A novel member of the GCN5-related *N*-acetyltransferase superfamily from *Caenorhabditis elegans* preferentially catalyses the *N*-acetylation of thialysine [*S*-(2-aminoethyl)-L-cysteine]

Benjamin ABO-DALO*, Dieudonne NDJONKA*, Francesco PINNEN†, Eva LIEBAU* and Kai LÜERSEN*¹

*Department of Biochemistry, Bernhard Nocht Institute for Tropical Medicine, Bernhard-Nocht-Str. 74, D-20359 Hamburg, Germany, and †Dipartimento di Scienze del Farmaco, Università degli Studi G. D'Annunzio, Via dei Vestini, I-66100 Chieti, Italy

The putative diamine *N*-acetyltransferase D2023.4 has been cloned from the model nematode *Caenorhabditis elegans*. The 483 bp open reading frame of the cDNA encodes a deduced polypeptide of 18.6 kDa. Accordingly, the recombinantly expressed His₆-tagged protein forms an enzymically active homodimer with a molecular mass of approx. 44 000 Da. The protein belongs to the GNAT (GCN5-related *N*-acetyltransferase) superfamily, and its amino acid sequence exhibits considerable similarity to mammalian spermidine/spermine-*N*¹-acetyltransferases. However, neither the polyamines spermidine and spermine nor the diamines putrescine and cadaverine were efficiently acetylated by the protein. The smaller diamines diaminopropane and ethylenediamine, as well as L-lysine, represent better substrates, but, surprisingly, the enzyme most efficiently catalyses the *N*-acetylation of amino acids analogous with L-lysine. As determined by the k_{cat}/K_m values, the *C. elegans* *N*-acetyltransferase prefers thialysine [*S*-(2-aminoethyl)-L-cysteine], followed by *O*-(2-aminoethyl)-L-

serine and *S*-(2-aminoethyl)-D,L-homocysteine. Reversed-phase HPLC and mass spectrometric analyses revealed that *N*-acetylation of L-lysine and L-thialysine occurs exclusively at the amino moiety of the side chain. Remarkably, heterologous expression of *C. elegans* *N*-acetyltransferase D2023.4 in *Escherichia coli*, which does not possess a homologous gene, results in a pronounced resistance against the anti-metabolite thialysine. Furthermore, *C. elegans* *N*-acetyltransferase D2023.4 exhibits the highest homology with a number of GNATs found in numerous genomes from bacteria to mammals that have not been biochemically characterized so far, suggesting a novel group of GNAT enzymes closely related to spermidine/spermine-*N*¹-acetyltransferase, but with a distinct substrate specificity. Taken together, we propose to name the enzyme 'thialysine *N*^ε-acetyltransferase'.

Key words: GCN5-related *N*-acetyltransferase, lysine analogue, spermidine/spermine-*N*¹-acetyltransferase, thialysine.

INTRODUCTION

The GCN5-related *N*-acetyltransferase (GNAT) superfamily encompasses enzymes that catalyse the transfer of an acetyl group from acetyl-CoA to a primary amine of acceptor molecules. The members of the superfamily are characterized further by amino acid sequence similarity with generally four moderately conserved motifs termed C, D, A and B in N- to C-terminal order. All known crystal structures of GNAT proteins suggest that these homologous regions are involved in acetyl-CoA binding. Numerous functionally diverse GNATs have been reported that, for example, *N*-acetylate lysine residues of proteins, such as histones and transcription factors, or small molecules, like aminoglycosides, arylalkylamines or polyamines. Acetylation of the latter is catalysed by SSAT (spermidine/spermine-*N*¹-acetyltransferase) [1,2].

The polyamines putrescine, spermidine and spermine are ubiquitous, and represent essential constituents of cells that are involved in developmental processes such as cell proliferation and cell differentiation [3,4]. The highly regulated SSAT1 catalyses the rate-limiting step of polyamine degradation, leading to *N*¹-acetylspermidine and *N*¹-acetylspermine respectively. The *N*-acetylated forms are either excreted out of cells or serve as substrates for the FAD-dependent PAO (polyamine oxidase) in the interconversion pathway. The PAO converts *N*¹-acetylsper-

mine into spermidine and *N*¹-acetylspermidine into putrescine by respectively cleaving 3-acetaminopropanal [5,6]. Recently, a second SSAT has been reported of mammalian origin. However, the physiological function of SSAT2 was not clearly resolved, although an involvement in polyamine metabolism was proposed [7].

Here, we report on the *N*-acetyltransferase D2023.4 from the model nematode *Caenorhabditis elegans*, which was annotated as a putative diamine *N*-acetyltransferase, since its amino acid sequence exhibits considerable similarity to mammalian SSAT2 and SSAT1. Although nematodes possess a polyamine interconversion pathway, the presence of an SSAT was reported to be doubtful, at least for parasitic species, due to a PAO that accepts only non-acetylated polyamines [8,9]. Furthermore, diamine *N*-acetyltransferases have been purified from the parasitic nematodes *Ascaris suum* and *Onchocerca volvulus*. These enzymes accept only the diamines putrescine, cadaverine, diaminopropane and the biogenic amine histamine, but not spermidine and spermine [10–12]. Thus our goal was to elucidate whether the only SSAT homologue from *C. elegans* *N*-acetylates polyamines or diamines, or has another function. Polyamines were found to be poor substrates of the nematode enzyme. Biochemical characterization has revealed that *N*-acetylation of small diamines, as well as *N*^ε-acetylation of L-lysine and especially of thialysine [*S*-(2-aminoethyl)-L-cysteine] and *O*-(2-aminoethyl)-L-serine, is

Abbreviations used: ESI-MS, electrospray ionization mass spectrometry; GNAT, GCN5-related *N*-acetyltransferase; IPTG, isopropyl β-D-thiogalactoside; Ni-NTA, Ni²⁺-nitrilotriacetate; PAO, polyamine oxidase; RACE, rapid amplification of cDNA ends; SL, spliced leader; SSAT, spermidine/spermine-*N*¹-acetyltransferase.

¹ To whom correspondence should be addressed (email luersen@bni.uni-hamburg.de).

preferred instead. In addition, our data show that *Escherichia coli* cells that express the *C. elegans* enzyme are able to cope with much higher concentrations of the lysine analogue thialysine. On the basis of these data, we propose to name the enzyme thialysine *N*^ε-acetyltransferase, a novel member of the GNAT superfamily.

MATERIALS AND METHODS

Chemicals

N^α- and *N*^ε-acetylated forms of thialysine were synthesized as described by Hermann et al. [13]. L-Lanthionine and S-(aminoethyl)-D,L-homocysteine were synthesized and kindly provided by L. Pecci and co-workers (Università di Roma "La Sapienza", Rome, Italy). *N*¹,*N*⁸-Diacetylspermidine and *N*¹,*N*¹²-diacetylspermine were kindly given by N. Seiler (IRCAD, Strasbourg, France). [¹⁴C]Acetyl-CoA was purchased from Amersham, Biosciences. All other chemicals were from Sigma except putrescine, spermidine and spermine that were from Fluka/BioChemica and O-(2-aminoethyl)-L-serine that was purchased from Aldrich.

C. elegans culture and nucleic acid preparation

The *C. elegans* strain Bristol N2 was cultured on nematode growth medium plates at 25 °C under xenic conditions in the presence of *E. coli* strain OP50. *C. elegans* were separated from bacteria by sucrose flotation [14]. Genomic DNA was prepared from homogenized worms as described previously [15]. Total RNA was extracted by homogenizing worms in the presence of TRIzol™ reagent according to the manufacturer's instructions (Invitrogen).

