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Polyamine Metabolism in Taeniid Metacestodes

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Abstract

Polyamine contents, ornithine decarboxylase (ODC) activity and polyamine oxidase activity were examined in the metacestode stages of family Taeniidae, including *Taenia crassiceps, T. hydatigena, T. taeniaeformis* and *Echinococcus multilocularis*. These larval taeniids contained significant levels of three major polyamines: putrescine, spermidine and spermine. The putrescine/spermidine ratios were much higher than those reported for some parasitic nematodes or adult cestodes. ODC levels of the parasites were negligible by measurement of released $^{14}CO_2$ from L-[1-^{14}C]ornithine, although these parasites were all in growing stages. Significant activity of polyamine oxidase was, however, detected in the larval taeniids, suggesting the presence of a reverse pathway of polyamines in the parasites. These results suggest that the larval taeniids possess novel polyamine metabolism.

Introduction

Polyamines such as putrescine, spermidine and spermine are ubiquitous in all eukaryotic cells and play an essential role in cell division and differentiation (Tabor and Tabor, 1984; Pegg, 1986). Ornithine decarboxylase (ODC), which catalyzes the conversion of ornithine to putrescine, is a key enzyme of polyamine biosynthesis (Tabor and Tabor, 1984; Pegg, 1986). Levels of ODC activity are normally under tight control by eukaryotic cells; although basal levels are very low, high levels of ODC activity are detected in the cells with a high multiplication rate, such as tumor cells and protozoa.

Srivastava *et al.* (1980) reported that spermidine and spermine were the major polyamines in some nematodes, cestodes and trematodes, whereas putrescine was present only in a small amount. In adult filarial worms, it was confirmed that spermidine and spermine were the major polyamine components, while putrescine level was much lower (Wittich *et al.*, 1987; Singh *et al.*, 1989). Furthermore, ODC activity has never been detected in adult parasitic nematodes (Wittich *et al.*, 1987; Sharma *et al.*, 1989, 1991; Singh *et al.*, 1989) nor in adult cestodes (Sharma *et al.*, 1991). Recently, ODC has been demonstrated in the membrane fraction of free-living nematode *Caenorhabditis elegans* (Schaeffer and Donatelli, 1990).

Little has been reported on polyamine metabolism in the larval stages of Taeniidae (Cestoda). Some of these parasites cause significant medical and veterinary problems. In particular, the meta-

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cestodes of *Echinococcus multilocularis* inhabit human livers and show a proliferative and metastatic tumor-like growth. There is an urgent need for effective chemotherapy against human echinococcosis.

In the present study we show the polyamine levels of several species of larval taeniids including *E. multiocularis*. In addition, we examined enzyme activities of ODC and polyamine oxidase which were related to polyamine metabolism.

Materials and Methods

Parasites

The Alaskan strain of *E. multilocularis* was supplied by Dr. Robert L. Rausch, University of Washington, USA. This strain of *E. multilocularis* has been maintained by intraperitoneal passages of larval tissue in Mongolian gerbils (*Meriones unguiculatus*). The cysticerci of *Taenia crassiceps* isolated in Japan from a *Microtus montebelli* in 1985 (Miyaji *et al.*, 1990; Uchida *et al.*, 1990) have been maintained by serial peritoneal passages of metacestodes in Mongolian gerbils. The strain of *T. taeniaeformis* originally isolated from a brown rat (*Rattus norvegicus*) in Japan has been maintained by using the normal cat-rat cycle. The cysticerci of *T. hydatigena* were obtained from a pig experimentally infected with the eggs.

