

Lipase Catalyzed Si-O Bond Scission in Trialkylsilyl Ethers

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Lipases from *Candida rugosa* (CRL), *Pseudomonas cepacia* (PCL) and *Porcine pancreas* (PPL) were found out to have an ability to scissor a Si-O bond in triethylsilyl phenylethyl ether in phosphate buffer (0.1 M, pH 7.2) despite that triethylsilyl-oxygen bond was known to be considerably resistant to nucleophilic scission.

Key Words: Lipase, Si-O bond, Trialkylsilyl ether, Hydrolysis, Enantioselective

Lipases are usually used for hydrolysis or transesterification of esters to obtain useful carboxylic acids or alcohols. Especially, production of optically active acids⁽¹⁾ or alcohols⁽²⁾ by lipase catalyzed reaction has been studied on laboratory and industry scale. Reaction mechanism of lipase-mediated hydrolysis and transesterification was investigated in detail, revealing that driving force of those reactions was attributed to increased nucleophilicity of hydroxyl group of serine(Ser)-residue and other nucleophiles, such as water molecule or alcohols, in active site in corporation with histidine- and aspartic acid-residues which were located near Ser-residue. Although study on chemistry of lipase has long history, application of its catalytic activity is limited in C-O bond scission in ester yet, which reaction proceeds in addition-elimination mechanism. On the other hand, since nucleophile in catalytic site get strongly increased nucleophilicity, it can be believed to exist a possibility that substitution reaction of silyl ethers occurs in two step mechanism like hydrolysis of esters and transesterification, in the first stage hydroxyl group of Ser-residue attacks at a Si atom in the silyl ether, forming Ser-silyl ether and in the second stage this Ser-silyl ether is resolved by attack of other nucleophile activated in the active site of the enzyme, for example activated water. However, a possibility of substitution reaction of silyl ethers is very low since several disadvantageous items are enumerated; 1) reactive center(Si atom) in the silyl ether is *sp*³ structure which is bulky relative to carbonyl carbon (*sp*²) of ester and this bulkiness leads to difficulty of nucleophilic attack by Ser-residue, 2) since this substitution reaction(*S*_N2 type reaction) proceeds in the course of change of structure at reactive center(Si atom) from *sp*³ to *sp*³ by way of trigonalbipyramid structure in the transition state, considerably large space is demanded for the active site of the enzyme and 3) stabilization effect in transition state of the reaction by oxyanion hole of the lipase can not be expected.

Though those disadvantageous items against Si-O bond scission by lipase catalyzed substitution reaction were enumerated, the fact that the nucleophilicity of hydroxyl group of Ser-residue in the active site of lipase was recognized to be very strong motivated us to try lipase catalyzed hydrolysis of trialkylsilyl ethers in order to confirm whether lipases possessed or not an ability of Si-O bond scission⁽³⁾ in phosphate buffer.

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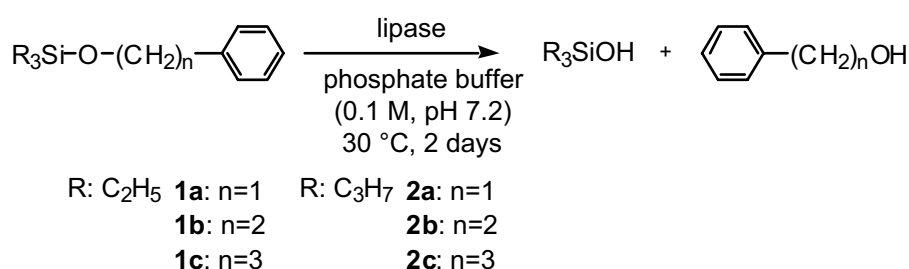
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Since trimethylsilyl ethers were highly sensitive to alkaline condition, needless to say to acidic condition, we chose more bulky triethylsilyl ethers **1** and tripropylsilyl ones **2** which would strongly resist to nucleophilic attack and then, their trialkylsilyl groups were usually used as a protective groups for alcohols. Benzyl, 2-phenylethyl and 3-phenyl-1-propyl alcohol were chosen as alcohol parts in trialkylsilyl ethers (**1a**, **b**, **c** and **2a**, **b**, **c**, respectively). Hydrolytic activities toward **1a–c** and **2a–c** were tested with lipases from *Candida rugosa* (CRL),⁽⁴⁾ *Pseudomonas cepacia* (PCL),⁽⁴⁾ *Porcine pancreas* (PPL) and without enzyme (non) in phosphate buffer.

We carried out hydrolysis of **1a–c** and **2a–c** under following procedure: 0.1 mmol of trialkylsilyl ether was suspended in phosphate buffer (pH 7.2, 5 mL) and 200 mg of lipase was added. Reaction mixture was stirred vigorously at 30 °C for 2 days. Silyl ether and resulting alcohol were extracted fully with ethyl ether. The ratio of recovered trialkylsilyl ether and resultant alcohol was determined by HPLC analysis.⁽⁵⁾

In the presence of CRL triethylsilyl ethers (**1a–c**) were hydrolyzed in 19.0–27.6% and PPL catalyzed hydrolysis of the same ethers proceeded in 9.6–18.1% conversion which were half compared with the case of CRL except for the case of **1b**. It seemed that hydrolytic activities of PCL for **1a–c** were negligible or slight judging from the results without enzyme(non). CRL hydrolyzed even **2a** and **b** in 12.4 and 10.8% whose bulky tripropylsilyl group seemed to prevent the Si–O bond scission, whereas **2c** which might be the largest of the three was resistant to hydrolysis, resulting in 5.7% conversion. Nevertheless CRL catalyzed Si–O bond scission in **2c** judging from the result with non-CRL. PCL and PPL proved to be invalid for hydrolysis of **2b** and **c**, and their activities against **2a** seemed that even if valid, it might be negligible small (1.8% with PCL and 3.0% with PPL, respectively). All results were summarized in Table 1.

