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Cloning, expression, and biochemical characterization of a new histone deacetylase-like protein from *Thermus caldophilus* GK24

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Abstract

Histone deactylases (HDACs) are members of an ancient enzyme family found in eukaryotes as well as in prokaryotes such as archaebacteria and eubacteria. We here report a new histone deacetylase (*Tca* HDAC) that was cloned from the genomic library of *Thermus caldophilus* GK24 based on homology analysis with human histone deacetylase1 (HDAC1). The gene contains an open reading frame encoding 375 amino acids with a calculated molecular mass of 42,188 Da and the deduced amino acid sequence of *Tca* HDAC showed a 31% homology to human HDAC1. The *Tca* HDAC gene was over-expressed in *Escherichia coli* using a Glutathione-S transferase (GST) fusion vector (pGEX-4T-1) and the purified protein showed a deacetylase activity toward the fluorogenic substrate for HDAC. Moreover, the enzyme activity was inhibited by trichostatin A, a specific HDAC inhibitor, in a dose-dependent manner. Optimum temperature and pH of the enzyme was found to be approximately 70 °C and 7.0, respectively. In addition, zinc ion is required for catalytic activity of the enzyme. Together, these data demonstrate that *Tca* HDAC is a new histone deacetylase-like enzyme from *T. caldophilus* GK24 and will be a useful tool for deciphering the role of HDAC in the prokaryote and development of new biochemical reactions.

Keywords: Histone deacetylase; *Thermus caldophilus*; *Tca* HDAC; Protein expression; Trichostatin A

Histone deactylases (HDACs) are members of an ancient enzyme family found in plant, animal, and fungi, as well as archaebacteria and eubacteria [1]. Class I and II HDACs [2], acetoin utilization proteins that are necessary for efficient utilization of the carbon source, acetoin, and acetylpolyamine amidohydrolases that deacetylate polyamines such as spermine are members of the same superfamily [3] and considered to have a common ancestor [4]. Supporting this idea, sequence comparison reveals that all three classes of proteins share a number of common motifs. Additionally, they show significant functional similarities such as recognition of acetylated aminoalkyl groups and the removal of the acetyl moiety by cleaving an amide bond.

In eukaryotes, it has been well known that HDACs play a key role in transcription regulation as well as cell proliferation through the post-translational modification of histones and other proteins [5]. The fact that acetylation is a key component in the regulation of gene expression has stimulated the study of HDACs in relation to the aberrant gene expression often observed in cancer. Although no direct alteration in the expression of HDACs has yet been demonstrated in cancer, HDACs are known to associate with a number of well characterized cellular oncogenes and tumour-suppressor genes [e.g. Mad and retinoblastoma protein (Rb)], leading to an aberrant recruitment of HDAC activity, which in turn results in changes in gene expression [6,7]. Consequently, HDACs have become key targets for anti-cancer drug development and purified HDACs are valuable as screening tools. Unfortunately, however, expression of human HDACs has proven to be difficult. Indeed, human HDAC1 is inactive when produced by recombinant techniques, implying that cofactors are necessary for HDAC activity. *In vivo*, HDAC1 only displays activity within a complex of

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proteins. These complexes consist of proteins necessary for modulating their deacetylase activity and for binding DNA, together with proteins that mediate HDACs to the promoters of particular genes [8]. Thus, isolation of individually purified HDACs by recombinant methodology without the associated complex components or cofactors has generally yielded inactive forms of the enzymes that are not a useful means for high throughput screen of inhibitors of HDACs as well as other biocatalytic purposes [9–14].

Reports demonstrate that not only eukaryotes but also eubacteria and archaea have proteins with deacetylase activity toward proteins or small molecules such as acetylputrescince and polyamines [15]. Indeed, the first crystal structure of HDAC was obtained from *Alcaligenes aeolicus*, a thermophilic bacterium, and has provided valuable information for rationale of catalytic mechanism of the enzyme as well as structure based drug design [16]. These results have prompted us to find a new histone deacetylase-like enzyme from prokaryotes that may exhibit its biochemical activity by conventional recombinant techniques. As a source of a new HDAC gene, *Thermus caldophilus* GK24 was chosen since archaebacteria and eubacteria were known to have the enzymatic activity and the genomic library of the bacterium has been established [17]. Based on homology analysis of the gene, we identified a cosmid clone from the genomic library of *T. caldophilus* GK24 and successfully cloned the gene by PCR. Here, we report for the first time on the cloning and nucleotide sequence of the HDAC gene from *T. caldophilus* GK24, its expression in *Escherichia coli*, and biochemical characterization of the purified enzyme.

Materials and methods

Bacterial strains and cultivation. Thermus caldophilus GK24 [17] was used as the source for cloning of the gene encoding *Tca* HDAC. *E. coli* BL21 (DE3) was used as the host for plasmid preparations and gene expression.

