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Purification and characterization of polyamine oxidase from *Ascaris suum*

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The interconversion of polyamines in the parasite nematode *Ascaris suum* by a novel type of polyamine oxidase was demonstrated. The nematode enzyme was clearly distinguishable from monoamine and diamine oxidases as well as from the mammalian polyamine oxidase, as shown by the use of the specific inhibitors pargyline, aminoguanidine and MDL 72527 respectively. All three inhibitors had no effect on the parasite polyamine oxidase, and the enzyme did not accept diamines such as putrescine, cadaverine or histamine as substrates. The parasite polyamine oxidase selectively oxidizes spermine and spermidine but not *N*-acetylated polyamines, whereas the mammalian tissue-type polyamine oxidase shows preference for the *N*-acetylated polyamines. These results suggest a regulatory function of the nematode polyamine oxidase in the degradation and interconversion of polyamines in parasite nematodes. The enzyme was purified to homogeneity by gel filtration, preparative isoelectric focusing and subsequent affinity chromatography on spermine- and berenil–Sepharose 4B. With respect to reaction type, the prosthetic group FAD, the molecular mass (66 kDa) and the contents of thiol and carbonyl groups, the polyamine oxidase from *A. suum* is similar to the isofunctional enzyme of mammalian tissue.

INTRODUCTION

The polyamines putrescine, spermidine and spermine are ubiquitous in the animal and plant kingdoms as well as in bacteria and viruses. They are known to be essential for cell growth and cell differentiation (Pegg & McCann, 1982; Tabor & Tabor, 1984, 1985). Former investigations have revealed that the polyamine content of nematodes is similar to that of vertebrates, where in both cases spermidine and spermine constitute the main polyamines. Putrescine is detectable only in very low amounts in vertebrates as well as in worm tissues (Wallace, 1987; Wittich *et al.*, 1987; Sharma *et al.*, 1989; Singh *et al.*, 1989).

The polyamine degradation and interconversion pathway shows some peculiar characteristics in nematode parasites. Acetylation of spermidine and spermine has not yet been detected in nematode parasites, whereas in mammalian cells the acetylation of spermidine and spermine is the rate-limiting step of the interconversion pathway (Seiler *et al.*, 1981, 1985; Seiler & Bolkenius, 1985). Studies on *Ascris suum* and *Onchocerca volvulus* by Wittich & Walter (1989, 1990) showed the presence of a novel type of acetylase acting selectively on diamines, e.g. putrescine, cadaverine and histamine. The polyamine oxidase is the second enzyme involved in polyamine interconversion in mammals and has no regulatory role, as shown by studies on its inhibition *in vitro* and *in vivo* (Seiler & Bolkenius, 1985; Bolkenius & Seiler, 1987). In nematodes, since the polyamine *N*-acetyltransferase is lacking, the polyamine oxidase is solely responsible for polyamine interconversion and degradation. Inhibition of polyamine oxidase by berenil in filarial worms maintained *in vitro* has been shown to be lethal (Müller *et al.*, 1988). Here we report on the polyamine oxidase of *A. suum*, which is a convenient model for nematode parasites in general. The enzyme was purified, and its characterization revealed substrate specificities which confirm the regulatory role of the polyamine oxidase in degradation and interconversion of polyamines in parasitic nematodes.

MATERIALS AND METHODS

Chemicals

[1,4-^{14}C]Spermidine trihydrochloride (sp. radioactivity 117 mCi/mmol) and [1,4-^{14}C]spermine tetrahydrochloride (sp. radioactivity 110 mCi/mmol) were from Amersham Buchler, Braunschweig, Germany. [*terminal methylenes*-^{3}H(n)]Spermidine (sp. radioactivity 29.7 Ci/mmol) was from Du Pont de Nemours (Deutschland) G.m.b.H., NEN Division, Dreieich, Germany. Horseradish peroxidase type II and catalase from bovine liver were from Sigma Chemicals, Deisenhofen, Germany. Homovanillic acid was from Serva, Heidelberg, Germany. SDS/PAGE standards were from Bio-Rad, München, Germany. All other chemicals used were of highest purity.

