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Research paper

DspA from *Strongylocentrotus purpuratus*: The first biochemically characterized haloalkane dehalogenase of non-microbial origin

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ABSTRACT

Haloalkane dehalogenases are known as bacterial enzymes cleaving a carbon–halogen bond in halogenated compounds. Here we report the first biochemically characterized non-microbial haloalkane dehalogenase DspA from *Strongylocentrotus purpuratus*. The enzyme shows a preference for terminally brominated hydrocarbons and enantioselectivity towards β-brominated alkanes. Moreover, we identified other putative haloalkane dehalogenases of eukaryotic origin, representing targets for future experiments to discover dehalogenases with novel catalytic properties.

1. Introduction

Haloalkane dehalogenases (EC 3.8.1.5.) are enzymes that catalyze hydrolytic cleavage of carbon–halogen bonds, thereby producing the corresponding alcohol, a proton and a halide. They belong to the α/β-hydrolase superfamily [1–3]. Haloalkane dehalogenases were initially detected in bacteria colonizing environments contaminated by halogenated hydrocarbons. These bacteria can grow on 1-chloro-*n*-alkanes, 1-bromo-*n*-alkanes and α,ω-dihalo-*n*-alkanes as the only carbon and energy source [4–10]. Genome sequence analyses significantly contributed to the identification of genes encoding putative haloalkane dehalogenases in organisms with no obvious connection to degradation of halogenated compounds. The group of haloalkane dehalogenases from haloalkane-utilizing organisms has been enriched by enzymes obtained from pathogenic bacteria [11–13], marine bacteria [14], cold-adapted bacteria [15], plant symbionts [16] and plant parasites [17]. Currently, all experimentally confirmed haloalkane dehalogenases are of bacterial origin. However, putative haloalkane dehalogenases from HLD-II subfamily have been previously identified in the genome of purple sea urchin *Strongylocentrotus purpuratus* by phylogenetic analysis [18]. The California purple sea urchin *S. purpuratus* has been favorite model organism for developmental and molecular biology. Because of its importance in the biomedical research, the sea urchin 814-megabase genome was completely sequenced and annotated in 2006 [19]. Here we report expression and biochemical characterization of DspA from *S. purpuratus* as the first experimentally confirmed haloalkane dehalogenase of eukaryotic origin.

2. Materials and methods

2.1. Gene synthesis

The *dspA* gene was synthesized artificially (Mr. Gene, Regensburg, Germany) according to the sequence deposited in the NCBI Protein database [20] under accession number XP_794172. The codon usage was automatically adapted to the codon bias of *Escherichia coli* genes by Mr. Gene's website service. For expression purposes, the gene was subcloned to the expression vector pET21b (Novagen, San Diego, USA) between restriction sites *Nde*I and *Hin*dIII.

Abbreviations: CD, circular dichroism; IPTG, isopropyl β-D-thiogalactopyranoside; PCA, Principle Component Analysis; T_m, melting temperature.

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2.2. Protein expression and purification

To overproduce haloalkane dehalogenase DspA in *E. coli*, the gene was expressed under the control of the T7*lac* promoter and the gene expression was induced by the addition of isopropyl β-D-thiogalactopyranoside (IPTG). *E. coli* BL21(DE3) cells containing recombinant plasmid pET21b-*dspa* were grown in 4 l of Luria broth medium with ampicillin (100 μg/ml) at 37 °C. When the culture reached an optical density 0.6 at 600 nm, the induction of enzyme expression (at 30 °C) was initiated by the addition of IPTG to a final concentration of 0.5 mM. The cells were harvested, disrupted by sonication using an ultrasonic processor UP200S (Hielscher, Teltow, Germany) and centrifuged for 1 h at 4 °C and 21,000*g*. DspA was purified on a Ni-NTA Superflow Cartridge (Qiagen, Hilden, Germany). His-tagged enzyme was bound to the resin in equilibrating buffer (20 mM potassium phosphate buffer, pH 7.5, containing 0.5 M sodium chloride, and 10 mM imidazole). Unbound and weakly bound proteins were washed out. His-tagged enzyme was eluted by a buffer containing 300 mM imidazole. The eluted protein was pooled and dialyzed overnight against 50 mM potassium phosphate buffer (pH 7.5) and then stored at 4 °C. The protein concentration was determined by the Bradford reagent (Sigma–Aldrich, St. Louis, MO, USA) and the protein purity was checked by SDS-PAGE.

