerobiosis, gluconeogenesis, glycolysis, intermediary metabolism, nematode, *Steinernema carpocapsae*.

Steinernema carpocapsae is an insect-parasitic nematode with great potential as a commercial insect-control agent due to its broad host range, high virulence, ease of mass production, storage, and application in the field (11). The nematode has to survive in two different environments: the adult and early juvenile stages live inside the carcass of the dead insect larva host, whereas the infective third-stage juveniles live in the soil and search for a host (24).

The metabolism of *S. carpocapsae* was recently examined by Thompson et al. (30), who observed rapid reversible transitions between aerobic and anaerobic metabolism in nematodes alternately exposed to aerobic and hypoxic conditions, respectively. Exposure to anaerobic conditions was accompanied by the formation of succinate, which was released into the medium. In contrast, succinate or other anaerobic end-products were not detected in nematodes maintained under aerobic conditions. On this basis, those authors hypothesized that *S. carpocapsae* is a facultative aerobe, thereby distinguishing it from many other animal-parasitic nematodes considered facultative anaerobes because they produce and excrete anaerobic end-products under anaerobic and aerobic conditions (15,31).

Although the oxidation of glucose by the glycolytic pathway has been studied extensively in helminths, the regulation of the pathway is not yet fully understood (15,31). Two major control points in glycolysis are catalyzed by phosphofructokinase (PFK) and pyruvate kinase (PK) (19). PFK is the principal regulatory enzyme under short-term metabolite control and operates together with the glucogenic enzyme fructose-1,6-bisphosphatase in directing net carbon flow through the glycolytic-glucogenic pathway. The PK-catalyzed formation of pyruvate from phosphoenolpyruvate (PEP) precedes anaerobic lactate formation and (or) aerobic tricarboxylic acid metabolism. In this case, lactate dehydrogenase (LDH) activity is important for maintenance of the [NAD]/[NADH] redox balance, and isocitrate dehydrogenase (ICDH) is critical in regulating metabolism of substrate through the tricarboxylic acid cycle. During glycolysis in many parasitic helminths, however, PK competes with phosphoenolpyruvate carboxykinase (PEPCK) for

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PEP at the so-called PEP branchpoint. In this metabolism, PEPCK-catalyzed carboxylation to oxaloacetate is followed by reduction to malate, catalyzed by malate dehydrogenase, formation of fumarate and finally succinate synthesis (15,31).

This investigation examines the activities of the above enzymes in *S. carpocapsae* to better understand the glycolytic oxidation of glucose in this species. Moreover, our aim was to compare the properties of these enzymes in *S. carpocapsae* with those from other parasitic nematodes.

Materials and Methods

All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) or from Fisher Scientific (Tustin, CA), unless noted otherwise. Stock solutions were stored at 4, −20, or −80 °C (as necessary) to maintain maximum stability.

Preparation of nematodes: Infective juveniles of *S. carpocapsae* were provided by Stephen Manweiler of biosys (Columbia, MD) on a monthly basis and stored at 4 °C. Approximately 5×10^6 nematodes in calcium-alginate-supporting medium (11) were released by the addition of 200 ml aerated deionized water and the content of one biosys activator pack (containing approximately 21 g of a proprietary mixture of Na-EDTA and Na-citrate) in a 1-liter plastic bottle. The bottle was shaken every 10 minutes for about 3 hours until the alginate support had dissolved. The solubilized alginate was separated and removed from the nematodes by three washes with aerated deionized water followed by centrifugation in an IEC Model PR-J for 10 minutes at 1,450*g*. Further procedures were carried out at 0–4 °C. Live parasites were separated from the dead nematodes and other detritus by flotation on high-density sucrose solution (29). Nematodes were suspended in cold 40% (w/w) sucrose (2 ml packed nematodes in 38 ml 40% sucrose). After centrifugation (10 minutes at 1,450*g*), live nematodes floated on top of the sucrose solution as a pellicle (with the detritus and dead worms in a pellet on the bottom of the centrifuge tube). The pellicle was gently removed by first tilting the original centrifuge tube, then sliding the pellicle out with a wooden applicator into a clean centrifuge tube containing aerated deionized water. The nematodes were washed once and placed in cold 15% (w/w) sucrose (2 ml packed worms in 38 ml 15% sucrose). After centrifugation (10 minutes at 1,450*g*), the worms were washed thrice more with aerated deionized water. Cleaned nematodes were suspended in 0.1M Tris-HCl/0.25 M sucrose, pH 7.0 (2 ml packed worms in a total of 10 ml) and homogenized by three passes through an ice-cold French pressure cell (SLM/Aminco, Urbana, IL) at 128 MPa. The homogenates were centrifuged in a Beckman Model J-21C at 45,650*g* for 45 minutes. The supernatant was withdrawn by a glass pipet without disturbing the lipid layer and used as the enzyme source.