Parasites and purification of the polyamine oxidase from *A. suum*

A. suum were collected from a local slaughterhouse and maintained in RPMI 1640 medium until dissection. Reproductive tissue was excised from the worms, washed in ice-cold phosphate-buffered saline (pH 7.4) containing 0.1 mM-PMSF and stored at −80 °C until further preparation (Wittich & Walter, 1989).

The following procedures were carried out at 4 °C. The reproductive tissue of *A. suum* was homogenized in 1 vol. of 50 mM-potassium phosphate buffer (pH 8.0) containing 0.02% Brij 35, 10 μM-dithiothreitol (DTT) and 0.1 mM-PMSF (buffer A) at 1400 rev./min with a glass/glass homogenizer. After centrifugation at 15000 *g* (SS34, Sorvall) for 20 min, the resulting supernatant was re-centrifuged at 100000 *g* (TFT 55.38, Centrikon T 1065) for 1 h. The resulting supernatant (crude extract) was used for further purification of polyamine oxidase.

Crude extract (12 ml; 180 mg) was applied to a Sephacryl S-200 column (2.6 cm × 95 cm), previously equilibrated with buffer A containing 0.1 M-NaCl. The enzyme was eluted with the same buffer at a flow rate of 12 ml/h, and 9 ml fractions were collected.

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; DTE, dithioerythritol; DTT, dithiothreitol; IEF, isoelectric focusing; NEM, *N*-ethylmaleimide.

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Fractions containing polyamine oxidase activity were pooled, concentrated 8-fold by Aquacide II (Calbiochem) and used for isoelectric focusing (IEF).

Preparative IEF in Sephadex-IEF (Pharmacia) with a pH gradient in the range of 4–9 (Servalyte, Serva) was carried out in accordance with the instructions of the manufacturer (Pharmacia). Briefly, 13 g of the gel was swollen in 280 ml of a 6.4% Ampholine solution overnight. The gel was prefocused for 500 V·h at 8 W constant power; subsequently the sample was loaded and focused for 12000 V·h at 40 W constant power. The gel was fractionated and gel fractions were eluted with 50 mM-potassium phosphate buffer (pH 8.5) containing 0.02% Brij 35, 10 μM-DTT and 0.1 mM-PMSF (buffer B), and those containing polyamine oxidase activity were pooled and applied to a spermine–Sepharose 4B column.

The spermine–Sepharose 4B column (0.9 cm × 10 cm) was equilibrated with buffer B. After loading the sample, the column was washed with 4 vol. of buffer B and eluted with a linear gradient of 0.1–0.8 M-NaCl in buffer B. Fractions containing polyamine oxidase activity were pooled, desalted by repeated dilution with buffer A and subsequent concentration.

The desalted spermine–Sepharose fraction was applied to a berenil–Sepharose 4B column (0.9 cm × 5 cm) equilibrated with buffer A. The column was washed with 4 vol. of buffer A and eluted with a linear gradient of 0–0.2 M-NaCl in buffer A.

Preparation of mammalian polyamine oxidase

Liver was isolated from *Mastomys coucha*. The polyamine oxidase was isolated as described by Bitonti *et al.* (1990), and the DEAE-cellulose-purified enzyme was used for further studies.

Protein determination

Protein was determined by the method of Bradford (1976), with BSA as a standard.

Silver staining after SDS/PAGE was carried out as described by Heukeshoven & Dernick (1986).

Enzyme-activity assays

The standard assay for polyamine oxidase, which depends on the demonstration of H_2O_2 as reaction product, was carried out as described by Snyder & Hendley (1968). The incubation mixture contained: 8 μg of homovanillic acid, 20 μg of horseradish peroxidase, 10 μM-DTT, 5 mM-benzaldehyde, 1 mM-spermine, enzyme and 50 mM-potassium phosphate buffer (pH 8.5) containing 0.02% Brij 35 in a total volume of 1 ml. After 30 min at 37 °C the reaction was stopped by chilling the tubes on ice, and the relative fluorescence was determined with an excitation wavelength of 315 nm and an emission wavelength of 425 nm. For the blanks the substrate was omitted. One unit of polyamine oxidase activity is defined as 1 nmol of H_2O_2/30 min.

