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Regioselective Acylation of Nucleosides Catalyzed by *Candida Antarctica* Lipase B: Enzyme Substrate Recognition

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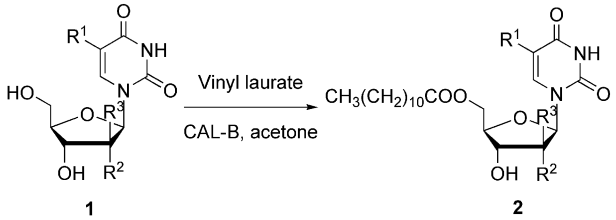
The substrate recognition of *Candida antarctica* lipase B (CAL-B) in the acylation of nucleosides was revealed through rational substrate engineering for the first time. CAL-B displayed lower activities and excellent 5′-regioselectivities (94 to >99 %) in the acylation of ribonucleosides **1f**–**1j** as compared to those in the acylation of 2′-deoxynucleosides **1a**–**1e**. The excellent regioselectivities might be attributed to the favorable steric hindrance of the 2′-hydroxy group present in ribonucleosides **1f**–**1j**, which destabilizes the conformation of the 3′-acylation transition state in the acylation of **1f**–**1j** and thus reduces the amount of the minor regioisomer. The study of the acyl chain-length specificity showed that CAL-B was more specific toward short-chain fatty acid vinyl esters, especially vinyl butyrate.

Introduction

Nucleosides and their analogs, an important group of polyhydroxy compounds, have been used extensively in the treatment of various diseases.[1] For example, floxuridine[2] and idoxuridine[3] have served as antitumor and antiviral agents for many years. Nevertheless, these nucleoside drugs displayed low oral bioavailability and various side effects in the clinical use.[4] The regioselective acylation of the sugar moiety is one of the successful approaches to the lipophilic prodrugs with enhanced clinical efficacy.[5] Moreover, the monoprotected nucleosides are the key building blocks for the synthesis of oligonucleotides, an emerging class of important chemotherapeutic agents.[6] The enzymatic acylation of nucleosides has received increasing attention in synthetic chemistry due to its simplicity, exquisite selectivity, high efficiency and environmental friendliness.[7]

Candida antarctica lipase B (CAL-B) is a versatile biocatalyst with a broad range of pH optima and excellent thermostability.[8] X-ray crystallographic studies have indicated that the active site of CAL-B is composed of a large hydrophobic pocket into which the acyl moiety fits and a medium-sized pocket in which the alcohol moiety lies.[9] CAL-B has been demonstrated to be one of the most useful lipases for the resolution of racemic alcohols, amines and acids, owing to its unique enantioselectivity.[10] In addition, the lipase displayed a high regioselectivity toward the 5′-hydroxy group in the regioselective acylation of natural or non-natural nucleosides.[11] Recently, Lavandera et al. proposed a molecular basis of this regioselectivity through molecular modeling, and they attributed the preferential acylation of the 5′-hydroxy group over the 3′-hydroxy one to favorable interactions, due to the appropriate orientation of the 2′-deoxynucleoside in the 5′-acylation pathway.[12] Nevertheless, they just focused their interest on the enzymatic acylation of 2′-deoxynucleosides, such as β-thymidine (**1c**). In the present work, the substrate recognition of CAL-B in the acylation of nucleosides, including ribonucleosides and 2′-deoxynucleosides, was revealed by rational substrate engineering (Scheme 1). Particularly, we attempted to gain



	R^1	R^2	R^3		R^1	R^2	R^3
a	H	H	H	**f**	H	OH	H
b	F	H	H	**g**	F	OH	H
c	CH_3	H	H	**h**	CH_3	OH	H
d	Br	H	H	**i**	Br	OH	H
e	I	H	H	**j**	I	OH	H
k	H	F	H	**m**	H	H	OH

Scheme 1. Enzymatic lauroylation of nucleosides and their analogs.

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a deep insight into the molecular basis for the higher regioselectivities in the enzymatic acylation of ribonucleosides (**1f**–**1j**) as compared to those in the acylation of 2′-deoxynucleosides (**1a**–**1e**).

