*Sulfatases*

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# Highly Enantioselective *sec*-Alkyl Sulfatase Activity of the Marine Planctomycete *Rhodopirellula baltica* Shows Retention of Configuration**

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The molecular mechanisms of the commonly employed hydrolases involve nucleophilic attack onto the carbonyl group of carboxylic acids or their derivatives.[1,2] As this group is a planar entity, any stereochemical alterations of the substrate caused by enzymatic catalysis are impossible, and as a consequence, enantiomers of the transformed substrate and product are usually homochiral (with the exception of prochiral or *meso* esters), that is, they have the same absolute configuration. Although the stereochemical features of the substrate, such as stereogenic centers (in racemates) or enantiotopic groups (in prochiral or *meso* compounds), are "recognized" by the enzyme, which gives rise to differences in $k_{cat}$ and/or $K_M$ values, they remain unchanged during catalysis.

Biocatalysts, which elicit the more complex potential to affect the stereochemistry of the substrate in a controlled fashion during catalysis, are rather rare and encompass haloalkane dehalogenases,[3] epoxide hydrolases,[4] and (alkyl) sulfatases.[5,6] In each case, a C($sp^3$) atom could potentially be involved in the catalysis and therefore open the possibility of stereocomplementary pathways. These enzymes do not only display enantioselectivity (through the transformation of one substrate enantiomer faster than the other) but also stereoselectivity (with retention or inversion of configuration). This therefore makes them important catalytic tools for the development of so-called enantioconvergent processes in which each enantiomer from a racemic mixture is transformed into the same product through independent pathways, that is, through retention and inversion of configuration.[7] As a consequence, a racemate can be

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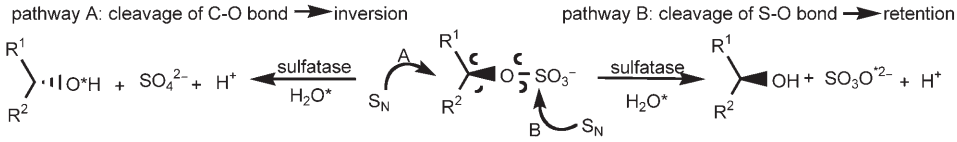
converted, in principle, into a single stereoisomeric product without the occurrence of an undesired stereoisomer.

Sulfatases catalyze the hydrolytic cleavage of the sulfate ester bond (Scheme 1). Depending on the enzyme subtype, the stereochemical course of sulfate ester hydrolysis can be




***Scheme 1.*** Enzymatic stereodivergent hydrolysis of sulfate esters catalyzed by sulfatases.

controlled by an appropriate enzyme. On the one hand, inverting sulfatases[8,9] were shown to act on *sec*-alkyl sulfate esters by breaking the C–O bond of the sulfate ester, which resulted in inversion of configuration (Scheme 1, pathway A). However, nothing is known about their mechanism of action. Based on limited knowledge of the stereospecific and enantioselective hydrolysis of alkyl sulfate esters,[8] we recently reported an inverting alkyl sulfatase (termed "RS2") from *Rhodococcus ruber* DSM 44541.[10–12] Although the enzyme exhibited absolute stereospecificity through strict inversion of configuration of simple *sec*-alkyl sulfate esters, its enantioselectivity was less than perfect and its substrate tolerance was rather narrow. These limitations could be circumvented through the use of aerobically grown sulfur metabolizers. In particular, *Sulfolobus* spp., which exhibited not only enhanced enantioselectivities but also a broader substrate spectrum,[13] showed a stereoselectivity identical to that of "RS2", that is, inversion of configuration was observed.