To demonstrate the formation of spermidine and putrescine from spermine and spermidine respectively, radiolabelled polyamines were used and the reaction products were analysed by h.p.l.c. The enzyme assay had the following composition: 25 mM-potassium phosphate buffer (pH 8.5) containing 0.02% Brij 35, 5 mM-benzaldehyde, 50 μM-DTT, 20 μg of catalase (220 units), 50 μM-[^{14}C]-spermidine or -spermine (50–250 nCi; sp. radioactivity 117 or 110 mCi/mmol respectively) and enzyme in a total volume of 200 ml. The reaction was incubated at 37 °C for 60 min and stopped by addition of 20 μl of 2 M-$HClO_4$. Protein was precipitated overnight at 4 °C and sedimented by centrifugation at 10000 rev./min (Eppendorf centrifuge 5415 C). Polyamines in the supernatant were dansylated and analysed by reversed-phase h.p.l.c. (Wittich *et al.*, 1987). The radioactivity was monitored by a LB 506 D radioactive detector (Berthold), and the gradient was controlled by Data System 450 MT 2 (Kontron).

To identify the aldehyde resulting from the polyamine oxidase reaction, the enzyme assay was carried out as described by Tabor *et al.* (1964) with 3.2 mM-[^{3}H]spermidine (33 nCi; sp. radioactivity 29.7 Ci/mmol) as the substrate and subsequent $NaBH_4$ reduction of the aldehydes formed. Then 10 μl of the reduced and concentrated sample was applied to a silica-gel 60 t.l.c. plate and developed for 8 h in ethoxyethanol/propionic acid/NaCl-saturated water (14:3:3, by vol.); 5 μl portions of 20 mM-putrescine, -spermidine, -spermine and -3-aminopropanol were used as standards. The plate was dried and stained with 0.1% ninhydrin spray. The spots were scraped from the plate, and their radioactivity was determined by liquid-scintillation spectrometry in a Tri-Carb 2000 instrument (Packard).

Molecular-mass determination

The molecular mass of the native polyamine oxidase was determined as described by Andrews (1964) on a Pharmacia HiLoad Superdex 200 column (1.6 cm × 60 cm), equilibrated with buffer A containing 0.1 M-NaCl and calibrated with cytochrome *c* (12.5 kDa), myoglobin (17.8 kDa), ovalbumin (45 kDa), BSA (67 kDa), phosphorylase *b* (97 kDa) and Blue Dextran 2000 (2000 kDa).

The molecular mass of the purified polyamine oxidase was determined as described by Weber & Osborn (1969) by SDS/PAGE on a 7.5% gel with a 3% stacking gel.

RESULTS

Purification of polyamine oxidase

A summary of the purification procedure is given in Table 1. Chromatography on Sephacryl S-200 revealed that inhibitory compounds must have been present in the crude extract, since this initial purification step resulted in an increase in the total activity by 1.8-fold. After preparative IEF, the enzymically active fractions were directly applied to the spermine–Sepharose column to eliminate the Ampholines. The enzyme bound strongly to spermine–Sepharose and was eluted at 0.55 M-NaCl in buffer B (Fig. 1). Berenil–Sepharose 4B was used for further purification; the enzyme was eluted at 0.15 M-NaCl in buffer A. This procedure resulted in about 12000-fold purification. The specific activity of the purified enzyme was about 2300 nmol of H_2O_2/min per mg of protein.