2.3. Circular dichroism spectroscopy and thermal denaturation

Circular dichroism (CD) spectra were recorded at room temperature using Jasco J-810 spectropolarimeter (Jasco, Tokyo, Japan). Data were collected from 185 to 260 nm, at 100 nm min^{-1}, 1 s response time and 2 nm bandwidth. A 0.1 cm quartz cuvette containing DspA in 50 mM potassium phosphate buffer (pH 7.5) was used for measurement. Presented spectra represented the average of ten individual scans and were corrected for absorbance caused by the buffer. CD data were expressed in terms of the mean residue ellipticity (Θ_{MRE}) using the equation:

$$\Theta_{MRE} = (\Theta_{obs} M_w 100)/(nlc)$$

where Θ_{obs} is the observed ellipticity in degrees, M_w is the protein molecular weight, n is number of residues, l is the cell path length (0.1 cm), c is the protein concentration and the factor 100 originates from the conversion of the molecular weight to mg $dmol^{-1}$.

Thermal unfolding of DspA was followed by monitoring the ellipticity at 220 nm over the temperature range of 20–80 °C, with a resolution 0.1 °C, at a heating rate 1 °C min^{-1}. Recorded thermal denaturation curves were roughly normalized to represent signal changes between approximately 1 and 0 and fitted to sigmoidal curves using Origin 8.0 software (OriginLab, Massachusetts, USA). The melting temperature (T_m) was evaluated as a midpoint of the normalized thermal transition.

2.4. Analysis of oligomeric state

The native structure of DspA was examined by native polyacrylamide gel electrophoresis and gel filtration chromatography. Native polyacrylamide gel electrophoresis was performed with 10% gels lacking sodium dodecyl sulfate. The electrophoresis tank was maintained at 4 °C during the experiment. Gels were stained with Coomassie brilliant blue R-250 dye (Fluka, Buchs, Switzerland). The molecular mass of DspA was estimated by comparison of its mobility with values for three molecular weight standards, ovalbumin (43 kDa), albumin (67 kDa) and conalbumin (75 kDa), and two haloalkane dehalogenases LinB (33 kDa, monomer) and DbjA (68 kDa, dimer).

Gel filtration chromatography was performed with Superdex™ 200 10/300 GL column (GE Healthcare, Freiburg, Germany) calibrated with aldolase (158 kDa), conalbumin (75 kDa), ovalbumin (43 kDa), carbonic anhydratase (29 kDa) and ribonuclease A (14 kDa). Total volume 100 μl of each protein sample was applied to the column and separated at a constant flow rate 0.5 ml min^{-1}, using an elution buffer consisting of 50 mM Tris–HCl and 150 mM of NaCl, pH 7.5.

2.5. Activity assays

The specific activity of DspA towards thirty halogenated substrates was assayed by the method of Iwasaki et al. [21]. The release of halide ions was measured spectrophotometrically at 460 nm using microplate reader SUNRISE (Tecan, Grödig/Salzburg, Austria) after reaction with mercuric thiocyanate and ferric ammonium sulfate. The dehalogenation reactions were performed at 37 °C in 25 ml Reacti-flasks closed by Mininert valves. The reaction mixtures were composed of 10 ml of 100 mM glycine buffer pH 8.6 and 10 μl of appropriate substrate in concentration 0.1–10 mM, depending on the substrate solubility. The reactions were initiated by addition of 0.2 ml of enzyme in a concentration 0.4–0.8 mg ml^{-1}. The reactions were monitored by withdrawing 1 ml samples at periodic intervals from the reaction mixtures and immediate mixing of the samples with 0.1 ml of 35% nitric acid to terminate the reaction. Dehalogenation activity data were quantified as the rate of product formation with time and corrected for the abiotic hydrolysis.

Luminescence activity of DspA and *Renilla* luciferase RLuc with coelenterazine was determined using luminometer FLUOStar OPTIMA (BMG Labtech, Ortenberg, Germany). Sample of 25 μl of purified enzyme (0.1 mg ml^{-1}) was placed into the microtiter well. After baseline collection for 10 s, the luminescence reaction was initiated by addition of 225 μl of 0.22 μM coelenterazine in reaction buffer consisting of 100 mM potassium phosphate buffer and 1.5 M Na_2SO_4 (pH 7.5). Luminescence was recorded for 20 s. Height of the luminescence peak was evaluated. Each sample was measured in at least three independent experiments.