Analysis of polyamine contents

Metacestodes of *T. crassiceps, T. hydatigena, T. taeniaeformis* and *E. multilocularis* were recovered from infected animals and homogenized using a glass homogenizer in 2 parts by volume of 0.25 M Tris-HCl buffer (pH 7.4). The homogenate of each parasite was stored at −80°C until used. After addition of perchloric acid (2% final), the parasite homogenates were centrifuged at 10,000g for 20 min at 4°C. The resultant supernatants were analyzed for their polyamine contents using HPLC with the Shimadzu ISC-05/S0504 cation exchange system (Murakami *et al.*, 1989). Polyamines were eluted with a solution containing 0.16 M sodium citrate and 2.0 M sodium chloride, and they were derivatized with 0.08% *o*-phthalaldehyde in 0.3 M borate buffer (pH 10.5) containing 0.1% Brij 35 and 28 mM 2-mercaptoethanol. The flow rates of the buffer and the *o*-phthalaldehyde reagent were 0.7 ml/min and 0.3 ml/min, respectively. The derivatized eluate was measured for fluorescence by a Shimadzu RF-540 spectrofluorophotometer with an excitation wavelength of 345 nm and an emission wavelength of 430 nm. As the standard polyamine mixture, a solution containing 20 μM each of putrescine dihydrochloride, spermidine trihydrochloride and spermine tetrahydrochloride (Nakarai Chemicals Ltd. Kyoto, Japan) was used. The protein content of the homogenate was determined by the method of Lowry *et al.* (1951).

ODC assay

Metacestodes (fresh or frozen at −80°C) of *T. crassiceps, T. hydatigena, T. taeniaeformis* and *E. multilocularis* were homogenized at 4°C in a 25 mM Tris-HCl buffer (pH 7.4) containing 1 mM dithiothreitol and 0.1 mM EDTA. The homogenate was centrifuged at 10,000g for 1 hr. The larval homogenates and the resulting supernatants were used for ODC assay. In a case of *E. multilocularis,* fresh homogenate dialyzed against homogenizing buffer was also used.

ODC activity was assessed by the CO_2-trapping method (Nishiyama *et al.*, 1988). The standard assay mixture contained 62.5 nCi of L-[1-^{14}C]ornithine (ICN Biochemicals), 0.4 mM L-ornithine, 40 μM pyridoxal phosphate, 4 mM dithiothreitol, 40 mM Tris-HCl buffer (pH 7.4) and parasite sample (350–650 μg protein) in a final volume of 0.125 ml. The mixture was incubated at 37°C for 60 min in a 1.5 ml Eppendorf 3810 micro-test tube with filter paper (Toyo No. 50; 9 mm in diameter), attached to the inside of the cap and moistened with 10 μl of 10% KOH. The reaction was terminated by the addition of 50 μl of 6 N HCl. Incubation was then continued for further 15 min to collect $^{14}CO_2$. The radioactivity on the filter paper was measured by a scintillation counter. Partially purified ODC from the kidney of mice was used as a positive control (Nishiyama *et al.*, 1988).

To examine if the *E. multilocularis* sample

contains any unknown factors disturbing ODC assay, the inhibitory effect of the sample on standard ODC was assessed. The standard mouse kidney ODC (42.5 pmol CO_2/hr/μl) was assayed in the absence or presence of dialyzed supernatant (stored at −80°C) of *E. multilocularis* (400 μg of protein).

Polyamine oxidase assay

Polyamine oxidase in the homogenates of the parasites was assayed according to the method described by Suzuki *et al.* (1984). Briefly the assay mixture (total 600 μl) contained 0.1 M Tris-HCl buffer (pH 9.0), 1.0 mM semicarbazide-HCl, 40 μg of horseradish peroxidase (Type I), 0.92 mM homovanillic acid, 0.1 mM pargyline-HCl, 0.2 mM N^1-monoacetylspermine and sample protein (about 1 mg of protein). The mixture was incubated at 37°C for 30 min. The enzyme reaction was stopped by addition of 2.0 ml of 0.1 N NaOH and centrifuged at 4000g and the fluorescence was measured at an excitation wavelength of 323 nm and emission wavelength of 426 nm.

Results

Polyamine content of larval taeniids

HPLC separation detected three major peaks in all the larval taeniids examined. A typical separation of polyamines in *T. taeniaeformis* is shown in Fig. 1. These peaks were identified as putrescine, spermidine and spermine, respectively, since retention time of each peak in the sample was identical to that in the polyamine standard (Fig. 1). Similar results were obtained when different elution buffers were used in HPLC separation (data not shown).