Preparation of DNA libraries from T. caldophilus GK24. Chromosomal DNA was isolated from *T. caldophilus* GK24 as described previously [18]. For construction of a whole-genome shotgun library, genomic DNA from *T. caldophilus* was sheared with a HydroShear (GeneMachines) to yield fragments with estimated sizes of about 5.0 kbp. The fragmented DNA was separated in a low-melting-point agarose gel, and the dominant fraction of the desired size range was isolated. The fragments were purified with a gel extraction kit and cloned using a TOPO shotgun subcloning kit (Invitrogen Corp. CA). Positive clones were identified by their ampicillin resistance in combination with their white colony phenotype in an X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) screening.

DNA sequencing and computer-assisted analysis. The isolated vectors served as templates in sequencing reactions using the primers M13uni(−43) (5′-AGGGTTTTCCCAGTCACGACGTT-3′) and M13rev(−49) (5′-GAGCGGATAACAATTTCACACAGG-3′). The sequence data were collected using an ABI Prism DNA System 377 apparatus and an ABI Prism 3700 DNA analyzer and processed and assembled with Phred/Phrap/Consed (http://www.phrap.org) and CAP3 software [19]. Gaps are being closed where needed by direct sequencing using chromosomal DNA as a template currently.

Construction of the pGEX-4T-1 for expression of Tca HDAC gene. The *Tca* HDAC gene was amplified by PCR using forward primer 5′-GCATGAATTCATGGTGATCTACCGGGAG-3′ with an EcoRI site (underlined) and reverse primer 5′-ATATGTCGACCTAGTGTAGCAGGTGGGG-3′ with a SalI site (underlined). The PCR cycling parameters consisted of an initial denaturation at 94 °C for 10 min; 30 cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min; and a final extension step at 72 °C for 10 min. After digestion with EcoRI and SalI, the PCR products were ligated into the plasmid pGEX-4T-1 (Amersham Biosciences, Uppsala, Sweden) and then transformed into *E. coli* BL21 (DE3) for protein expression [20]. Both strands of inserted fragment and vector cloning sites were sequenced to confirm the identity of the construct. The nucleotide sequence of the *Tca* HDAC gene of *T. caldophilus* GK24 and the encoded amino acid sequence have been deposited in the NCBI GenBank under Accession No. AY253853.

Expression and purification of the GST-tagged Tca HDAC in E. coli. BL21 (DE3) cell strains transformed with pGEX-4T-1 harboring *Tca* HDAC gene were grown in LB (Luria–Bertani) medium containing ampicillin (50 μg/ml) at 37 °C to an absorbance of 0.6–0.7 at 600 nm. The protein expression was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and then cells were grown for an additional 16 h. The cells were collected by centrifugation suspended in buffer [150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4 (pH 7.3), 1% Triton, 0.1% β-mercaptoethanol, 2 mM EDTA, protease inhibitor cocktail tablets (Roche, Mannheim, Germany), and 1 mM phenylmethylsulfonylfluoride (PMSF)], then disrupted by sonication. Sonication was performed five times on ice for 0.5 min with 1 min intervals for cooling. Following centrifugation at 15,000 rpm, 4 °C for 30 min, and the supernatant was subjected to purification by affinity chromatography using Glutathione agarose (Sigma, MI) according to the manufacturer's instructions. Bound proteins were eluted with 50 mM Tris–HCl, pH 8.0, and 10 mM reduced glutathione (Sigma). The protein concentrations of these elution fractions were determined by using the BCA protein assay kit (Pierce, Rockford, IL).

Electrophoresis and western blotting analysis. The eluted fractions were analyzed by SDS–PAGE and gels were stained with Coomassie brilliant blue R-250. For western blotting analysis, proteins resolved by SDS–PAGE were transferred onto polyvinylidene fluoride (PVDF) membranes (Immobilon-P, Millipore, Bellerica, MA). The membranes were incubated with polyclonal anti-HDAC1 antibody (Abcam, Cambridge, UK). The western blots were visualized with horseradish peroxidase-linked whole anti-rabbit IgG antibody (Amersham Biosciences, Uppsala, Sweden) and the blots were detected with an enhanced West-pico chemiluminescence kit (ECL) as described by the manufacturer (Pierce).

HDAC enzymatic assay. HDAC enzymatic assay was performed with HDAC Fluorescent Activity Assay kit (Biomol, Plymouth Meeting, PA) according to the manufacturer's instructions, and a GENios microplate fluorometer was used with the Magellan software system (TECAN, Austria) with excitation at 360 nm and emission at 465 nm. HDAC activity was measured three times per one sample, and the mean value was collected in three independent samples.

Statistical analysis. Results are expressed as the means ± standard error (SE). Student's t test was used to determine statistical significance between control and test groups. A P value of <0.05 was considered statistically significant.