Enzyme assays: Kinetic studies were performed at pH 7.5, the in vivo cytoplasmic pH of the nematode (30). All assays, except MDH, were performed in a Gilford 240 spectrophotometer (Ciba Corning Diagnostics, Norwood, MA) with a Model 6050 chart recorder and a Lauda/Brinkman RM 3 water bath-circulator (Westbury, NY). MDH assays were performed in a Beckman DU-64 spectrophotometer. Temperature of all the assays was maintained at 26.5 °C. Temperature was monitored prior to the start of each assay with a BAT-8 thermocouple (Physitemp Instruments, Clifton, NJ). Reaction rates were determined as change in absorbance at 340 nm. Controls lacking substrate were included with each assay to account for activity due to endogenous substrates in the enzyme source. All specific activities reported are means of three or more determinations on independently prepared homogenates. Each determination of specific activity was the average of three replicates and reported as substrate-cofactor consumed-produced per minute per mg protein.

Standard assay mixtures in 1.0 ml final volume were as follows (the buffer pHs decribed below generated a final assay pH of 7.5):A. Dehydrogenases

1. *Isocitrate dehydrogenase* ($NADP^+$-dependent) (*threo*-D_s-isocitrate: NADP oxidoreductase (decarboxylating), E.C. 1.1.1.42): 100 mM HEPES, pH 7.7, 2 mM $MnCl_2$, 0.8 mM NADP, 5 mM DL-isocitric acid, and 50μl enzyme source. The reactions were started with the addition of isocitrate. Enzyme activity was determined by the rate of NADP reduction (10).

2. *Lactate dehydrogenase* (L-lactate:NAD oxidoreductase, EC 1.1.1.27): 100 mM KH_2PO_4-K_2HPO_4 buffer, pH 7.2, 0.4 mM NADH, 0.5 mM pyruvate, and 50 μl enzyme source. Reactions were started with the addition of pyruvate. Enzyme activity was determined by the rate of NADH oxidation (14).

3. *Malate dehydrogenase* (L-malate:NAD oxidoreductase, EC 1.1.1.37): (a) oxaloacetate reduction. 100 mM bis-tris propane, pH 7.5, 0.2 mM NADH, 1 mM oxaloacetate (OAA, prepared in 0.5 N HCl), and 10 μl enzyme extract (diluted 4:1 with homogenizing buffer). (b) malate oxidation. 100 mM tris-Cl, pH 7.25, 0.2 mM NAD, 1.0 mM malate, and 10 μl enzyme source. Reactions were started with the addition of the OAA in (a) and malate in (b). Enzyme activity was determined by the rate of NADH oxidation in (a) and NAD reduction in (b) (14). B. Kinases

1. *Phosphofructokinase* (ATP:D-fructose-6-P-1-phosphotransferase, E.C. 2.7.1.11): 100 mM HEPES, pH 7.5, 1 mM EDTA, 2.5 mM DTT, 0.2 mM NADH, 1.0 mM ATP, 8 mM fructose-6-phosphate, 5 mM $MgCl_2$, 5 mM NH_4Cl, 25 mM KCl, 1 unit of aldolase, 2 units of triosephosphate isomerase, 1 unit of a-glycerophosphate dehydrogenase, and 50 μl enzyme source. The reactions were started with the addition of ATP. Enzyme activity was determined by the rate of coupled NADH oxidation (1).

2. *Phosphoenolpyruvate carboxykinase* (GTP:oxaloacetate carboxylase (transphosphorylating), EC 4.1.1.32): 100 mM HEPES, pH 7.5, 60 mM $NaHCO_3$, 2.5 mM $MnCl_2$, 0.2 mM NADH, 4.0 units MDH, 5 mM phosphoenolpyruvate, 1.0 mM IDP, and 50 μl enzyme source. The reactions were started with the addition of IDP. Enzyme activity was determined by the rate of coupled NADH oxidation (14).

3. *Pyruvate kinase* (ATP:pyruvate phosphotransferase, EC 2.7.1.40): 100 mM tris-maleate buffer, pH 7.75, 100 mM KCl, 5 mM $MgSO_4$, 0.2 mM NADH, 4.0 units LDH, 4 mM ADP (in 10 mM Tris, pH 7.5), 5 mM PEP, and 50 μl enzyme source. Reactions were started by the addition of ADP. Enzyme activity was determined by the rate of coupled NADH oxidation (14). C. Hydrolase

Fructose-1,6-bisphosphatase (D-fructose-1,6-bisphosphate 1-phosphohydrolase, E.C. 3.1.3.11): 100 mM HEPES, pH 7.60, 5 mM $MgCl_2$, 5 mM EDTA, 0.4 mM NADP, 14 units of phosphoglucose isomerase, 14 units of glucose-6-phosphate dehydrogenase, 1.5 mM of fructose-1,6-bisphosphate, and 50 μl enzyme source. The reactions were started with the addition of FBP. Enzyme activity was determined by the rate of coupled NADP reduction (1,23).