Cloning of *C. elegans* *N*-acetyltransferase D2023.4 cDNA

A BLAST search using the human and mouse SSAT1 sequences (EMBL accession nos. P21673 and P49431) as the query revealed two identical *C. elegans* sequences with the EMBL accession nos. CAB02871 and NP_505978. The corresponding gene D2023.4, which is annotated as a putative diamine *N*-acetyltransferase, was identified on chromosome V (www.sanger.ac.uk). The open reading frame of D2023.4 was amplified by PCR using *C. elegans* cDNA as template and gene specific oligonucleotides NATeXS (5'-GCGCCTGCAGCTATGAAAACTTCGAAATTGTC-3') and NATeXAS (5'-GCGCAAGCTTCTATTCATCAGCAAATT-TATTT-3'; introduced restriction sites for *Pst*I and *Hind*III are shown underlined). PCR was performed as follows: 95 °C for 2 min, 55 °C for 1 min and 68 °C for 1 min for 30 cycles, using the Elongase amplification system (Invitrogen). The PCR product was subcloned for sequence analysis into pCRII™ vector using T/A cloning. To determine the 5' and 3' untranslated regions, a *C. elegans* λ-ZapII cDNA library (Stratagene) was screened using a digoxigenin-labelled probe of the obtained PCR fragment (Random Primed DNA Labelling Kit; Roche) according to standard plaque and hybridization procedures [15,16]. Furthermore, the 5' region of the D2023.4 mRNA was analysed by RACE (5' rapid amplification of cDNA ends) with total *C. elegans* RNA, as described by the manufacturer (Invitrogen). In the reverse transcriptase reaction, the oligonucleotide NATeXAS was used. Subsequently, PCR was performed using the obtained cDNA as template and the gene-specific oligonucleotide 5'-CTTCCACA-GAGTTCCTGGTAGTCCCATTCGTCG-3' (A96P-antisense) as antisense primer. The sense primer corresponds either to SL1 (spliced leader 1; sequence 5'-GGTTTAATTACCCAAGTTTGAAG-3') or to SL2 (sequence 5'-GGTTTAAACCCAGTTACTCAAG-3'). In parallel, negative control PCRs were performed on *C. elegans* genomic DNA with the same primer pairs. PCR pro-

ducts were only obtained with the cDNA and cloned into pCRII™.

Expression and purification of *C. elegans* *N*-acetyltransferase D2023.4

The PCR product of the *C. elegans* D2023.4 coding region obtained with the oligonucleotides NATeXS and NATeXAS was purified, digested and subcloned into *Pst*I/*Hind*III-cleaved pTrcHisB vector (Invitrogen) to produce a His₆-tag fusion protein. The recombinant expression plasmid pTrcHisB:CeNAT was sequenced, before being transformed into the *E. coli* strain BL21(DE3). A fresh overnight culture from a single colony was diluted 1:100 in Luria-Bertani medium supplemented with 100 µg·ml⁻¹ ampicillin and grown at 37 °C until the *D*₆₀₀ reached 0.5. Expression was initiated with 1 mM IPTG (isopropyl β-D-thiogalactoside). The cells were grown for additional 3 h at 37 °C and harvested by centrifugation at 10 000 g for 15 min at 4 °C. The cell pellet was resuspended in lysis buffer supplemented with 0.1 mM PMSF, sonified, and then centrifuged at 100 000 g for 1 h at 4 °C (TFT 55.38; Centricon T-1065, Kontron, Neufahrn, Germany). Recombinant *C. elegans* *N*-acetyltransferase D2023.4 was purified from the supernatant by chelating chromatography on Ni-NTA (Ni²⁺-nitrilotriacetate) agarose (Qiagen) according to the manufacturer's instructions. For further purification, and in order to determine the molecular mass of the *C. elegans* *N*-acetyltransferase, the eluate was subjected to fast protein liquid chromatography on a calibrated Superdex S-200 column (2.6 cm × 60 cm). The column was equilibrated with buffer A (50 mM Tris/HCl, pH 7.8, containing 1 mM EDTA, 0.02 % Brij 35, 0.1 mM PMSF and 150 mM NaCl). Elution was performed at a flow rate of 2 ml·min⁻¹. Protein concentration was determined by the method of Bradford [17]. The homogeneity of the enzyme preparation was analysed by SDS/PAGE. Proteins were revealed by Coomassie Blue staining [15].

N-Acetyltransferase enzyme assay

The enzyme assay for *C. elegans* *N*-acetyltransferase D2023.4 was performed as described by Bode et al. [18]. The method is based on the endpoint determination of free CoA with 5,5'-dithiobis-(2-nitrobenzoate) ('DTNB'). The standard assay mixture contained 100 mM Tris/HCl buffer, pH 7.8, supplemented with 1 mM acetyl-CoA, 10 mM of acceptor and 20–4000 ng of purified *C. elegans* *N*-acetyltransferase in a final volume of 50 µl. The reaction was stopped after 5 min by the addition of 150 µl of ethanol. Subsequently, 500 µl of 0.2 mM 5,5'-dithiobis-(2-nitrobenzoate) in 100 mM Tris/HCl, pH 8.0, was added. The absorption at 412 nm was measured in a spectrophotometer (Uvicon; Kontron).

Alternatively, *N*-acetyltransferase activity was assayed by measuring the transfer of a ¹⁴C-labelled acetyl group from acetyl-CoA to an acceptor molecule. In a final volume of 50 µl, the reaction mixture contained 50 mM Tris/HCl, pH 7.8, 1 mM EDTA, 50 µM [1-¹⁴C]acetyl-CoA (25 nCi; 57 mCi·mmol⁻¹) and 10 mM acceptor. The reaction was initiated by the addition of 0.5–500 ng of purified enzyme. Following incubation at 37 °C for 10 min, the reaction was terminated by the addition of 0.2 M HClO₄. Reaction products were identified by TLC or HPLC analysis. For TLC separation on silica gel 60 sheets (Merck), ethylene glycol monomethyl ether, propionic acid and water saturated with NaCl (140:30:30, by vol.) was used as the mobile phase. An aliquot (10 µl) of the assay, together with 2 µl of 50 mM of the respective standard, were applied on to the sheets and run for approx. 4 h. Reaction products were visualized either

by ninhydrin staining at 60 °C or by autoradiography (Biomax; Kodak). Spots were cut out, and radioactivity was measured in a Packard–Tricarb 2000 liquid scintillation counter using 4 ml Packard UltimaGold™ liquid scintillation cocktail.

For the determination of the corresponding K_m values, concentrations of acetyl-CoA were varied from 4 to 100 μ M; those of spermidine from 1 to 50 mM, of ethylenediamine from 0.5 to 50 mM, of diaminopropane from 1 to 50 mM, of L-lysine from 1 to 40 mM, of thialysine from 0.025 to 1 mM, of *O*-(2-aminoethyl)-L-serine from 0.050 to 2 mM, and of *S*-(aminoethyl)-D,L-homocysteine from 0.5 to 15 mM. K_m values were calculated by Lineweaver–Burk plots using the program GraphPad Prism 1.02 (GraphPad, San Diego).

HPLC analysis of reaction products

HPLC analysis of L-lysine, thialysine and their N^α - and N^ϵ -acetyl-derivatives was performed as described by Patchett et al. [19]. *o*-Phthalaldehyde derivatives were detected using a fluorescence spectrophotometer (excitation wavelength 338 nm, emission wavelength 425 nm, SFM 25; Kontron) and a flow-through radiodetector (LB 506; Berthold). For HPLC analysis of *N*-acetylated diamines and polyamines, the method of Seiler and Knödgen [20] was applied. The dansylated products were monitored using a fluorescence spectrophotometer (excitation wavelength 365 nm, emission wavelength 485 nm) and a flow-through radiodetector.