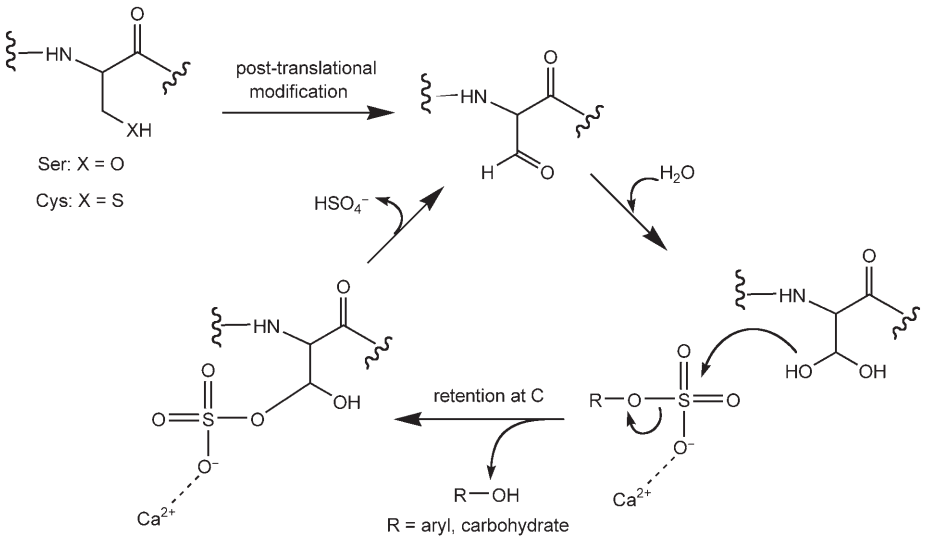
To exploit the full potential of the enzymatic hydrolysis of alkyl sulfate esters, stereocomplementary sulfatases that act with retention of configuration were needed. In contrast to inverting sulfatases, the mechanism of action of retaining sulfatases is well understood (Scheme 2).[14,15] The mechanism was elucidated during structural studies on human aryl sulfatase A[16] and was shown to proceed through cleavage of the S–O bond.[17] The latter is effected by nucleophilic attack of an aldehyde hydrate (formed from a Cys or Ser residue by post-translational modification[18]) onto the sulfur atom; the result is liberation of the corresponding alcohol and retention of its stereochemistry (Scheme 2).[19]

The same principle of catalysis was shown to occur in sulfatases from *Pseudomonas aeruginosa*[20] and *Klebsiella pneumoniae*.[21] As the natural substrates of these enzymes are believed to be glycosyl and aryl sulfate esters,[16] nothing is known about their enantioselectivities. Our search for a suitable microbial source for retaining (alkyl) sulfatase activity was led by the fact that all known sulfatases that act through the above-mentioned mechanism have the canonical sulfatase consensus motif C/S-X-P-X-R-$X_4$-T-G.[18,22,23,27] This sequence motif encompasses sulfatases from mammals, lower eukaryotes, and prokaryotes,[24] but is absent in the inverting alkyl sulfatase RS2 from *Rhodococcus ruber*.[25]

A recently published complete genome sequence of the marine planctomycete *Rhodopirellula baltica* DSM 10527 (formerly denoted as *Pirellula* sp. 1)[26,27] revealed the existence of an unexpectedly high number of 110 genes putatively encoding sulfatases. It is believed that, in *R. baltica*, these enzymes play a vital role in the metabolism of sulfated carbohydrates, such as chondroitin. Of the 110 potential sulfatase sequences, 85 show the conserved "core" of the sulfatase consensus motif C-X-P-X-R, which is required in post-translational modification to render activated sulfatase proteins. Furthermore, each of the 85 sequences contains the adjacent "auxiliary" part of the consensus motif, $X_4$-T-G, either fully conserved or only slightly modified. This part of the sulfatase consensus motif has been implicated in modulating the efficiency of the post-translational modification step.[24] According to the sequence–function relationships proposed for both eukaryotic and prokaryotic sulfatases,[20,22,24] the 85 putative sulfatases from *R. baltica* were expected to act through retention of configuration.

To prove this hypothesis, a set of *sec*-alkyl sulfate esters, *rac*-**1a**–**9a** and the primary sulfate ester **10a** were screened by using resting whole cells of *R. baltica* DSM 10527 (Table 1). In general, the enantiopreference was shown to be *R*, that is, *R* enantiomers were preferentially hydrolyzed from the racemate, while *S* enantiomers remained untouched.

*R. baltica* displayed excellent activities on linear (ω-1)-sulfate esters *rac*-**1a**–**3a** (Table 2, entries 1–3), which were readily accepted as substrates. Surprisingly, even sterically demanding sulcatyl sulfate (*rac*-**4a**), which was accepted by neither sulfatase RS2 nor *Sulfolobus* spp., was converted with good rates. Concerning the




***Scheme 2.*** Schematic representation of the mechanism of action of retaining (aryl) sulfatases.


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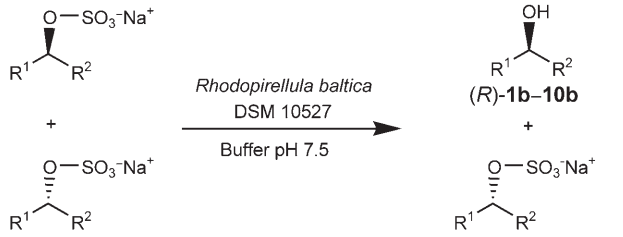
***Table 1:*** Enantioselective hydrolysis of *sec*-alkyl sulfates with retention of configuration.