Results and discussion

Library construction, shotgun sequencing, and primer walking

As a source of a new HDAC gene, *T. caldophilus* GK24 was chosen since archaebacteria and eubacteria were known to have the enzymatic activity and the genomic library of the bacterium has been established [17]. The thermophilic aerobacteria *T. caldophilus* GK24 was analyzed using a low-coverage shotgun-sequencing approach.

A total of 1.91 Mbp are reported, giving insights into the genome of *T. caldophilus*. The genome assembly used for the annotation of the genes described in this paper consists of 8448 sequence reads and patch-seqs from *T. thermophilus* HB27 assembled in 40 contigs; the largest contig is 893 kbp in length. Contigs smaller than 2 kbp were excluded from the analysis. The total length of the contigs adds up to 1.91 Mbp, which represents about 96% of the estimated genome size of 1.98 Mbp. The average gap size is estimated to be about 2 kbp.

Nucleotide sequence of the Tca HDAC gene and its deduced amino acid sequence

Based on homology analysis of the gene, we identified a cosmid clone having a high homology to human HDAC1 from genomic library of *T. caldophilus* GK24. Sequence data were submitted for analysis by searching for sequence homology against the non-redundant Genbank database using BLASTX. The *Tca* HDAC gene contains 1128 bp coding for a polypeptide with 375 amino acids and a

