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Cloning, expression and purification of D-Tyr-tRNA^{Tyr}-deacylase from *Thermus thermophilus*

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D-Tyr-tRNA^{Tyr}-deacylase (DTD) is a conservative enzyme, found in all domains of life, which ensures an additional checkpoint in the recycling of misaminoacylated D-Tyr-tRNA^{Tyr}. DTD is capable of accelerating the hydrolysis of the ester linkage of D-Tyr-tRNA^{Tyr} producing a free tRNA and D-tyrosine, thereby preventing an incorrect incorporation of D-amino acids into proteins. Deacylase distinguishes between D- and L-aminoacyl moieties and does not hydrolyze L-aminoacylated tRNA. The structural bases of this specificity and the mechanism of D-aminoacyl-tRNA hydrolysis are poorly understood. **Aim.** To clone D-Tyr-tRNA^{Tyr}-deacylase from *T. thermophilus* (DTDTT), optimize the conditions for its expression in *E. coli* and develop an efficient purification procedure yielding the high quality enzyme suitable for the structural and functional studies. **Methods.** For amplification of *DTD* gene from *T. thermophilus* genomic DNA and its cloning into the pProEXHTb expression vector modern techniques were applied. Purification of the recombinant DTD protein was done with three types of column chromatography. His-tag was cleaved out from DTD by TEV protease. The cleavage was confirmed by Western blot analysis with anti-His-tag antibodies. Molecular weight of purified DTDTT was determined by the gel-filtration. **Results.** The expression construct pProEXHTb, containing DTD sequence from *T. thermophilus*, was obtained and successfully expressed in the BL21(DE3)pLysS *E. coli* strain. The protein of interest was purified to homogeneity by the combination of affinity (Ni-NTA), anion-exchange (Q-Sepharose) and size-exclusion (Superdex S 200) chromatographies. 2 mg of more than 90% pure recombinant DTD can be obtained from 1 L of bacterial culture. Molecular weight of purified DTD from *T. thermophilus* was determined to be 32 kDa, suggesting its dimeric structure. **Conclusions.** The pProEXHTb expression vector can be used for expression of DTD from *T. thermophilus*. The preparative amounts of DTD can be obtained after the three-step chromatographic procedures and used for further functional and structural studies.

Keywords: D-amino acids, D-Tyr-tRNA^{Tyr}-deacylase from *T. thermophilus*, cloning, expression, purification.

Introduction

D-amino acids are present in the cells of various species from bacteria to mammals. In the bacterial walls D-amino acids contribute to the resistance to proteolytic digestion [1]. They also could be considered as bacterial growth inhibitory factors that prevent a biofilm formation [2]. Furthermore, D-amino acids have also been found in the proteins extracted from aged human tissues [3]. These are the myelin basic pro-

tein, erythrocyte proteins, and L-amyloid peptides from Alzheimer disease brains [4]. D-amino acids are shown to have toxic effects on the cells in both prokaryotes and eukaryotes [5, 6, 7, 8].

Aminoacyl-tRNA-synthetases (aaRS), being specific to L-amino acids, ensure the first step of D-amino acids' exclusion from protein synthesis. However, the stereospecificity of these enzymes is not absolute: several aaRS have been found to charge tRNAs with D-amino acids [9, 10]. D-Tyr-tRNA^{Tyr}-deacyla-

se is an enzyme responsible for the recycling of mis-aminoacylated D-Tyr-tRNA^{Tyr}, hydrolyzing an ester bond between the amino acid and tRNA. However, this enzyme has a broad specificity [11], and may accommodate different D-aminoacyl moieties, for example, D-Tyr, D-Trp, D-Asp and D-Phe [9, 10].

The first observations of the deacylase editing activity in the extracts of *E.coli*, *S.cerevisiae*, rabbit reticulocytes and rat liver were reported by Calendar and Berg [9]. Later Soutourina *et al.* purified D-Tyr-tRNA^{Tyr}-deacylase from *E.coli* [10, 12] and *S.cerevisiae* [10, 13]. Plant DTD was discovered as a product of *GEK1* gene that is involved in the ethanol tolerance in *Arabidopsis thaliana* [14]. The identification of DTD in other groups of organisms, including human [15], confirms its widespread distribution in all kingdoms of life and may be considered as an important checkpoint of the translation machine specificity. In addition, the DTD amino acid sequences identity among prokaryotes and eukaryotes are highly conservative [16, 17], suggesting a high conservative function of this enzyme in all living organisms.

