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HALOALKANE DEHALOGENASES: DMXA FROM *MARINOBACTER SP.* ELB 17 AND DPCA FROM *PSYCHROBACTER CRYOHALOLENTIS* K5, FROM THE CRYSTALLIZATION TO THE STRUCTURE ANALYSIS

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The selected enzymes, belonging to the family of haloalkane dehalogenases (EC 3.8.1.5; HLDs), catalyzing the hydrolytic conversion of halogenated aliphatic compounds to their corresponding alcohols, were isolated from *Psychrobacter* cryohalolentis K5 (DpcA) and from *Marinobacter sp.* ELB 17 (DmxA).

Both are exctremoenzymes, exhibiting height enantioselectivity, however reveals the highest activity at low and height temperatures, respectively, what highlights them among another HLDs.

The enzymes were crystallized and obtained diffraction data of the crystals was refined to the resolution 1.05 Å for DpcA and 1.45 Å for DmxA. Diffraction data were collected at the beamline 14.2, Helmholtz-Zentrum Berlin (HZB) (Germany) at the BESSY II electron storage ring, equipped with a detector Rayonics MX-225 CCD, wavelength 0.978 Å and on the beamline MX- ID29 at the ESRF electron-storage ring (Grenoble, France), with Pilatus 6M-F detector at the wavelengths of 0.972 Å.

Crystals of DpcA belonged to $P2_1$ the primitive monoclinic space group with unit-cell parameters: a = 41.3, b = 79.4, c = 43.5 Å, = 90 , = 95.0 and contained one molecule in the asymmetric unit. Crystals of DmxA belonged to the primitive orthorhombic $P2_12_12_1$ space group, with unit-cell parameters: a = 43.371, b = 78.343, c = 150.51Å; = = 90 and contained 2 molecules in the asymmetric unit. The structures were solved by molecular replacement with *MOLREP* from the CCP4 software suite. The coordinates of *Xanthobacter autotrophicus* (PDB code: 1B6G; 40% sequence identities for 121 residues and 53% sequence similarity was used as search model for DpcA structure and for DmxA from *Rhodococcus rhodochrous* (PDB entry 4E46; 48% sequence identity for 142 residues and 63% sequence similarity). Refining was carried out manually with REFMAC5 and WinCOOT 6.4 from the CCP4 suit.

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COMPARISON OF CATALYTIC PERFORMANCE OF HALOALKANE DEHALOGENASES IN DEEP EUTECTIC AND ORGANIC CO-SOLVENTS

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Haloalkane dehalogenases (HLDs, EC 3.8.1.5) make up an important class of enzymes that cleave carbon-halogen bonds in a broad range of halogenated aliphatic compounds with potential utilization in biodegradation, biocatalysis

and biosensing [1]. Application potential of HLDs could be further improved by adding appropriate co-solvent to the reaction media, leading to enhanced solubility of their hydrophobic substrates and suppression of the substrates spontaneous hydrolysis. Here we focus on biotransformation by HLDs in the presence of novel and green deep eutectic solvent ethaline, consisting of ethylene glycol and choline chloride [2]. Catalytic performance of three haloalkane dehalogenases, DbjA, DhaA and LinB, in aqueous solutions of ethaline was systematically and critically compared with the enzyme reactivity in the presence of the individual components of ethaline and two conventional organic solvents, methanol and acetone [3]. The activities of studied enzymes responded differently to the different co-solvents. All of the enzymes tolerated ethaline and ethylene glycol significantly better than methanol and acetone. Haloalkane dehalogenase DhaA was found to be the most tolerant of ethaline. Its excellent compatibility with the solvent was demonstrated by the enzyme's enhanced thermal stability, and by its retention of detectable catalytic activity even at very high ethaline concentrations (? 90% v/v). In contrast, the activities of DbjA and LinB were higher in ethylene glycol than in ethaline. Moreover the activity of DbjA was 1.5 times higher in 50% (v/v) ethylene glycol than in a pure buffer. Additionally, the enantioselectivity of DbjA increased more than 4-fold in the presence of ethaline or ethylene glycol. Our results demonstrate that ethylene glycol and deep eutectic solvent ethaline can have beneficial effects on catalysis by haloalkane dehalogenases, broadening their usability in "green" biotechnologies.

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MODIFICATION OF SUBSTRATE SPECIFICITY AND CATALYTIC ACTIVITY OF HALOALKANE DEHALOGENASE LINB BY OPENING *DE NOVO* ACCESS TUNNELS

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Enzymes are natural catalysts accelerating the rate of chemical reactions in living organisms. Some of the enzymes posses the active site deeply buried inside the protein interior, which is connected with surrounding environment by access pathways, called tunnels. The size, shape and flexibility of the tunnels play a significant role in the entry of substrates and solvent into the enzyme active site and egress of reaction products [1]. Haloalkane dehalogenase LinB from *Sphingobium japonicum* UT26 is an enzyme that catalyzes hydrolytic conversion of halogenated aliphatic hydrocarbons to their corresponding alcohols and halide anions [2]. Its active site is located in predominantly buried hydrophobic cavity that is linked with the protein surface by several tunnels [3]. Several variants of haloalkane dehalogenases LinB with modified tunnels were rationally designed and constructed by site directed and site saturation mutagenesis. Constructed LinB variants with closed and re-opened *de novo* tunnels were expressed in *Escherichia coli*, purified to homogeneity by metallo-affinity chromatography and kinetically characterized. Substrate specificity of the enzymes was determined spectrophotometrically towards a set of thirty different halogenated substrates and compared with substrate specificity profiles of the wild type dehalogenase enzymes. Multivariate statistical analysis of collected data revealed significant changes in substrate specificity profiles of tested enzymes. The LinB variants with closed tunnels formed a substrate specifity novel group, whereas the variants possessing *de novo* tunnels were classified into the same substrate specificity group as the LinB wt. All variants with closed tunnels showed considerably lower activities towards majority of tested substrates compared to the LinB wt. On the contrary, LinB variants with *de novo* tunnels showed comparable or even better activity than the wild type enzyme. Rational engineering of protein tunnels thus represents powerful approach for modification of substrate specificity and catalytic activity of the enzymes with buried active sites.

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