Activity and stability of the polyamine oxidase

The polyamine oxidase activity in the crude extract of reproductive and muscle tissue of *A. suum* was in the range 40–100 pmol of H_2O_2/min per mg of protein. Under standard assay conditions, the enzyme activity was linear for 30 min at 37 °C. The optimum pH for the enzymic reaction was pH 8.5, which was shown for both the crude enzyme and the purified polyamine oxidase.

The purified enzyme was stable for 2 days at −20 °C with minimal loss of activity; 14 days at −20 °C led to a total loss of enzyme activity.

Preincubations of the purified polyamine oxidase at 30, 37, 42 or 56 °C resulted in a total loss of activity after 60, 30, 20 and 10 min respectively.

Molecular mass and pI

The molecular mass of the polyamine oxidase was determined by gel filtration on Superdex 200 and SDS/PAGE. The molecular mass of the native polyamine oxidase was calculated to be 65.7 ± 1.3 kDa ($n = 3$). SDS/PAGE analysis of the purified

Table 1. Purification of polyamine oxidase from *A. suum*

1 unit is 1 nmol of H_2O_2/30 min.

Step	Total activity (units)	Total protein (mg)	Sp. activity (units/mg)	Purification factor (fold)	Yield (%)
Crude extract	315	180	1.75	—	—
Sephacryl S-200	566	103	5.5	1	100
Preparative IEF	360	—	—	—	—
Spermine–Sepharose	195	0.11	1755	320	34
Berenil–Sepharose	180	0.0026	69230	12587	32

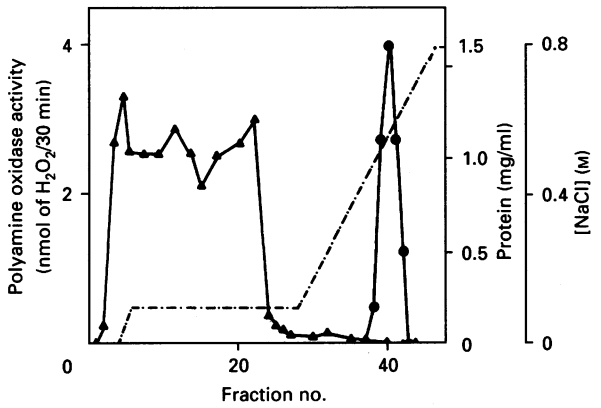

Fig. 1. Purification of the *A. suum* polyamine oxidase by spermine–Sepharose 4B chromatography

Protein from preparative IEF was loaded on a spermine–Sepharose 4B column, washed with buffer B and eluted with a linear gradient (–·–·–) of 0.1–0.8 M-NaCl in buffer B. ▲, Protein; ●, polyamine oxidase activity.

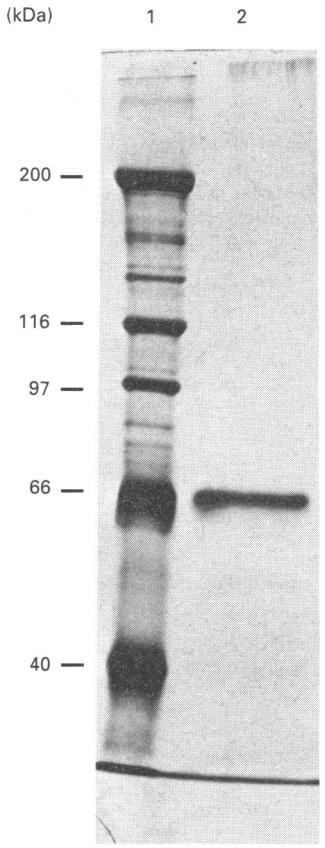

Fig. 2. SDS/PAGE of the purified *A. suum* polyamine oxidase

Purified polyamine oxidase was subjected to SDS/PAGE on a 7.5% gel. Lane 1, high-molecular-mass standards (Bio-Rad); lane 2, 0.25 μg of purified polyamine oxidase. The gel was silver-stained in accordance with Heukeshoven & Dernick (1986).

polyamine oxidase showed a major band with a molecular mass of 66 kDa (Fig. 2).