Protein determination: Protein concentrations were determined spectrophotometrically using the Bradford method (5), with the Coomassie Blue reagent obtained from Bio-Rad Laboratories (Richmond, CA). A bovine serum albumin concentration series from 0 mg/ml to 20 mg/ml in 5-mg/ml increments was used to construct the standard curve. Measurements were perfomed in a 96-well plate, which was read using an ELISA plate reader from Bio-Rad Laboratories.

Dialysis and determination of ion requirements: One batch of the enzyme source, after final centrifugation as described above, was placed into a dialysis bag and dialyzed against 1 liter of the homogenizing buffer (0.1 M Tris/0.25 M sucrose, pH 7.0) for 24 hours at 4 °C. The buffer was changed every 6 hours. The dialyzed enzyme source was then used in enzyme assays that re-

quire the addition of monovalent and (or) divalent ions to determine absolute requirements.

Results

A. Specific activities of ICDH, MDH, PEPCK, PFK, PK, and FBPase in third-stage juveniles: All of the enzymes were found in extracts prepared from the third-stage juveniles obtained from biosys (Table 1). With the exception of LDH, all enzymes were stable when the enzyme source was kept at −20°C or 4°C overnight. LDH was unstable (activity decreased by 50% in about 1 hour), despite attempts to inhibit possible proteases and stabilize the enzyme with benzamidine, E-64, EDTA, EGTA, PMSF, and TPCK (data not shown). In addition, attempts to partially purify LDH with either oxamate affinity chromatography and ammonium sulfate fractionation failed to produce stable LDH for kinetic assays. The only reliable data obtained for LDH was the pH profile in one enzyme preparation. The specific activity of that preparation was 3.5 nmoles substrate · min^{-1} · mg $protein^{-1}$ (data not shown). Activities for ICDH, MDH, PEPCK, PFK, PK, and FBPase at pH 7.5 are in Table 1.

B. Reaction Requirements and pH Optima: Malate dehydrogenase rapidly reduced OAA in the presence of NADH, but the rate of the reverse reaction was extremely low. The pH optima for LDH and MDH were 7.3 (in 0.1 M K_2HPO_4-KH_2PO_4) and 8.8 (in 0.1 M CHES), respectively (Fig. 1A). The activity of MDH was also studied in 0.1 M bis-tris propane and 0.1 M Tris-HCl, and similar pH responses were found. The pH optimum of ICDH was 7.5 (in 0.1 M HEPES, Fig. 1A). Addition of Mn^{2+} was essential for activity of ICDH.

The two enzymes that metabolized PEP had specific nucleotide and ionic requirements. PK required K^+, Mg^{2+}, and ADP for activity, whereas PEPCK required HCO_3^-, Mn^{2+}, and IDP. Equimolar replacement of IDP with ADP resulted in a 95% decrease in activity. However, equimolar replacement of IDP with GDP resulted in only a 32% decrease in activity. PEPCK had a higher (six-fold) requirement for HCO_3^- than the standard reaction mixture reported for other nematodes (14,25). The pH optimum for PK was 7.3 (0.1 M Tris-maleate), whereas the optimum for PEPCK was 6.5 (0.1 M MES, Fig. 1B).

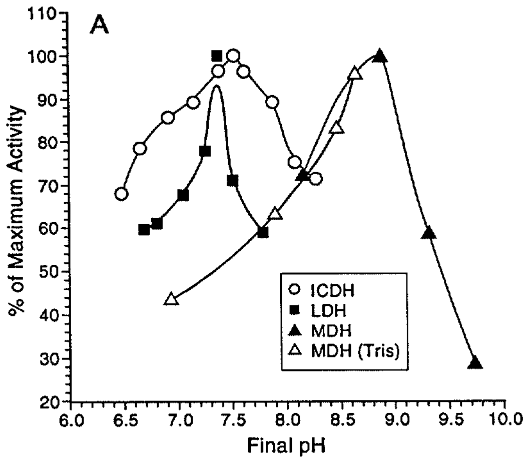
The pH optima for PFK and FBPase (in 0.1 M HEPES) were 7.3 and 7.5, respectively (Fig. 1B). PFK had an absolute requirement for ATP and Mg^{2+} · NH_4^+ and K^+ enhanced the activity of PFK. Omission of NH_4^+ and K^+ resulted in a decrease in PFK activity by 70% and 84%, respectively. FBPase had an absolute requirement for the presence of EDTA and Mg^{2+}.

C. Kinetic Analysis: 1. Dehydrogenases. At the physiological pH of 7.5, ICDH exhibited a sigmoidal response to substrate with an estimated $S_{0.5}$ of 4.18 and a Hill constant of 0.5 (Fig. 2A). However, its response to cofactor $NADP^+$ was hyperbolic

TABLE 1. Specific activity and pH optima of ICDH, MDH, PEPCK, PFK, PK, and FBPase in *Steinernema carpocapsae.*

Enzyme	Activity[a]	pH Optima
Isocitrate dehydrogenase	4.8 ± 0.21 (5)	7.5 ± 0.02 (3)
Malate dehydrogenase	1,300 ± 98.0 (9)	8.8 ± 0.02 (3)
Phosphoenolpyruvate carboxykinase	22 ± 2.4 (5)	6.5 ± 0.01 (3)
Phosphofructokinase	25 ± 2.1 (4)	7.3 ± 0.04 (3)
Pyruvate kinase	35 ± 7.7 (5)	7.2 ± 0.01 (3)
Fructose 1,6-bisphosphatase	6.8 ± 0.28 (4)	7.5 ± 0.05 (3)

[a] Activity expressed as nmoles of substrate utilized · min^{-1} · mg $protein^{-1}$ at pH 7.5 and 26.5 °C. Mean ± SEM (number of determinations).