Three classes of deacylases have been identified: class DTD1 has been found in most bacteria and all eukaryotes [12], class DTD2 has been discovered in archaea and plants [14, 18], class DTD3 – in most cyanobacteria [11]. The species with DTD1 have the *yihZ* and *dtd* orthologous genes, responsible for the deacylase activity. Despite the fact that the homologues of *dtd* were found in different pro- and eukaryotic genomes, another type of DTD (*dtd2*) was identified in archaea and subsequently in plants. In contrast to the mainly dimeric DTD1 proteins, DTD2 has a monomer structure. In addition, the activity of deacylases from the second class depends on the presence of Zn²⁺ ions. The third type of D-Tyr-tRNA^{Tyr}-deacylases has been reported to be encoded by the *dtd3* gene (homologous to *dtd1*). DTD3 is a metal-enzyme with two active sites for metal ions binding: the first one binds only Zn²⁺, the second – Ni²⁺, Mn²⁺ and Co²⁺ ions.

Some functional investigations of *E.coli* [12], *S. cerevisiae* [10, 13], archaeal [18] D-Tyr-tRNA^{Tyr}-deacylases were performed, but profound structural research that may explain the mechanism of D-aa-

tRNA hydrolysis by DTD, is still to be carried out. Unfortunately, the proposed catalytic mechanism of DTD based on the crystal structures available at this time is controversial and remains to be clarified [17, 19].

In order to investigate the structural and functional properties of deacylase, one needs to have the preparative quantities of this enzyme of a high purity. In this work we describe the expression and purification of DTD from *T. thermophilus* (DTD^{TT}).

Materials and Methods

Cloning of DTD^{TT} gene. Genomic DNA from *T. thermophilus* cells were obtained according to [20]. Based on the sequence information of the *DTD* gene from *T. thermophilus* (152 amino acid residues) (http://www.ncbi.nlm.nih.gov/protein/WP_01_1173028.1) two PCR primers were designed Dtyr TT-N (5'-CCA TGG CGG GCG GTG GTG CAG CGG GTC TCC) and DtyrTT-C (5'-AAG CTT ATT AGC GTG GGC GG AAG CGT TCC TCC GAG TCC). Sites for NcoI and Hind III restriction enzymes were included in forward and reverse primers, respectively. The primers were previously phosphorylated by 10 unites of T4-polynucleotide kinase («Thermo Scientific», Lithuania) in the mixture containing 200 pmol of primer, 50 mM Tris-HCl (pH 7.5), 5 mM DTT («Euromedex», France), 0.1 mM ATP («Sigma», USA), 10 mM MgCl₂ («Sigma», USA) for 1 h at 37 °C with further kinase inactivation during 20 min at 65 °C.

Amplification of the DTD^{TT} gene was performed as follows: denaturation – 1 min, 94 °C; annealing – 1 min, 50 °C; elongation – 1 min, 72 °C (totally – 30 cycles) in 50 mM Tris-HCl (pH 9.0), 1.5 mM MgCl₂, 20 mM ammonium sulfate («Merck», Germany), 1 µl of genomic DNA, 0.2 mM dNTP («Sigma», USA), 40 pmol of each primer and 2.5 units of Taq DNA-polymerase («Stratagen», USA). The PCR fragment of about 460 bp was obtained and cloned first into pCRII-TOPO vector («Invitrogen», USA) using Zero-Blunt-TOPO PCR cloning Kit («Life Technologies», USA). Top10 *E.coli* cells («Invitrogen», USA) were used for the transformation. Screening for the positive clones was performed by GeneJET Plasmid Miniprep Kit («Thermo Scientific», Lithuania). The positive clones were identified by NcoI and HindIII

restriction. The correct sequence of *DTDTT* gene was confirmed by DNA sequencing. Then the *DTDTT* gene was excised by NcoI and Hind III Fast Digest («Thermo Scientific», Lithuania) restrictases and ligated into pProEXHTb vector (EMBL, France), previously dephosphorylated by 1 unit of shrimp phosphatase in 1^x reaction buffer («Roche», Switzerland). 1 unit of T4-DNA-ligase in 1^x ligase buffer («Thermo Scientific», Lithuania), supplemented with 1mM spermidine («Sigma», USA) and 1 mM ATP (for 20 h at 14 °C) was used for ligation. Top10 *E. coli* cells were transformed by ligation mixture using «BioRad» (USA) electroporation system.