The identification of putrescine in the sample was confirmed using putrescine oxidase reaction as follows. Cyst homogenate of *E. multilocularis* was incubated with putrescine oxidase (from *Micrococcus*: Polyamine Test-Enzyme: Tokuyama Soda) at 37°C for 40 min, and analyzed by HPLC. After the enzyme treatment, the peak of putrescine and spermidine disappeared, confirming that the peak of putrescine contained no other components such as amino acids (data not shown).

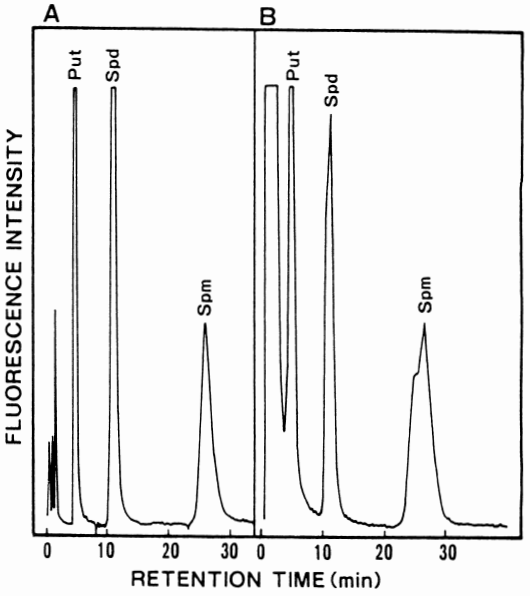

Fig. 1 Separation of polyamines in the metacestodes of *Taenia taeniaeformis* by HPLC with a cation exchange column, using 0.16 M sodium citrate and 2.0 M sodium chloride as mobile phase. A, standard polyamine mixture containing 20 μM putrescine (Put), spermidine (Spd) and spermine (Spm); B, polyamines of *T. taeniaeformis*.

Table 1 shows the polyamine levels of four different species of larval taeniid in the present study and those reported for other helminths and protozoa. In the larval taeniids, although there were significant levels of putrescine, spermidine and spermine, the level of spermidine was the lowest, and the putrescine/spermidine ratio was over 1.5. These polyamine levels in the larval taeniids differ from the published values for other parasites.

ODC activity in larval taeniids

Table 2 shows profiles of ODC activity in the larval taeniids. A very low and questionable radioactivity was detected in each species, whereas the partially purified mouse kidney ODC showed a reasonable activity (74,000 pmol CO_2/min/mg protein). Freshly prepared *E. multilocularis* also showed a negligible ODC

Table 1 Polyamine levels of larval taeniids and other parasites (nmol/mg protein)

Parasite	Putrescine	Spermidine	Spermine	Put/Spd*	References
Echinococcus multilocularis (Larva)	1.56	0.580	1.37	2.7	This paper
Taenia crassiceps (Larva)	2.43	0.970	2.98	2.5	This paper
Taenia hydatigena (Larva)	2.73	1.79	2.98	2.5	This paper
Taenia taeniaeformis (Larva)	3.21	1.60	3.05	2.0	This paper
Onchocerca volvulus (Male)	0.28	34	168	0.008	(21)
(Female)	0.22	39	61	0.006	(21)
Setaria cervi (Adult)	0.009	0.455	1.236	0.020	(14)
Ancylostoma ceylanicum (Adult)	0.090	2.250	0.240	0.040	(13)
Acanthocheilonema viteae (Male)	2.25	4.17	8.61	0.54	(12)
(Female)	1.99	3.41	17.73	0.58	(12)
Hymenolepis diminuta (Adult)	0.319	3.252	19.317	0.091	(12)
Hymenolemip nana (Adult)	0.481	6.792	8.841	0.070	(12)
Trypanosoma brucei	1.6	31.1	n.d.†	0.05	(1)
Leishmania donovani (Amastigote)	1.85	18.80	3.47	0.10	(6)
(Promastigote)	35.20	37.10	0	0.95	(6)
Trichomonas vaginalis	57	9.7	9.5	5.9	(9)
Entamoeba histolytica	92.45	2.63	0.03	35	(3)
Giardia lamblia	9.60	9.57	0.77	1.0	(3)

