

CLONING, EXPRESSION AND PURIFICATION OF HUMAN TRUNCATED SIRT1

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Abstract- Sirtuins belong to an evolutionarily conserved class of proteins that regulate a variety of cellular functions such as genome maintenance, longevity, and metabolism. The conserved part of amino acids residues 244-498 of full length human SIRT1 have the functional activity. To construct a functional form of SIRT1 by DNA recombinant technology, we have expressed and purified truncated protein of 420 (193 to 611) amino acids of MW 47 kDa in prokaryotic system, *Ecoli* BL21 (DE3) strain. This functional protein has a potential to be used as a diagnostic tool and for the development of new therapeutic agent for age related disease. **Key words-** Truncated SIRT1; Protein expression; Mass Spectroscopy; Western blot.

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Introduction

Sirtuin or silence information regulator comprises an universal conserved family of NAD+ (Nicotinamide adenine dinucleotide) dependent deacetylases conserved from bacteria to human and play a role in a wide variety of important biological processes including development, heterochromatin formation, transcriptional silencing, DNA recombination and repair, genomic stability, apoptosis, axonal protection, fat metabolism, metabolic regulation and longevity [1,2]. This enzyme was first isolated from yeast, and later it was found to be present in mammals including humans. There are seven human sirtuins (1-7) that display diversity in cellular localization and function. They contain a conserved catalytic core domain comprising of approximately 275 amino acids. SIRT1 is most studied among the 7 sirtuin family member, is highly expressed in several adult tissue such as brain, heart and skeletal muscle [3]. Calorie restriction, the only known mechanism which has been reported to delay ageing is mediated by sirtuin gene family which were shown to have anti ageing function in yeast [4], Caenorhabtidis [5], Drosophila [6] and mammals including rodents [7], and perhaps primates [8, 9]. SIRT1 is localized in the nucleus [10, 11] and cytoplasm [12]. It interacts and deacetylates many proteins like p53 [10, 11], the forkhead transcription factor FOXO3A [13], BCL6 [14], TAF68 [15], HES1 [16] and CTIP2 [17]. It is an important mediator of longevity through induction of cell cycle arrest and resistance to oxidative stress by deacetylating FOXO3. Sirtuins have emerged as a therapeutic target to treat age related diseases. SIRT1 controls insulin secretion from pancreatic beta cells via UCP2, FOXO1 and NAD metabolism and can help on the development of new therapeutic tool for insulin resistance and type2 diabetes [18]. It promotes fatty acid mobilization in white adipose tissues by deacetylating and repressing PPARy (Peroxisome proliferator activated receptor y) [19]. In skeletal muscles, it results in increased aerobic capacity [20, 21] while in liver it increases gluconeogenisis [22] by deacetylating and activating PGC1a (PPAR1y coactivator 1a). Three different mRNA sequence of human SIRT1 are known from Gene bank NM_012238 (4110 bp), NM_001142498.1 (3604 bp) and BC012499.1 (3691 bp) respectively, with their respective frames encoding 747, 452 and 555 amino acids residues. The preliminary crystal structure of truncated SIRT1 from 140-747 amino acid residues is also reported [23]. In the present study, we report the cloning, expression and purification of truncated human sirtuin constructed from 193-611 amino acid residues.

Materials and methods Bio-informatics Analysis

mRNA sequence of human SIRT1 gene was obtained from NCBI Gen Bank (www.ncbi.nlm.nih.gov/genbank/) under accession No. NM_012238. NCBI ORF finder (www.ncbi.nlm.nih.gov/projects/ gorf/) was used to find out the ORF and respective amino acid sequence. The deduced amino acids sequence was analyzed by Expasy MW/pI (web.expasy.org/compute_pi/) to calculate the molecular weight and pl of the protein. Homologues were explored by BLAST programme (blast.ncbi.nlm.nih.gov/Blast.cgi) in NCBI Gen Bank database and multiple sequence alignment was performed using Clustal W programme. (www.ebi.ac.uk/Tools/msa/ clustalw2/).

Cloning PCR

Truncated SIRT1 gene was amplified by using Pfu Taq Polymerase (Phusion). The forward and reverse primers used for the amplification were CGGGATCATGATTGGCACAGATCC and CCGCTCGAGCTATTCATTTTTCTCCCC respectively having BamH1 and Xho1 sites.