Test of DTDTT expression in different media. *E. coli* BL21(DE3)pLysS cells were electroporated by pProEXHTb-DTD plasmid. Analysis of an expression level was performed in LB (Lauria-Broth) medium (10 g/l tryptone, 5 g/l yeast extract («Difco», USA), 10/l g NaCl («Helicon», Russian Federation), TB (Terrific-Broth) (12 g/l tryptone, 24 g/l yeast extract, 2.5 % glycerol, 2.31 g/l KH₂PO₄ and 12.54 g/l K₂HPO₄ («Helicon», Russian Federation), P (phosphate-medium) (10 g/l yeast extract, 0.4 % glucose («Helicon», Russian Federation), 5.6 g/l KH₂PO₄ and 28.9 g/l K₂HPO₄), 2x TY (16 g/l tryptone, 10 g/l yeast extract, 5 g/l NaCl) supplemented with ampicillin and chloramphenicol («Euromedex», France). Pre-culture (5 ml) was grown overnight at 37 °C. Culture (50 ml of each medium) was inoculated with pre-culture in dilution 1:100. Bacterial growth was continued to A₆₀₀ = 0.6 and 0.6 mM IPTG («Thermo Scientific», Lithuania) induction was performed for 5 h. The extract of soluble proteins was prepared from 1 ml of culture volume according to the Zerbs *et al.* protocol for the analysis of protein solubility [21].

DTDTT purification by affinity, anion exchange and size-exclusion chromatography. Pre-culture of *E. coli* BL21(DE3)pLysS cells (50 ml), carrying the recombinant plasmids, was grown overnight at 37 °C in Terrific-Broth with appropriate antibiotics. The culture (2.5 L) was inoculated with pre-culture in dilution 1:100 and grown at 37 °C on TB medium supplemented with ampicillin (100 µg/ml) and chloramphenicol (35 µg/ml). The expression of *DTDTT* was induced by the addition of 0.6 mM IPTG at A₆₀₀ = 0.6

and the culture growth continued for 4 h at 37 °C.

The cells were harvested by the centrifugation for 15 min at 6000 × g (4 °C). The bacterial cell pellet was resuspended in 70 ml of 25mM Tris-HCl (pH 7.5), 1mM PMSF, 10 mM β-mercaptoethanol supplemented with 1.5 tablets of EDTA free protease inhibitors cocktail. The cells were incubated on ice for 30 min and then disrupted by sonication 8 × 30 sec with 1 min breaks (4 °C). All subsequent steps were conducted at 4 °C. The cell debris was precipitated by centrifugation at 20 000 × g. The clear supernatant was recovered and concentrations of sodium chloride and imidazole («Sigma», USA) were adjusted to 300 mM and 10 mM, respectively. The obtained solution was mixed with Ni-NTA Sepharose Fast Flow resin (5 ml of 50 % slurry, «GE Healthcare», Sweden), pre-equilibrated with the same buffer, and incubated for 1.5 h on the rotor shaker at 130 rpm. The resin was washed with buffer A (25 mM Tris-HCl, pH 7.5, 0.1 mM PMSF, 1 mM β-mercaptoethanol, 300 mM NaCl, 10 mM imidazole) and then with buffer A containing 600 mM NaCl. DTDTT was eluted from the column by 400 mM imidazole in buffer A. The collected fractions were analyzed by SDS-PAGE. The purest fractions were combined and dialyzed overnight against an appropriate buffer for TEV protease digestion – buffer B (50 mM Tris-HCl (pH 7.5), 0.1 mM PMSF, 1 mM DTT, 0.5 mM EDTA). After the dialysis His-tag-residues were cut off from DTDTT by recombinant TEV protease as follows: 1 A₂₈₀ of TEV per 5 A₂₈₀ of DTD during overnight digestion at 4 °C [22]. The resulting solution from the first purification step was diluted to 1 A₂₈₀ unites/ml and applied on Q-Sepharose Fast Flow column («Pharmacia», Sweden) (1.35 × 4 cm, V = 6 ml), pre-equilibrated by buffer B. A column was washed by the same buffer. The elution was performed at a flow rate of 0.6 ml/min with a linear gradient of 200-800 mM NaCl (70 ml).

The protein-containing fractions were detected by Bradford assay, the DTD-containing fractions were analyzed by 15 % SDS-PAGE. The collected fractions containing DTDTT were dialyzed overnight against 25 mM Tris-HCl (pH 7.5), 1 mM DTT at 4 °C and concentrated on 10 kDa Centricon («Merck», Ger-

many) at 5500 rpm to $4.54 A_{280}$ unites/ml (≈ 12 mg/ml).