Table 1. Conversions^a of lipase catalyzed hydrolysis of silyl ethers

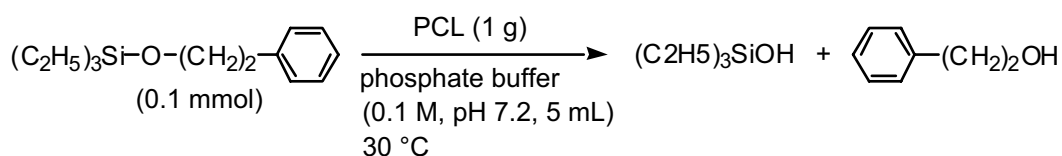


Silyl ether	CRL	PCL	PPL	None
1a	27.6	3.0	14.5	2.0
1b	19.0	5.7	18.1	0.39
1c	21.1	0.68	9.6	1.3
2a	12.4	1.8	3.0	0.29
2b	10.8	0	0.13	0.68
2c	5.7	0.72	0.22	0.77

^aConversions were expressed in %

We observed that in the reaction of **1b** with PCL the hydrolysis proceeded in 5.7%, and in order to confirm whether addition of PCL was meaningful or not, PCL-hydrolysis reaction was performed again under the same conditions except for increase in the addition of PCL from 200 mg to 1 g and extension of reaction time. Two days reaction with 1 g of PCL (5-fold to the usual reaction condition mentioned above) proceeded in 24.9% which conversion corresponded to 4.4-fold compared with the reaction with 200 mg of PCL. Extension of the reaction time increased conversions to 52.6% (4 days) and 80.3% (7 days) in monotone as shown in Table 2.

Table 2. PCL catalyzed hydrolysis of silyl ether (**1b**)



Reaction time (d)	conversion (%) ^a
2	24.9
4	52.6
7	80.3

^a Conversions were determined by HPLC analysis.

From the result above, catalytic activity of PCL to scissor a Si-O bond in **1b** could be recognized even though very low.

Next the turnover rates⁽⁶⁾ of CRL and PCL for **1b** were calculated, resulting in 14.2 and 8.8 times a day, respectively. These values were extremely low compared with ester hydrolysis with lipases, whereas large differences of the ability of Si-O bond scission between these two lipases were not observed as only 1.6-folds, though conversions were different by a factor of 3.3 folds.

Finally, we attempted stereoselective hydrolysis of racemic triethylsilyl 1-phenylethyl ether (**3**) with the three lipases at pH 7.0 and 30 °C. PCL catalyzed hydrolysis of **3** afforded (*R*)-1-phenylethylalcohol preferentially in 9.8% ee at 54.9% conversion after 21 d.⁽⁷⁾ Even though enantioselectivity was very low, enantiopreference of PCL for this racemic silyl ether hydrolysis corresponded to that for hydrolysis of this racemic alcohol ester.⁽⁸⁾ In the trials of enantioselective hydrolysis of **3** with CRL⁽⁸⁾ and PPL,⁽⁹⁾ clear enantioselectivities could not be observed as less than 3% ee, though proceeding in the (*R*)-alcohol preferential manner same as hydrolysis of 1-phenylethylalcohol esters.

In conclusion, we could observe lipase catalyzed hydrolysis of several triethyl and/or tripropylsilyl ethers even though it was very slow, and PCL proved to scissor Si-O bond in triethylsilyl 1-phenylethyl ether even though slightly but (*R*)-alcohol selectively which selectivity was synchronized with the one of hydrolysis of the same alcohol esters.

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- (4) CRL was purchased from Meito Sangyo Co., Ltd. as lipase OF and PCL was offered from Amano Pharmaceutical Co., Ltd. as lipase PS, respectively.
- (5) Trialkylsilyl ethers (**1a-c**, **2a-c**) and corresponding alcohols were identified by HPLC (TRI ROTER III, JASCO, Japan) analysis with a DEVELOSIL 100-3 column (Nomura Chemical, Japan) under the following conditions: mobile phase, *n*-hexane/2-propanol (9/1); column temperature, 30 °C; detection, 254 nm; flow rate, 0.5 mL/min. Retention times of **1a**, **b**, **c** and **2a**, **b**, **c** were 6.9, 6.9, 6.7 and 6.8, 6.8, 6.6 min, respectively. Retention times of benzyl alcohol, 2-phenylethyl alcohol and 3-phenyl-1-propanol were 11.4, 11.7 and 10.5 min, respectively. The ratio, measured by HPLC, of trialkylsilyl ether and corresponding alcohol were corrected by comparing the intensity of one to one mixture of each silyl ether and alcohol.
- (6) Turnover rates were calculated based on the following information; Lipase OF and Lipase PS contain 20% (w/w) and 5% (w/w) of protein as CRL and PCL whose molecular weight are 60,000 and 33,128, respectively.
- (7) The enantiomers of 1-phenylethyl alcohol and **3** were separated by HPLC (Gulliver, JASCO, Japan) with Daicel Chiralcel OB-H (Daicel, Japan) under the same condition depicted in ref 5. Retention times of (*R*)- and (*S*)-alcohols and racemic **3** were 14.8, 11.4 and 6.3 min, respectively.
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