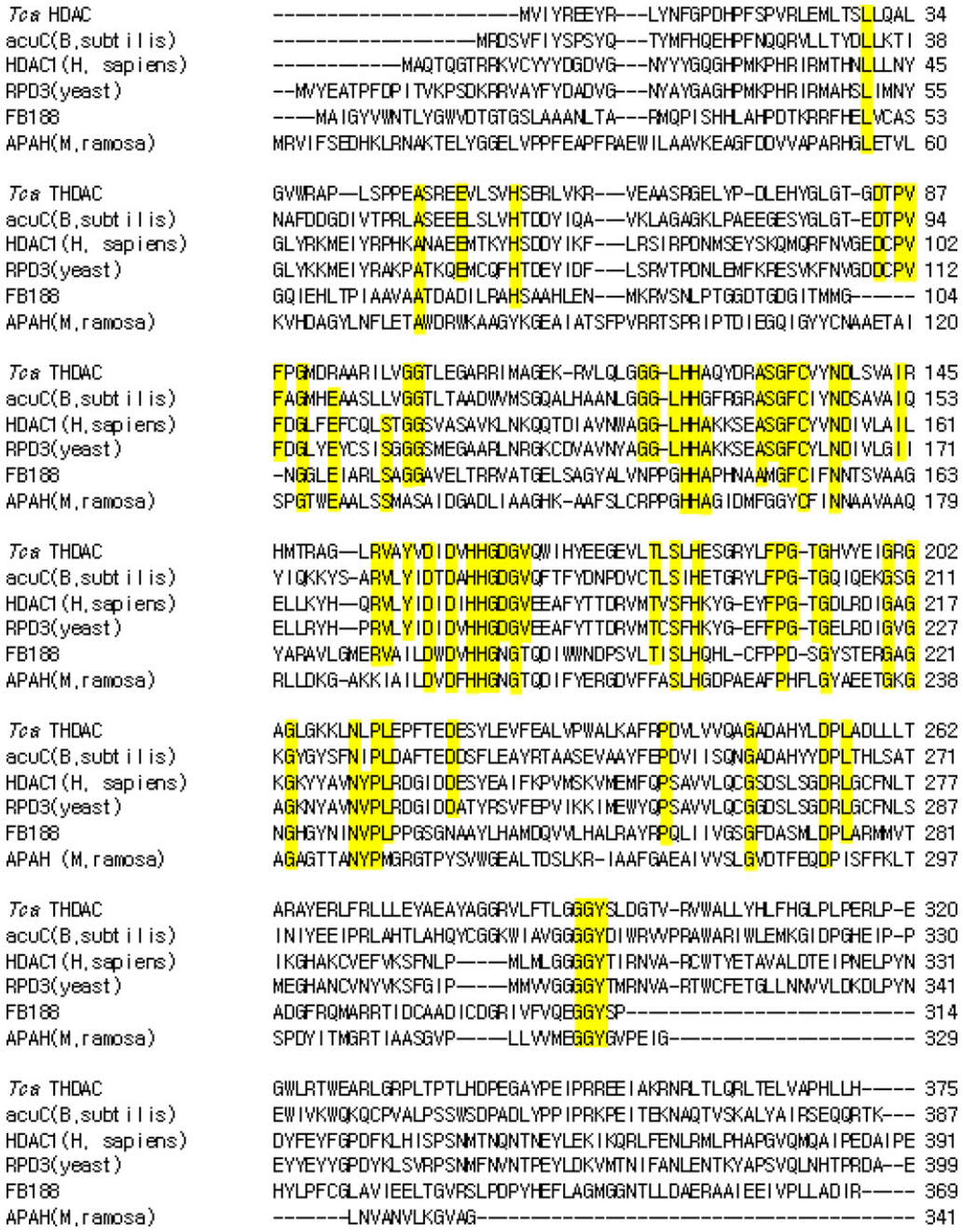


Fig. 1. Multiple sequences of the regions of significant similarity in *Tca* HDAC, acuC gene product, FB188 HDAH, and human HDAC1, and APAH. Consensus residues are indicated in shaded boxes. The following sequences are included in the alignment: *Tca* HDAC (*T. caldophilus* GK24), acuC (*Bacillus subtilis*), HDAC1 (*Homo sapiens*), RPD3 (*Saccharomyces cerevisiae*), HDAH (FB188), HDAH from *Bordetella/Alcaligenes*; APAH (*Mycoplana ramosa*).

calculated molecular mass of 42,188 Da. Multiple sequence alignment of the amino acid sequences of thermophilic *acuC* and several other mesophilic or hyperthermophilic enzymes showed that the putative function of *acuC* from *T. caldophilus* could be histone deacetylase. The H124, H125, D158, D160, H162, and Y293 have been identified as catalytically important residues [21,22]. The sequence analysis revealed that such residues are also conserved in motifs: GG[LM]HHX(4)[RK]AXGFC, D[IL]D[AV]HHX DG[VI]Q, X(2)GXDX(4)DPL and GGGY, respectively, in thermostable *acuC* from *T. caldophilus* GK24. In addition, the *Tca* HDAC showed high identity to *acuC* amino acid sequences of *T. thermophilus* (98% identical residues), *B. subtilis* (42% identical residues), and *Rubrobacter xylanophilus* (47% identical residues). The deduced amino acid sequence of *Tca* HDAC exhibited a 31% identity with human HDAC1, 35% FB188 HDAH [15], 32% yeast RPD3 [23], and 28% APAH [24] (Fig. 1).

Expression and purification of recombinant Tca HDAC

The pGEX-4T-1 vector has a strong *tac* promoter and a GST cluster, which was used to accelerate the protein purification. The *Tca* HDAC gene was amplified from the genomic DNA of *T. caldophilus* GK24 through the PCR by primers specific for *Tca* HDAC. The amplified 1128kb DNA fragment was ligated into an expression vector pGEX-4T-1 through EcoRI and SalI sites to obtain pGEX-4T-1/*Tca* HDAC. The *E. coli* BL21 harboring pGEX-4T-1/*Tca* HDAC was cultured in the presence of IPTG. The recombinant construct pGEX-4T-1/*Tca* HDAC was over-expressed in the soluble fraction at 20 °C, and the cell lysates were analyzed by SDS–PAGE. The GST-*Tca* HDAC proteins were purified from the cell lysates by Glutathione affinity chromatography, as described in Materials and methods, resulting in a single band at 70 kDa (Fig. 2A). Since the GST-*Tca* HDAC has a 31% homology with human HDAC1, we examined whether the HDAC can be recognized by anti-HDAC1 antibody specific for human and mouse HDAC1. Western blot analysis using the antibody showed that the GST-*Tca* HDAC can be detected by the commercially available antibody, implying cross-immunity of these two proteins from different origins (Fig. 2B).

Biochemical characterization of the recombinant Tca HDAC

To investigate whether the purified GST-*Tca* HDAC exhibits the enzymatic activity of deacetylases, we conducted conventional HDAC assay using a fluorogenic