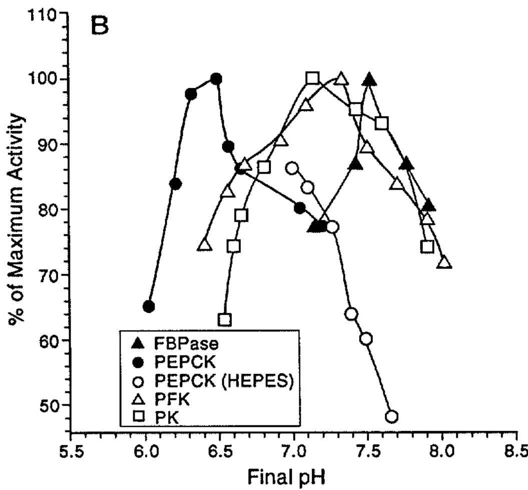
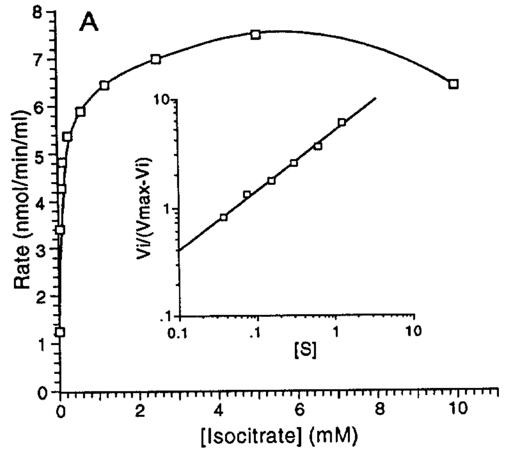


FIG. 1. The pH profiles for dehydrogenases, kinases, and a hydrolase determined in third-stage infective juveniles of *Steinernema carpocapsae*. Activities are expressed as percentage of maximum obtained at the optimum pH. Each point is the mean of three replicates: A) ICDH, LDH, and MDH; B) FBPase, PEPCK, PFK, and PK.



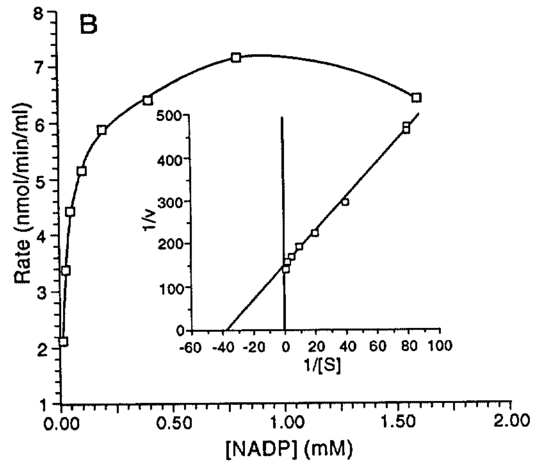


FIG. 2. Substrate and coenzyme affinity plots for isocitrate dehydrogenase from *Steinernema carpocapsae* third-stage infective juveniles at pH 7.5. Each point is the mean of three replications: A) Isocitrate saturation curve with Hill plot (inset); B) $NADP^+$ saturation curve with double-reciprocal plot (inset).

(Fig. 2B), and apparent K_m and V_{max} were determined (Table 2).

LDH activity was unstable in most homogenates as described above, and kinetics could not be determined. MDH activity in the direction of OAA reduction was high. Hyperbolic saturation curves were obtained for both substrates and cofactors (Fig. 3A,B) at the physiological pH of 7.5. At the optimal pH of 8.8 (in 0.1M CHES), however, MDH exhibited a sigmoidal saturation curve with regard to the cofactor NADH, which was indicative of cooperative allosteric kinetics. When Hill plots were made, the Hill constant was 1.5 (data not shown). The apparent K_m values obtained for OAA and NADH at pH 7.5 are in Table 2. The rate of malate oxidation was extremely low, and kinetic analysis was not attempted.

2. Kinases. At the physiological pH of 7.5, PEPCK exhibited hyperbolic responses to both IDP (Fig. 4A) and PEP (Fig. 4B), and Lineweaver-Burk double-

TABLE 2. Kinetic parameters of isocitrate dehydrogenase, malate dehydrogenase, phosphoenolpyruvate carboxykinase, phosphofructokinase, pyruvate kinase, and fructose 1,6-bisphosphatase from *Steinernema carpocapsae*.