Cloning into pGEMT easy vector

The amplified product was purified using PCR cleanup kit (Promega) and adenylated by Taq polymerase followed by cloning into pGEMT easy vector (Promega). The ligation mixture was transformed into *E.coli* DH5 α competent cells and positive clones were identified by blue white screening. The sequence of positive clones was confirmed by PCR-based sequencer.

Sub-cloning into expression vector

Plasmid was extracted from the positive clones by using Plasmid Purification Kit (Promega). Directional cloning was performed by using BamH1 and Xho1 as a restriction sites to clone truncated SIRT1 gene into pET28a⁺ vector using T4DNA ligase. Ligation mixture was then transformed into expression host *E.coli* BL21 (DE3) competent cells.

Expression

E.coli BL21 cells possessing recombinant plasmid were allowed to grow in 100 ml of LB media containing Kanamycin (50 mg/L) at 37°C until absorbance at 600 nm reached 0.6 and then induced with 1 mM IPTG(Isopropyl β -D-1-thiogalactopyranoside). The cells were harvested after 5 hrs of induction and weighed. The induced and uninduced cultures were analysed by SDS- PAGE to ensure the recombinant protein expression.

Protein purification and on column refolding

Inclusion bodies obtained after sonication of the 0.3 grams of harvested cells in 1.2 ml of 50 mM Tris-HCl were re-suspended in Buffer A (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0 and 8 M urea), centrifuged and the supernatant was loaded on 1 ml of Ni-NTA resin (Qiagen) pre equilibrated with Buffer A. The column was successively washed with Buffer B (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 8 M urea and 20 mM imidazole), Buffer C (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, and 20 mM imidazole), Buffer D (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol) and Buffer E (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol) and Buffer E (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol) and Buffer E (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol) and Buffer E (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole and 10% glycerol) and Buffer E (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole phosphate pH 8.0, Tris-HCl pH 8.0, 20 mM imidazole phosphate pho

azole, 10% Glycerol and 300 mM NaCl). Finally, the recombinant protein was eluted with Buffer F (100 mM sodium phosphate pH 8.0, Tris-HCl pH 8.0, 300 mM imidazole and 10% glycerol and 300 mM NaCl) in a refolded state.

Characterization of truncated SIRT1 Western Blotting

Purified protein along with human serum sample was separated by SDS PAGE and transferred onto PVDF membrane (MDI). Membrane was then blocked with 3% Bovine Serum Albumin (Bio Basic Inc.) prepared in TBS1(150 mM NaCl and 10 mM Tris.HCl pH 7.5) for 2 hrs followed by overnight incubation in 1:500 rabbit antihuman SIRT1 polyclonal antibody(Enzo Life Sciences). The blot was then washed with TBS-T (500 mM NaCl, 20mM Tris HCl pH 7.5 and 0.05% Tween) 3 times for 10mins and incubated with 1:5000 HRP (Horse Radish Peroxidase) conjugated goat secondary antibody (Genescript). The membrane was washed as above with TBS-T and visualized using Enhanced Chemiluminescent system (Thermo Scientific).

Surface Plasmon resonance

All SPR measurements were performed at 25°C using the BIAcore-2000 apparatus (Pharmacia Biosensor AB, Uppsala, Sweden) which is a biosensor-based system for real time specific interaction analysis. The IgG SIRT1 of human origin (ENZO Biotechnology, CA) was immobilized on the CM5 sensor chip using the amine coupling kit (Pharmacia Biosensor AB, Sweden). The running buffer used was 10 mM HBS-EP (10 mM HEPES pH 7.4, 150 mM Sodium Chloride, 3 mM EDTA, 0.005% Polysorbate 20) containing 0.005% surfactant P20. The dextran on the chip was equilibrated with running buffer and was activated with an equal volume (110 µl) of EDC (N-ethyl-N'-3 diethylaminopropyl carbodiimide) (15 mg in 200 µl of water) and NHS (Nhydroxysuccinimide) (2.3 mg in 200 µl of water) mixture. This mixture was passed at a flow rate of 5 µl/min for 15 mins. Then 250 µl of antibody with 100 µl in 10 mM sodium acetate (pH 4.7) was injected at a flow rate of 5 µl/min and the unreacted groups were blocked by ethanolamine (pH 8.5).