Results and Discussion

The nucleoside recognition of CAL-B was investigated with the lauroylation as a model reaction. As shown in Table 1, CAL-B displayed high activities and moderate 5′-regioselectivities (71–83%) in the acylation of 2′-deoxynucleosides **1a**–**1e** (Entries 1–5). Besides, the structure of 2′-deoxynucleosides, particularly the R^1 group, had a significant effect on the reaction rate and regioselectivity. For example, CAL-B showed a higher catalytic activity in the lauroylation of 2′-deoxyuridine (**1a**) than in the lauroylation of **1b**–**1e**. This might be due to steric hindrance with the increasing size of the R^1 group. The 5′-regioselectivity was highest for the lauroylation of 2′-deoxyuridine (**1a**) (83%, Entry 1), lower for the acylation of β-thymidine (**1c**) (80%, Entry 3), even lower for the acylation of floxuridine (**1b**) (77%, Entry 2) and idoxuridine (**1e**) (77%, Entry 5), and lowest for the acylation of 5-bromo-2′-deoxyuridine (**1d**) (71%, Entry 4). It was proposed that there exists a hydrophobic interaction between the propyl group of acyl chain and the hydrophobic side (C_5–C_6–R^1) of the thymine in the conformation of the 5′-butanoylation of β-thymidine.[12] According to the model, the 5′-regioselectivity should increase with an enhancement of the hydrophobicity of the R^1 group. However, we found that the 5′-selectivity did not increase, but decreased in waved form with enhancing hydrophobicity of the R^1 group (Table 1, Entries 1–5). It is worthy to note that the hydrophobic side of the thymine lies close to the propyl group in the conformation of the 5′-butanoylation of β-thymidine. The lauroyl group is much more bulky than the butanoyl group used in the molecular modeling. A steric clash, therefore, might occur if the size of the acyl moiety (from the butanoyl group to the lauroyl group) and/or R^1 group (from H to I atom) increases. The reason for the unexpected facts might be due to the simultaneous presence of a favorable hydrophobic interaction and an unfavorable steric hindrance between the lauroyl group and the hydrophobic side of the base moiety.

High substrate conversions (99%) and excellent 5′-regioselectivities (94 to >99%) were achieved in the CAL-B-catalyzed acylation of ribonucleosides **1f**–**1j** (Table 1, Entries 6–10). Interestingly, the effects of the R^1 group on the reaction rate and the regioselectivity in the acylation of ribonucleosides **1f**–**1j** closely resembled those in the acylation of 2′-deoxynucleosides **1a**–**1e** (Entries 1–10). For instance, the catalytic activities of CAL-B decreased with increasing bulk of the R^1 group in the acylation of ribonucleosides **1f**–**1j**, except for 5-fluorouridine (**1g**) [corresponding to floxuridine (**1b**) among **1a**–**1e**]. Furthermore, CAL-B exhibited the highest 5′-regioselectivity (>99%) toward uridine (**1f**) among **1f**–**1j** [corresponding to 2′-deoxyuridine (**1a**) among **1a**–**1e**], whereas the lowest regioselectivity (94%) was found toward 5-bromouridine (**1i**) [corresponding to 5-bromo-2′-deoxyuridine (**1d**)]. All these facts suggested that the orientations of the ribonucleosides in the active site of CAL-B might be analogous to those of the 2′-deoxynucleosides. Surprisingly, lower reaction rates and higher 5′-regioselectivities were observed in the acylation of ribonucleosides **1f**–**1j** as compared to those in the acylation of 2′-deoxynucleosides **1a**–**1e**. The unique difference of the structures between the compounds **1a**–**1e** and **1f**–**1i** lies in the 2′-substituent (R^2 group). The molecular modeling has revealed that the C2′ atom lies above the medium-sized pocket in the conformation of the 5′-acylation transition state, whereas it fits into the medium-sized pocket in the conformation of the 3′-acylation.[12] X-ray crystallographic studies have shown that the medium-sized pocket, into which the sugar moiety binds, has not enough room for a substituent larger than the propyl group.[9,12] As a result, the presence of the R^2 group as a hydroxy group might cause a steric clash and destabilize the conformation of the 3′-acylation transition state, thus enhancing the 5′-regioselectivity and decreasing the reaction rate. Interestingly, the hypothesis was confirmed by the results in the enzymatic acylation of 2′-fluoro-2′-deoxyuridine (**1k**) and 1-β-D-arabinofuranosyluracil (**1m**) (Entries 11 and 12). For example, CAL-B displayed an excellent 5′-regioselectivity (98%) in the acylation of 2′-fluoro-2′-deoxyuridine (**1k**) with the R^2 group being an F atom, which is similar in size to the hydroxy group. Likewise, a high 5′-regioselectivity (97%) was achieved in the CAL-B-mediated acylation of 1-β-D-arabinofuranosyluracil (**1m**) with the R^3 group as a hydroxy group. The favorable steric clash, despite the different conformations of the C2′ position between **1f**–**1j** and **1m**, would still exist in the acylation of 1-β-D-arabinofuranosyluracil (**1m**), due to the very narrow space of the medium-sized pocket of the enzyme.

