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

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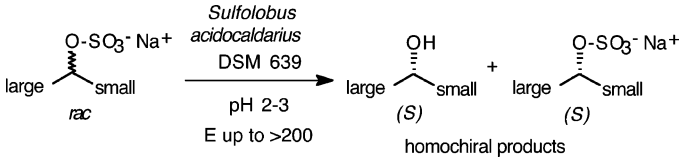
ABSTRACT



***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 Fe^{3+})[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

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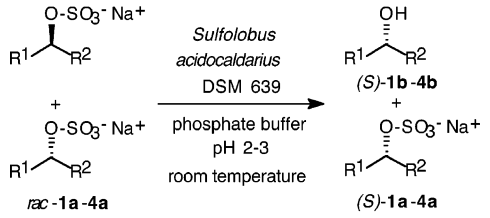
Table 1. Enantioselectivitites of Enzymatic Sulfate Ester Hydrolysis

substrate	conversion [%][a]	product/ee [%][a]	enantiomeric ratio (E)	
			Sulfolobus acidocaldarius DSM 639	*Rhodococcus sulfatase* RS2[b]
rac-**1a**	32	(*S*)-**1b**/>99	>200	21
rac-**2a**	40	(*S*)-**2b**/59	6	4
rac-**3a**	43	(*S*)-**3b**/55	5	~1
rac-**4a**	42	(*S*)-**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 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-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.

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



compound	R^1	R^1
1a,b	CH_3	*n*-C_6H_{13}
2a,b	C_2H_5	*n*-C_5H_{11}
3a,b	*n*-C_3H_7	*n*-C_4H_9
4a,b	CH_3	CH_2-Ph

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(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.

Overall, the enantiopreference 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 enantioselectivites and a wider substrate spectrum as compared to *Rhodococcus* sulfatase RS2. The full substrate-selectivity pattern of this novel biocatalyst is currently being studied.

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

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