

Highly Enantioselective *sec*-Alkyl Sulfatase Activity of *Sulfolobus acidocaldarius* DSM 639

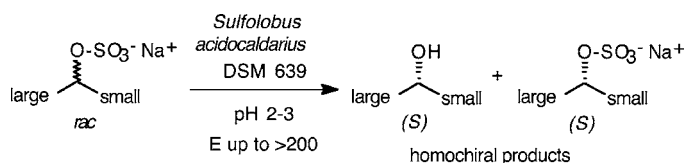
Sabine R. Wallner, Bettina M. Nestl, and Kurt Faber*

Department of Chemistry, Organic and Bioorganic Chemistry, University of Graz,
Heinrichstrasse 28, A-8010 Graz, Austria

kurt.faber@uni-graz.at

Received October 29, 2004

ABSTRACT



rac-sec-Alkyl sulfate esters **1a–4a** were resolved in high enantioselectivities with *E*-values up to >200 using whole cells of aerobically grown *Sulfolobus acidocaldarius* DSM 639. The stereochemical course of this biohydrolysis was shown to proceed with strict inversion of configuration; thus, the preferred (*R*)-enantiomers were converted into the corresponding (*S*)-*sec*-alcohols to furnish a *homochiral* product mixture.

Sulfatases catalyze the hydrolytic cleavage of the sulfate ester bond.¹ In contrast to the majority of hydrolytic biotransformations catalyzed by lipases, esterases, and proteases, which do not alter the stereochemistry of the substrate, the stereochemical course of sulfate ester hydrolysis can be controlled by the choice of the appropriate subtype of sulfatase enzyme: Thus, whereas aryl sulfatases generally act through *retention* of configuration at the sulfated carbon atom by cleavage of the S–O bond,² alkyl sulfatases lead to *inversion* of configuration by acting on the C–O bond.^{3,4} This rarely observed phenomenon makes them particularly attractive for the design of so-called deracemization processes, which allow the transformation of a racemate into a single stereochemical product in 100% theoretical yield.⁵ This can be accomplished by removal of the sulfate ester moiety

from the remaining nonconverted sulfate ester by acid-catalyzed hydrolysis with retention of configuration.⁶

On the basis of vague hints on the stereospecific and enantioselective hydrolysis of alkyl sulfate esters,³ we recently reported an alkyl sulfatase (termed “RS2”) from *Rhodococcus ruber* DSM 44541.^{7,8} On one hand, the enzyme displayed absolute *stereospecificity* by acting with strict inversion of configuration of simple *sec*-alkyl esters, but its *enantioselectivity* was less than perfect: although 2-octyl sulfate (*rac-1a*) was resolved with an acceptable *E*-value of 21, no appreciable enantioselectivities were observed for 3- and 4-octyl sulfate (*E* < 5).⁷ Attempts to enhance selectivities by enzyme inhibition (e.g., addition of Fe³⁺)⁹ were successful but (as usual in this technique) led to a significant loss of catalytic activities. Furthermore, the substrate tolerance of sulfatase RS2 was rather narrow, as substrates bearing bulky aryl groups (e.g., *rac-4a*) were not accepted.⁷

Our search for novel (and more selective) alkyl sulfatases was led by the idea that organisms known to possess a rich

* Corresponding author. Phone: +43-316-380-5332. Fax: +43-316-380-9840.

(1) Dodgson, K. S.; White, G. F.; Fitzgerald, J. W. *Sulfatases of Microbial Origin*; CRC Press: Boca Raton, FL, 1982; Vols. 1 and 2.

(2) Boltes, I.; Czapińska, H.; Kahnert, A.; von Bülow, R.; Dierks, T.; Schmidt, B.; von Figura, K.; Kertesz, M. A.; Uson, I. *Structure* **2001**, *9*, 483–491.

(3) Shaw, D. J.; Dodgson, K. S.; White, G. F. *Biochem. J.* **1980**, *187*, 181–196.

(4) Pogorevc, M.; Kroutil, W.; Wallner, S. R.; Faber, K. *Angew. Chem., Int. Ed. Engl.* **2002**, *41*, 4052–4054.

(5) Faber, K. *Chem. Eur. J.* **2001**, *7*, 5004–5010.

(6) Wallner, S. R.; Nestl, B.; Faber, K. *Tetrahedron* **2004**, in press.

(7) Pogorevc, M.; Faber, K. *Tetrahedron: Asymmetry* **2002**, *13*, 1435–1441.

(8) Pogorevc, M.; Faber, K. *Appl. Environ. Microbiol.* **2003**, *69*, 2810–2815.

(9) Pogorevc, M.; Strauss, U. T.; Riermeier, T.; Faber, K. *Tetrahedron: Asymmetry* **2002**, *13*, 1443–1447.