MS analysis of reaction products

Reaction mixtures containing 1 μ g of purified *C. elegans* *N*-acetyltransferase D2023.4 in 25 mM Tris/HCl, pH 8.0, containing 10 mM thialysine and 1 mM acetyl-CoA were incubated at 30 °C for 1 h. Control reactions run in parallel included either heat-inactivated enzyme or active enzyme and 1 mM acetyl-CoA in the absence of thialysine. ESI–MS (electrospray ionization mass spectrometry) was performed in positive-ion mode using a Quattro II mass spectrometer (Micromass, Beverly, MA, U.S.A.). Aliquots (20 μ l) of each sample were mixed with 20 μ l of HPLC grade acetonitrile (Fisher Scientific), and 10 μ l volumes were introduced into the mass spectrometer by flow injection with a 100 μ l $\cdot$ min⁻¹ flow of acetonitrile/water (1:1, v/v). Full-scan spectra were obtained over the range of m/z 50–500 at 2 s/scan. Product ion MS/MS spectra were obtained under identical ionization conditions, except that 1.8×10^{-3} mbar of argon was used as the collision gas, with a collision cell potential of –20 V.

Site-directed mutagenesis

Two oligonucleotides (A96P-sense: 5'-CGACGAATGGGACTA-CCAAGAATTCTGTGGAAG-3' and A96P-antisense: 5'-CTTC-CACAGAGTTCTTGGGTAGTCCCATTCGTCG-3'; mutated triplets are shown underlined) were designed to replace Ala⁹⁶ of *C. elegans* *N*-acetyltransferase D2023.4 with proline. PCR-based mutagenesis was performed as described previously [21] using the plasmid construct pTrcHisB:CeNAT as template. The mutation was verified by nucleotide sequencing and a positive construct, pTrcHisB:CeNAT-A96P, was used to transform *E. coli* BL21(DE3) cells. Recombinant expression and purification was performed as described for the wild-type enzyme above.

E. coli growth curves, cell viability determination and disc diffusion assay

The effect of heterologous expression of *C. elegans* *N*-acetyltransferase D2023.4 in *E. coli* BL21(DE3) cells on the suscepti-

Figure 1 Genomic organization of *C. elegans* *N*-acetyltransferase D2023.4

Coding regions and introns are represented by black and white boxes respectively. Lines indicate intergenic regions. The orientation of the ABC1 protein, rhodanese (TST) and *N*-acetyltransferase (NAT) are shown according to the sequence data of cosmid D2023 from the *C. elegans* genome project, EMBL accession no. Z81052.

bility to the anti-metabolite thialysine was tested. Cells were freshly transformed with pTrcHisB, pTrcHisB:CeNAT and pTrcHisB:CeNAT-A96P respectively. Single colonies were inoculated in M9 minimal medium [15] supplemented with 0.4 % glucose, 0.3 mM L-methionine, 0.3 mM L-proline, 0.1 mM L-leucine, 50 μ g $\cdot$ ml⁻¹ thiamin and 100 μ g $\cdot$ ml⁻¹ ampicillin. Subsequently, overnight cultures were diluted 1:100 in the same medium and allowed to grow at 37 °C with shaking at 150 rev./min until a D_{600} of 0.1 was reached. Various concentrations of thialysine (0.25–2 mM) were added to aliquots of the cultures. Further growth was monitored by the increase in attenuation at D_{600} . Since turbidimetric determination cannot discriminate between viable and dead cells, the number of viable cells was determined at certain time points (0, 1, 4 and 7 h) after the addition of thialysine by spreading serial dilutions of the cultures on Luria–Bertani agar plates supplemented with 100 μ g $\cdot$ ml⁻¹ ampicillin. Plates were incubated for 16 h at 37 °C before colonies were counted.

Susceptibility of *E. coli* cells to thialysine was additionally determined by disc diffusion assays. Approx. 5×10^8 cells of the respective cultures were mixed with 3 ml of M9 top agar, and placed on to M9 agar plates. Where indicated, 1 mM IPTG was added to the top agar to enhance expression of recombinant proteins. Aliquots (5 μ l) of increasing thialysine concentrations (1, 5, 20 and 50 mM) were spotted on to Whatman paper disks (diameter 0.6 cm) that were placed on the surface of the top agar. Cells were allowed to grow for 24 h at 37 °C, before measuring the inhibition zones around the paper disks.

RESULTS

Characterization of *C. elegans* *N*-acetyltransferase D2023.4 cDNA

The 483 bp open reading frame of the putative diamine *N*-acetyltransferase D2023.4 was amplified from *C. elegans* cDNA using gene-specific oligonucleotides based on the EMBL genomic sequence accession number Z81052. The PCR product was used to screen a *C. elegans* λ -ZapII cDNA library. Several clones were obtained that encompass the entire 3' untranslated region of 84 bp, including a poly(A)⁺ (polyadenylated) tail and a typical polyadenylation signal sequence (AATAAA) 48 bp downstream of the translational stop. Furthermore, 5'-RACE revealed an SL2 sequence one nucleotide upstream of the initiator ATG of the *C. elegans* *N*-acetyltransferase. Consistent with this, the *N*-acetyltransferase gene contains the sequence GTTAAAGT-ATG, with a splice acceptor site 2 bp upstream of the translational start site (shown in bold). In *C. elegans*, SL2 is *trans*-spliced to downstream genes of polycistronic precursor mRNAs [22], indicating that the *N*-acetyltransferase D2023.4 forms an operon together with the closely spaced putative thiosulphate sulphurtransferase (rhodanese) D2023.5 (Figure 1). The mature *C. elegans* *N*-acetyltransferase cDNA sequence consists of 589 bp

Figure 2 Multiple sequence alignment of *C. elegans* N-acetyltransferase D2023.4 with other N-acetyltransferases

Amino acid residues that are identical in at least six sequences are shaded in black. Similar amino acid residues are shaded in grey. Gaps (—) were introduced to maximize homology. The four motifs A, B, C and D that characterize N-acetyltransferases of the GNAT superfamily [2] are indicated by appropriately labelled horizontal bars. CeNAT, *C. elegans* N-acetyltransferase D2023.4; MmAT2, putative mouse SSAT2; HsAT2, putative human SSAT2; DmNAT, putative *D. melanogaster* N-acetyltransferase; SpNAT, putative *S. pombe* N-acetyltransferase; PaNAT, putative *P. aeruginosa* N-acetyltransferase; HsSAT, human SSAT; MmSAT, mouse SSAT.

encoding a polypeptide of 160 amino acids with a deduced molecular mass of 18.6 kDa. Comparison with the genomic sequence of *C. elegans* N-acetyltransferase D2023.4 revealed three short introns of 49, 41 and 48 bp respectively (Figure 1).

