ESRF	**Experiment title:** Crystal structures of the mutant and substrate complexes of the haloalcane dehalogenase from Sphingomonas paucimobilis UT26	**Experiment number**: LS-2063
Beamline: ID14 2	**Date of experiment**: from: 03 December 2001 to: 04 December 2001	**Date of report**: 21 August 2002
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Report:

Haloalkane dehalogenase LinB is the enzyme isolated from a γ-hexachlorocyclohexane degrading bacterial strain *Sphingomonas paucimobilis* UT26. The enzyme converts a broad range of halogenated cyclic and aliphatic compounds to their corresponding alcohols [1]. The active site of haloalkane dehalogenase LinB includes a catalytic triad similar to the haloalkane dehalogenases from *Xanthobacter autotrophicus* GJ10 and *Rhodococcus rhodochrous* NCIMB13064. The dehaloganation reaction is catalysed without oxygen or any other cofactor [2].

The purposes of the project are the re-construction of the active site of the putative haloalkane dehalogenase from *Mycobacterium tuberculosis* H37Rv (protein Rv2579) and the study of the dehalogenation reactions on the other halogenated ligands. Rv2579 protein is very difficult to manipulate, i.e. purify and crystallize, due to high pathogenicity of *M. tuberculosis*. Therefore, six cumulative single-point mutations were introduced in the closely related protein LinB. Mutations were designed by comparison of the crystallographic structure of LinB [2] and homology model of Rv2579 [3].

The native single crystals of average size 0.4 x 0.3 x 0.04 mm were soaked in the reservoir solutions containing the 25mM halogenated ligands. There were used 1-chlorobutan, 1,2,3-trichloropropan and chlorocyclohexan. The best crystals belong to space group $P2_12_12$ with unit cell parameters a=50.6, b=72.1, c=73.3 Å. There is one molecule per asymmetric unit. The crystals of LinB mutant have unit cell parameters

a=50.6, b=72.1, c=73.3 Å and belong to space group $P2_12_12$. The statistics of the best datasets are summarized in Table. For the structure solution has been used molecular replacement with coordinates of native LinB.

	LinB mutant	1-chlorobutan	1,2,3-trichloropropan	chlorocyclohexan
Beamline	ID14 2	ID14 2	ID14 2	ID14 2
Detector	ADSC Q4	ADSC Q4	ADSC Q4	ADSC Q4
Distance	124 mm	125 mm	120 mm	125 mm
Wavelength	0.933Å	0.933Å	0.933Å	0.933Å
Exposure	10s	12s	10s	11s
Resolution	30-1.5Å	30-1.65Å	30-1.6Å	30-1.65Å
Total reflections	760645	390584	325309	353193
Unique reflections	43708	33142	36132	32936
Completeness	99.9%	99.3%	99.8%	98.1%
R-merge (last shell)	0.064 (0.258)	0.047 (0.149)	0.052 (0.174)	0.057 (0.170)

[1] Nagata, Y., Miyauchi, K., Damborsky, J., Manova, K., Ansorgova, A. and Takagi, M. (1997) Appl. Environ. Microbiol. 63, 3707-3710.

[2] Marek, J., Vévodová, J., Kutá Smatanová, I., Nagata, Y., Svensson, L.A., Newman, J., Takagi, M., Damborsky, J., 2000: Crystal Structure of the Haloalkane Dehalogenase from *Sphingomonas paucimobilis* UT26. *Biochemistry* 39: 14082-14086.

[3] Damborsky, J., Koca, J., 1999: Analysis of the Reaction Mechanism and Substrate Specificity of Haloalkane Dehalogenases by Sequential and Structural Comparisons. *Protein Engineering* 12: 989-998.