[MS32-P06] Crystal structure of haloalkane dehalogenase DpcA from psychrophilic *Psychrobacter cryohalolentis* K5

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Haloalkane dehalogenases (EC 3.8.1.5; HLDs) are microbial enzymes that catalyze the hydrolytic conversion of halogenated aliphatic compounds to their corresponding alcohols [1, 2], which is The hydrolytic dehalogenation accomplished by these enzymes is one of the most important steps in the biodegradation of 1-halo-n-alkanes and α,ω-dihalo-n-alkanes, which are serious halogenated pollutants [3]. HLDs have a broad substrate specificity [4] and a high enantioselectivity [5], which makes these enzymes applicable in bioremediation [6], in biosensing [7,8], biocatalysis [5,9], cellular imaging, and protein analysis [10, 11] . Understanding of the structural bases of the enzyme extremophilicity allows for the construction of HLD variants with improved activity and stability at low and high temperatures and thus enlarges their applicability in environmental and biosynthetic applications.A novel HLD enzyme, DpcA, exhibiting unique temperature profiles with exceptionally high activities at low temperature, was recently isolated from Gram-negative psychrophilic bacteria *Psychrobacter cryohalolentis* K5 [12] and crystallized by sitting-drop and hanging-drop vapour-diffusion techniques. Diffraction data were collected at the beamline 14.2, Helmholtz-Zentrum Berlin (HZB) (Germany) at the BESSY II electron storage ring, detector Rayonics MX-225 CCD [13]. Crystals of DpcA enzyme diffracted to the 1.05 Å resolution and crystals of DpcA complexes with 1-bromohexane and 1,2-dichloroethane diffracted to the 1.35 Å and 1.65 Å resolutions, respectively, belonged to *P*21 space group [14]. Structurally DpcA belongs to the superfamily of α/βhydrolase, determined by solving by molecular replacement with *MOLREP* [15] from the CCP4 software suite [16]. 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. DpcA protein has a globular shape and is composed of two domains. The core domain shows composed of eight β-strands, within one is antiparallel (β2). The central β-sheet is flanked on both sides by α-helices: four are on one side and two are on the other side of the sheet. The second domain, the cap structure is located at the C-terminal end of the β-sheet and is composed of α-helices and covers the active site. . The structure of DpcA is consimilar to the others structurally known HLD.

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[1] Damborsky, J, Rorije, E., Jesenska, A., Nagata, Y., Klopman, G., Peijnenburg, W.J.G.M. (2001). Environ. Toxicol. Chem. 20, 2681–2689.
[2] Janssen, D. B., Dinkla, I. J., Poelarends, G. J. & Terpstra, P. (2005). Environ.
[3] Poelarends, G. J., Zandstra M., Bosma T., Kulakov L. A., Larkin M. J., Marchesi J. R., Weightman A. J., and Janssen D. (2000). J. Bacteriol. 188, 2725-2731.
[4] Koudelakova, T., Chovancova, E., Brezovsky, J., Monincova, M., Fortova, A., Jarkovsky, J., Damborsky, J. (2011). Biochem. J. 435, 345-354.
[5] Prokop, Z., Sato, Y., Brezovsky, J., Mozga, T., Chaloupkova, R., Koudelakova, T., Jerabek, Stepankova, P., V., Natsume, R., van Leeuwen J. G. E. Janssen, D. B., Florian, J., Nagata, Y., Senda, T., Damborsky, J. (2010). Angew. Chem. Int. Ed. 49, 6111-6115.
[6] Stucki, G., Thuer, M. (1995). Environ. Sci. Technol. 29, 2339–2345.
[7] Campbell, D.W., Muller, C., Reardon, K.F. (2006). Biotechnol. Lett. 28, 883–887.
[8] Bidmanova, S., Chaloupkova, R., Damborsky, J., Prokop, Z. (2010). Anal. Bioanal. Chem. 398, 1891–1898.
[9] Westerbeek, A., Szymanski, W., Feringa, B.L., Janssen, D. B. (2011). ACS Catal. 1, 1654–1660.
[10] Los, G.V., Wood, K. (2007). Methods Mol. Biol. 356, 195–208.
[11] Ohana, R.F., Encell, L.P., Zhao, K., Simpson, D., Slater, M.R., Urh, M., Wood, K.V. (2009). Protein Expr. Purif. 68, 110–120.
[12]Drienovska,I.,Chovancova,E.,Koudelakova, T., Damborsky, J. and Chaloupkova, R. (2012). Appl. Environ. Microbiol. 78 (14), 4995-8.
[13] Mueller, U., Darowski, N., Fuchs, M.R., Förster, R., Hellmig, M., Paithankar, K.S., Pühringer, S., Steffien, M., Zocher, G., Weiss, M.S. (2012). J. Synchrotron Rad. 19, 442-449.
[14] Tratsiak,K., Degtjarik, O., Drienovska, I., Chrast, L., Rezacova, P., Kuty, M., Chaloupkova, R., Damborsky, J. and Kuta Smatanova, I. (2013). Acta Cryst. F69, 683-688.
[15] Vagin, A. and Teplyakov, A. (2010). Acta Cryst. D66, 22–25.
[16] Winn, M. D., Ballard, C. C., Cowtan, K. D., Dodson, E. J., Emsley, P., Evans, P. R., Keegan, R. M., Krissinel, E. B., Leslie, A. G. W., McCoy, A., McNicholas, S. J., Murshudov, G. N., Pannu, N. S., Potterton, E. A., Powell, H. R., Read, R. J., Vagin, A. and K. S. Wilson. (2011). Acta Cryst. D67, 235-242.

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