Enzyme		K_m (mM)	V_{max}[a]
ICDH	Isocitrate	4.18 ± 0.279 (4)[c]	H[b]
	NADP	0.04 ± 0.004 (4)	9.8 ± 1.1 (4)
MDH	OAA	0.039 ± 0.0035 (4)	1,600 ± 40 (4)
	NADH	0.052 ± 0.0029 (4)	84 ± 0.94 (4)
PEPCK	PEP	0.48 ± 0.03 (4)	44 ± 3.8 (4)
	IDP	0.32 ± 0.063 (4)	50 ± 3.5 (4)
PFK	F-6-P	1.45 ± 0.119 (4)[c]	H[b]
	ATP	0.11 ± 0.026 (4)	39 ± 1.6 (4)
PK	PEP	2.4 ± 0.66 (4)	41 ± 9.9 (4)
	ADP	1.0 ± 0.26 (4)	24 ± 2.8 (4)
FBPase	FBP	0.34 ± 0.048 (4)	9.3 ± 0.21 (4)

[a] Expressed as nmoles of substrate utilized · min^{-1} · mg $protein^{-1}$. Mean ± SEM (number of determinations).
[b] H indicates that the enzyme kinetics does not follow Michaelis-Menten kinetics but instead follows cooperative allosteric kinetics (as described by the Hill equation).
[c] Estimated $S_{0.5}$ value obtained from saturation plot.

reciprocal plots were used to obtain apparent K_m and V_{max} values (Table 2).

At pH 7.5, PFK exhibited a sigmoidal response to fructose-6-phosphate, with an estimated $S_{0.5}$ of 1.45 and a Hill constant of 1.7 (Fig. 5A). However, at the same pH, PFK exhibited a hyperbolic response to ATP (Fig. 5B), and Lineweaver-Burk double-reciprocal plots were used to obtain apparent K_m and V_{max} values (Table 2).

At pH 7.5, PK exhibited a hyperbolic response toward both PEP (Fig. 6A) and ADP (Fig. 6B), and Lineweaver-Burk double-reciprocal plots were used to obtain apparent K_m and V_{max} values (Table 2).

3. Hydrolase. FBPase exhibited a hyperbolic response to FBP at the physiological pH of 7.5 (Fig. 7), and Lineweaver-Burk double-reciprocal plots were used to determine the apparent K_m and V_{max} (Table 2).

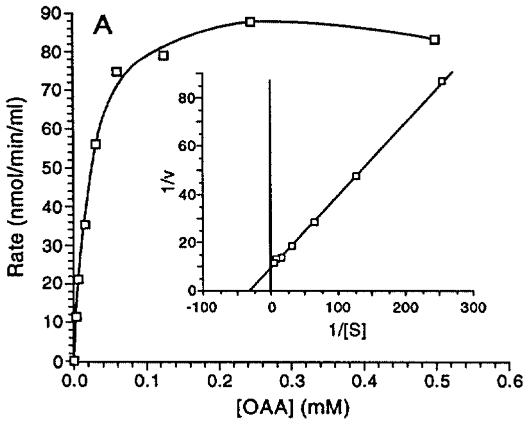
DISCUSSION

LDH activity varies widely among animal-parasitic nematodes, from 1,301 in *Brugia pahangi* (a lactate-producing nematode) (3) to 88 moles substrate · min^{-1} · mg $protein^{-1}$ in *Ascaris lumbricoides* (18). The single-value specific activity recorded for *S. carpocapsae*, 3.5 nmoles substrate · min^{-1} · mg $protein^{-1}$, is similar to that reported for another insect-parasitic nematode, *Romanomermis culicivorax* (14). The latter nematode produces succinate and can survive extensive anaerobiosis in the presence of carbon dioxide (14).

The K_m of MDH for OAA and NADH of *S. carpocapsae* did not differ significantly from that of *R. culicivorax* (14) and *Toxocara canis* (22). Under standard thermodynamic conditions, the equilibrium of MDH strongly favors the reduction of OAA rather than oxidation of malate. However, during aerobic conditions the rapid depletion of OAA by an active citrate synthase causes a mass action effect that drives the equilibrium of MDH into malate oxidation (19).

The cofactor and cation requirement of PK activity in *S. carpocapsae* were similar to those of the enzyme from other sources, both vertebrate and invertebrate. Monovalent cations, divalent cations, and nucleotide diphosphates are universally required by PKs from other sources (15,19, 31).