The pI of the *A. suum* polyamine oxidase was determined to be 7.8±0.2 (Fig. 3).

Substrate specificity

Spermine, spermidine, norspermidine, norspermine, tryptamine and benzylamine were found to be substrates of the polyamine oxidase from *A. suum*, whereas *N*-acetylated polyamines and diamines, e.g. N^1-acetylspermidine, N^8-acetylspermidine, *N*-acetylspermine, N^1N^{12}-diacetylspermine, putrescine, cadaverine and histamine, were not accepted as substrates. K_m and $V_{max.}$ values of the substrates determined in the presence of 5 mM-benzaldehyde are given in Table 2. In the absence of benzaldehyde, the enzyme activity was decreased about 10-fold. The enzyme showed the highest affinity for the natural polyamines spermine and spermidine, with K_m values of 0.35 mM and 0.66 mM respectively. The other amines were only poor substrates, with K_m values in the range 2–5 mM. Bis(benzyl)polyamines, such as MDL 27695 and 27391, were not substrates for the polyamine oxidase from *A. suum*, but they had an inhibitory effect on the enzyme activity. As shown in Fig. 4, MDL 27695 was a competitive inhibitor of the enzyme from the parasites, with a K_i value of 20 μM.

Reaction products of the polyamine oxidase

The polyamine oxidase of *A. suum* acts on the secondary amino group of the polyamines. Spermine and spermidine are converted into spermidine and putrescine respectively (Fig. 5). As additional products H_2O_2 and 3-aminopropanal are formed. The production of H_2O_2 was determined by the standard assay, and the reduced aminopropanol was identified by t.l.c. analysis on silica gel 60.

Cofactors and essential groups of the polyamine oxidase

The presence of FAD as a prosthetic group of the *Ascaris* polyamine oxidase was shown by treatment of the enzyme with acidic $(NH_4)_2SO_4$ in the presence of KBr, as described by Hölttä (1977). The dissociated FAD was monitored from the spectrofluorimetric emission spectrum, with FAD as the standard. It was not possible to re-activate the apoenzyme by addition of

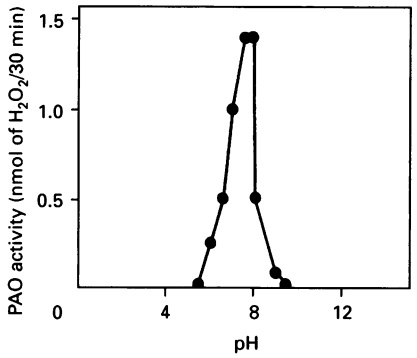

Fig. 3. pI of the *A. suum* polyamine oxidase

Protein from gel filtration on Sephacryl S-200 was subjected to IEF on Sephadex. After focusing for 12000 V·h with 40 W constant power, the gel fractions were eluted and tested for enzyme activity by the standard assay.

Table 2. Substrates of polyamine oxidase from *A. suum*

The kinetic studies were carried out under standard assay conditions with various substrate concentrations (0.075–5 mM). The K_m and $V_{max.}$ values were determined by use of Lineweaver–Burk plots.

Substrate	K_m (mM)	$V_{max.}$ (units/mg of protein)
Spermine	0.35	4.2
Spermidine	0.66	3.0
Norspermine	2.0	3.6
Norspermidine	5.0	6.3
Tryptamine	2.5	5.5
Benzylamine	1.6	5.0

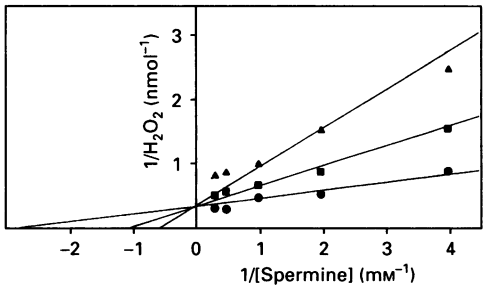

Fig. 4. Inhibition of the polyamine oxidase from *A. suum* by the bis(benzyl)polyamine MDL 27695

The purified polyamine oxidase was incubated without inhibitor (●) or with addition of 10 μM- (■) or 100 μM- (▲) MDL 27695 under standard assay conditions at various substrate concentrations (0.25–5 mM). Reciprocal of initial velocity was plotted against reciprocal of variable spermine concentrations.