2.6. Principle Component Analysis

The data matrix containing the specific activities for DspA and haloalkane dehalogenases LinB, DbjA, DhaA, DhlA, DmbA, DbeA [22], DmbC, DrbA [12] and DatA [17] towards 30 halogenated substrates was analyzed by means of Principle Component Analysis (PCA) [23] using Statistica 10.0 software package (StatSoft, Tulsa, USA) as described previously by Koudelakova et al. [22]. Individual enzymes were considered as cases, whereas their substrates as variables. Two PCAs were performed. In the first analysis, the raw data concerning individual enzymes' specific activities towards particular substrates were used as the primary input data. This analysis compared the overall activity of DspA with overall activity of other characterized enzymes in the score–contribution plot t_1. In the second analysis, the raw data were log-transformed and weighted relative to the individual enzyme's activity towards other substrates prior to analysis. Each specific activity value was incremented by 1 unit; the log of this new value was taken and divided by the sum of all the log values for a given enzyme. The log-transformed, weighted measure of that enzyme's activity towards that specific substrate (relative to its activity towards all of the other substrates) was thus obtained. Such transformed data were used to compare the individual specificity profiles of characterized haloalkane dehalogenases, without regard to their overall specific activity. The coordinates of individual enzymes in the space defined by the three biologically significant principle components arising

from PCA with transformed dataset were used to calculate a matrix of Euclidean distances. To characterize the similarities of individual haloalkane dehalogenases in terms of their substrate specificity profiles, the matrix of Euclidean distances was in turn used to construct a dendrogram. The dendrogram was generated in Statistica 10.0 using the complete linkage amalgamation rule.

2.7. Steady-state kinetic analysis

Steady-state kinetic parameters for the reaction of DspA with 1,3-dibromopropane were evaluated by measuring substrate concentrations prior to initiation of the enzymatic reaction using the Trace GC 2000 gas chromatograph (Finnigen, San Jose, USA) equipped with a flame ionization detector and a capillary column DB-FFAP 30 m × 0.25 mm × 0.25 μm (J&W Scientific, Folsom, USA), and the colorimetric method described by Iwasaki et al. [21]. Analysis of the product, 3-bromo-1-propanol, formed during enzymatic conversion of 1,3-dibromopropane, was carried out using the Agilent 7890A gas chromatograph equipped with a mass spectrometer detector 5970C (Agilent, Wilmington, Delaware, USA) and a capillary column ZB-FFAP 30 m × 0.25 mm × 0.25 μm (Phenomenex, Torrance, USA). The dehalogenation was performed at 37 °C in 25 ml Reacti-flasks closed by Mininert valves in a shaking water bath. The reaction mixture consisted of 10 ml of glycine buffer (100 mM, pH 8.6) and various concentrations of substrate. The enzymatic reaction was initiated by addition of 0.1 ml of DspA in a concentration 0.7 mg ml^{-1}. The reaction was terminated by the addition of 0.1 ml of 35% nitric acid at different times after initiation (0, 10, 20, 30, 40 and 50 min). All data points corresponded to the mean of 3 independent replicates. Kinetic parameters were determined by non-linear curve fitting the data points using the software Origin 8.0 (OriginLab, Massachusetts, USA).

2.8. Enantioselectivity assay

Kinetic resolution experiments were performed at 21 °C. Racemic substrates (2-bromobutane, 2-bromopentane, ethyl 2-bromobutyrate, methyl 2-bromobutyrate) were added in a final concentration of 1 mM to reaction vessel containing 25 ml Tris–sulfate buffer (50 mM, pH 8.2). Enzymatic reaction was initiated by addition of 1.0 ml of enzyme in a concentration 4.0 mg ml^{-1}. The progress of each reaction was monitored by periodical withdrawing samples from the reaction mixture and analysis using a Hewlett–Packard 6890 gas chromatograph (Agilent, Wilmington, Delaware, USA) equipped with a flame ionization detector and a Chiraldex G-TA chiral capillary column (Alltech, Deerfield, Illinois, USA). The enantiomeric ratio was calculated using the following equation [24]:

$$E = (k_{cat,R}/K_{m,R})/(k_{cat,S}/K_{m,S})$$

where k_{cat}/K_m represents the specificity constant. To estimate *E*-values, the equations describing competitive Michaelis–Menten kinetics were fitted by numerical integration to time courses of changes in substrate concentrations obtained from the kinetic resolution experiments using MicroMath Scientist for Windows (ChemSW, Fairfield, California, USA).

