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Supporting information for article:

Structural and functional analysis of a novel haloalkane dehalogenase with two halide-binding sites

Radka Chaloupkova, Tatyana Prudnikova, Pavlina Rezacova, Zbynek Prokop, Tana Koudelakova, Lukas Daniel, Jan Brezovsky, Wakako Ikeda-Ohtsubo, Yukari Sato, Michal Kuty, Yuji Nagata, Ivana Kuta Smatanova and Jiri Damborsky

Table S1 Specific activities of DbeA wt and its variant DbeA ΔCl towards the set of 30 different halogenated substrates**.**

Substrate	Specific activity (μmol s^{-1} mg^{-1} of enzyme)	
	DbeA wt	DbeA ΔCl
1-chlorobutane	0.0010	0.0008
1-chlorohexane	0.0211	0.0006
1-bromobutane	0.0297	0.0011
1-bromohexane	0.0176	0.0006
1-iodopropane	0.0274	0.0019
1-iodobutane	0.0236	0.0005
1-iodohexane	0.0155	0.0005
1,2-dichloroethane	-[a]	-[a]
1,3-dichloropropane	-[a]	0.0011
1,5-dichloropentane	0.0247	0.0006
1,2-dibromoethane	0.0102	0.0098
1,3-dibromopropane	0.0357	0.0034
1-bromo-3-chloropropane	0.0416	0.0033
1,3-diiodopropane	0.0926	0.0021
2-iodobutane	0.0017	0.0010
1,2-dichloropropane	-[a]	-[a]
1,2-dibromopropane	0.0033	0.0023
2-bromo-1-chloropropane	0.0013	0.0028
1,2,3-trichloropropane	-[a]	0.0002
bis(2-chloroethyl)ether	0.0012	0.0031
chlorocyclohexane	-[a]	-[a]
bromocyclohexane	0.0054	0.0010
(1-bromomethyl)cyclohexane	0.0012	0.0001
1-bromo-2-chloroethane	0.0094	0.0010
chlorocyclopentane	0.0033	0.0012
4-bromobutyronitrile	0.0412	0.0045
1,2,3-tribromopropane	0.0059	0.0057
1,2-dibromo-3-chloropropane	0.0025	0.0033
3-chloro-2-methylpropene	0.0111	0.0050
2,3-dichloropropene	0.0025	0.0032

[a] - activity not detectable under tested conditions.

Table S2 Steady-state kinetic constants of DbeA and other HLDs with 1-chlorobutane, 1-bromobutane and 1,3-dibromopropane.

	k_{cat} (s^{-1})	$K_{0.5}$ (mM)	*n*	K_{si} (mM)	*m*
1-chlorobutane					
DbeA	0.17 ± 0.01	3.23 ± 0.39	-[a]	-[a]	-[a]
DbjA[b]	1.40 ± 0.42	5.62 ± 0.41	-[a]	-[a]	-[a]
DhaA	0.48 ± 0.01	0.24 ± 0.01	-[a]	-[a]	-[a]
LinB[c]	1.11 ± 0.03	0.23 ± 0.02	-[a]	-[a]	-[a]
DmbA[b]	0.08 ± 0.01	0.16 ± 0.04	-[a]	-[a]	-[a]
1-bromobutane					
DbeA	3.91 ± 0.16	0.51 ± 0.02	1.33 ± 0.09	27.06 ± 3.05	-[a]
DbjA[b]	1.14 ± 0.08	0.01± 0.004	-[a]	2.40 ± 0.79	-[a]
DhaA[d]	0.98 ± 0.05	0.35 ± 0.02	-[a]	-[a]	-[a]
LinB	2.26 ± 0.01	0.12 ± 0.01	0.44 ± 0.01	-[a]	-[a]
DmbA[b]	0.24 ± 0.03	2.71 ± 0.97	-[a]	-[a]	-[a]
1,3-dibromopropane					
DbeA	7.62 ± 1.48	1.99 ± 0.49	-[a]	1.64 ± 0.31	2.29 ± 0.21
DbjA[b]	3.60 ± 0.49	0.22 ± 0.07		6.98 ± 2.91	
DhaA	2.50 ± 0.32	0.14 ± 0.06	-[a]	1.70 ± 0.40	-[a]
LinB	40.9 ± 5.20	24.1 ± 3.23	-[a]	0.49 ± 0.06	-[a]
DmbA[b]	9.20 ± 1.17	4.52 ± 0.71	-[a]	2.65 ± 0.49	-[a]