Table 1. Effect of the substrate structure on the CAL-B-catalyzed lauroylation of nucleosides.[a]

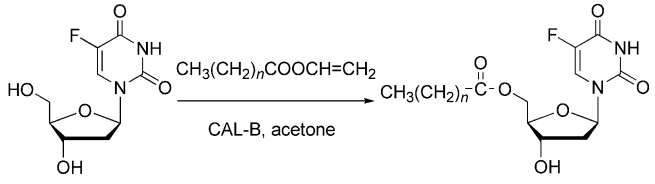
Entry	Nucleoside	Time [h]	Conversion (%)	5′-Regioselectivity (%)[b]
1	**1a**	2.0	99	83
2	**1b**	5.0	99	77
3	**1c**	3.0	99	80
4	**1d**	3.0	>99	71
5	**1e**	4.0	99	77
6	**1f**	3.0	99	>99
7	**1g**	8.0	99	94
8	**1h**	5.0	99	97
9	**1i**	12.0	99	94
10	**1j**	12.0	99	95
11	**1k**	2.0	99	98
12	**1m**	8.0	99	97

[a] The reaction was initiated by adding 200 U of CAL-B into 2 mL of anhydrous acetone containing 0.04 mmol of nucleosides and 0.24 mmol of vinyl laurate at 40 °C and 250 rpm. [b] Defined as the ratio of the concentration of the desired product to that of all the products, and determined by HPLC analysis using an SB-C18 column.

Finally, the acyl chain-length specificity of CAL-B was explored with floxuridine (**1b**) as a model acyl acceptor

(Table 2). As shown in Table 2, CAL-B displayed the highest catalytic activity in the butanoylation of floxuridine (Entry 2). Moreover, the initial reaction rate dropped markedly with the elongation of acyl chain from C_4 to C_8 (Table 2, Entries 2–4) and then remained constant (Table 2, Entries 5–9). Pleiss et al. described the orientation and binding of the acyl group in the hydrophobic pocket of CAL-B through molecular modeling.[13] An acyl group of less than C_4 binds into a narrow cleft at the bottom of the active site. Sharp distortion would occur if the acyl chain would extend further from C_4, resulting in an increase of the activation energy and a lower reaction rate. Up to C_7, the binding site is still a narrow cleft, but then it becomes spacious. Likewise, it was reported that CAL-B was more specific toward short chain fatty acids or acyl donors in the synthesis of glucose esters and in the acylation of β-thymidine.[12,14]

Table 2. Effect of the acyl chain length on the CAL-B-catalyzed acylation of floxuridine.[a]



Entry	Acyl chain length	v_0 [mM/h]	Time [h]	Conversion (%)	5′-Regioselectivity (%)[b]
1	C2	44.6	2	99	65
2	C4	48.5	2	99	80
3	C6	38.2	3	99	77
4	C8	22.0	4	99	76
5	C10	19.6	5	99	75
6	C12	18.9	5	99	76
7	C14	19.1	5	99	77
8	C16	18.8	5	98	77
9	C18	19.0	5	98	77

[a] The reaction was initiated by adding 200 U of CAL-B into 2 mL of anhydrous acetone containing 0.04 mmol of floxuridine and 0.24 mmol of vinyl esters at 40 °C and 250 rpm. [b] Defined as the ratio of the concentration of the desired product to that of all the products, and determined by HPLC analysis using an SB-C18 column.

As can be seen in Table 2, the highest 5′-regioselectivity was observed in the butanoylation (80%, Entry 2). As mentioned above, there exists a hydrophobic interaction between the hydrophobic side of the base moiety (C5–C6–R^1) and the acyl portion in the conformation of the 5′-acylation transition state.[12] Compared with the butanoyl group, the acetyl group would display a weaker interaction, leading to a lower 5′-regioselectivity (65%, Entry 1). CAL-B displayed slightly lower 5′-regioselectivities (75–77%, Entries 3–9) for the acylation of floxuridine with acyl donors of the chain length of more than C_4. The reason for this might be that the hydrophobic side of the base moiety of floxuridine is smaller than the acyl group of more than C_4, and cannot interact with the entire chain. Moreover, a larger acyl group might cause a steric clash.

Conclusions

A deep insight was gained into the interactions between the enzyme and the substrates (nucleosides) in the catalytic pathway by rational substrate engineering. The results improved and extended the model for the CAL-B-catalyzed acylation of 2′-deoxynucleosides proposed by Lavandera et al. Besides, the findings provide a guide to control and maximize the regioselectivity of the synthetically useful enzyme by chemical modification or protein engineering approaches.