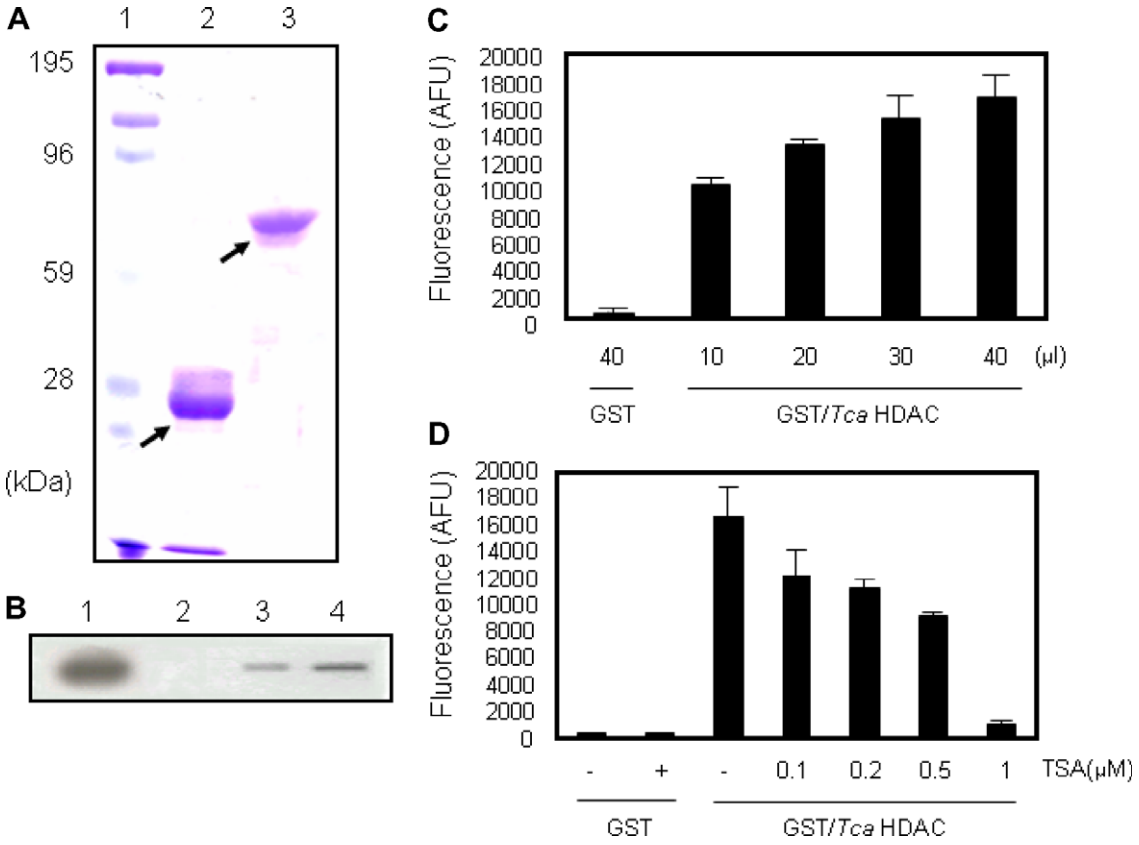


Fig. 2. SDS gel electrophoretic analysis and western blotting of purified enzyme fractions and its HDAC enzymatic actibity. (A) SDS gel electrophoretic analysis. lane 1, molecular weight markers; lane 2, purified GST; lane 3, purified GST-*Tca* HDAC. Protein bands were visualized by Coomassie staining and arrows indicating the purified protein, respectively. (B) Western blotting using the anti-HDAC1 antibody. Lane 1, HeLa lysate; lane 2, GST; lanes 3, 4, GST-*Tca* HDAC. (C) The deacetylase activity of the purified protein toward the fluorogenic substrate for HDAC. (D) A dose-dependent enzyme activity inhibition by trichostatin A, a specific HDAC inhibitor.

substrate for HDAC. As shown in Fig. 2C, the purified protein showed a deacetylase activity toward the HDAC substrate. Moreover, the enzyme activity was inhibited by trichostatin A, a specific HDAC inhibitor, in a dose-dependent manner (Fig. 2D). To determine K_m value for *Tca* HDAC, a kinetics analysis was conducted with increasing substrate concentrations. As shown in Fig. 3A, a typical Michaelis–Menten substrate saturation pattern was obtained for the fluorogenic HDAC substrate. Lineweaver-Burk plot analysis revealed that the apparent K_m value of *Tca* HDAC was 143 μM (Fig. 3B) whereas that of positive control (a HeLa nuclear extract, rich in HDAC activity) was 54 μM. We, next, investigated the optimum temperature and pH conditions for the enzymatic activity GST-*Tca* HDAC. The effect of temperature on *Tca* HDAC activity was determined within a range 40–90 °C. Interestingly, optimum temperature was found to be approximately 70 °C (Fig. 4A). This thermostable feature of the enzyme can be accounted by sequence-based computational analyses. Table 1 demonstrates a statistical comparison of the *Tca* HDAC and human HDAC1 calculated from ProtParam tool (http://kr.expasy.org/tools/protparam.html). *Tca* HDAC shows not only a higher aliphatic index [25], and a higher hydropathicity (GRAVY) [26], but also a lower instability index [27] than human HDAC1, supporting an elevated thermostability of the enzyme.

The dependence of *Tca* HDAC activity on the pH was determined between pH 4.0–12.0. The buffers used were 50 mM sodium citrate (pH 4.0–5.0), 50 mM MES (pH 5.0–7.0), 50 mM Tris–HCl (pH 7.0–9.0), 50 mM Glycine–NaOH (pH 9.0–10.0) and sodium acetate (pH 10.0–12.0). The optimum pH for *Tca* HDAC activity was observed at 7.0 (Fig. 4B). Since class I and II HDACs contain Zn^{2+} in the active site, we subjected the *Tca* HDAC to thorough analysis concerning possible metal cofactors. The enzymes were incubated for 30 min at room temperature in the presence of 1 mM EDTA. The deacetylase activity of the *Tca* HDAC was completely abolished by chelation with EDTA and restored by the addition of $ZnCl_2$, $MgCl_2$, and $MnCl_2$. As a shown in Fig. 4C, the *Tca* HDAC essentially requires divalent metal ions for its enzymatic activity and zinc is the most favorable ion among the ions tested.