To separate our target protein from the high molecular weight contaminations we used a size-exclusion chromatography on Hi-Load 16/60 Superdex 200 (150 ml, «Pharmacia Biotech», Sweden) co-column, pre-equilibrated with 25 mM Tris-HCl (pH 7.5), 1 mM DTT, 150 mM NaCl, 0.003 % NaN_3 , with a flow rate of 0.5 ml/min.

The eluted fractions were collected and analyzed by 15 % SDS-PAGE. The deacylase containing fractions were combined and concentrated to 8 mg/ml. The enzyme was supplemented by 50 % glycerol and stored at -20°C .

The protein concentrations were determined by the Bradford assay using Roti®-Quant («Roth», Germany) [23]. Light absorption coefficient at 280 nm ($\epsilon_{280} = 5960 \text{ M}^{-1} \text{ cm}^{-1}$) and absorbance of 0.1 % solution ($A_{280} (1 \text{ mg/ml}) = 0.354 \text{ unites} \cdot \text{mg}^{-1} \cdot \text{ml}$) were calculated from the amino acid sequence of the DT-DTT (ProtParam tool, ExPASy, Swiss Port) and used for determination of the enzyme concentration.

Analytical gel filtration of proteins

To determine the approximate molecular weight of DT-DTT the gel filtration on Hi-Load 16/60 Superdex S 200 (150 ml, «Pharmacia Biotech») was used. The column was pre-equilibrated with 25 mM Tris-HCl (pH 7.5), 1 mM DTT, 150 mM NaCl, 0.003 % NaN_3 . All samples were run at 1 ml/min flow rate. The void column volume (V_0) was determined by blue dextran (2 MDa). A set of proteins were used for the column calibration: ferritin (450 kDa), catalase (240 kDa), β -amilase (200kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), carboanhydrase (29 kDa), cytochrome c (12,4 kDa).

The molecular weight of DT-DTT was determined by a comparison of its V_e / V_0 index with those of the known protein standards. The logarithms of the molecular weights of marker proteins were plotted against their appropriate ratios of the elution volume to the column void volume (V_e / V_0). Calibration curve is shown in Fig. 6.

Western blot analysis of DT-DTT before and after TEV protease treatment

The proteins were separated in 15% SDS-PAGE and transferred onto a prepared $0.45 \mu\text{m}$ polyvinyl difluoride (PVDF) membrane (incubated for 1 min with MeOH and rinsed once by Towbin buffer («Bio-Rad», USA)) on Trans-Blot Semi-Dry electrophoretic transfer system («Bio-Rad», USA). The membrane was blocked overnight by 5 % non-fat milk in PBST buffer solution (PBS plus 0.5 % Tween-20). After blocking, membrane was incubated with mouse anti-His mono-clonal antibodies («Sigma», USA) in dilution 1 : 6000 for 1 h at room temperature. Then, the membrane was extensively washed by PBST buffer (4 times \times 5 min) and treated with secondary anti-mouse antibodies (Jackson Immuno Research Inc., USA), conjugated to peroxidase, at 1 : 10000 working dilution for 1 h. After this incubation the extensive (4 times \times 5 min) washing with PBST was performed. The immune complexes were detected by ECL detection kit (EMD Millipore Immobilon Western Chemiluminescent HRP Substrate) («Millipore», USA) using X-ray film.

Results and Discussion

Creation of DTD expressing construction and expression of recombinant protein in different media. Previously, we tried to express the DTD gene from *T. thermophilus* in pET15b, pET28b and pET29b vectors (under control of T7-promotor and lac-operator), but it resulted in low expression level of the target protein even after 24 h of IPTG induction. To overcome this problem we decided to switch to pProEXHTb expression vector, which possess' Trc promoter. pProEXHTb was earlier shown to produce large quantities of the target proteins, during a short time IPTG induction [24].

The expression level of DT-DTT in *E. coli* BL21 (DE3)pLysS cells was checked under varied IPTG concentrations and in several media (LB, TB, P, 2xTY). The best conditions obtained were as follows: 4–5 h of 0.6 mM IPTG induction at 37°C in Terrific Broth medium (Fig. 1). These conditions were further used for the preparative DT-DTT expression.