*rac*-**1a**–**10a** (*R*)-**1b**–**10b** (*S*)-**1a**–**10a**

| Compound | $R^1$ | $R^2$ |
|---|---|---|
| **1a**,**b** | $CH_3$ | $n$-$C_6H_{13}$ |
| **2a**,**b** | $CH_3$ | $n$-$C_5H_{11}$ |
| **3a**,**b** | $CH_3$ | $n$-$C_7H_{15}$ |
| **4a**,**b** | $CH_3$ | $(CH_3)_2C{=}CH(CH_2)_2$ |
| **5a**,**b**[a] | $CH_3$ | $PhCH_2$ |
| **6a**,**b**[a] | $CH_3$ | $Ph(CH_2)_2$ |
| **7a**,**b** | $n$-$C_2H_5$ | $n$-$C_5H_{11}$ |
| **8a**,**b** | $n$-$C_3H_7$ | $n$-$C_4H_9$ |
| **9a**,**b**[b] | $CH_2{=}CH$ | $n$-$C_5H_{11}$ |
| **10a**,**b** | H | $n$-$C_7H_{15}$ |

[a] No conversion. [b] Opposite absolute configuration due to a switch in Cahn—Ingold priority (CIP) assignment of $R^1$ and $R^2$.

***Table 2:*** Hydrolysis of sulfate esters using glucose-grown *Rhodopirellula baltica* DSM 10527.

| Entry | Substrate | Conversion [%] | Product | *ee* [%] | Enantioselectivity (*E* value) |
|---|---|---|---|---|---|
| 1 | *rac*-**1a** | 16 | (*R*)-**1b** | >99 | >200 |
| 2 | *rac*-**2a** | 13 | (*R*)-**2b** | >99 | >200 |
| 3 | *rac*-**3a** | 18 | (*R*)-**3b** | >99 | >200 |
| 4 | *rac*-**4a** | 11 | (*R*)-**4b** | >99 | >200 |
| 5 | *rac*-**5a** | n.c. | – | – | – |
| 6 | *rac*-**6a** | n.c. | – | – | – |
| 7 | *rac*-**7a** | 3 | (*R*)-**7b** | 48 | 3 |
| 8 | *rac*-**8a** | 10 | (*R*)-**8b** | 29 | 2 |
| 9 | *rac*-**9a** | 6 | (*S*)-**9b**[a] | 42 | 3 |
| 10 | **10a** | 26 | **10b** | n.a. | n.a. |

[a] Absolute configuration is *S* owing to a switch in CIP priority rules, however, it is homochiral to (*R*)-**1b**–**4b**, **7b**, **8b**. n.c. = no conversion; n.a. = not applicable. *E* values were calculated from $E = \{\ln[1-c(1+ee_p)]\}/\{\ln[1-c(1-ee_p)]\}$ where c = conversion and $ee_p$ = enantiomeric excess of product.

enantioselectivities, all four substrates (Table 2, entries 1–4) showed perfect *E* values (>200) and the corresponding *R* alcohols **1b**–**4b** were obtained with excellent *ee* values (>99%). However, in the case of phenyl-substituted substrates (Table 2, entries 5 and 6), no conversion was observed. As $R^1$ and $R^2$ became similar in size, yielding near-symmetrical compounds (Table 2, entries 7–9), the enantioselectivities decreased as was observed in previous studies on inverting sulfatases.[9,10,13] As *R. baltica* has more than 100 genes that encode putative sulfatases, it is not surprising that the primary sulfate ester **10a** was readily converted as well.

Stereochemical analysis of the products suggested that the hydrolysis pathway proceeded with retention of configuration. For unambiguous proof, enantiopure (*R*)-2-octyl sulfate was used as the substrate, which yielded (*R*)-2-octanol without racemization in >99% *ee*; analogously, enantiopure (*S*)-2-octyl sulfate remained untouched. Screening of the extracellular medium did not show any activity, which suggests that these sulfatases are intracellular. Using *R. baltica* grown on various C sources—glucose, chondroitin sulfate A,[28] and chondroitin sulfate C—did not lead to significant differences in either activity or selectivity, thus indicating that no enzyme induction takes place. This suggests that the observed activity can, most likely, be associated with a "broad-spectrum sulfatase" rather than with separate primary- or *sec*-alkyl sulfatases.

In summary, the first highly enantioselective *sec*-alkyl sulfatase that acts with strict retention of configuration was detected in *R. baltica* DSM 10527 through a sequence-similarity approach. The use of this sulfatase in combination with a stereocomplementary inverting enzyme from *Sulfolobus* spp. in a deracemization strategy is currently being investigated.[29]

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