2.9. Database searches and sequence analysis

Putative haloalkane dehalogenases of eukaryotic origin were identified using the previously published methodology [18]. Six haloalkane dehalogenase family members—LinB [6], DhaA [25], DhlA [5], DmbB [13], DrbA [12] and DmbC [12]—served as queries for the PSI-BLAST [26] search against the NR database of NCBI (version September 2012) [20] using the *E*-value threshold of 10^{-10} for the initial BLAST search and the threshold of 10^{-15} for inclusion of the sequence in the position specific matrix. Sequences collected after three iterations of PSI-BLAST were clustered with CLANS [27] to distinguish haloalkane dehalogenases from other related sequences. The main haloalkane dehalogenase cluster including all experimentally characterized haloalkane dehalogenases was identified at the *P*-value of 10^{-36}. Additional cluster containing putative haloalkane dehalogenases from *Aspergillus* and *Neosartorya* species was identified by a text search. Sequences from these two clusters were aligned with MUSCLE [28] and classified into haloalkane dehalogenase subfamilies based on the composition of their catalytic pentads. All incomplete, degenerated or diverged sequences were removed from the dataset.

3. Results and discussion

3.1. Analysis of correct folding, stability and oligomeric state

The sequence of *dspA* gene was optimized for heterologous expression in *E. coli* (Supplementary Fig. S1) and artificially synthesized (Mr. Gene, Regensburg, Germany). The protein was expressed in *E. coli* BL21(DE3) and purified to homogeneity with a yield of 6.5 mg per litre of cell culture. Proper folding and secondary structure of DspA was verified by CD spectroscopy. Similarly to other related haloalkane dehalogenases, DspA exhibited CD spectrum with one positive peak at 195 nm and two negative maxima at 208 and 220 nm, a characteristic of α-helical content, suggesting correct folding of the enzyme (Supplementary Fig. S2). Thermally induced denaturation of DspA was parallelly tested by monitoring ellipticity at 220 nm at elevated temperature. Determined melting temperature of the enzyme, $T_m = 44.2 \pm 0.3$ °C, was in good agreement with the range of melting temperatures observed for other haloalkane dehalogenases [29].

The size of the native structure of DspA was estimated by native electrophoresis and by gel filtration chromatography. DspA, with an expected molecular mass of 36 kDa, existed as a dimer in the absence of the salt (Fig. 1A), but was predominantly monomeric in the presence of 150 mM NaCl (Fig. 1B). Similar behavior was observed for haloalkane dehalogenase DatA, which forms monomer and dimer under high- and low-salt conditions, respectively [17]. Other haloalkane dehalogenases display predominantly monomeric form [5,6,15]. The exceptions are DmbC and DrbA, forming the oligomeric complexes of size larger than 2000 kDa [12] and DbjA from *Bradyrhizobium japonicum* USDA110 forming monomer, dimer, and tetramer in a buffer of different pH [30].

3.2. Analysis of substrate specificity and steady-state kinetics

The substrate specificity profile of DspA was investigated with the set of thirty different halogenated hydrocarbons (Table 1). DspA exhibited better activities towards terminally halogenated substrates with following substituent preference: brominated > iodinated >> chlorinated. The highest activity of DspA was observed with 1-bromohexane (0.031 μmol s^{-1} mg^{-1}), 1,3-dibromopropane (0.023 μmol s^{-1} mg^{-1}) and 1-bromo-3-chloropropane (0.014 μmol s^{-1} mg^{-1}). Additionally, PCA was performed to quantitatively compare the overall activity and substrate specificity of DspA with other characterized haloalkane dehalogenases. Compared to the most experimentally characterized haloalkane dehalogenases, the overall activity of DspA was lower (Supplementary Fig. S3A). The enzyme exhibited up to 500 times lower activity than the most active haloalkane dehalogenases DbjA and LinB, but up to 170 times higher activity than the least active haloalkane dehalogenases DmbC and DrbA. Due to its preference