The absolute requirement of *S. carpocapsae* PEPCK for HCO_3^-, divalent metal ion (Mn^{2+}), and NDP (IDP or ADP) are common characteristics for this enzyme, as has been reported for *A. suum* (32). PEPCKs generally have an acidic pH optima (pH 5 to 6) and are generally restrictive in NDP



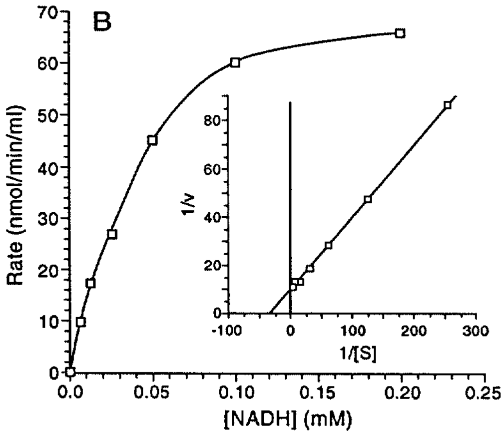
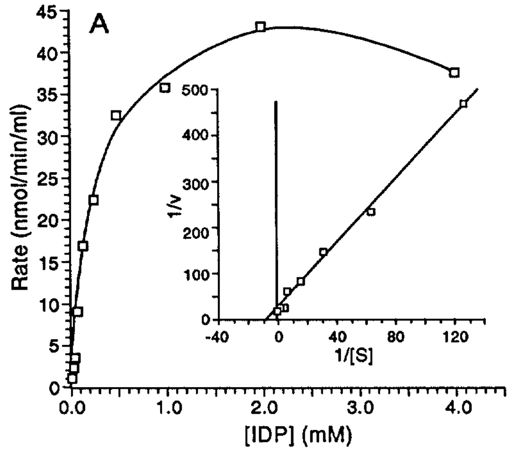


FIG. 3. Substrate and coenzyme affinity plots for malate dehydrogenase from *Steinernema carpocapsae* third-stage infective juveniles at pH 7.5. Each point is the mean of three replications: A) Saturation curve for oxaloacetate (inset = double reciprocal plot); B) Saturation curve for NADH with double reciprocal plot (inset).



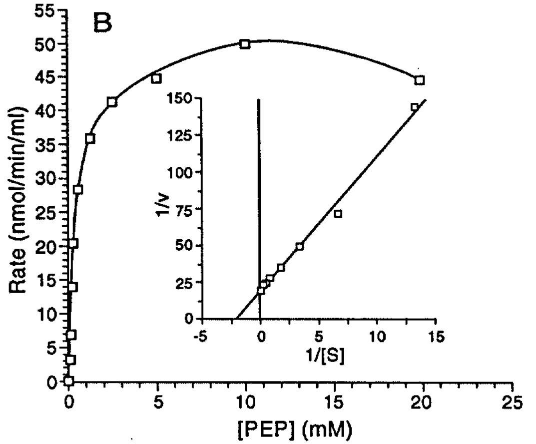
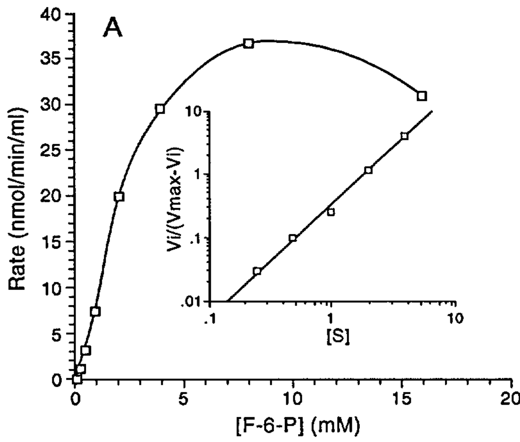


FIG. 4. Substrate and coenzyme affinity plots for phosphoenolcarboxykinase from third-stage infective juveniles of *Steinernema carpocapsae* at pH 7.5. Each point is the mean of three replicates: A) Saturation curve for IDP with double-reciprocal plot in the inset; B) Saturation and double-reciprocal (inset) plots for PEP.

requirement. In *S. carpocapsae,* IDP was the optimal cofactor for PEPCK activity, equimolar GDP was 32% less effective, and ADP was virtually ineffective. In *Ascaris suum* the K_m for GDP was almost 6 to 8 times lower than that for IDP and, hence, was the better nucleotide substrate (25,32). The affinity of PEPCK from *S. carpocapsae* for PEP was similar to that of *R. culicivorax* and *A. lumbricoides* (25). The pH optimum of 6.5 for PEPCK in *S. carpocapsae* was higher than other helminths. However, it was similar to that of *Trichinella spiralis* (4).