$K_{0.5}$ - concentration of substrate at half maximal velocity, k_{cat} - catalytic constant, *n* - Hill coefficient K_{si} - substrate inhibition constant, *m* - Hill coefficient in inhibitory mode. All measurements were performed at pH 8.6 and 37 °C.

[a] - not applicable

[b] data from (Monincova, 2007)

[c] data from (Chaloupkova *et al.*, 2003)

[d] data from (Schindler *et al.*, 1999)

Table S3 Thermal stability of DbeA and other biochemically characterized HLDs[a] quantified by melting temperatures.

HLD enzyme	T_m (°C)
DbeA	58.5 ± 0.2
DbjA	53.6 ± 0.6
DhaA	50.4 ± 0.3
LinB	48.0 ± 0.5
DmbA	52.7 ± 0.2
DhlA	39.2 ± 0.0
DatA	48.3 ± 0.2
DmbB[b]	57.4 ± 0.6
DmbC[b]	45.8 ± 0.4
DrbA[b]	39.4 ± 0.1

[a] data from (Koudelakova *et al.*, 2011)

[b] thermal stability of DmbB, DmbC and DrbA were determined under different experimental conditions (heating rate 0.5 °C/min) compare to other HLDs (heating rate 1.0 °C/min).

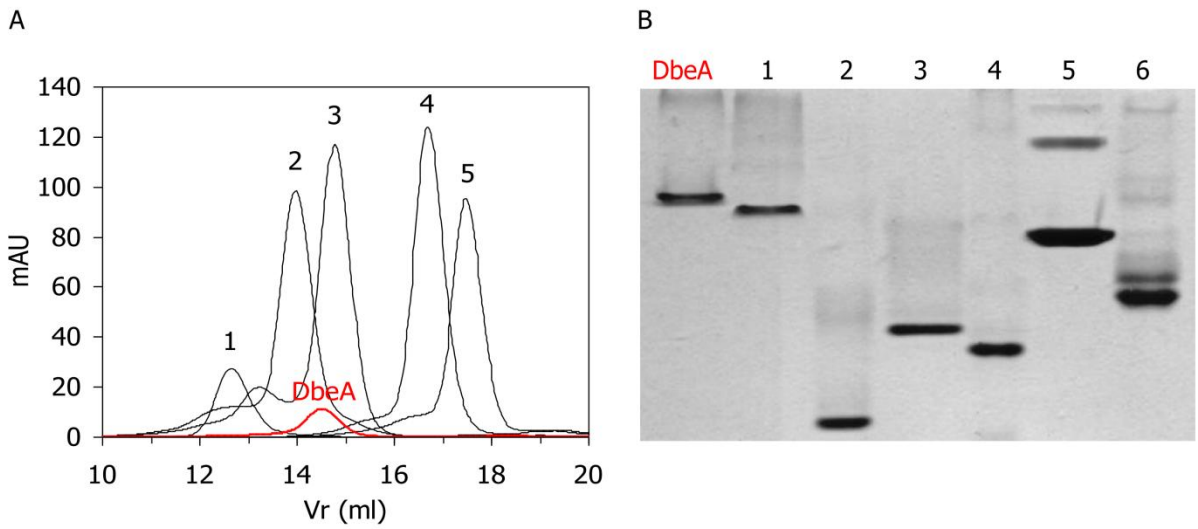

Figure S1 (*A*) Gel filtration chromatogram of DbeA and molecular weight calibration standards. Red line, DbeA; line 1, aldolase (158 kDa); line 2, conalbumin (75 kDa); line 3, ovalbumin (43 kDa); line 4, chymotripsinogen A (25 kDa); line 5 ribonuclease A (14 kDa). (*B*) Native electrophoresis of DbeA, molecular weight standards and other haloalkane dehalogenases. Red lane, DbeA; lane 1, DbjA (68 kDa); lane 2, DhaA (33 kDa); lane 3, LinB (33 kDa); lane 4, DmbA (34 kDa); lane 5, albumin (67 kDa); lane 6, ovalbumin (43 kDa). Theoretical molecular weight (M_W) of DbeA monomer and dimer is 34 and 68 kDa, respectively. Experimentally determined M_W of DbeA is 64 kDa, suggesting that DbeA forms a dimer under tested conditions.

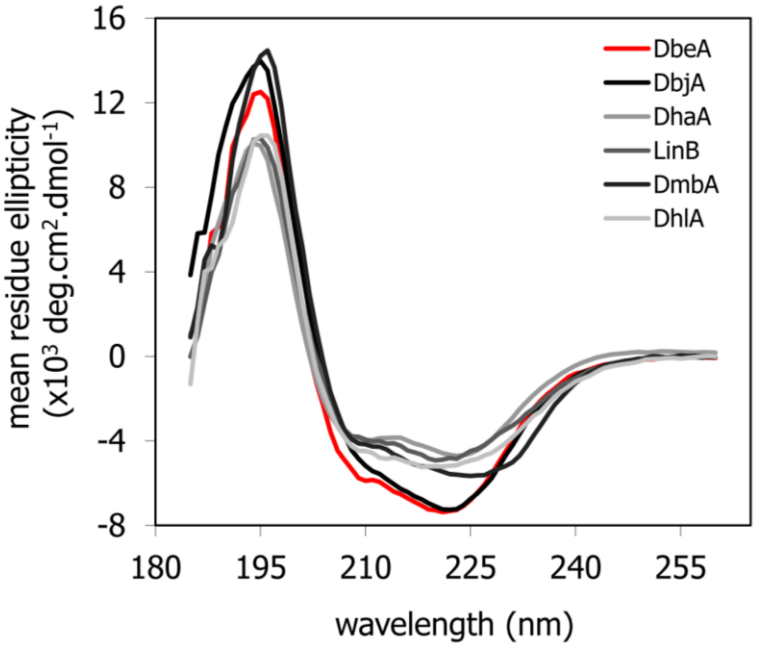

Figure S2 Far-UV CD spectra of DbeA and other related HLDs.