Analysis of the deduced amino acid sequence

Pairwise sequence alignment using BCM search launcher (<http://searchlauncher.bcm.tmc.edu/>) illustrates that the amino acid sequence of *C. elegans* N-acetyltransferase D2023.4 exhibits a moderate similarity to known human and mouse SSAT1 enzymes (Figure 2) with a sequence identity of 28% and 26% respectively. In contrast, only 11 amino acid residues of *E. coli* spermidine N-acetyltransferase (EMBL accession number BAA15308) are conserved in the *C. elegans* N-acetyltransferase sequence, resulting in a sequence identity of 7% (results not shown). A BLAST search of the SwissProt database (<http://www.ncbi.nlm.nih.gov:80/BLAST/>) with *C. elegans* N-acetyltransferase D2023.4 as the query revealed best scores with members of the GNAT superfamily from mammalian, invertebrate, fungal and prokaryotic sources. The e-value of 1^{-23} for the human SSAT2, for example, is considerably higher than the score obtained for human SSAT1, with an e-value of 5^{-14} . Similar to the *C. elegans*

N-acetyltransferase, some sequences were termed putative diamine N-acetyltransferases or SSAT. However, except for human SSAT2 [7], none of these putative N-acetyltransferases have been characterized so far, and hence their function is still unknown. Pairwise sequence alignment with *C. elegans* N-acetyltransferase D2023.4 revealed slightly higher values of 30% sequence identity with the human SSAT2 (EMBL accession number NP_597998), 29% with a putative *Pseudomonas aeruginosa* N-acetyltransferase (EMBL accession number NP_249169) and 28% with putative N-acetyltransferases from *Drosophila melanogaster* (EMBL accession number AAM51135) and *Schizosaccharomyces pombe* (EMBL accession number NP_593494) respectively (Figure 2). Despite the wide distribution of putative *C. elegans* N-acetyltransferase D2023.4 homologues in prokaryotes and eukaryotes, it is noteworthy that a BLAST search revealed no D2023.4 homologue in the genome of *E. coli* (http://www.ncbi.nlm.nih.gov/sutils/genom_table.cgi).

Members of the GNAT superfamily are characterized by four conserved regions A, B, C and D. In *C. elegans* N-acetyltransferase D2023.4, motifs A, B and C are present, while motif D is partly deleted (Figure 2). The alignment in Figure 2 revealed that the regions between these GNAT motifs are also moderately conserved in SSAT and SSAT homologues.

Figure 3 SDS/PAGE analysis of *C. elegans* N-acetyltransferase D2023.4

A Coomassie Blue-stained SDS/PAGE gel of *E. coli* BL21 cells overexpressing *C. elegans* N-acetyltransferase D2023.4 is shown; the lanes represent the following: cell lysate (lane 2), 100 000 g supernatant (lane 3) and pellet (lane 4). Lane 5 shows the recombinant *C. elegans* N-acetyltransferase purified by Ni-NTA-chelating chromatography and subsequent gel filtration. The size of protein standards (lane 1) is given on the left in kDa.

Characterization of the recombinant *C. elegans* N-acetyltransferase D2023.4

C. elegans N-acetyltransferase D2023.4 was recombinantly expressed in *E. coli* BL21(DE3) cells as a His₆-tag fusion protein and purified by affinity chromatography on Ni-NTA-agarose. One litre of bacterial culture yielded approx. 20 mg of purified protein. SDS/PAGE analysis of the purified recombinant enzyme revealed a single band corresponding to a molecular mass of approx. 23 kDa, including the His-tag of approx. 4.25 kDa (Figure 3). This is in good accordance with the predicted molecular mass of 18.6 kDa on the basis of the deduced amino acid sequence of the cDNA. The protein was subjected to gel filtration on a calibrated Superdex S-200 column. N-Acetyltransferase activity eluted as a single peak corresponding to a molecular mass of approx. 44 000, indicating a dimeric structure of the enzymically active *C. elegans* N-acetyltransferase D2023.4. The enzyme was found to be very stable; after storage for 10 months at -20°C in buffer A, the specific activity was unchanged.

To determine the substrate specificity of *C. elegans* N-acetyltransferase D2023.4, several amines were tested as acetyl acceptor molecules in two different assays. As shown in Table 1, for all acceptors the release of free CoA (product 1) determined in assay A corresponds very well with the amount of radio-labelled product (product 2) revealed by TLC in assay B. The naturally occurring polyamines putrescine, cadaverine, spermidine and spermine, which are substrates of mammalian SSAT [23] or known nematode diamine acetyltransferases [10–12], were only poor substrates of the nematode enzyme. The reaction rates with the 'rare' polyamines norspermidine [bis-(3-aminopropyl)amine] and norspermine [bis-(3-aminopropyl)-1,3-propanediamine] were slightly higher. Testing *N*^ε-acetylated spermidine with a remaining free aminopropyl group and *N*¹-acetylated spermine as substrates did not change the rate of N-acetylation compared with the unmodified polyamines, whereas *N*¹-acetylspermidine with a free aminobutyl group was not derivatized by *C. elegans* N-acetyltransferase D2023.4. These data indicate that N-acetylation of the polyamines occurs at the aminopropyl group.

Concerning diamines, the specific activity increased with decreasing lengths of the methylene backbone, whereby the $k_{\text{cat}}/K_{\text{m}}$ values revealed that ethylenediamine was by far the best

Table 1 Acceptor specificity of *C. elegans* N-acetyltransferase D2023.4

Acceptor specificity of *C. elegans* N-acetyltransferase D2023.4 was determined by two assays (see the Materials and methods section). In assay A, the production of CoA was measured; in assay B, ¹⁴C-acetylated products were separated by TLC. The relative rates of N-acetyltransferase activity are expressed as the percentage of the values obtained with thialysine (131.9 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}$ of protein⁻¹ and 98.4 $\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}$ of protein⁻¹ respectively). The specific activities were determined under standard assay conditions in the presence of 1 mM (assay A) or 100 μM acetyl-CoA (assay B) and 10 mM of the respective acceptor molecules. Results are means $\pm$ S.D. for four independent duplicate determinations (asterisks mark results where acetylated standards were not available).

Acceptor (10 mM)	Relative specific activity (%)	
	Assay A	Assay B
Thialysine [S-(2-aminoethyl)-L-cysteine]	100	100
O-(2-aminoethyl)-L-serine	120 $\pm$ 6	126 $\pm$ 11*
S-(2-Aminoethyl)-D,L-homocysteine	60.8 $\pm$ 1.8	62.9 $\pm$ 4.4*
L-Lysine	4.0 $\pm$ 0.0	3.5 $\pm$ 0.4
D-Lysine	0.27 $\pm$ 0.06	0.23 $\pm$ 0.07
(2-Ethylthio)ethylamine	0.05 $\pm$ 0.02	0.08 $\pm$ 0.02*
Spermidine	0.51 $\pm$ 0.08	0.36 $\pm$ 0.08
Spermine	0.30 $\pm$ 0.01	0.14 $\pm$ 0.02
<i>N</i> ^ε -Acetylspermidine	0.50 $\pm$ 0.08	0.60 $\pm$ 0.02
<i>N</i> ¹ -Acetylspermine	0.24 $\pm$ 0.02	0.20 $\pm$ 0.02
Norspermidine	1.5 $\pm$ 0.1	1.3 $\pm$ 0.1*
Norspermine	1.7 $\pm$ 0.2	1.3 $\pm$ 0.1*
Ethylenediamine	16.3 $\pm$ 1.6	18.0 $\pm$ 1.2*
Diaminopropane	3.2 $\pm$ 0.7	3.1 $\pm$ 0.8*
Putrescine	0.19 $\pm$ 0.08	0.11 $\pm$ 0.01
Cadaverine	0.09 $\pm$ 0.04	0.02 $\pm$ 0.00
Cystamine	0.19 $\pm$ 0.06	0.12 $\pm$ 0.02*
(2-Hydroxyethyl)ethylenediamine	10.4 $\pm$ 2.7	11.0 $\pm$ 1.7*
Ethanolamine	0.18 $\pm$ 0.06	0.21 $\pm$ 0.03*

substrate, being approx. 145 times more efficiently acetylated than spermidine (Table 2). Hydroxy groups cannot replace an amino group, since ethanolamine was poorly acetylated and longer amino alcohols, with carbon-chain lengths from three to six, did not represent substrates of *C. elegans* N-acetyltransferase D2023.4 (results not shown).