FAD, FMN or riboflavin. The flavin content of the purified enzyme was also determined spectrofluorimetrically as described by Bessey *et al.* (1949). The emission maximum was found to be 530 nm with excitation at 430 nm. The absorption spectrum of the purified enzyme showed three bands with peaks at 275, 380 and 456 nm, identical with that of the FAD used as a standard.

Copper and iron are not cofactors of the polyamine oxidase from *A. suum*. Addition of diethyl dithiocarbamate and 1,10-phenanthroline up to 1 mM had no effect on the polyamine

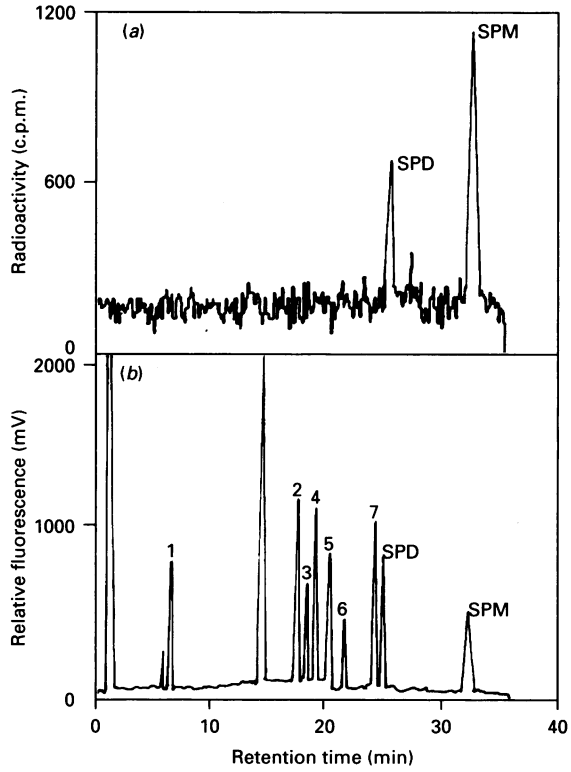

Fig. 5. Reaction product of polyamine oxidase from *A. suum*

[^{14}C]Spermine was used as substrate for the polyamine oxidase of *A. suum*. The reaction products were dansylated and analysed by reversed-phase h.p.l.c. (data system MT 2, Kontron). The relative fluorescence was determined with a spectrofluorimeter (SFM 25, Kontron). The radioactive peaks were detected and integrated by a radioactive monitor (LB 506 D; Berthold). (*a*) Formation of spermidine after spermine oxidation by polyamine oxidase from *A. suum* (SPD, spermidine; SPM, spermine). (*b*) Polyamine standards detected fluorimetrically after dansylation and separation on h.p.l.c.: 1, *N*-acetylputrescine; 2, N^1-acetylspermidine, N^8-acetylspermidine; 3, 1,3-diaminopropane; 4, putrescine; 5, cadaverine; 6, 1,6-diaminohexane as internal standard; 7, *N*-acetylspermine; SPD, spermidine; SPM, spermine.

oxidase activity of *A. suum*. The same was found for EDTA and EGTA up to 1 mM.

Thiol groups are essential for the activity of the polyamine oxidase of *A. suum*. Addition of dithiothreitol (DTT) and dithioerythritol (DTE) up to 10 μM increased the enzyme activity, whereas higher concentrations of both compounds inhibited it. Without addition of DTT or DTE to the preparation buffers, the enzyme activity was lost within 24 h. Preincubations with *N*-ethylmaleimide (NEM), isoniazid and iproniazid strongly inhibited the polyamine oxidase activity (Table 3).