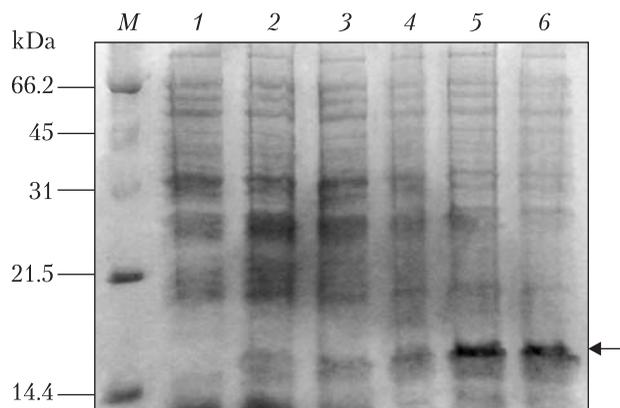


Fig. 1. DTDTT expression in TB medium. 15 % SDS-PAGE of the soluble protein extracts from BL21(DE3)pLysS: *M* – protein marker (SDS-PAGE Standards, Low 6-200 «Bio-Rad»); *1* – extract from non-induced bacteria; *2–6* – extracts after 1–5 hours of 0.6 mM IPTG induction. The position of His-DTDTT is indicated by arrow

Purification of His-DTDTT

The first step of the His-DTDTT purification was the affinity chromatography on Ni-NTA. The result is presented in Fig. 2. The enzyme, eluted from the column by 400 mM imidazole, contained the contaminations of higher molecular weight proteins. Washing the column with a buffer supplemented with 20 mM imidazole slightly increased the DTDTT purity but decreased its yield. In addition, washing the column with 1 M NaCl did not significantly diminish the amount of impurities (data not shown). Unfortunately, we could not improve the quality of the DTDTT preparation after this step of purification.

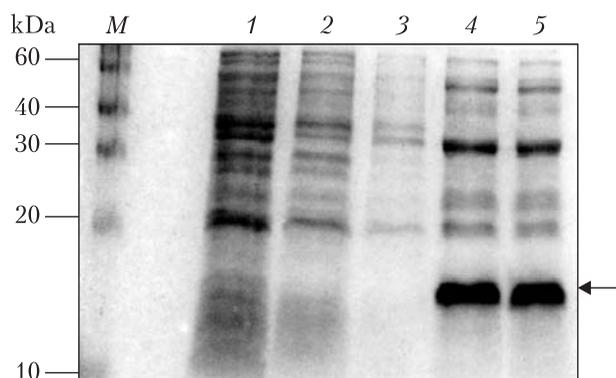


Fig. 2. Affinity purification of His-DTDTT on Ni-NTA column. 15 % SDS-PAGE of fractions: *M* – protein marker (Roti-Mark 10–150); *1* – soluble protein lysate; *2* – flow-through fraction after Ni-NTA column; *3* – wash Ni-NTA column; *4* – combined eluted DTDTT fraction; *5* – His-DTDTT after dialysis. The position of His-DTDTT on the gel is indicated by arrow

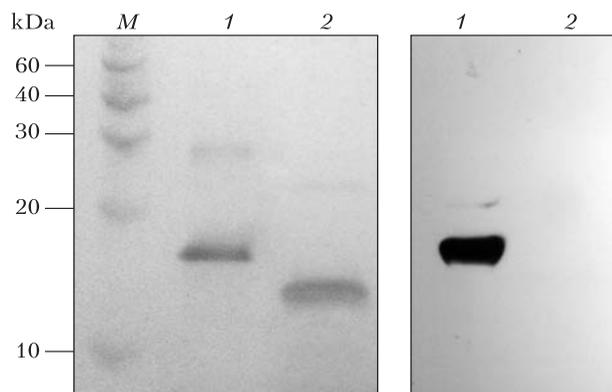


Fig. 3. 15 % SDS-PAGE (left panel) and Western blot (right panel) analysis of His-DTDTT before (*1*) and after (*2*) TEV protease treatment