Six different concentrations of the purified human truncated SIRT1 (0.62, 3.12, 6.25, 18.75, 31.25, 62.25 ng) were passed over the immobilized SIRT1 antibody. As the analyte (pure human truncated SIRT1) binds with the immobilized anti-IgG SIRT1 (ligand) over the CM5 sensor chip, an increase in SPR signal (expressed in response units, RU) was observed. The binding of the pure human truncated SIRT1was monitored by the change in RU values of the sensogram and the corresponding RUs were obtained.

Mass Spectrometry Analysis

The purified protein band was excised from the gel and then subjected to in gel reduction, alkylation and tryptic digestion. The MS/ MS was used to determine the mass and sequence of the protein. Each of the peptide was used to BLAST search to confirm that the protein identified by Mascot search programme was the only relevant match in the non redundant protein database for a particular peptide sequence and statistically significant hits were recorded together with the number of peptides and percentage coverage of the protein.

Activity assay for SIRT1

Synthesis of Acetylated N terminal tail of histone H3(AcH3)

Acetyl lysine peptide based on the sequence of N terminal tail of histone H3(AcH3) having sequence H₂N-KSTGGK(COCH₃) APRKQ-OH was synthesised by solid phase peptide synthesis using automated peptide synthesizer (PS3 Protein technology, USA) using Fmoc and Wang resin (G.L. Biochem, China) chemistry. The solvent used for synthesis was DMF (dimethylformamide). HBTU (2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) was used as an activator of the Fmoc amino acids. Fmoc was deprotected by 20% piperidine and Wang resin was cleaved by TFA (trifluoroacetic acid). The peptides were precipitated from dry ether.

Purification of Nicotinamidase

E.coli BL21(DE3) cells harboring plasmid pPNC2 which encodes nicotinamidase protein were allowed to grow until absorbance at 600 nm reached 0.6 and then induced with 1 mM IPTG. Harvested cells were dissolved in buffer1 (20 mM sodium phosphate buffer pH 7.5, 500 mM NaCl and 20 mM imidazole), sonicated and the cell free extract was loaded on Ni-NTA resin (Qiagen) pre equilibrated with buffer1, followed by washing with the same buffer and finally the protein was eluted with buffer2 (20 mM sodium phosphate buffer pH 7.5, 500 mM NaCl and 500 mM imidazole). [24] *Enzyme coupled assay*

Reaction mixture for the coupled enzymatic assay consisted of 3.3 mM AcH3, 3 mM NAD⁺, 2.4 mM NAD(P)H (Nicotinamide adenine dinucleotide phosphate), 13 mM DTT (Dithiothreitol), 13.7 mM α KG (α Ketoglutarate), 2 units of GDH (Glutamate dehydrogenase) and 2 μ M Nicotinamidase. All assay components except SIRT1 were incubated until absorbance at 340 nm gets stabilized and the reaction was initialized by the addition of truncated SIRT1 protein [25].

Results

Bioinformatics Analysis

The sequence of a 4110 bp mRNA of full length human SIRT1 was obtained from Gen Bank under accession no. NM_012238 which contain an ORF of 2244 bp encoding a protein of 747 amino acid residues. Since the catalytic core of full length human SIRT1 spans a region of 244-498 amino acid residues, forward and reverse primers were designed to amplify a1260 bp fragment starting 577 bp downstream of the original ORF initiation site encoding amino acid sequences from 193-612 residues (Fig1). The gene bank accession No. is JQ768366.The deduced protein was found to be 47 kDa having a pl value of 5.1 by using Bioinformatics tool.

Cloning

Truncated SIRT1 gene was amplified using Pfu polymerase and the PCR product was found to be of 1260 bp. The purified PCR product was then cloned into pGEMT easy vector successfully and sequencing results showed that there was no nucleotide error in the truncated SIRT1 gene.

Prokaryotic expression vector pET28a⁺ which possesses a high stringency T7 promoter and an N- terminal His Tag has been recognized as one of the most powerful tool for producing recombinant proteins in *E. coli*. The vector also possesses a thrombin cleavage site. The truncated SIRT1 gene was directionally sub-

cloned into pET28a⁺ and transformed into E.coli BL21(DE3) cells.