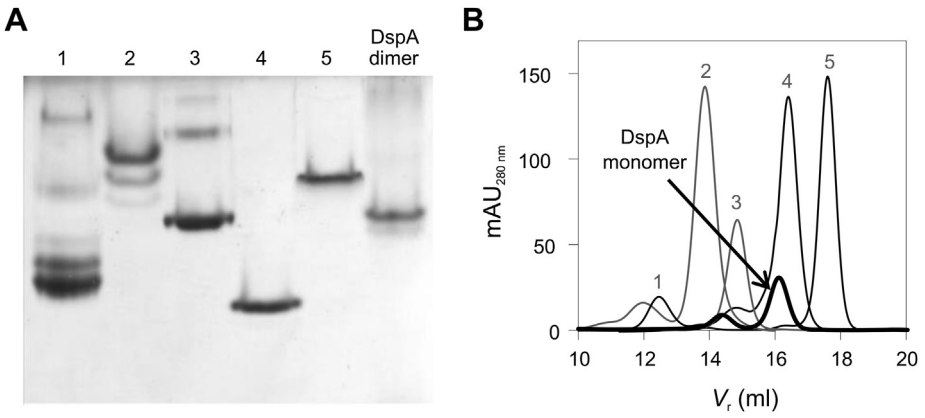

Fig. 1. A) Native electrophoresis of DspA, molecular weight standards and other haloalkane dehalogenases. (1) Ovalbumin (43 kDa), (2) conalbumin (75 kDa), (3) albumin (67 kDa), (4) monomeric haloalkane dehalogenase LinB (33 kDa) and (5) dimeric haloalkane dehalogenase DbjA (68 kDa). B) Gel filtration chromatogram of DspA (bold line) and calibration standards. (1) Aldolase (158 kDa), (2) conalbumin (75 kDa), (3) ovalbumin (43 kDa), (4) carbonic anhydrase (29 kDa) and (5) ribonuclease A (14 kDa).

for brominated and iodinated compounds, DspA was assigned to the substrate specificity group IV (Supplementary Fig. S3B and S3C), together with the haloalkane dehalogenases DatA, DmbC and DbeA [22]. Since the activity assay was performed in the absence of salts, we assume that DspA existed predominantly in dimeric form during the measurement.

Steady-state kinetic analysis was performed with the substrate 1,3-dibromopropane, for which the enzyme exhibited one of the highest activities. Kinetics of 1,3-dibromopropane conversion to 3-bromo-1-propanol by DspA ($K_{0.5}$ = 0.92 ± 0.27 mM; k_{cat} = 1.57 ± 0.32 s^{-1}) follows a mechanism with positive cooperative substrate binding and Hill coefficient (n_H) equal to 1.3 ± 0.3. Comparison of DspA kinetic parameters with other haloalkane dehalogenases is summarized in Supplementary Table S1.

Table 1
Specific activities of haloalkane dehalogenase DspA towards thirty halogenated substrates.[a]

Substrate	Specific activity [μmol s^{-1}/mg of enzyme]
1-Chlorobutane	NA[b]
1-Chlorohexane	NA[b]
1-Bromobutane	0.013
1-Bromohexane	0.031
1-Iodopropane	0.006
1-Iodobutane	0.008
1-Iodohexane	0.010
1,2-Dichloroethane	NA[b]
1,3-Dichloropropane	NA[b]
1,5-Dichloropentane	0.003
1,2-Dibromoethane	0.002
1,3-Dibromopropane	0.023
1-Bromo-3-chloropropane	0.014
1,3-Diiodopropane	0.005
2-Iodobutane	0.002
1,2-Dichloropropane	NA[b]
1,2-Dibromopropane	0.001
2-Bromo-1-chloropropane	0.001
1,2,3-Trichloropropane	NA[b]
Bis(2-chlorethyl)ether	NA[b]
Chlorocyclohexane	NA[b]
Bromocyclohexane	NA[b]
(Bromomethyl)cyclohexane	0.010
1-Bromo-2-chloroethane	0.003
Chlorocyclopentane	0.001
4-Bromobutyronitrile	0.006
1,2,3-Tribromopropane	0.001
1,2-Dibromo-3-chloropropane	0.001
3-Chloro-2-methylpropene	0.002
2,3-Dichloropropene	0.001

[a] Each specific activity was measured in three independent replicates with the standard error of less than 10%.
[b] No activity detected under tested conditions.