C. elegans N-acetyltransferase D2023.4 catalyses the modification of L-lysine. Qualitative HPLC analyses revealed that L-lysine was acetylated exclusively at the ϵ -amino group (Figure 4A). It is remarkable that neither L-ornithine, whose side chain contains one methylene group less than L-lysine, nor 5-aminovaleric acid, which lacks the α -amino group of L-lysine, nor the decarboxylated form of L-lysine, cadaverine, are acetylated by the *C. elegans* N-acetyltransferase (results not shown). Taken together, these data suggest a crucial role of the carboxy and amino groups and the distance to the side chain ϵ -amino group for substrate recognition and/or catalysis. Furthermore, *N*^ε-acetylation of lysine by *C. elegans* N-acetyltransferase D2023.4 was stereoisomer-specific, since D-lysine was not as effectively acetylated as L-lysine (Table 1). *N*^ε-Acetyl-L-lysine and *N*^α-acetyl-L-lysine were not accepted.

However, the lysine analogues thialysine, O-(2-aminoethyl)-L-serine and S-(aminoethyl)homocysteine were determined to be the best substrates of *C. elegans* N-acetyltransferase D2023.4 (Tables 1 and 2). As indicated by the $k_{\text{cat}}/K_{\text{m}}$ values (Table 2) the catalytic efficiency for N-acetylation of thialysine is approx. 2-fold higher than with the second best substrate [O-(2-aminoethyl)-L-serine] and 1000-fold higher than with L-lysine itself. The related diamino acids L-lanthionine [(2-amino-2-carboxyethyl)-L,L-cysteine] and L-cystathionine [(2-amino-2-carboxyethyl)-L-homocysteine], as well as S-ethylcysteine and cystine, were not substrates for the *C. elegans* N-acetyltransferase (results

Table 2 Kinetic parameters of *C. elegans* *N*-acetyltransferase D2023.4 for different substrates

Kinetic parameters were determined using assay B. Where the corresponding acetylated standards were not available, K_m values were determined additionally by the spectrophotometric assay A. The K_m values of *O*-(aminoethyl)-L-serine (0.53 mM), *S*-(aminoethyl)-D,L-homocysteine (3.2 mM), ethylenediamine (1.5 mM) and diaminopropane (10.2 mM) are consistent with those obtained with assay B (results of assay A are the mean of two independent duplicate determinations).

	K_m (mM)	k_{cat} (s^{-1})	k_{cat}/K_m ($M^{-1} \cdot s^{-1}$)
Thialysine	0.18 ± 0.03 ($n=4$)	38.5 ± 3.1 ($n=5$)	213 992
<i>O</i> -(2-Aminoethyl)-L-serine	0.56 ± 0.07 ($n=3$)	48.9 ± 3.4 ($n=3$)	87 258
<i>S</i> -(2-Aminoethyl)-D,L-homocysteine	3.7 ($n=2$)	27.7 ($n=2$)	7475
L-Lysine	6.7 ± 1.5 ($n=5$)	1.4 ± 0.3 ($n=4$)	210
Ethylenediamine	1.6 ± 0.2 ($n=4$)	5.8 ± 0.3 ($n=4$)	3623
Diaminopropane	12.4 ± 1.5 ($n=6$)	1.2 ± 0.1 ($n=4$)	95
Spermidine	5.5 ± 0.9 ($n=3$)	0.14 ± 0.01 ($n=3$)	25

Figure 4 Qualitative HPLC analysis of the reaction product of *C. elegans* *N*-acetyltransferase D2023.4

The elution profile of OPA-derivatized amino acids is shown. (A) Standard mixture of L-lysine, N^{ϵ} -acetyl-lysine and N^{α} -acetyl-lysine (upper panel) and radiodetection of N^{ϵ} -acetylated lysine as the only product formed in the *C. elegans* *N*-acetyltransferase reaction when L-lysine is used as acceptor (lower panel). (B) Standard mixture of AEC (thialysine), N^1 -acetylthialysine and N^{ϵ} -acetylthialysine (upper panel) and radiodetection of N^{ϵ} -acetylthialysine as the only product formed when thialysine is used as acceptor (lower panel).

not shown). 2-(Ethylthio)ethylamine, which represents the side chain of thialysine, was hardly acetylated, with a reaction rate of only approx. 0.1 % (Table 1). Taken together, these data indicate a high specificity of *C. elegans* *N*-acetyltransferase D2023.4 for thialysine. As found for L-lysine, HPLC analysis revealed that *N*-acetylation occurs exclusively at the ϵ -amino group of thialysine (Figure 4B). In order to confirm this result, the products from the thialysine acetylation assay were subjected to ESI-MS, as described in the Materials and methods section. The full-scan spectrum identified ions at m/z 165 and 207, corresponding to thialysine and acetyl-thialysine respectively. To determine whether the *C. elegans* *N*-acetyltransferase D2023.4-induced acetylation of thialysine was occurring on either the N^{α} - or the N^{ϵ} -amino moiety, MS/MS fragmentation of the acetylated thialysine at m/z 207 was performed using matrix-free ESI-MS. The major fragment ions present in the MS/MS daughter spectrum of m/z 207 occurred at m/z 118 and 86, which is consistent with acetylation of the ϵ -amino group of thialysine (results not shown).

Other amino acids found in proteins, taurine and β -alanine, as well as the biogenic amines histamine, octopamine, serotonin, tyramine, tryptamine and agmatine, were not accepted by the *C. elegans* *N*-acetyltransferase (results not shown). Furthermore, the enzyme exhibits no acetyltransferase activity with phenylethylamine and *S*-benzyl-L-cysteine as acceptors. The K_m value for acetyl-CoA was found to be $22.7 \pm 3.0 \mu M$ ($n=5$) when 10 mM L-lysine was used as acceptor, and $23.8 \pm 5.2 \mu M$ ($n=3$) in the presence of 10 mM thialysine.

The amino acid residue Ala⁹⁶ of *C. elegans* *N*-acetyltransferase D2023.4, which is part of the well-conserved GNAT acetyl-CoA-binding motif A (Figure 2), was changed to proline by site-directed mutagenesis. Recombinant expression of the mutant in *E. coli* BL21(DE3) led to a completely inactive enzyme (results not shown). The Ala⁹⁶ → Pro mutant was used as a control when the effect of heterologous expression of *C. elegans* *N*-acetyltransferase D2023.4 in *E. coli* was investigated.

Heterologous expression of *C. elegans* *N*-acetyltransferase D2023.4 in *E. coli* leads to enhanced resistance against thialysine

Thialysine is a known potent anti-metabolite of L-lysine that inhibits growth of prokaryotic as well as eukaryotic cells [24–26]. To determine whether *C. elegans* *N*-acetyltransferase D2023.4 has the ability to modulate the inhibitory effect of the L-lysine analogue, the nematode protein was heterologously expressed in *E. coli* cells. The cells were cultured in minimal medium supplemented with various thialysine concentrations ranging from 0.25–1.00 mM. Following cell growth spectrophotometrically over 7 h demonstrated that expression of the *C. elegans* *N*-acetyltransferase D2023.4 wild-type enzyme, at least partly, abolished

the anti-proliferating effect of thialysine up to a concentration of 1 mM (results not shown). Cells that carry solely the pTrcHisB plasmid or those that express the inactive *C. elegans* N-acetyltransferase Ala⁹⁶Pro mutant or the *C. elegans* ornithine decarboxylase were similarly much more affected by the lysine analogue. The effect was even more pronounced when the number of viable cells was determined (Figure 5A). Except for those bacteria that express the *C. elegans* N-acetyltransferase D2023.4 wild-type enzyme, incubation in the presence of only 0.25 mM thialysine led to decreasing cell numbers visible after 4 h (results not shown for cells transformed with the pTrcHisB plasmid or those that express *C. elegans* ornithine decarboxylase). Since recombinant expression of the *C. elegans* N-acetyltransferase is not tightly regulated in the pTrcHisB/*E. coli* BL21(DE3) system, some protein is produced even in the absence of IPTG. Hence the effect was achieved without IPTG induction.