In general, the pH optima of PEPCK and PK do not overlap, but in the case of *S. carpocapsae* the more neutral PEPCK and PK resulted in a considerable overlap at pH 6.5, where both enzymes still retained significant activity (more than 80% of the optimal pH). The overlap in pH optima may explain the near unity of the PK/PEPCK ratio, as PEPCK would be significantly more active at the physiological pH of *S. carpocapsae* than the enzymes from other helminths with lower pH optima. In



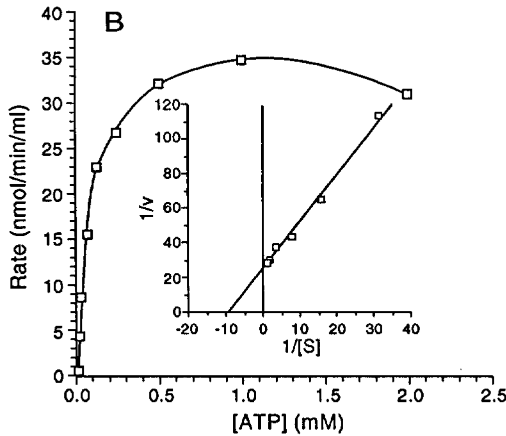
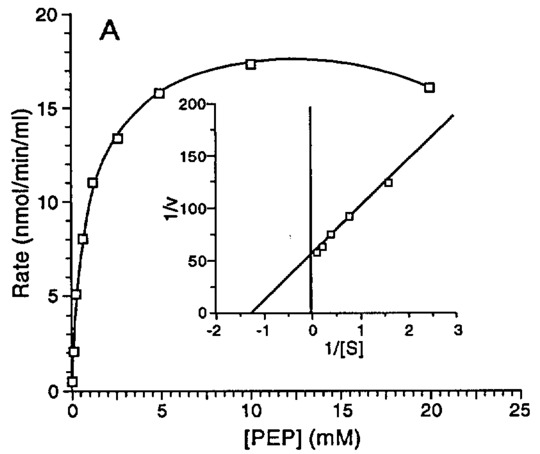


FIG. 5. Substrate and coenzyme affinity plots for phosphofructokinase from third-stage infective juveniles of *Steinernema carpocapsae* at pH 7.5. A) Saturation curve for fructose-6-phosphate (Hill plot in inset); B) Saturation curve for ATP with double-reciprocal plot (inset).



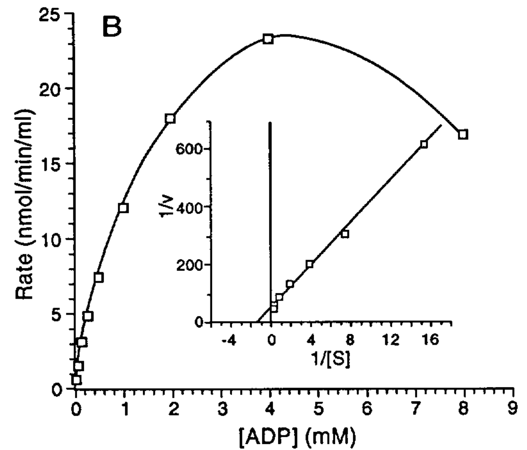


FIG. 6. Substrate and coenzyme affinity plots for pyruvate kinase from third-stage infective juveniles of *Steinernema carpocapsae* at pH 7.5. A) Saturation curve for phosphoenolpyruvate (double-reciprocal plot in inset); B) Saturation curve for ADP with double-reciprocal plot (inset).

S. carpocapsae at the physiological pH of 7.5, the K_m of PK for PEP was about fivefold that of PEPCK. The K_m of PK for ADP was also approximately three times that of PEPCK for IDP at pH 7.5.

The divalent metal ion and ATP requirement of PFK in *S. carpocapsae* are typical for helminths, as were the inhibitory effect of citrate and ATP at saturating concentrations. In *Dirofilaria immitis,* fructose-2,6-bisphosphate and AMP were reported to stimulate activity of PFK (28). Hofer et al. (13) reported that the purified PFK of *A. suum* exhibited biphasic kinetics in the saturation curve for fructose-6-phosphate. This effect was also seen in the PFK of *S. carpocapsae.* In *S. carpocapsae,* increasing concentration of F-6-P relieved the inhibition by ATP, as can NH_4^+ and K^+.

The requirement of *S. carpocapsae* FBPase for divalent metal ion (Mg^{2+}) and EDTA is similar to the enzyme from vertebrate sources. EDTA in nematode cell-free assays is used to chelate the heavy metal ions (such as Zn^{2+}) that are potent inhibitors of FBPase activity. Similar requirements have been reported in a variety of mammalian tissue sources, such as rab-

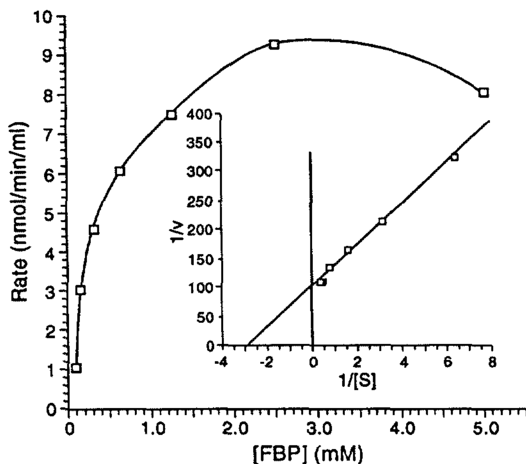


FIG. 7. Substrate (fructose-1, 6-bisphosphate) affinity curve (double-reciprocal plot in inset) for fructose bisphosphatase from third-stage infective juveniles of *Steinernema carpocapsae* at pH 7.5.