Fig. 1- The cDNA sequence and deduced 193-612 amino acid residues of human truncated SIRT1

Expression and Purification

The expression of truncated gene was induced with 1 mM IPTG. Induced and uninduced cultures were compared by SDS PAGE and a specific band of about 51 kDa corresponding to truncated SIRT1 protein was detected in induced culture (Fig 2).



Fig. 2- Expression of human truncated SIRT1 in *E. coli* BL21 cells. Lane 1: molecular weight marker, Lane2 and 3: uninduced and induced cells respectively

International Journal of Molecular Biology ISSN: 0976-0482 & E-ISSN: 0976-0490, Volume 3, Issue 2, 2012 In order to obtain highly purified recombinant protein the inclusion bodies were solubilised in 8M urea and passed through Ni-NTA resin. The purity of the eluted recombinant protein was analyzed by SDS PAGE which detected a single band of about 51 kDa (Fig 3). The protein sequence obtained of molecular weight 47 kDa showed 51 kDa band in SDS PAGE due to the presence of fused His tag along with extra amino acid residues provided by the vector. Recombinant truncated SIRT1 was produced in large quantities, greater than 5% of the total cell proteins.



Fig. 3- Purification of human truncated SIRT1 by Ni-NTA column: Lane1: Molecular weight marker, Lane2-5:flow through and washings, Lane 6-10: refolded purified protein

Western Blot

Purified truncated SIRT1 which reacted with rabbit anti SIRT1 polyclonal antibody was apparent as a single band. To compare the full length and truncated protein we used human serum sample and found that the molecular weight of full length human SIRT1 was of around 81KDa while the truncated protein was of only 51 kDa (Fig 4).



Fig. 4- Western blot showing the specificity of human truncated SIRT1 compared to SIRT1 present in human serum

Truncated SIRT1 assessment by SPR.

The SPR signal for immobilization of antibody of SIRT1 was found to be 14908.5 RU where 1 RU corresponds to immobilized protein concentration of 1 pg/mm². Six different amounts of pure recombinant SIRT1 in HBS-EP buffer were passed over the immobilized SIRT1 anti body and the RU obtained were 14918.4, 15021.1, 15115.5, 15473.6, 15768.3 and 16982.0 RU. The curve was plotted with RU obtained from the sensogram with different concentrations of pure truncated SIRT1 as mentioned above. The binding of the ligands i.e. truncated SIRT1 were in the linear range shown in Fig 5. The RU increased linearly as the concentration of truncated SIRT1 increases.



Fig. 5- RU values obtained by passing six different amounts (0.62 ng, 3.12 ng 6.25 ng, 18.75 ng, 31.25 ng and 62.25 ng) of truncated SIRT1 over immobilized antiSIRT1 antibody

MASS Spectrometry

The purified protein band was excised from gel and identified through Mass Spectrometry. The trypsanized fragments were analyzed through a mascot search program from matrix science shows candidate proteins as SIRT1 and a score of 116 based on probability analysis. The score of 116 for an MS/MS match in Mascot search depends upon the absolute probability that the selected match between reported data and the database sequence is a random event.

Enzyme Activity

The activity assay was performed as described by Smith et al in which the rate of nicotinamide formation was measured using a coupled enzyme system with nicotinamidase and glutamate dehydrogenase. Nicotinamidase hydrolyses nicotinamide to nicotinic acid and ammonia. Glutamate dehydrogenase then converts ammonia, α -ketoglutarate, and NAD(P)H to glutamate and NAD(P)⁺. NAD(P)H oxidation/consumption is measured spectrophotometrically at 340 nm. A linear response was obtained from the coupled enzyme reaction (Fig 6). Through this assay it was established that the purified truncated protein possess histone deacetylase activity.



Fig. 6- Activity assay of human truncated SIRT1: Linear response due to the consumption of NAD(P)H with time.

Discussion

The first gene discovered in the Sirtuin family was Sir2 from yeast, is able to extend the life span in various organisms. Seven human homologous of sirtuins (SIRT1-7) which share the catalytic domain with Sir2 have been characterized [26, 27]. The anti ageing effects of human homologous of sirtuins are also seen by animal and human associated studies.SIRT1, the most studied of 7 sirtuin family members, is a key mediator of the pathways down-

International Journal of Molecular Biology ISSN: 0976-0482 & E-ISSN: 0976-0490, Volume 3, Issue 2, 2012 stream of calorie restriction, dietary regimen that is known to delay the onset of ageing and reduce the incidence of age related diseases. SIRT1 has been proved to be a potential therapeutic target for age associated diseases.