These results were supported by disc diffusion assays using thialysine as the anti-proliferating agent. *E. coli* cells that express *C. elegans* N-acetyltransferase D2023.4 wild-type form smaller inhibition zones than those cells that express the inactive Ala⁹⁶Pro mutant (Figure 5B). Increasing the expression of the wild-type enzyme by the addition of 1 mM IPTG hardly affects the level of resistance. In conclusion, expression of *C. elegans* N-acetyltransferase D2023.4 in *E. coli* cells led to the efficient detoxification of the anti-metabolite thialysine.

DISCUSSION

A novel N-acetyltransferase of the GNAT superfamily has been cloned and characterized from *C. elegans*. Although its amino acid sequence exhibits considerable similarity with mammalian SSAT1, comparison of the substrate specificities revealed strong differences [23]. Most importantly, the naturally occurring polyamines spermidine and spermine represent poor substrates of *C. elegans* N-acetyltransferase D2023.4, with high K_m values and low reaction rates. Regarding polyamines and diamines, *C. elegans* N-acetyltransferase D2023.4 exhibits a preference for the smallest diamines, diaminopropane and ethylenediamine. In contrast, SSAT1 shows the highest reaction rates with amines of the $\text{NH}_2(\text{CH}_2)_3\text{NHR}$ type, preferring one primary and one secondary amino group, whereas diaminopropane is a relatively poor substrate of mammalian SSAT1 [23]. Diaminopropane is found in several organisms [27], but not in *C. elegans* (K. Lüersen, and R. D. Walter, unpublished work), and the shortest diamine (ethylenediamine), which is the best substrate among the diamines, is not reported to be a natural product.

C. elegans N-acetyltransferase D2023.4 was found to preferentially catalyse the *N*^ε-acetylation of the amino acid thialysine, a L-lysine analogue with the 4-methylene group substituted with a sulphur. Thialysine is generally regarded as an anti-metabolite of L-lysine. Owing to its structural similarity, thialysine was reported to compete with L-lysine for activation and transfer to tRNA^{lys} in prokaryotes and eukaryotes [28]. In this way, the lysine analogue can be incorporated into polypeptide chains resulting in the formation of inactive proteins. Thialysine is toxic against bacteria, as well as against eukaryotic cells [24–26]. The data presented here indicate that, at least in the prokaryote *E. coli*, cytotoxicity of thialysine is prevented by *N*^ε-acetylation mediated by the introduced *C. elegans* N-acetyltransferase D2023.4. It is noteworthy that *E. coli* does not possess a homologue for *C. elegans* N-acetyltransferase D2023.4. In conclusion, *C. elegans* N-acetyltransferase D2023.4 has the capability to detoxify the lysine anti-metabolite thialysine.

Although thialysine functions as an anti-metabolite, several investigations have indicated a biological significance of this

Figure 5 Effect of *C. elegans* N-acetyltransferase D2023.4 expression in *E. coli* on cytotoxicity by thialysine

(A) Cultures of *E. coli* BL21(DE3) cells transformed with pTrcHisB:CeNAT (WT) and pTrcHisB:A96P (A96P) respectively were supplemented with thialysine concentrations as indicated. Growth was followed without the addition of IPTG over a period of 7 h by monitoring D_{600} . The number of viable cells were determined at different time points from the respective cultures by spreading serial dilutions on Luria-Bertani agar plates supplemented with 100 $\mu\text{g} \cdot \text{ml}^{-1}$ ampicillin. Results are the means $\pm$ S.E.M. for at least three independent tests. (B) Disc diffusion assays: approx. 5×10^8 *E. coli* BL21(DE3) cells transformed with pTrcHisB:CeNAT (WT) and pTrcHisB:A96P (A96P) respectively were mixed with M9 top agar and spread on M9 agar plates. Where indicated, 1 mM IPTG was added to the top agar to enhance recombinant expression. Whatman paper discs that were placed on the M9 top agar were soaked with 5 μl of 0, 1, 5, 20 and 50 mM thialysine respectively. Representative disc diffusion plates spread with *E. coli* BL21(DE3) that express *C. elegans* N-acetyltransferase D2023.4 wild-type (left photo) and the Ala⁹⁶Pro mutant (right photo) respectively. The thialysine concentrations in mM are indicated on the Whatman paper discs. The diameters of the inhibition zones around the paper discs were measured after incubation at 37 °C for 24 h. Results shown in the lower panel are the means $\pm$ S.E.M. for at least three independent assays.

sulphur-containing amino acid, which has been reported to be present in human urine samples [29]. Furthermore, it has been demonstrated, at least *in vitro*, that thialysine can be synthesized enzymically from D-pantetheine and L-serine by the action of cystathionine β -synthase and pantetheinase [30], or by cystathionine β -synthase using cysteamine as substrate [31]. It was suggested that thialysine may be the precursor of the naturally occurring S-(2-aminoethyl)-L-cysteine ketimine and the corresponding decarboxylated dimer, the latter being reported to function as an antioxidant [32,33]. SSAT2 is the mammalian N-acetyltransferase with the highest similarity to the *C. elegans* N-acetyltransferase D2023.4. Expression of human SSAT2 after transient transfection of HEK-293 cells [7] or stable transfection of NIH-3T3 cells [34] did not affect polyamine content. These results and the *in vitro* determination of substrate specificity using purified recombinant human SSAT2 indicates that SSAT2 has negligible activity against polyamines, and greatly prefers thialysine as a substrate [34].

In previous studies, Tanaka, Soda and Yamamoto [35,36] purified a thialysine-inducible N-acetyltransferase from *Klebsiella (Aerobacter) aerogenes* that catalyses N^ε-acetylation of L-lysine and, more efficiently, N^ε-acetylation of its analogues thialysine and O-(2-aminoethyl)-L-serine. Therefore the enzyme was termed acetyl-CoA:S-(2-aminoethyl)-L-cysteine ω-N-acetyltransferase. To some extent these features resemble the results obtained with the *C. elegans* N-acetyltransferase D2023.4. However, in contrast with the *C. elegans* enzyme, the *Klebsiella* N-acetyltransferase exhibits no optical specificity and thus does not discriminate between D- and L-lysine. Furthermore, it also accepts L- and D-ornithine as substrates. The molecular mass of the *Klebsiella* protein was determined by gel filtration to be 100 000 Da, which is more than twice the mass of the *C. elegans* protein.