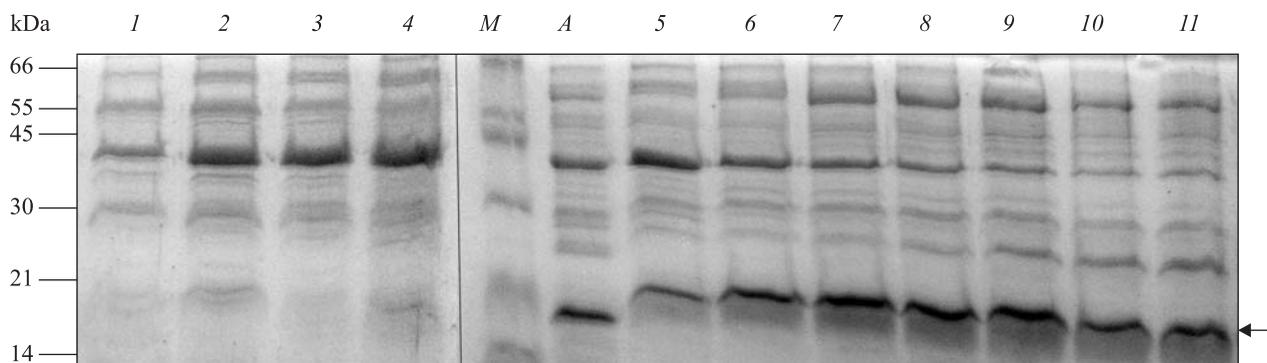


Fig. 4. 15 % SDS-PAGE of fractions after Q-Sepharose chromatography: *M* – protein marker (RPN 58100, «Amersham»); *A* – protein, loaded onto the column; lanes *1–11* – fractions obtained during the gradient elution from Q-Sepharose column. The position of DTDTT on the gel is indicated by arrow

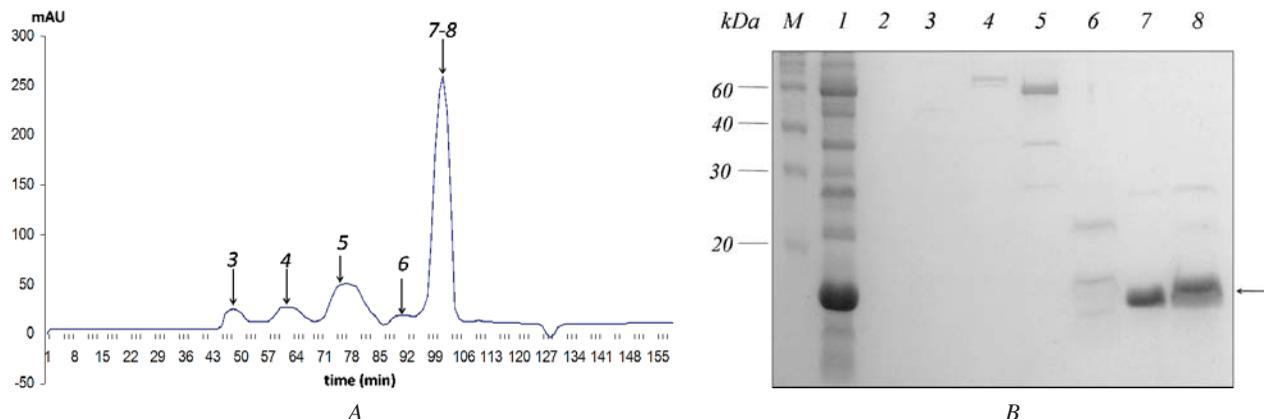


Fig. 5. Purification of DTDTT by size-exclusion chromatography: A – elution profile of DTDTT on Superdex S 200 column; B – 15 % SDS-PAGE of fractions after Superdex 200: M – protein marker (Roti-Mark 10–150); 1 – DTDTT applied onto Superdex 200 column; 2 – empty lane; lanes 3–8 – corresponding fractions obtained after the gel filtration. The position of DTDTT is indicated by arrow

To remove His-tag from the His-DTDTT enzyme we used the recombinant TEV protease as described in «Materials and methods». The efficiency of cleavage was checked by SDS-PAGE and Western blot analysis with anti-His antibodies (Fig. 3). The absence of a signal on the lane 2 of Western blot panel confirms complete His-tag removing from DTDTT. Moreover, there is a shift of the DTDTT

migration on SDS-PAGE (lane 2, left panel) after the TEV protease treatment. Thus, the His-tag cleavage from His-DTDTT can be simply monitored by SDS-PAGE.

It is worth noting that the ratio A_{260}/A_{280} of DTDTT after the affinity purification step and His-tag cleavage was about 1.0 that may reflect the presence of nucleic acid contaminations. To remove the nucleic acid fragments we applied an anion-exchange chromatography. Unfortunately, during this step we could not get rid of the protein contamination present in the DTDTT preparation. We applied various linear gradients (from 0 to 1M NaCl and from 50 mM to 800 mM NaCl), but this did not improve the quality of DTDTT. Finally, we used the gradient from 200 to 800 mM of NaCl, which allowed us to remove the nucleic acids and some protein contaminations. After Q-Sepharose column, DTDTT had typical absorbance ratio $A_{260}/A_{280} = 0.5–0.6$. SDS-PAGE of fractions obtained after Q-Sepharose is presented in Fig. 4.