Since the catalytic domain of full length human sirtuin consist of 255 conserved amino acids (244-498 aa), we have cloned and expressed a protein from 193 to 611 amino acids residues to keep the activity intact in expressed protein. The primers were designed based on the sequence of human SIRT1 mRNA sequence which was obtained from NCBI (NM_012238) of 4110 bp. The cloned gene includes an ORF of 1260 bp encoding 420 amino acids residues.

The protein was characterized by mass spectroscopy, SDS PAGE and Western Blot was found to be of 51 kDa and pl value of 5.1. The specificity of protein was identified by using its specific antibody by western blotting and SPR. In SPR the sensogram increases linearly as the mass of the SIRT1 increases over the antibody.

This sequence shows high similarity with other species' SIRT1. The amino acid sequence of truncated SIRT1 sequence shared 99% identity to SIRT1FL_human, 97% identity to Sus scrofa, 94% identity to Bos taurus, 93% to Cricetulus griseus, 93% to Mus musculus, 94% to Oryctolagus cuniculus, 92% to Callithrix jacchus, 91% to Ailuropoda melanoleuc, 93% to Mustela putorius furo, 51% to Drosophila melanogaster and 41% to Caenorhabditis elegans (Table 1). The Fig. 7 shows the high homology of truncated SIRT1 with the conserved catalytic domain of other mammalian SIRT1.