Inhibitors of amine oxidases

Inhibitors of various amine oxidases were tested for their effects on the polyamine oxidase from *A. suum*. The monoamine and diamine oxidase inhibitors pargyline and aminoguanidine at the appropriate concentrations of 10 μM did not inhibit the parasite enzyme activity. The polyamine oxidase inhibitor MDL 72527 [N^1,N^2-bis(buta-2,3-dienyl)butane-1,4-diamine], specific for the tissue-type polyamine oxidases in mammals, also had no effect on the parasite polyamine oxidase activity. Preincubation with the inhibitor was performed for 10 or 20 min at 37 °C in the concentration range 0.001–0.1 mM. As a positive control, the inhibition of the *Mastomys* liver polyamine oxidase was investigated. Preincubation of the vertebrate enzyme with either

Table 3. Essential groups of the polyamine oxidase from *A. suum*

The enzyme preparation was preincubated for 10 min with the indicated compounds, followed by determination of enzyme activity by the standard assay.

Addition	Concn. (μM)	Inhibition (%)
NEM	10	85
	100	100
Isoniazid	10	81
	100	100
Iproniazid	10	87
	100	100

0.001 mM- or 0.01 mM-MDL 72527 for 10 min resulted in a 35% or 75% inhibition of enzyme activity respectively, as determined under standard assay conditions.

DISCUSSION

The amine oxidases are classified as FAD- and copper-dependent oxidases. The mitochondrial monoamine oxidases and the tissue-type polyamine oxidases are FAD enzymes, whereas serum amine oxidases and diamine oxidases are Cu-dependent (Seiler, 1987; Mondovi *et al.*, 1988). The FAD-containing tissue-type polyamine oxidase acts on the secondary amino group of the polyamines, and the serum amine oxidases act on the primary amino group, which results in different products after polyamine oxidation (Mondovi *et al.*, 1988). In plants, bacteria and amoebae the polyamine oxidases catalyse the deamination of the secondary amino group, but this reaction does not result in an interconversion of polyamines as described for the vertebrate enzyme. In these organisms 1,3-diaminopropane and 4-aminobutyral are the reaction products of spermidine oxidation (Slocum *et al.*, 1984; Tabor & Tabor, 1984; Kim *et al.*, 1987; O. P. Shukla, S. Müller & R. D. Walter, unpublished work).

The *A. suum* polyamine oxidase resembles the tissue-type polyamine oxidase in that it has a similar reaction type and prosthetic group. The nematode enzyme oxidizes spermine and spermidine at the secondary amino group, and FAD was identified as the prosthetic group of the polyamine oxidase from *A. suum*.

The specific activity of the polyamine oxidase in the crude extract of *A. suum* was found to be in the range of 40–100 pmol of H_2O_2/min per mg of protein. The polyamine oxidase of mouse leukaemia cells was in the same range, with 97 pmol/min per mg of protein (Libby & Porter, 1987), whereas the polyamine oxidase activity of rat liver was higher, with 200–440 pmol/min per mg of protein (Hölttä, 1977; Seiler *et al.*, 1980). The specific activity of the purified polyamine oxidase from *A. suum* was about 2300 nmol of H_2O_2/min per mg, which is 2-fold higher than that reported for the enzyme from rat liver (Hölttä, 1977), which indicates a higher turnover rate of the parasite enzyme relative to that of the vertebrate enzyme.

The molecular mass of the purified active enzyme, as determined by gel chromatography on Superdex 200, was found to be 65.7 kDa, and that of the major band, determined by SDS/PAGE, was 66 kDa. These results indicate that the parasite enzyme is active as a monomer, as reported previously for the rat liver polyamine oxidase (Hölttä, 1977). In contrast, the serum amine oxidase exists as a polymer with subunits of 90 kDa (Achee *et al.*, 1968; Morgan, 1985).