The substrate spectrum of *C. elegans* N-acetyltransferase D2023.4 includes L-lysine. Several members of the GNAT superfamily, such as histone acetyltransferases or the p300/CBP (CREB-binding protein)-associating factor, modify their substrate proteins by N^ε-acetylation of specific lysine residues [1]. However, a GNAT that catalyses the N^ε-acetylation of the amino group of free lysine has not been reported. In the yeasts *Yarrowia lipolytica* and *Saccharomyces cerevisiae*, acetyl-CoA-dependent lysine-N^ε-acetyltransferases have been characterized, which catalyse the first step of the L-lysine degradation pathway [18,37]. The amino acid sequence of the 43 kDa *Yarrowia* protein has no significant similarity to the *C. elegans* N-acetyltransferase D2023.4, and the protein does not belong to the GNAT superfamily. The acceptor specificity of *Saccharomyces* lysine-N^ε-acetyltransferases includes thialysine, but the activity was only 24 % compared with L-lysine as acceptor, and the K_m for the lysine analogue was determined to be 100 mM compared with 5.8 mM for L-lysine [18]. In addition, a Ping Pong reaction mechanism has been determined for the *Y. lipolytica* enzyme [37], which can be excluded for the *C. elegans* N-acetyltransferase.

Generally, two distinct reaction mechanisms are possible for N-acetyltransferases: a Ping Pong mechanism or a direct acetyl transfer [1]. For the first type of reaction, a cysteine residue is required to form a covalently bound acetylated enzyme intermediate, as has been shown for arylamine N-acetyltransferases [38,39]. However, the *C. elegans* N-acetyltransferase D2023.4 does not possess any cysteine residue (Figure 3). Hence a Ping Pong mechanism can be excluded and a direct transfer of acetyl group on to the acceptor molecule via nucleophilic attack is very likely. A direct acetyl transfer has been suggested as the general mechanism for GNAT [1].

Members of the GNAT superfamily are characterized by four conserved regions termed A, B, C and D [1,2]. The crystal

structures of different GNATs indicate that these four regions are involved in acetyl-CoA binding [1]. In particular, the pantothenic and the pyrophosphate moieties of CoA interact with the highly conserved motif (R/Q)XXGX(G/A) (where 'X' represents any amino acid), which is also present in the *C. elegans* N-acetyltransferase D2023.4 (amino acid residues 91–96). Mutagenic studies on human SSAT1 also confirmed the involvement of this motif in acetyl-CoA binding [40,41] and, accordingly, mutation of Ala⁹⁶ of *C. elegans* N-acetyltransferase D2023.4 to proline results in an inactive enzyme.

The crystal structures of serotonin N-acetyltransferase complexed with a bisubstrate analogue and of *Tetrahymena* GCN5 complexed with CoA and a peptide substrate analogue showed that the more divergent regions between these motifs and the C-terminus participate in acceptor binding [42,43]. In SSAT and in the *C. elegans* N-acetyltransferase D2023.4 modifying similar acceptor molecules (i.e. small molecules with terminal amino groups), these regions contain several conserved amino acid residues. For mammalian SSAT1 it has been demonstrated that charged amino acid residues within these regions are involved in polyamine binding [41,44].

Polyamines are only poor substrates of *C. elegans* N-acetyltransferase D2023.4 and no other SSAT homologue is present in the genome of *C. elegans*. Hence, the data presented here give evidence that the polyamine interconversion pathway of free-living nematodes (as previously proposed for parasitic nematodes [9]) does not include an N-acetylation step. Furthermore, a diamine N-acetyltransferase that has been reported from *O. volvulus* and *A. suum* catalysing the N-acetylation of the diamines putrescine and cadaverine, as well as of the biogenic amine histamine but not of spermidine and spermine [10–12], is either absent in *C. elegans* or is evolutionarily not closely related to mammalian SSAT1.

In conclusion, based on the kinetic data and the thialysine-detoxifying capacity of *C. elegans* N-acetyltransferase D2023.4, we propose to name the enzyme thialysine N^ε-acetyltransferase. Putative orthologues of the *C. elegans* N-acetyltransferase D2023.4 are also present in the genomes of prokaryotes, invertebrates and mammals, and we have already determined a similar enzyme activity for the respective N-acetyltransferase from the human pathogen *Leishmania major* (K. Lüersen, unpublished work). Future studies will show whether more of these GNATs possess a comparable substrate specificity as the nematode enzyme and hence form a novel group of N-acetyltransferases within the GNAT superfamily.

We thank Rolf D. Walter for helpful discussions, Silke van Hoorn for excellent technical assistance, Laura Pecci for the generous gift of diamino acids and for helpful discussions, as well as Mark Wickham for detailed blast searches in *E. coli* genome data bases. MS analyses were kindly performed by Daniel Jones and Bruce Stanley. We are grateful to Catherine Coleman and Anthony Pegg for discussing and exchanging information prior to publication. This work was supported by the Deutsche Forschungsgemeinschaft (grant Lu 733/5-1).

REFERENCES

- 1 Dyda, F., Klein, D. C. and Hickman, A. B. (2000) GCN5-related N-acetyltransferases: a structural overview. *Annu. Rev. Biophys. Biomol. Struct.* **29**, 81–103.
- 2 Neuwald, A. F. and Landsman, D. (1997) GCN5-related histone N-acetyltransferases belong to a diverse superfamily that includes the yeast SPT10 protein. *Trends Biochem. Sci.* **22**, 154–155.
- 3 Pegg, A. E. (1986) Recent advances in the biochemistry of polyamines in eukaryotes. *Biochem. J.* **234**, 249–262.
- 4 Tabor, C. W. and Tabor, H. (1984) Polyamines. *Annu. Rev. Biochem.* **53**, 749–790.
- 5 Seiler, N. (1987) Functions of polyamine acetylation. *Can. J. Physiol. Pharmacol.* **65**, 2024–2035.