SIRTItruncated_human	I EDAVKLLQECKKIIVLTGAGVSVSCGIPDFRSRDGIYARLAVDFPDLPD	100
SIRIFL numan	I KDAVKLEQKCKKIIVEIGAGVSVSCGIPDFPSRDGI YARLAVDFPDEPD	292
Sirtuini_tailithrix_jacenus	I KDAVKLEQKCKKIIVEIGAGVSVSCGIPDFFSRDGI YARLAVDFPDEPD	297
Sircumi_Sus_scrora	I EDAVALLQECKETTVLTGAGVSVSCGTPDFFSRDGTTARLAVDFPDLPD	286
Sirtuini Bos caurus	T EDAUGI LOR CEVETIULECACUSUS COLDER DE DEDECTIVALI MENERLA	100
Sirtuin 1 Mus musculus	TEDAUNI LORCHYTTULTCACUSUS CCTEDE DEDDCTYADI AUDEDDI DD	122
Sircumi_nus_musculus	TEDAVALLQBCARTIVLICAGE SVSCGIPDF RSRDGITAR LAVDFPDLPD	123
cirtuin hilurenede relevalous	T EDAUNI LOR CUVITIVI TO ACUSUS COLIDER DEDOCTIVADI ATDEDDI DD	254
Sircular Antropoda metanoleuc	TEDAUGI LORCHATTULECACUCUCCCTEDERCEDECTVALLATEREDIDE	1.09
Sifeami_nascera_pacorias_taro	***************************************	100
SIRT ltruncated human	POAMEDI EYF RED PRP FFK FAK EIY PGO FOP SLCHKF IAL SDK EGKLLEN	1.50
SIRTIFL human	P QAMFDI EYF PRO PRP FFKFAKETY PGQ FQP SLCHRF IAL SOR EGRLLRN	342
sirtuinl Callithrix jacchus	P QAMFDIEYF FRD PRP FFRFARETY PGQ FQP SLCHRF IAL SDREGKLLRN	347
Sirtuinl Sus scrofa	P QAMFDIEYF FRD PRP FFKFARETY PGQ FQP SLCHFF IAL SDREGKLLRN	336
Sirtuinl Bos taurus	P QAMFDIEYF RED PRP FFKFAKETY PGQ FQP SLCHEF IAL SDEEGELLEN	327
Sirtuinl_Cricetulus_griseus	P QAMFDI EYF RED PRP FFE FAREIY PGQ FQP SLCHEF IAL SDE EGELLRN	150
Sirtuinl Mus_musculus	P QAMFDI EYF RED PRP FFEFAEETY PGQ FQP SLCHEF IAL SDE EGELLRN	173
Sirtuinl_Oryctolagus_cuniculus	P QAMFDI EYF RED PRP FFEFAEETY PGQ FQP SLCHEF IAL SDE EGELLRN	302
sirtuinl_Ailuropoda_melanoleuc	P QAMFDI EYF PRO PRP FFKFAKETY PGQ FQP SLCHKF IAL SDKEGKLLRN	304
Sirtuinl_Mustela_putorius_furo	P QAMFDI EYF PRD PRP FFKFAKEIY PGQ FQP SLCHKF IAL SDKECKLLRN	208
GTDE LA CALL A LA CALL		0.00
SIRIEruncaced numan	Y TONIDI DEQUACIONI QUE CENTROLLI CRITED CENTROLLE SOVV	200
sirtuinl Callithriv jacobus	VTONITTLEOUSCIOPTIOCHCSES SSCLICKVKUDCESUBCDIEMOUU	392
Sirtuinl Sus scrofe	YTON TOT LEOVAGTOD TTOCHGSRA TASCLT CKYKUD CRAUDEDTEMOUU	386
Sirtuinl Bos teurus	YTONIDT LEOVACTORITOCHCSEA TASCLICKYRUD CRAUDCDIEMOUU	377
Sirtuinl Cricetulus griseus	YTONTOT LEOVAGTOD TTOCHGSRA TASCLT CKYKUD CRTUDGDTRMOUV	200
Sirtuinl Mus musculus	YTON IDTLEOVAGIORILOCHOSFA TASCLICKYKVD CRAVEGDIFNOVV	223
Sirtuinl Orvetolagus cuniculus	YTON IDT LEOVAGIORIIOCHGSFATASCLICKYKVD CEAVEGDIFNOVV	352
sirtuinl Ailuropoda melanoleuc	Y TON IDT LEOVAGIORIIOCHGSFATASCLICKYRVD CEAVEGDIFNOVV	354
Sirtuinl_Mustela_putorius_furo	YTQNIDTLEQVAGIORIIQCHGSFATASCLICKYKVDCEAVRGDIFNQVV	2.58
STRT ltrimcated himan	PROPROPADE PLA THE PREVER GEN LPROPH PAMEYDED RUDI. LTW 1688	2.50
SIRTIFL human	P RCP RCPADE PLA THE PET VEF GEN LPROFH RAMEYDED RUDL LTV TGSS	442
sirtuinl Callithrix jacchus	PROPROPADE PLA IMK PEIVFF GENLPE OFHRAMKYDKDEADL LIVIGSS	447
Sirtuinl Sus scrofa	P RCP RCP ADE PLA IMK PEI VFF GEN LPE QFH RAMKYD KDE VDL LIVIGSS	436
Sirtuinl Bos taurus	P RCP RCP ADE PLA IMK PEI VFF GEN LPE OFH RAMKYD KDE VDL LIVIGSS	423
Sirtuinl_Cricetulus_griseus	PRCPRCPADE PLAIMK PEIVFF GENLPE OFHRAMKYDKDEVDLLIVIGSS	250
Sirtuinl Mus_musculus	PROPROPADE PLAINK PEIVFF GENLPE OF HRAMKYD KDEVDLLIVIGSS	273
Sirtuinl_Oryctolagus_cuniculus	PROPROPADE PLAINK PEIVFF GENLPE OF HRAMKYD KDEVDLLIVIGSS	402
sirtuinl_Ailuropoda_melanoleuc	PROPROPADE PLA INK PEIVFF GENLPE OF HRAMKYD KDEVDLLIVIGSS	404
Sirtuinl_Mustela_putorius_furo	PROPROPADE PLA IME PEI VFF GEN LPE OFHRAMEYDEDE VOL LIVIGSS	308
14-20-20-20-20-20-20-20-20-20-20-20-20-20-	****	-
SIRTitruncated_human	LEVE PVALIPSSIPHEVPQILINE PLPHLHFDVELLGDCDVIINELCHE	300
SIRTIFL_human	LEVEPVALIPSSIPHEVPQILINEEPLPHLHFDVELLGDCDVIINELCHE	492
sirtuinl_Callithrix_jacchus	LKVR PVALIPSSI PHEVPQILINRE PLPHLHFDVELLGDCDVIINELCHR	497
Sirtuni_Sus_scrofa	LEVEPVALIPSSIPHEVPUILINEEPLPHLHFDVELLGDCDVIINELCHE	486
Sircumi_Bos_caurus	I VID NUL I TREAT NURINO TI TIME DI DUI MEDIURI I COCOVI INELICIE	200
Sircumi_criceculus_griseus	I MID BUAL TD COT DURING TI THERE DI DUI HEDDIRI I CDCDVI INE BORK	222
Sircumi_nus_musculus	I VID BUAL TREAT DURING TI THER DI DULURDURI I COCNUT THEI CUD	4 520
sircumi_oryccoragus_cumcurus	I VID NUAL TROOT DURING TI THERE DI DUI MEDIZELI COCUVITINE I CHD	400
Sirtuin Mustele mutorius furo	LVUD DUALTD SST DHEUDO TI TNDE DI DHI H EDU ELL CDCDUT THE LCHD	350
birodin_nabotin_pacor na_nato	* * * * * * * * * * * * * * * * * * * *	000
SIRTItruncated_human	LGGEYAKLCCNPVKLSEITEKPPRTQKELAYLSELPPTPLHVSEDSSSPE	350
SIRTIFL_human	L CCEYAKLCONPUKLS BIT EKP PRT OKE LAYLSE LPP TPLHUS EDSSSPE	542
sirtuinl_Callithrix_jacchus	L GGE YAKLCONPVKLSEITEKP PRT QKE LAYLSE LPP TPLHISEDSSSPE	547
Sirtuinl_Sus_scrofa	L GGE YAKLCONPVKLS EIT EKP PRT OKE LAHLSE LPP TPLNIS EGSSSP E	536
Sirtuinl_Bos_taurus	L GGEYAKLCCNPVKLSEITEKP PRIQKELAHLSELPPTPLNISEGSSSPE	527
Sirtuinl_Cricetulus_griseus	LGGEYAKLCCNPVKLSEITERPPRTQKELVHLSELPPTPLHISEDSSSPE	350
Sirtuini_Mus_musculus	LGGEYAKLCONPURISEITERPPRPQKELUHLSELPPTPLHISEDSSSPE	373
Sirtuni_Uryctolagus_cuniculus	LGGEYARLCUNFIRLS BIT KRP PRT QRE LWHLSE LPP TPLHISEDSSSPE	502
Sircuni_Alturopoda_melanoleuc	LOGE TAR LCONPURES BIT ERP PRTUKE LAHLAE LPP TPLNIS EDSSSPE	504
sircuini_Mustela_putorius_furo	A GOR TARADOONPVKLS BIT KKP PRTUKE LAHDSE DPP TPLNIS KDS SSPE	408