* The value of putrescine/spermidine
† n.d. = not detected

activity, indicating that enzyme degradation during storage at −80°C is unlikely. Less ODC activity in a dialyzed homogenate sample of *E. multilocularis* eliminated the possibility of the presence of inhibitory substances with a low molecular weight such as ornithine, Na^+ and K^+ in the parasite homogenates.

Inhibitory effect of *E. multilocularis* on standard mouse kidney ODC is shown in Fig. 2. By addition of supernatant of *E. multilocularis* to the standard ODC, little decrease of the enzyme activity was detected.

Polyamine oxidase activity in larval taeniids

Using N^1-monoacetylspermine as the substrate, all the parasites sample showed considerable activity of polyamine oxidase (Table 3).

Table 2 Ornithine decarboxylase activity in larval taeniids (pmol CO_2/min/mg protein)

Parasite	Preparation	ODC activity
Echinococcus multilocularis	Fresh cysts homogenate	<1.0
	Fresh cysts homogenate (Dialyzed)	<1.0
	Frozen cysts supernatant	<1.0
Taenia crassiceps	Fresh cysts homogenate	<0.1
	Frozen cysts supernatant	<0.1
Taenia hydatigena	Frozen cysts supernatant	<0.1
Taenia taeniaeformis	Frozen cysts supernatant	<0.5

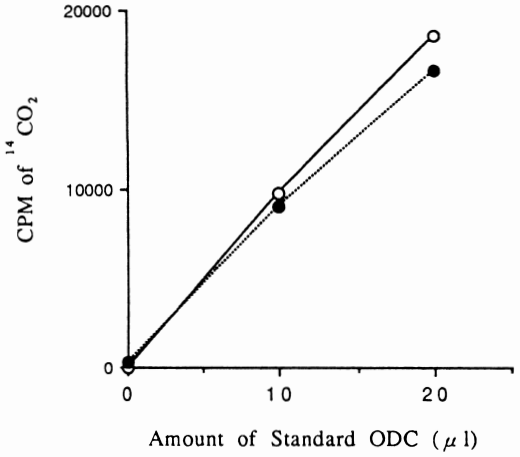

Fig. 2 Inhibitory effect of *Echinococcus multilocularis* against standard mouse kidney ODC (specific activity: 42.5 pmol CO_2/hr/μl). Standard mouse ODC was assayed for ODC activity in the absence (○) or presence (●) of *E. multilocularis* extract (400 μg of protein). Each point represents the mean of two determinants.

Table 3 Polyamine oxidase activity in larval taeniids (pmol/min/mg protein)

Parasite	Enzyme activity (n)
Echinococcus multilocularis	301.8 ± 59.2 (4)
Taenia crassiceps	250.2 ± 63.6 (5)
Taenia taeniaeformis	168.2 ± 3.9 (2)

Discussion

In the present study, we revealed that metacestode stages of taeniids contained significant levels of putrescine, spermidine and spermine as the major polyamines and that the putrescine/spermidine ratio in these larval taeniids was greater than 1.5. This value is extremely high compared to those reported for other helminths, in which the values were less than 0.58 (Table 1). With respect to the high ratio of putrescine/spermidine and the significant levels of all the three major polyamines, the polyamine distribution pattern in the larval taeniids appears peculiar compared with other helminths and protozoa. Higher eukaryotes, in general, contain spermidine and spermine as the major polyamines and very low levels of putrescine (Tabor and Tabor, 1984), while high concentrations of putrescine and spermidine and no spermine have been reported in most prokaryote species (Tabor and Tabor, 1985). In parasitic protozoa, the trypanosomatids apparently lack spermine (Bacchi *et al.*, 1977), and *Trichomonas vaginalis* possesses high levels of putrescine but low levels of spermidine and spermine (North *et al.*, 1986).