bit liver (9) and turkey liver (12). In *Fasciola hepatica,* Lloyd (21) reported that unlike PFK, which is modulated by a multitude of effectors, AMP is the only effector of FBPase. The main regulator is apparently the substrate, fructose 1,6-bisphosphate, as the enzyme exhibits substrate inhibition, as in *S. carpocapsae.* According to Lloyd (21), the phenomenon of substrate cycling between FBPase and PFK was thought to increase the sensitivity of the control signal, AMP. In *S. carpocapsae,* the activity of PFK was nearly four times higher than FBPase, and the fact that a high rate of substrate cycling is energetically unfavorable should discount the possibility that such cycling is operative in *S. carpocapsae.*

Beuding and Saz (6) first proposed the differentiation of lactate and succinate producers with the PK/PEPCK ratio. A ratio less than 1.0 would indicate a succinate producer, while a ratio well above 1.0 would indicate a lactate producer. However, the relevancy of this ratio has been in debate. The two enzymes are known to catalyze non-equilibrium reactions, and different metabolites (*e.g.* citrate, NADH, fructose 1,6,-bisphosphate, and malate) act as modulators in the control of the enzymes (8,15). Tielens and van den Bergh (31) questioned the relevance of the ratio measured in vitro, in that pyruvate cannot only be converted to lactate, but it could also be metabolized by the mitochondria of parasitic helminths (into acetate). Köhler (15) indicated that the factors determining the direction of carbon flow at the PEP branch point include the activities of the two enzymes, the affinities of these two enzymes for PEP, the concentrate of various substrates within the parasite (especially CO_2), and the activities of the enzymes that catalyze the subsequent reactions. Thus, the PK/PEPCK ratio, as used in determining the direction of the carbon flow from glucose, would seem to be an oversimplification. The ratio of LDH/PEPCK was also proposed as an indicator of direction of carbohydrate metabolism at the PEP branch point (16). However, this is unlikely in light of the newly found relationship between PK and PEPCK.

In *S. carpocapsae,* the PK/PEPCK ratio was 1.6, which suggests that it has a mixed metabolism, with both succinate and lactate being produced at the same time. However, this is not supported by in vivo flow NMR, which shows primarily the production of large amounts of succinate accompanied by trace amounts of acetate and lactate under anaerobic conditions in *S. carpocapsae* (30). This ratio is comparable to that of *Setaria digitata* (2) and *Nippostrongylus brasiliensis* (26). The presence of a high amount of succinate indicates that the fumarate reductase system is operational, and this requires the CO_2-fixing ability of PEPCK. It is possible that the in vitro assay conditions do not adequately simulate that of the in vivo environment, and that the combined effect of different modulators would produce a PK/PEPCK ratio different from the current determination in this study. In addition, the higher affinity of PEPCK for PEP would have a greater effect on the carbon flow under anaerobic conditions.

The activity of MDH in the direction of malate oxidation was extremely low, as was the NAD-dependent ICDH. Therefore, these two enzymes were not characterized thoroughly. The NADP-dependent form

of ICDH appears to be the predominate form in nematodes, as exemplified by *N. brasiliensis* and *Ancylostoma ceylanicum* (27). The enzymatic analysis showed a generally low activity of both MDH (malate oxidation) and ICDH and suggests that the TCA cycle in *S. carpocapsae* operates at a low level, or serves only to generate amino acids. However, the studies of Thompson et al. (30) showed that the nematodes must have an active TCA cycle in an aerobic environment. Further investigation of other enzymes and metabolites of the cycle (such as aconitase, alpha-ketoglutarate dehydrogenase, isocitrate, and succinate) in *S. carpocapsae* is necessary to evaluate the activity of the cycle and its significance. Earlier attempts by the senior author to assay the enzymes of the glyoxylate cycle gave equivocal results (data not shown). The low activity of FBPase would further suggest the non-functionality of the glyoxylate pathway and gluconeogenesis. Further studies on metabolites and carbon flow are required to evaluate the potential presence-absence of gluconeogenesis.

In summary, the infective juveniles of *S. carpocapsae* displayed metabolism typical of a facultative aerobe. Because the infective stage is non-feeding, all energy must be derived from internal storage. The ability to use both aerobic and anaerobic metabolism demonstrates the versatility of the nematode in surviving a myriad of environments. The transition from the aerobic environment of the top soil into the environment of the host hemocoel (where oxygen may be depleted by the flora of rapidly growing bacteria) would require an adaptable metabolism. The presence of both active PEPCK and PK indicates a dual usage of the PEP branch point pathways to generate energy and maintain the redox balance. In the absence of oxygen, as is the case with most anaerobic helminths, fumarate becomes the terminal electron acceptor, and succinate is produced (15). The requirement of oxygen for long-term survival (17,20) indicates the aerobic nature of the nematode. The ability to survive for more than 40 days under hypoxia (7) demonstrates its ability to use anaerobic metabolism to continue energy production and maintain redox balance.

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