In conclusion, the gene encoding *Tca* HDAC from the genus *T. caldophilus* was cloned and expressed. The thermostable HDAC was purified to homogeneity from a thermophilic bacterium, *T. caldophilus* GK24. This is the first report describing the cloning of a gene encoding HDAC from the bacterium. Comparison of the deduced amino acid sequence with those in the National Center for Biotechnology Information databases revealed a number of homologs, all belonging to the HDAC superfamily. The closest homolog of *Tca* HDAC among human HDACs is HDAC1. To facilitate biochemical characterization, the *Tca* HDAC has been cloned and over-expressed in *E. coli*. Purification has been expedited by introducing an N-terminal GST tags into the mature protein. The purified recombinant enzyme exhibits interesting biochemical properties in terms of temperature optimum, pH optimum, and Zn^{2+} cation requirements (Fig. 4). It is noteworthy that optimum temperature of the enzyme is extremely high, 70 °C, than that of human HDACs. This heat stability may contribute stability of the protein as well as provide

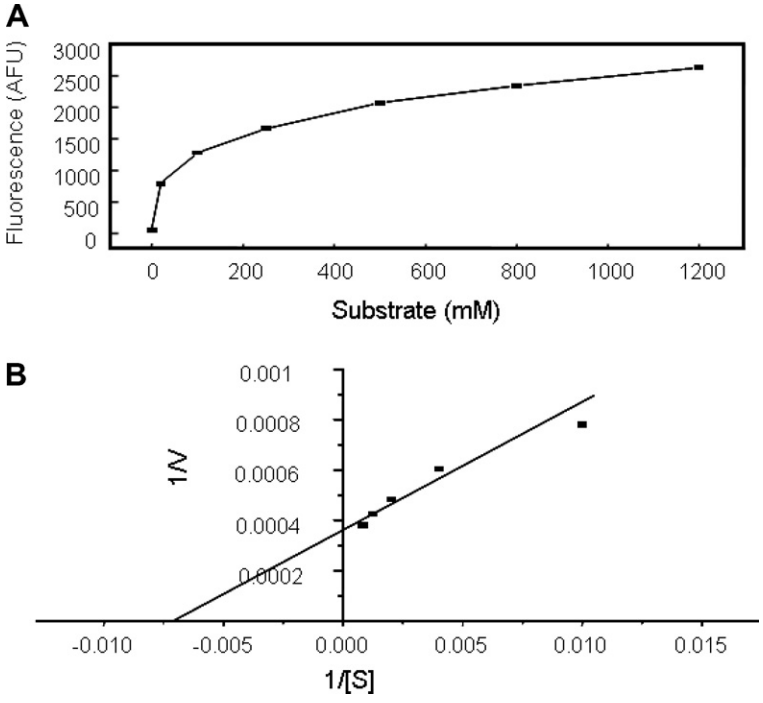
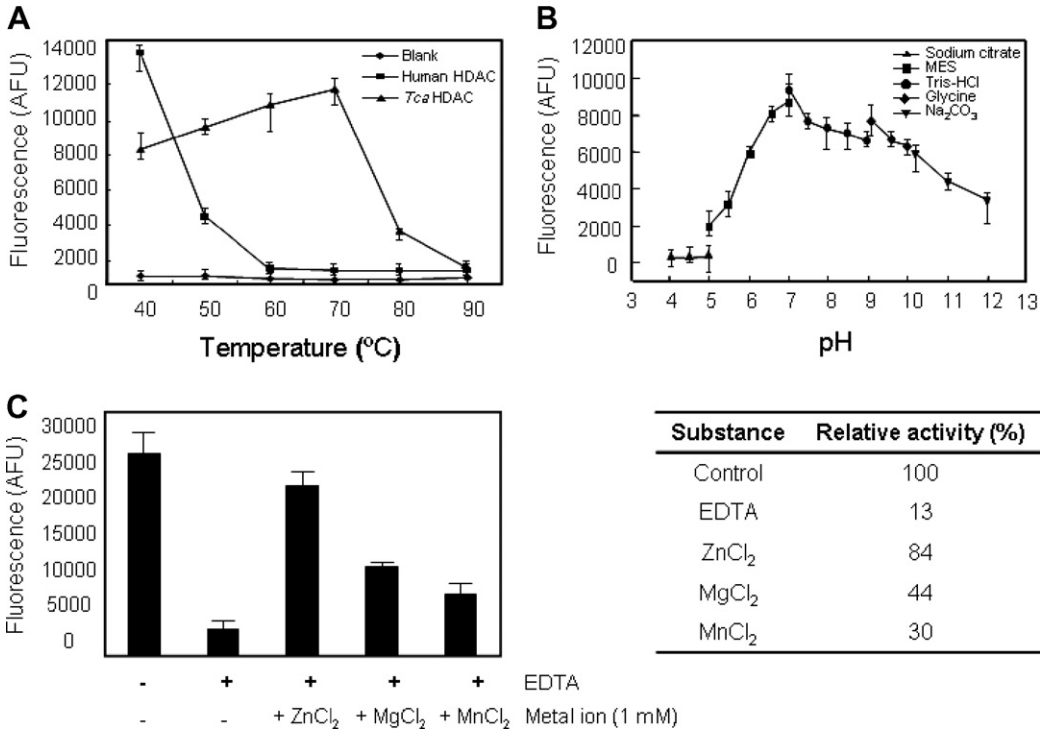