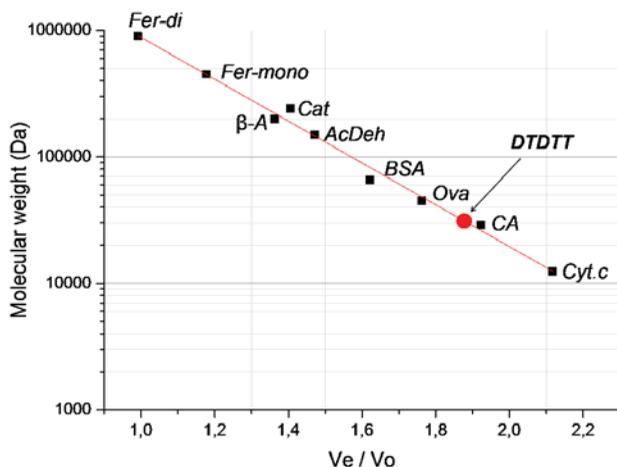


Fig. 6. Determination of DTDTT molecular weight by size-exclusion chromatography on Superdex S 200. Calibration of Superdex S 200 column was performed as described in «Materials and methods» (Fer-di – ferritin dimer; Fer-mono – ferritin monomer; β -A – β -amilase; Cat – catalase; AcDeh – alcohol dehydrogenase; BSA – bovine serum albumin; Ova – ovalbumin; DTDTT – D-Tyr-tRNA^{IV}-deacylase; CA – carboanhydrase; Cyt.c – cytochrome c). The position of DTDTT is indicated by a circle

To get rid of the higher molecular weight impurities in the DTDTT preparation we performed a size-exclusion chromatography as a final step of the purification procedure. The elution profile of DTDTT from the column is shown in Fig. 5, B. As can be judged from the elution profile, DTDTT was efficiently separated from the contaminating proteins and eluted from the column as a single peak (Fig. 5, A). According to SDS-PAGE

(Fig. 5, B) the purity of DTDTT may be more than 90 %.

Molecular weight determination of D-Tyr-tRNA^{Tyr}-deacylase

The molecular weight of the D-Tyr-tRNA^{Tyr} deacylase was deduced from a comparison of its elution time on Superdex S 200 column with the proteins of known molecular weight. Column was calibrated as described in «Materials and methods». The elution volume (V_e) of DTDTT from Superdex S 200 was determined to be 11 ml. V_e/V_0 index of DTDTT was calculated to be 1,888. According to the calibration curve (Fig. 6) the molecular weight of DTDTT was estimate to be 32 kDa. Based on the amino acid sequence of DTDTT (152 a. a. residues), its theoretical molecular weight is 16.7 kDa. Thus, the purified recombinant DTDTT most probably is a dimer in solution. However, a monomeric unglobular form of this protein with an elongated shape could not be excluded.

Conclusions

The cDNA encoding D-Tyr-tRNA^{Tyr}-deacylase from *T. thermophilus* was cloned into pProEXHTb vector and successfully expressed in BL21(DE3)pLysS strain in TB medium. The purification procedure described here allows obtaining 2 mg of the pure enzyme from 1 L of the bacterial culture. According to the gel filtration analysis recombinant DTDTT may exist as a dimer in solution. The obtained protein will be used for further structural and functional studies.

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Клонування, експресія та очистка