ODC levels were negligible in the metacestodes of *T. crassiceps, T. hydatigena, T. taeniaeformis* and *E. multilocularis* (Table 2). A very low level of ODC activity has been also reported in parasitic nematodes (Wittich *et al.*, 1987; Walter, 1988; Sharma *et al.*, 1989, 1991; Singh *et al.*, 1989) and *Hymenolepis* spp. (Sharma *et al.*, 1991). By the evidence that the enzyme activities in some parasitic nematodes were not inhibited by alpha-difluoromethylornithine and that glutamic acid strongly inhibited the liberation of CO_2, it was suggested that those nematodes and adult cestodes may lack ODC (Sharma *et al.*, 1989, 1991; Singh *et al.*, 1989). Recently, Schaeffer and Donatelli (1990) demonstrated a membrane-associated ODC in free living nematode, *Caenorhabditis elegans*. In plants, another putrescine synthesis pathway, which utilyzes arginine decarboxylase as a key enzyme, is well known (Tabor and Tabor, 1984). However, arginine decarboxylase has been detected in neither mammalians nor helminth parasites (Sharma *et al.*, 1989, 1991; Singh *et al.*, 1989; Tabor and Tabor, 1984; Wittich *et al.*, 1987).

Polyamine oxidase catalyses the degradation of N^1-acetylspermidine and N^1-acetylspermine to putrescine and spermidine, respectively (Bolkenius and Seiler, 1981). This enzyme is highly related to the reverse pathway of polyamines. As shown in Table 3, the larval taeniids contained significant levels of polyamine oxidase, which were comparable to those reported with other helminths and rat liver (Sharma *et al.*, 1991), suggesting the presence of a reverse pathway of polyamines in the parasites.

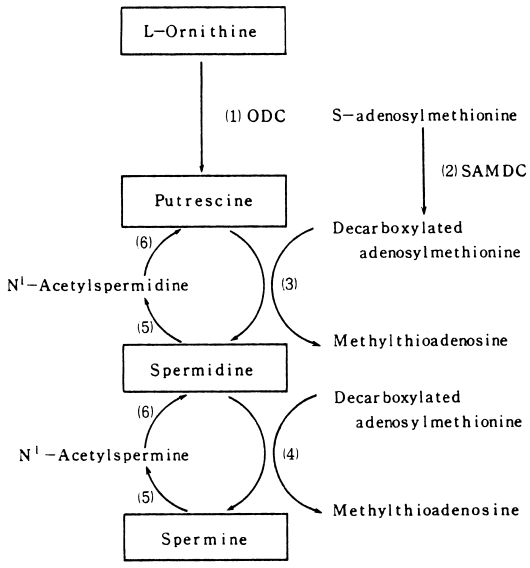

Fig. 3 Pathway for biosynthesis and interconversion of polyamines in mammals. The enzymes involved are (1) ornithine decarboxylase; (2) S-adenosylmethionine decarboxylase; (3) spermidine synthase; (4) spermine synthase; (5) spermidine/spermine-N^1-acetyltransferase; (6) polyamine oxidase.

Fig. 3 shows general pathway of polyamine synthesis and interconversion in mammalian cells. ODC is a key enzyme of polyamine synthesis and polyamine oxidase is related to polyamine reverse pathway. In the present study, we revealed that the ODC levels of the larval taeniids were negligible, while the parasites contained significant levels of polyamine oxidase. These results suggest that the larval taeniids may have unique pathway for polyamine metabolism. Although it is still unknown why these larval taeniids lack ODC, it seems that the larval taeniids may depend on their host and/or utilize an ODC-independent pathway for a supply of the essential polyamines. Such a novel polyamine metabolism in larval taeniids may serve as a potential target for chemotherapy against cysticercoses.

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