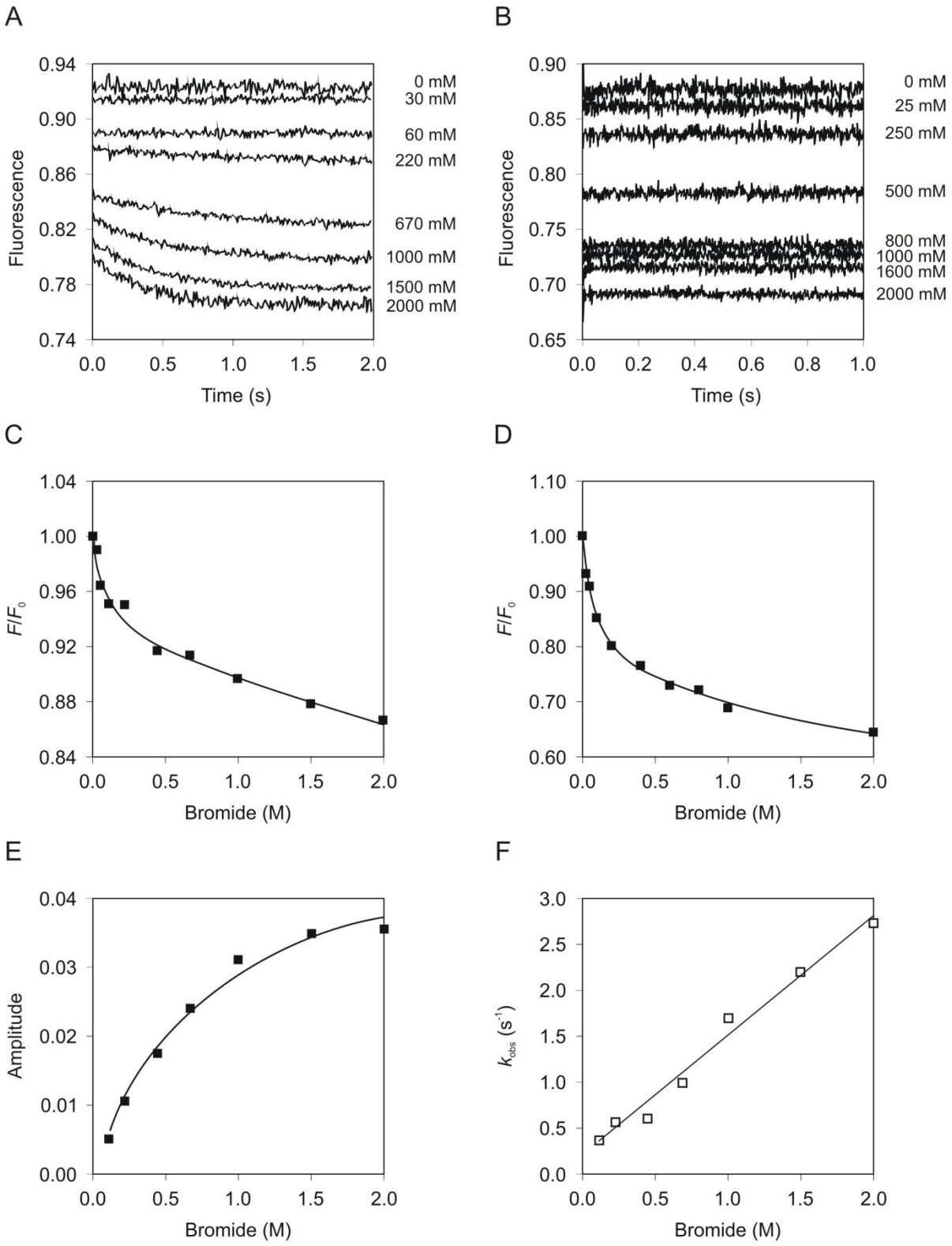

Figure S3 Stopped flow fluorescence analysis of bromide binding to DbeA wt and DbeA ΔCl. (*A*) Fluorescence traces obtained upon mixing 30 μM DbeA wt with bromides. (*B*) Fluorescence traces obtained upon mixing 30 μM DbeA ΔCl with bromides. (*C*) Bromide concentration dependence of rapid equilibrium fluorescence quench of DbeA wt with equilibrium constant (K_{d1}) 0.33 ± 0.08 M. (*D*)

Bromide concentration dependence of rapid equilibrium fluorescence quench of DbeA ΔCl with equilibrium constant (K_d) 0.09 ± 0.01 M. (*E*) Bromide dependence of the amplitude of the slow exponential fluorescence quench of DbeA wt with equilibrium constant (K_{d2}) 0.84 ± 0.13 M. Solid lines represent best fits to the data based on Stern-Volmer equation $F/F_0 = (1+(fK_q[Br^-]))/(1+K_{Br}[Br^-])$ in which F/F_0 is the relative fluorescence; f is the relative fluorescence intensity of enzyme-bromide complex; K_{Br} is the association equilibrium constant of specific binding of bromide; K_q is the quenching constant which is the apparent association equilibrium constant of the non-specific quenching interaction between bromide and the fluorophore. (*F*) Bromide dependence of the observed rate constants of the slow exponential fluorescence quench of DbeA wt. Solid line represents the best fit to the data using the equation $k_{obs} = k_{assoc}[Br^-]/k_{dissoc}$ where k_{assoc} and k_{dissoc} are association ($1.30 \pm 0.08\ M^{-1}\ s^{-1}$) and dissociation ($0.21 \pm 0.08\ s^{-1}$) rate constants, respectively.