Table 1. Enantioselectivities of Enzymatic Sulfate Ester Hydrolysis

substrate	conversion [%] ^a	product/ee [%] ^a	enantiomeric ratio (E)	
			<i>Sulfolobus acidocaldarius</i> DSM 639	<i>Rhodococcus sulfatase</i> RS2 ^b
<i>rac-1a</i>	32	(<i>S</i>)- 1b / >99	>200	21
<i>rac-2a</i>	40	(<i>S</i>)- 2b /59	6	4
<i>rac-3a</i>	43	(<i>S</i>)- 3b /55	5	~ 1
<i>rac-4a</i>	42	(<i>S</i>)- 4b / >99	>200	no conversion

^a Data for *Sulfolobus acidocaldarius* DSM 639. ^b Data from ref 7.

inorganic sulfur metabolism (encompassing sulfur species of oxidation states from sulfide to sulfate)¹⁰ might also show sulfated *organic* species such as alkyl sulfates. From a screening among anaerobic and aerobic Archaea,¹¹ we identified whole cells of *Sulfolobus acidocaldarius* DSM 639 as a convenient source of highly selective alkyl sulfatase activity. When alkyl sulfate esters *rac-1a–4a* were subjected to aerobically grown *Sulfolobus* cells at pH 2–3, which corresponds to that of their natural habitats, hydrolysis of (*R*)-enantiomers from the racemate provided the corresponding (*S*)-configured *sec*-alcohols; in other words, a *homochiral* product mixture was generated from the racemate (Scheme 1, Table 1).¹² In each case, the absence of undesired

octyl sulfate (ee $>97\%$), which yielded (*S*)-2-octanol as the sole product in $>97\%$ ee. Excellent enantioselectivity was observed for 2-octyl sulfate (*rac-1a*, $E > 200$); in comparison, native sulfatase RS2 showed only $E = 21$ in the absence of inhibitors. When the sulfate ester moiety was gradually moved toward the center of the molecule (3-octyl- and 4-octyl sulfate, *rac-2a*, *rac-3a*), the enantioselectivities decreased, which is due to the fact that the alkyl groups flanking the sulfate ester group became similar in size, thus making the chiral recognition process more difficult. We were particularly pleased to see that the sterically demanding substrate *rac-4a* (which was not accepted by sulfatase RS2) was not only well accepted but also displayed excellent enantioselectivity.

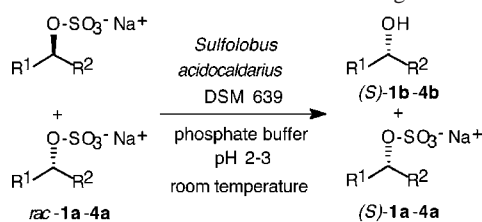
Overall, the enantioselectivity of *Sulfolobus acidocaldarius* DSM 639 proved to be identical to that of sulfatase RS2, i.e., (*R*)-*sec*-alkyl sulfate esters were hydrolyzed with inversion of configuration to form the corresponding (*S*)-*sec*-alcohols, while (*S*)-sulfate ester enantiomers remained untouched.

In summary, whole cells of *Sulfolobus acidocaldarius* DSM 639 were identified as a convenient source of alkyl sulfatase activity with enhanced enantioselectivities and a wider substrate spectrum as compared to *Rhodococcus* sulfatase RS2. The full substrate-selectivity pattern of this novel biocatalyst is currently being studied.

Acknowledgment. This study was performed in cooperation with Degussa AG (Frankfurt) within the Competence Center *Applied Biocatalysis* and financial support by the TIG, the City of Graz, and the Province of Styria is gratefully acknowledged. G. Antranikian and his crew is cordially thanked for their excellent expertise in the microbiology of Archaea.

Supporting Information Available: Experimental biocatalytic procedures and growth conditions. This material is available free of charge via the Internet at <http://pubs.acs.org>.

OL0477778

Scheme 1. Enantioselective Microbial Hydrolysis of *sec*-Alkyl Sulfate Esters with Inversion of Configuration

compound	R ¹	R ²
1a,b	CH ₃	<i>n</i> -C ₆ H ₁₃
2a,b	C ₂ H ₅	<i>n</i> -C ₅ H ₁₁
3a,b	<i>n</i> -C ₃ H ₇	<i>n</i> -C ₄ H ₉
4a,b	CH ₃	C ₂ H ₅ -Ph

spontaneous hydrolysis was verified in the absence of biocatalyst. The stereochemical course (i.e., clear inversion of configuration) was proven by the hydrolysis of (*R*)-2-

(10) Huber, R.; Huber, H.; Stetter, K. O. *FEMS Microbiol. Rev.* **2000**, *24*, 615–623.

(11) Details will be published in a full paper.

(12) For the synthesis of substrates and determination of the absolute configuration of products see ref 7.