3.3. Determination of enantioselectivity

The enantioselectivity of DspA was assessed by determining the kinetic resolution of racemic β-brominated alkanes and α-brominated esters. Medium enantioselectivity was observed with 2-bromobutane (*E*-value = 52, Fig. 2A) and 2-bromopentane (*E*-value = 29, Fig. 2B), while none or very low enantioselectivity was observed with methyl 2-bromobutyrate (*E*-value = 1) and ethyl 2-bromobutyrate (*E*-value = 2). To the best of our knowledge, DspA is the most enantioselective of all biochemically characterized haloalkane dehalogenases towards 2-bromobutane and thus may be attractive for biocatalysis [17,31,32].

3.4. Identification of putative eukaryotic haloalkane dehalogenases

DspA represents the first biochemically characterized member of the haloalkane dehalogenase family originating from eukaryotic organism. Growing number of completed genome sequencing projects shows that haloalkane dehalogenases are rather common enzymes. Database searches followed by cluster analysis of the 15,155 sequences of α/β-hydrolases similar to haloalkane dehalogenases led to the identification of two haloalkane dehalogenase clusters: (i) the main cluster composed of 622 non-unique sequences originating from 496 different sources, including unclassified organisms and artificial constructs; and (ii) cluster containing 8 putative haloalkane dehalogenase sequences from *Aspergillus* and *Neosartorya* species. After excluding incomplete, degenerated and diverged sequences, putative haloalkane dehalogenases were found in the genomes of 16 eukaryotic organisms: *Aspergillus clavatus* NRRL 1, *Aspergillus fumigatus* Af293, *Aspergillus nidulans* FGSC A4, *Aspergillus niger* ATCC 1015, *A. niger* CBS 513.88, *Branchiostoma floridae*, *Caligus rogercresseyi*, *Ciona intestinalis*, *Ectocarpus siliculosus* Ec 32, *Lepeophtheirus salmonis*, *Neosartorya fischeri* NRRL 181, *Saccoglossus kowalevskii*, *S. purpuratus, Trichoderma atroviride* IMI 206040, *Trichoderma virens* Gv29-8 and *Trichoplax adhaerens* (Table 2). The identified proteins were further classified to the haloalkane dehalogenase subfamilies based on the composition of their catalytic pentads. The catalytic pentad of haloalkane dehalogenases involves Asp (nucleophile), His (base), Asp or Glu (catalytic acid) and two halide-stabilizing residues, Trp—

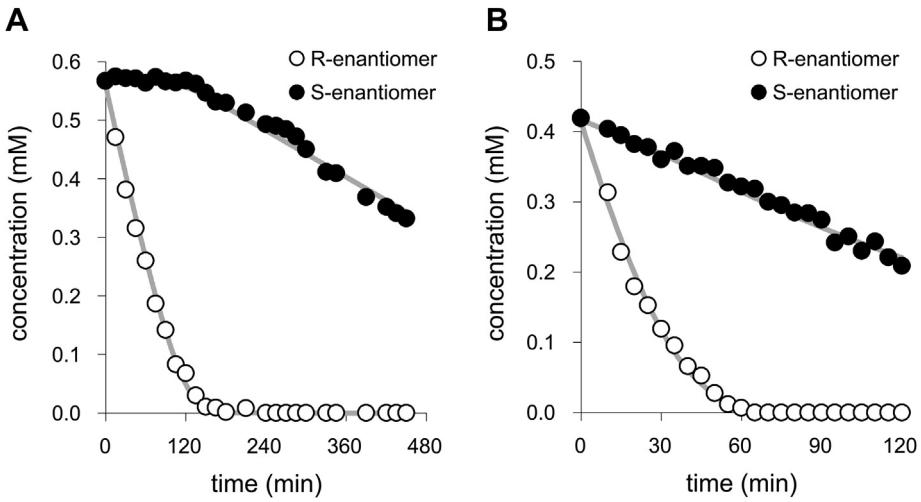

Fig. 2. Kinetic resolution of A) 2-bromobutane and B) 2-bromopentane by DspA.