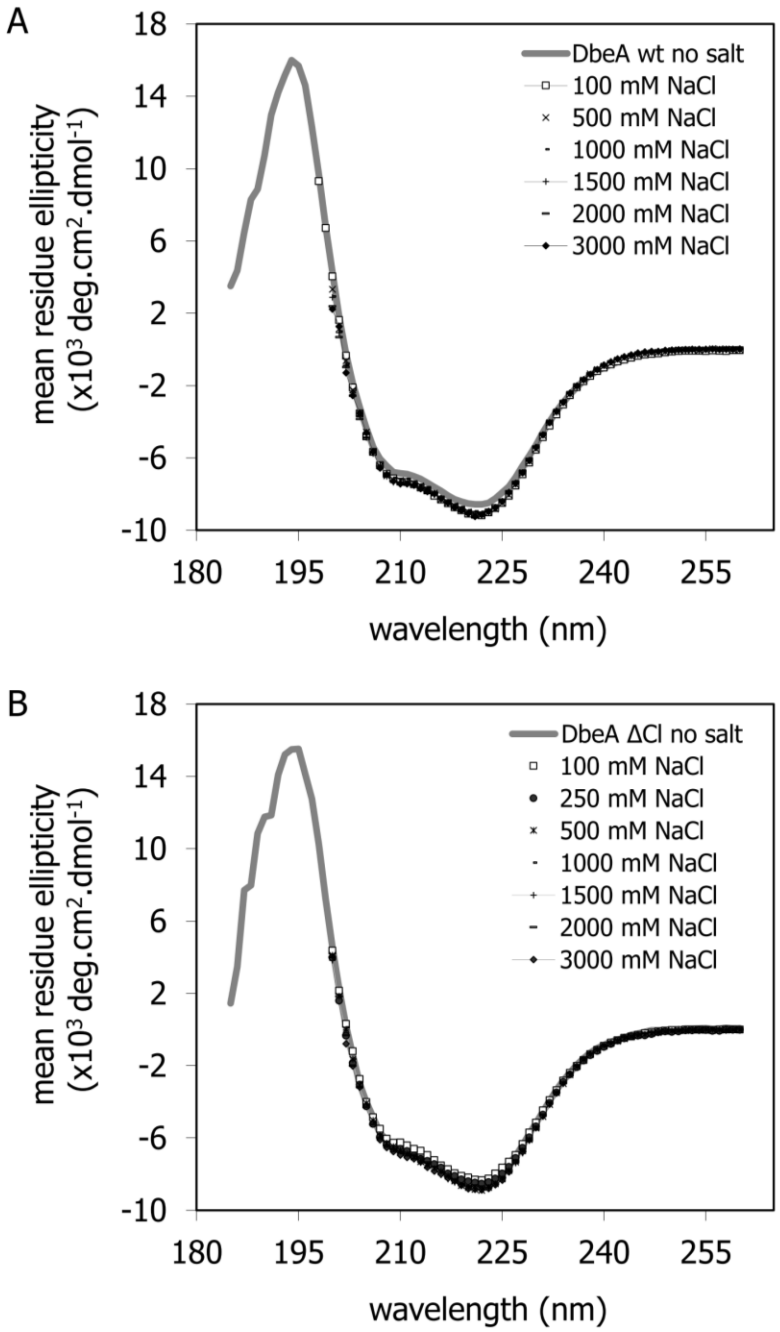

Figure S4 Far-UV CD spectra of DbeA wt (*A*) and its variant DbeA ΔCl (*B*) in the presence and absence of various concentrations of sodium chloride.

Supplementary References

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