D-Тир-тРНК^{Тир}-деацилази *Thermus thermophilus*

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D-Тир-тРНК^{Тир}-деацилаза (DTD) є консервативним білком, що забезпечує додатковий корегульований етап гідролізу помилково аміноацильованих субстратів D-Тир-тРНК^{Тир}. DTD прискорює гідроліз ефірного зв'язку в комплексі D-Тир-тРНК^{Тир}, утворюючи вільну тРНК та D-Тир, таким чином попереджуючи включення D-амінокислот до білків. Деацилаза розрізняє D- та L-аміноацильні залишки в аміноацильованій тРНК та не гідролізує останні. Структурні основи такої специфічності та механізм гідролізу D-аміноацил-тРНК за участі DTD є недостатньо зрозумілими. **Мета.** Клонувати D-Тир-тРНК^{Тир}-деацилазу з *T. thermophilus* (DTDГТ), оптимізувати умови її експресії в *E. coli*, розробити ефективний метод очищення з високим виходом високоякісного ферменту для структурно-функціональних досліджень. **Методи.** Ампліфікацію та клонування гена *DTD* з геномної ДНК *T. thermophilus* в рProEXHTб експресуючий вектор проведено стандартними молекулярно-біологічними методами. Очищення рекомбінантного DTD білка проведено хроматографічно. Залишки гістидину відщеплено від DTD протеазою вірусу тютюну (TEV), ефективність реакції перевірено Вестерн-блот аналізом з анти-His-антитілами. Молекулярну масу очищеної DTDГТ визначено гелі-фільтрацією. **Результати.** Отримана конструкція рProEXHTб, що містить *DTD* послідовність з *T. thermophilus*, експресовано в *E. coli* штаму BL21(DE3)pLysS. Цільовий рекомбінантний білок виділено і очищено комбінацією хроматографій: афінної (Ni-NTA), аніон-обмінної (Q-Sepharose) та гелі-фільтрації (Superdex S 200) до чистоти 90%. Розроблений метод дозволяє отримати до 2 мг цільового білка з 1 л бактеріальної культури. Визначена молекулярна маса очищеної DTD з *T. thermophilus* – 32 кДа свідчить на користь її димерної структури. **Висновки.** Отриманий вектор рProEXHTб, що містить *DTD* з *T. thermophilus* і підібрані умови очищення дозволяють отримати рекомбінантний DTD в кількостях достатніх для

подальших структурно-функціональних досліджень.

Ключові слова: D-амінокислоти, D-Тир-тРНК^{Тир}-деацилаза *Thermus thermophilus*, клонування, експресія, очистка.

Клонирование, экспрессия и очистка D-Тир-тРНК^{Тир}-деацилазы *Thermus thermophilus*

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D-Тир-тРНК^{Тир}-деацилаза (DTD) является консервативным белком, обеспечивающим дополнительный корректирующий этап гидролиза ошибочно аминацилированных субстратов D-Тир-тРНК^{Тир}. DTD ускоряет гидролиз эфирной связи в комплексе D-Тир-тРНК^{Тир}, предупреждая, таким образом, ошибочное включение D-аминокислот в белки. Деацилаза различает D- и L-аминоацильные остатки в аминацилированной тРНК и не гидролизует последние. Структурные основы такой специфичности и механизм гидролиза D-аминоацил-тРНК при участии DTD являются недостаточно изученными. **Цель.** Клонировать D-Тир-тРНК^{Тир}-деацилазу из *T. thermophilus* (DTDГТ), оптимизировать условия её экспрессии в *E. coli* и разработать эффективный метод очистки с высоким выходом высококачественного фермента для дальнейших структурно-функциональных исследований. **Методы.** Для амплификации и клонирования гена *DTD* из геномной ДНК *T. thermophilus* в рProEXHTб экспрессирующий вектор использованы стандартные молекулярно-биологические методы. Очищение рекомбинантного DTD белка проводили хроматографически. Остатки гистидина отщепили протеазой вируса табака (TEV), эффективность реакции проверили Вестерн-блот анализом с анти-His-антителами. Молекулярный вес очищенной DTDГТ определили гелі-фільтрацией. **Результаты.** Полученная конструкция рProEXHTб, содержащая *DTD* последовательность *T. thermophilus*, экспрессирована в клетках *E. coli* штамма BL21(DE3)pLysS. Последовательными хроматографиями: афінної (Ni-NTA), аніон-обмінної (Q-Sepharose) и гелі-фільтрації (Superdex S 200) получен гомогенный белок с чистотой более 90 %. Разработанная методика позволяет получить до 2 мг целевого белка / 1 л бактериальной культуры. Определенный молекулярный вес очищенной DTD из *T. thermophilus* – 32 кДа свидетельствует о ее димерной структуре. **Выводы.** Полученная конструкция рProEXHTб, содержащая *DTD* последовательность *T. thermophilus* и подобранные условия очистки позволяют получить рекомбинантный DTD для дальнейших структурно-функциональных исследований.

Ключевые слова: D-аминокислоты, D-Тир-тРНК^{Тир}-деацилаза *Thermus thermophilus*, клонирование, экспрессия, очистка.

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