Fig. 3. Measurements of kinetic parameters of *Tca* HDAC. The reaction initial rates of the *Tca* HDAC under a variety of different substrate concentrations were plots against substrate concentrations to obtain the K_m values of the enzyme. (A) Origin computer program was use to fit the kinetic data using Michaelis–Menten equation. (B) Lineweaver–Burk plot of purified recombinant *Tca* HDAC activity.



Substance	Relative activity (%)
Control	100
EDTA	13
$ZnCl_2$	84
$MgCl_2$	44
$MnCl_2$	30

Fig. 4. Determination of the enzymatic activity of the *Tca* HDAC. (A) Effects of temperature on *Tca* HDAC activity was determined within a range 40–90 °C. (B) The dependence of *Tca* HDAC activity on the pH was determined between pH 4.0 and 12.0. The enzymatic activity was measured with 30 μl purified enzyme for 1.5 h at 37 °C. (C) Effect of bivalent cations on deacetylase activity. The deacetylase activity of the *Tca* HDAC was removed by chelation with EDTA and treated metal ions of 1 mM $ZnCl_2$, $MgCl_2$, and $MnCl_2$.

Table 1
Statistical comparison of *Tca* HDAC and Human HDAC1 calculated from ProtParam

Parameters	*Tca* HDAC	HDAC1
Aliphatic index[a]	100.13	65.33
Hydrophathicity (GRAVY[b])	−0.170	−0.710
Instability index[c]	40.22	40.48

These parameters were calculated through ProtParam tool of Expasy website (http://kr.expasy.org/tools/protparam.html).

[a] Relative volume occupied by aliphatic side chains of given protein (alanine, valine, isoleucine, and leucine).

[b] Grand average of hydropathy. The sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence.

[c] An estimate of the stability of protein in a test tube.

new biocatalytic applications such as deacetylation of acetylated amino acids and high throughput screen using the enzyme [15]. In addition, a high homology of *Tca* HDAC with human HDAC1 suggests its conserved biological functions from prokaryotes to eukaryotes. Thus, it would be interesting to investigate whether biological functions of *Tca* HDAC are conserved in eukaryotes. Collectively, the present study provides a new means for functional studies of HDAC in biological evolution and for biocatalytic applications using the enzyme.

Acknowledgments

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References

[1] W.M. Leipe, S.C. Tsai, Y.D. Wen, G. Fejer, E. Seto, Functional domains of histone deacetylase—3, J. Biol. Chem. 277 (2002) 9447–9454.
[2] R. Marmorstein, Structure of histone deacetylases: insights into substrate recognition and catalysis, Structure 9 (2001) 1127–1133.
[3] D.D. Leipe, D. Landsman, Histone deacetylases, acetoin utilization proteins and acetylpolyamine amidohydrolases are members of an ancient protein superfamily, Nucl. Acids Res. 25 (1997) 3693–3697.
[4] S. Khochbin, A.P. Wolffe, The origin and utility of histone deacetylase, FEBS Lett. 419 (1997) 157–160.
[5] D.E. Ayer, Histone deacetylase: transcriptional repression with SINers and NuRDs, Trends Cell Biol. 9 (1999) 193–198.
[6] W.D. Cress, E. Seto, Histone deacetylases, transcriptional control, and cancer, J. Cell Physiol. 184 (2000) 1–16.
[7] S. Timmermann, H. Lehrmann, A. Polesskaya, A. Harel-Bellan, Histone acetylation and disease, Cell. Mol. Life Sci. 58 (2001) 728–736.
[8] Y. Zhang, H.H. Ng, H. Erdjument-Bromage, P. Tempst, A. Bird, D. Reinberg, Analysis of the NuRD subunits reveals an histone deacetylase core complex and a connection with DNA methylation, Genes Dev. 13 (1999) 1924–1935.
[9] W. Fischle, F. Dequiedt, M.J. Hendzel, M.G. Guenther, M.A. Lazar, W. Voelter, E. Verdin, Enzymatic activity associated wih calss-II HDACs is dependent on a mutiprotein complex containing HDAC3 and SMRT/N-CoR, Mol. Cell 9 (2002) 45–57.
[10] M.G. Guenther, O. Barak, M.A. Lazar, The SMRT and N-CoR corepressors are activating cofactors for histone deacetylase-3, Mol. Cell. Biol. 21 (2001) 6091–6101.