Trp or Trp–Asn [18,33,34]. Most of the putative eukaryotic haloalkane dehalogenases were classified to the HLD-II (Asp–Glu–His + Asn–Trp) or the HLD-III (Asp–Asp–His + Asn–Trp) subfamily. Only two putative eukaryotic members were assigned to the HLD-I subfamily (Asp–Asp–His + Trp–Trp) (Table 2).

The biological role of haloalkane dehalogenases in the organisms that do not grow on haloalkanes is not clear [11–17,22]. The data currently available in the genome databases do not provide any obvious indication of potential biological role of DspA. The low catalytic efficiency of DspA with tested substrates suggests that the set of 30 halogenated compounds commonly used for characterization of haloalkane dehalogenases may not contain the natural substrate of DspA or that this enzyme may have another function. Alternatively, the DspA enzyme heterologously produced in *E. coli* may lack important post-translational modifications. DspA has a typical catalytic pentad of HLD-II subfamily members—nucleophile Asp120, catalytic acid Glu144, base His285 and two halide-stabilizing residues Asn53 and Trp121. Previous studies showed that it is closely evolutionary related to the experimentally characterized haloalkane dehalogenases LinB (40% amino acid sequence identity) [6] and DmbA (42%) [13], but also to *Renilla* luciferase RLuc (46%) [18,35]. In order to explore whether DspA possesses luciferase activity, the enzyme was tested with coelenterazine, which is the natural substrate of *Renilla* luciferase RLuc. However, the luciferase activity of DspA was not detected under used conditions (Supplementary Fig. S4). Many simple as well as complex natural organohalogens are biosynthesized by various marine plants and sponges presumably for their chemical defense [36]. We speculate that DspA isolated from marine sea urchin *S. purpuratus* might be involved in metabolism of these halogenated compounds to enable growth of the organism on marine plants producing such feeding-deterrent substances. Identification of biological role of DspA as well as many other haloalkane dehalogenases remains a challenge for their future research.

Table 2
Putative haloalkane dehalogenases identified in the genomes of eukaryotic organisms.

Accession No.[a]	Source organism	Subfamily
Microorganism origin		
XP_001275122	*Aspergillus clavatus* NRRL 1	HLD-III
XP_747036	*Aspergillus fumigatus* Af293	HLD-III
XP_660404	*Aspergillus nidulans* FGSC A4	HLD-III
EHA28085	*Aspergillus niger* ATCC 1015	HLD-III
XP_001401350	*Aspergillus niger* CBS 513.88	HLD-III
CAK46720		
XP_001261805	*Neosartorya fischeri* NRRL 181	HLD-III
EHK43615	*Trichoderma atroviride* IMI 206040	HLD-II
EHK50390 [b]		
EHK20782	*Trichoderma virens* Gv29-8	HLD-II
EHK22813		
EHK18062 [b]		
Non-microorganism origin		
XP_002611539	*Branchiostoma floridae*	HLD-II
ACO10768	*Caligus rogercresseyi*	HLD-I
XP_002127127	*Ciona intestinalis*	HLD-II
CBJ34072 [b]	*Ectocarpus siliculosus* Ec 32	HLD-II
ADD38698	*Lepeophtheirus salmonis*	HLD-I
XP_002738321	*Saccoglossus kowalevskii*	HLD-II
XP_002730984		
XP_794172	*Strongylocentrotus purpuratus*	HLD-II
XP_788943		
XP_798042		
XP_794218		
XP_792159		
XP_002116678	*Trichoplax adhaerens*	HLD-II
XP_002116677		

[a] Accession number to Protein database of NCBI.
[b] Halide-binding asparagine replaced by valine.

4. Conclusions

The novel haloalkane dehalogenase DspA from *S. purpuratus* described in this paper serves as a paradigm for non-microbial haloalkane dehalogenases. The interesting properties of DspA include its unique enantioselectivity with 2-bromobutane. The identification of other putative haloalkane dehalogenases in eukaryotic genomes suggests that there might be many other enzymes of non-microbial origin with haloalkane dehalogenase activity. Altogether, our study demonstrates the power of genome sequencing projects and bioinformatic analyses for exploration of the diversity of enzyme families and discovery of biocatalysts with novel catalytic properties.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2013.07.025.

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