Fig. 7- The sequence alignment of catalytic domain of human truncated SIRT1 with other mammalian SIRT1

Table1- The sequence similarity of human truncated SIRT1 with
sirtuin of other species

Species name	Gen bank accession no.	Percentage identity
Homo sapiens	NP_036370.2	99
Callithrix jacchus	XP_002756358.1	92
Sus scrofa	NP_001139222.1	97
Bos taurus	NP_001179909.1	94
Cricetulus griseus	EGW03730.1	93
Mus musculus	AAI52315.1	93
Oryctolagus cuniculus	XP_002718507.1	94
Ailuropoda melanoleuc	XP_002913805.1	91
Mustela putorius furo	AES06615.1	93
Drosophila melanogaster	NP_477351	51
Caenorhabditis elegans.	CBK19468.1	41

The enzymes that produce the nicotinamide from the catabolism of NAD⁺ are the mediator of many physiological processes [28]. Sirtuin protein used NAD⁺ as a co-substrate during catalysis for deacetylation to remove the acetyl group from the acetyl lysine residues of protein forming deacetylated protein, o-acetyl-ADPribose and nicotinamide. Sirtuin use NAD⁺ to form one molecule of nicotinamide for every lysine residue deacetylated. The cloned truncated SIRT1protein in the present study binds with both substrates AcH3 and cosubstrate NAD⁺ to produce nicotinamide.

Conclusion

It can be concluded that the truncated sirtuin 1 of 420 amino acids which is homologous to full length SIRT1 can be easily expressed in *E. coli*. The protein is purified and refolded in active form and binds to both substrate and co substrate to deacetylate the acetylated protein. It can be used for the in vitro study for the development of new therapeutic agent.

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