The polyamine oxidase of *A. suum* contains thiol and carbonyl groups, both of which are essential for enzyme activity. The presence of thiol groups was shown by use of DTT, DTE and NEM. DTT and DTE activated, whereas the thiol reagent NEM inhibited, the enzyme activity. Similar results were reported for tissue-type polyamine oxidase as well as for the serum amine oxidases (Hölttä, 1977; Morgan, 1985). The carbonyl reagents isoniazid and iproniazid do not affect the tissue-type polyamine oxidase (Hölttä, 1977), but both compounds were found to inhibit the nematode enzyme as well as the serum amine oxidases (Tabor *et al.*, 1954; Buffoni, 1966; Hölttä *et al.*, 1975).

Neither copper nor iron participates in the reaction of the nematode polyamine oxidase. Diethyl dithiocarbamate, a Cu chelator which has been reported to inactivate serum amine oxidases and diamine oxidases (Buffoni, 1966), did not affect the parasite polyamine oxidase. 1,10-Phenanthroline, an iron chelator, which has been reported to be an inhibitor of the tissue-type polyamine oxidase (Hölttä, 1977) also did not inhibit the parasite enzyme.

The polyamine oxidase of *A. suum* is clearly distinguishable from monoamine and diamine oxidase, as shown by its insensitivity to pargyline and aminoguanidine, specific inactivators of these enzymes (Buffoni, 1966; Fowler *et al.*, 1982). Similarly, the polyamine oxidase activity of rat tissue is not inhibited by pargyline and aminoguanidine (Hölttä, 1977).

Although the *Ascaris* enzyme is similar to the tissue-type polyamine oxidase with respect to the reaction type and the prosthetic group FAD, the specifically designed polyamine oxidase inhibitor MDL 72527 did not affect the parasite polyamine oxidase activity (Bey *et al.*, 1985; Seiler & Bolkenius, 1985; Bolkenius & Seiler, 1987). In addition, the nematode enzyme showed different substrate specificities from those of the tissue-type polyamine oxidase. The parasite enzyme selectively oxidized spermidine and spermine as substrates, whereas *N*-acetylated polyamines were not accepted as substrates. Benzylamine was also accepted as a substrate, as reported for the vertebrate polyamine oxidase (Bitonti *et al.*, 1990). The overlapping substrate specificity indicates that the vertebrate enzyme may function as a broad-specificity oxidase (Bitonti *et al.*, 1990). The higher affinity of the parasite enzyme for polyamines compared with benzylamine points to the significance of the polyamine oxidase for the polyamine interconversion pathway in the parasites. The bis(benzyl)polyamines that were reported to be substrates of the mammalian polyamine oxidase (Bitonti *et al.*, 1990) were not oxidized by the parasite enzyme. These polyamine derivatives were found to be competitive inhibitors of the parasite polyamine oxidase, as shown for MDL 27695. The tissue-type polyamine oxidase of vertebrates shows preference for the N^1-acetylated polyamines, which are discussed as the physiological substrates (Hölttä, 1977; Della Ragione & Pegg, 1982; Bolkenius & Seiler, 1981). Reports on the *N*-acetyltransferase of *A. suum* and *O. volvulus* (Wittich & Walter, 1989, 1990), which acts exclusively on diamines, and the results presented here on the substrate specificity of the polyamine oxidase, provide evidence that the nematodes lack a polyamine *N*-acetyltransferase. Thus the polyamine oxidase is suggested as the sole and regulatory enzyme in the polyamine-interconversion and degradation pathway in nematode parasites, whereas in vertebrates the *N*-acetyltransferase is the rate-limiting step in the polyamine-interconversion pathway (Seiler *et al.*, 1981, 1985; Seiler & Bolkenius, 1985).

In conclusion, the enzyme of *A. suum* is clearly distinguishable from monoamine oxidases and diamine oxidases and has a similar reaction type, prosthetic group and molecular mass to the tissue-type polyamine oxidase. The parasite enzyme, however, shows different substrate specificities compared with those of the

vertebrate polyamine oxidase, and is likely to have a regulatory function in the degradation and interconversion of polyamines in these nematodes.

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