[11] M.G. Guenther, W.S. Lane, W. Fischle, E. Verdin, M.A. Lazar, R. Shiekhatter, A core SMRT corepressor complex containing HDAC3 and TBL1, a WD40-repeat protein linked to deafness, Genes Dev. 14 (2000) 1048–1057.
[12] S.I. Imai, C.M. Armstrong, M. Kaeberlein, L. Guarente, Transcriptional silencing and longevity protein Sir2 id an NAD-dependent histone-deacetylase, Nature 403 (2000) 795–800.
[13] J. Landry, A. Sutton, S.T. Tafrov, R.C. Heller, J. Stebbins, L. Pillus, R. Sternglanz, The silencing protein SIR2 and its homology are MAD-dependent protein deacetylase, Proc. Nat. Acad. Sci. 97 (2000) 5807–5811.
[14] T. Lechner, M.J. Carrozza, Y.X. Yu, P.A. Grant, A. Eberharter, D. Vannier, G. Brosch, D.J. Stillman, D. Shore, J.L. Workman, Sds3 (suppressor of defective silencing-3) is an integral component of the yeast Sin3/Rpd3 histone deacetylase complex and is required for histone deacetylase activity, J. Biol. Chem. 275 (2000) 40961–40966.
[15] C. Hildmann, M. Ninkovic, R. Dietrich, D. Wegener, D. Riester, T. Zimmermann, O.M. Birch, C. Bernegger, P. Loidl, A. Schwienhorst, A new amidohydrolase from *Bordetella* or *Alcaligenes* strain FB188 with similarities to histone deacetylases, J. Bacteriol. 186 (2004) 2328–2339.
[16] M.S. Finnin, J.R. Donigian, A. Cohen, V.M. Richon, R.A. Rifkind, P.A. Marks, R. Breslow, N.P. Pavletich, Structures of a histone deacetylase homologue bound to the TSA and SAHA inhibitors, Nature 401 (1999) 188–193.
[17] H.S. Hoe, I.G. Jo, H.J. Shin, H.K. Kim, J.S. Lee, Y.S. Kim, D.S. Lee, S.T. Kwon, Cloning and expression of the gene for inorganic pyrophosphatase of *Thermus caldophilus* GK24 and properties of the enzyme, J. Microbiol. Biotechnol. 12 (2002) 301–305.
[18] J. Marmur, A procedure for isolation of deoxyribonucleic acid from micro-organism, J. Mol. Biol. 3 (1961) 208–218.
[19] X. Huang, A. Madan, CAP3: a DNA sequence assembly program, Genome Res. 9 (1999) 868–877.
[20] J. Sambrook, E.F. Fritsch, T. Maniatis, Molecular Cloning. A Laboratory Manual, second ed., Cold Spring Harbor Laboratory press, Cold Spring Harbor, New York, 1989.
[21] C.A. Hassig, J.K. Tong, T.C. Fleischer, T. Owa, P.G. Grable, D.E. Ayer, S.L. Schreiber, A role for histone deacetylase activity in HDAC1-mediated transcriptional repression, Proc. Natl. Acad. Sci. 95 (1998) 3519–3524.
[22] A.K. Ingram, D. Horn, Histone deacetylases in *Trypanosoma brucei*: two are essential and another is required for normal cell cycle progression, Mol. Microbiol. 45 (2002) 89–97.
[23] M. Vidal, R.F. Gaber, RPD3 encodes a second factor required to achieve maximum positive and negative transcriptional states in *Saccharomyces cerevisiae*, Mol. Cell. Biol. 11 (1991) 6317–6327.
[24] K. Sakurada, T. Ohta, K. Fujishiro, M. Hasegawa, K. Aisaka, Acetylpolyamine amidohydrolase from *Mycoplana ramosa*: gene cloning and characterization of the metal-substituted enzyme, J. Bacteriol. 178 (1996) 5781–5786.
[25] A. Ikai, Thermostability and aliphatic index of globular proteins, J. Biochem. 88 (1980) 1895–1898.
[26] J. Kyte, R.F. Doolittle, A simple method for displaying the hydropathic character of a protein, J. Mol. Biol. 157 (1982) 105–132.
[27] K. Guruprasad, B.V.B. Reddy, M.W. Pandit, Correlation between stability of a protein and its dipeptide composition: a novel approach for predicting in vivo stability of a protein from its primary sequence, Protein Eng. 4 (1990) 155–161.