- 6 Casero, Jr, R. A. and Pegg, A. E. (1993) Spermidine/spermine N^1 -acetyltransferase – the turning point in polyamine metabolism. *FASEB J.* **7**, 653–661
- 7 Chen, Y., Vujcic, S., Liang, P., Diegelman, P., Kramer, D. L. and Porter, C. W. (2003) Genomic identification and biochemical characterization of a second spermidine/spermine N^1 -acetyltransferase. *Biochem. J.* **373**, 661–667
- 8 Wittich, R. M., Kilian, H. D. and Walter, R. D. (1987) Polyamine metabolism in filarial worms. *Mol. Biochem. Parasitol.* **24**, 155–162
- 9 Müller, S. and Walter, R. D. (1992) Purification and characterization of polyamine oxidase from *Ascaris suum*. *Biochem. J.* **283**, 75–80
- 10 Wittich, R. M. and Walter, R. D. (1989) A novel type of putrescine (diamine)-acetylating enzyme from the nematode *Ascaris suum*. *Biochem. J.* **260**, 265–269
- 11 Wittich, R. M. and Walter, R. D. (1990) Putrescine N -acetyltransferase in *Onchocerca volvulus* and *Ascaris suum*, an enzyme which is involved in polyamine degradation and release of N -acetylputrescine. *Mol. Biochem. Parasitol.* **38**, 13–17
- 12 Davids, G. L., Aisien, S. O. and Walter, R. D. (1994) Characterization of the N -acetyltransferases respectively responsible for arylalkylamine and diamine acetylation in *Ascaris suum*. *Mol. Biochem. Parasitol.* **64**, 341–344
- 13 Hermann, P., Stalla, K., Schwimmer, J., Willhardt, I. and Kutschera, I. (1969) Synthesis of some sulfur-containing amino acid analogs. *Journal fuer Praktische Chemie (Leipzig)* **311**, 1018–1028
- 14 Sulston, J. and Hodgkin, J. (1988) *The Nematode Caenorhabditis elegans* (Wood, W. B., ed.), pp. 587–606, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY
- 15 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) *Molecular Cloning: a Laboratory Manual*, 2nd edn., Cold Spring Harbor Press, Cold Spring Harbor, NY
- 16 Benton, D. W. and Davis, R. W. (1977) Screening λ gt recombinant clones by hybridization to single plaque *in situ*. *Science* **196**, 180–182
- 17 Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**, 248–254
- 18 Bode, R., Thureau, A. M. and Schmidt, H. (1993) Characterization of acetyl-CoA: L-lysine N^6 -acetyltransferase, which catalyses the first step of carbon catabolism from lysine in *Saccharomyces cerevisiae*. *Arch. Microbiol.* **160**, 397–400
- 19 Patchett, M. L., Monk, C. R., Daniel, R. M. and Morgan, H. W. (1988) Determination of agmatine, arginine, citrulline and ornithine by reversed-phase liquid chromatography using automated pre-column derivatization with *o*-phthalaldehyde. *J. Chromatogr.* **425**, 269–276
- 20 Seiler, N. and Knödgen, B. (1978) Determination of di- and poly-amines by high-performance liquid chromatographic separation of their 5-dimethylaminonaphthalene-1-sulfonyl derivatives. *J. Chromatogr.* **145**, 29–39
- 21 Ndjonka, D., Da'dara, A., Walter, R. D. and Lüersen, K. (2003) *Caenorhabditis elegans* S-adenosyl-methionine decarboxylase is highly stimulated by putrescine but exhibits a low specificity for activator binding. *Biol. Chem.* **384**, 83–91
- 22 Blumenthal, T. and Gleason, K. S. (2003) *Caenorhabditis elegans* operons: form and function. *Nat. Rev. Genet.* **4**, 112–120
- 23 Della Ragione, F. and Pegg, A. E. (1983) Studies of the specificity and kinetics of rat liver spermidine/spermine N^1 -acetyltransferase. *Biochem. J.* **213**, 701–706
- 24 Di Girolamo, M., Busiello, V., Cini, C., Foppoli, C. and De Marco, C. (1982) Thialysine utilization by *E. coli* and its effects on cell growth. *Mol. Cell. Biochem.* **46**, 43–48
- 25 Di Girolamo, M., Di Girolamo, A., Coccia, R., Foppoli, C. and Blarzino, C. (1985) Effects of thialysine on CHO cells growth. *Microbiologica* **8**, 367–377
- 26 Jun do, Y., Rue, S. W., Han, K. H., Taub, D., Lee, Y. S., Bae, Y. S. and Kim, Y. H. (2003) Mechanism underlying cytotoxicity of thialysine, lysine analog, toward human acute leukemia Jurkat T cells. *Biochem. Pharmacol.* **66**, 2291–2300
- 27 Cohen, S. S. (1998) *A Guide to the Polyamines*, Oxford University Press, NY
- 28 De Marco, C., Busiello, V., Di Girolamo, M. and Cavallini, D. (1976) Selenalysine and protein synthesis. *Biochim. Biophys. Acta* **454**, 298–308
- 29 Yu, S., Sugahara, K., Zhang, J., Ageta, T., Kodama, H., Fontana, M. and Dupre, S. (1997) Simultaneous determination of urinary cystathionine, lanthionine, S-(2-aminoethyl)-L-cysteine and their cyclic compounds using liquid chromatography-mass spectrometry with atmospheric pressure chemical ionization. *J. Chromatogr. B Biomed. Sci. Appl.* **698**, 301–307
- 30 Pitari, G., Maurizi, G., Flati, V., Ursini, C. L., Spera, L., Dupre, S. and Cavallini, D. (1992) Enzymatic synthesis of S-aminoethyl-L-cysteine from pantetheine. *Biochim. Biophys. Acta* **1116**, 27–33
- 31 Costa, M., Pecci, L., Pensa, B., Fontana, M. and Cavallini, D. (1989) High-performance liquid chromatography of cystathionine, lanthionine and aminoethylcysteine using *o*-phthalaldehyde precolumn derivatization. *J. Chromatogr.* **490**, 404–410
- 32 Pecci, L., Montefoschi, G., Antonucci, A. and Cavallini, D. (1995) Antioxidant properties of the decarboxylated dimer of aminoethylcysteine ketimine. *Physiol. Chem. Phys. Med. NMR* **27**, 223–229
- 33 Fontana, M., Pecci, L., Maccone, A. and Cavallini, D. (1998) Antioxidant properties of the decarboxylated dimer of aminoethylcysteine ketimine: assessment of its ability to scavenge peroxynitrite. *Free Radicals Res.* **29**, 435–440
- 34 Coleman, C., Stanley, B. A., Jones, A. D. and Pegg, A. Spermidine/spermine N^1 -acetyltransferase-2 (SSAT2) acetylates thialysine and is not involved in polyamine metabolism. *Biochem. J.* **383**, 139–148
- 35 Soda, K., Tanaka, H. and Yamamoto, T. (1970) Enzymic N -acetylation of a sulfur analog of L-lysine, S-(beta-aminoethyl)-L-cysteine. *FEBS Lett.* **8**, 301–303
- 36 Tanaka, H. and Soda, K. (1974) Enzymatic N -acetylation of lysine analogs. Purification and properties of acetyl coenzyme A:S-(beta-aminoethyl)-L-cysteine omega- N -acetyltransferase. *J. Biol. Chem.* **249**, 5285–5289
- 37 Beckerich, J. M., Lambert, M. and Gaillardin, C. (1994) LYS1 is the structural gene for lysine N -6-acetyltransferase in yeast. *Curr. Genet.* **25**, 24–29
- 38 Dupret, J. M. and Grant, D. M. (1992) Site-directed mutagenesis of recombinant human arylamine N -acetyltransferase expressed in *Escherichia coli*. Evidence for direct involvement of Cys68 in the catalytic mechanism of polymorphic human NAT2. *J. Biol. Chem.* **267**, 7381–7385
- 39 Watanabe, M., Igarashi, T., Kaminuma, T., Sofuni, T. and Nohmi, T. (1994) N -Hydroxyarylamine O -acetyltransferase of *Salmonella typhimurium*: proposal for a common catalytic mechanism of arylamine acetyltransferase enzymes. *Environ. Health Perspect.* **102** (suppl 6), 83–89
- 40 Lu, L., Berkey, K. A. and Casero, Jr, R. A. (1996) RGFGIGS is an amino acid sequence required for acetyl coenzyme A binding and activity of human spermidine/spermine N^1 -acetyltransferase. *J. Biol. Chem.* **271**, 18920–18924
- 41 Coleman, C. S., Huang, H. and Pegg, A. E. (1996) Structure and critical residues at the active site of spermidine/spermine- N^1 -acetyltransferase. *Biochem. J.* **316**, 697–701
- 42 Hickman, A. B., Klein, D. C. and Dyda, F. (1999) Melatonin biosynthesis: the structure of serotonin N -acetyltransferase at 2.5 Å resolution suggests a catalytic mechanism. *Mol. Cell.* **3**, 23–32
- 43 Rojas, J. R., Trievel, R. C., Zhou, J., Mo, Y., Li, X., Berger, S. L., Allis, C. D. and Marmorstein, R. (1999) Structure of *Tetrahymena* GCN5 bound to coenzyme A and a histone H3 peptide. *Nature (London)* **401**, 93–98
- 44 Coleman, C. S., Huang, H. and Pegg, A. E. (1995) Role of the carboxyl terminal MATEE sequence of spermidine/spermine N^1 -acetyltransferase in the activity and stabilization by the polyamine analog N^1,N^{12} -bis(ethyl)spermine. *Biochemistry* **34**, 13423–13430

Received 12 May 2004/8 July 2004; accepted 29 July 2004

Published as BJ Immediate Publication 29 July 2004, DOI 10.1042/BJ20040789