Supporting Information (see footnote on the first page of this article): General procedure for the acylation of nucleosides, HPLC analysis conditions, retention times, and characterization data.

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[1] a) J. A. Montgomery, S. Ananthan, W. B. Parker, J. A. Secrist, C. G. Temple, *Cancer Chemotherapy Agents – Series: ACS Professional Reference Book* (Ed.: W. O. Foye), American Chemical Society, Washington DC, **1995**, pp. 47–110; b) A. Matsuda, T. Sasaki, *Cancer Sci.* **2004**, *95*, 105–111.
[2] C. Heidelberger, F. J. Ansfield, *Cancer Res.* **1963**, *23*, 1226–1243.
[3] W. H. Prusoff, *Biochim. Biophys. Acta* **1959**, *32*, 295–296.
[4] B. A. Chabner, D. P. Ryan, L. Paz-Area, R. Garcia-Carbonero, P. Calabresi, *Goodman & Gilman's The Pharmacological Basis of Therapeutics*, 10th ed. (Ed.: G. Hardman, L. E. Limbird), Mc-Graw Hill, New York, **2001**, pp. 1389–1459.
[5] a) E. De Clercq, H. J. Field, *Br. J. Pharmacol.* **2006**, *147*, 1–11; b) C. P. Landowski, X. Q. Song, P. L. Lorenzi, J. M. Hilfinger, G. L. Amidon, *Pharm. Res.* **2005**, *22*, 1510–1518; c) B. S. Vig, P. J. Lorenzi, S. Mittal, C. P. Landowski, H. C. Shin, H. I. Mosberg, J. M. Hilfinger, G. L. Amidon, *Pharm. Res.* **2003**, *20*, 1381–1388.
[6] a) A. Diaz-Rodriguez, S. Fernandez, Y. S. Sanghvi, M. Ferrero, V. Gotor, *Org. Process Res. Dev.* **2006**, *10*, 581–587; b) J. Garcia, S. Fernandez, M. Ferrero, Y. S. Sanghvi, V. Gotor, *J. Org. Chem.* **2002**, *67*, 4513–4519; c) J. Garcia, S. Fernandez, M. Ferrero, Y. S. Sanghvi, V. Gotor, *Tetrahedron: Asymmetry* **2003**, *14*, 3533–3540.
[7] a) M. Ferrero, V. Gotor, *Monatsh. Chem.* **2000**, *131*, 585–616; b) M. Ferrero, V. Gotor, *Chem. Rev.* **2000**, *100*, 4319–4347; c) N. Li, D. Ma, M. H. Zong, *J. Biotechnol.* **2008**, *133*, 103–109; d) N. Li, M. H. Zong, X. M. Liu, D. Ma, *J. Mol. Catal. B: Enzym.* **2007**, *47*, 6–12.
[8] O. Kirk, M. W. Christensen, *Org. Process Res. Dev.* **2002**, *6*, 446–451.
[9] a) J. Uppenberg, M. T. Hansen, S. Patkar, T. A. Jones, *Structure* **1994**, *2*, 293–308; b) J. Uppenberg, N. Ohrner, M. Norin, K. Hult, G. J. Kleywegt, S. Patkar, V. Waagen, T. Anthonsen, T. A. Jones, *Biochemistry* **1995**, *34*, 16838–16851.
[10] a) E. M. Anderson, M. Karin, O. Kirk, *Biocatal. Biotransform.* **1998**, *16*, 181–204; b) V. Gotor-Fernandez, E. Busto, V. Gotor, *Adv. Synth. Catal.* **2006**, *348*, 797–812.
[11] a) A. Diaz-Rodriguez, S. Fernandez, I. Lavandera, M. Ferrero, V. Gotor, *Tetrahedron Lett.* **2005**, *46*, 5835–5838; b) J. Garcia,

S. Fernandez, M. Ferrero, Y. S. Sanghvi, V. Gotor, *Tetrahedron Lett.* **2004**, *45*, 1709–1712; c) X. F. Li, W. Y. Lou, T. J. Smith, M. H. Zong, H. Wu, J. F. Wang, *Green Chem.* **2006**, *8*, 538–544; d) X. F. Li, M. H. Zong, H. Wu, W. Y. Lou, *J. Biotechnol.* **2006**, *124*, 552–560; e) X. F. Li, M. H. Zong, R. D. Yang, *J. Mol. Catal. B: Enzym.* **2006**, *38*, 48–53.

[12] I. Lavandera, S. Fernandez, J. Magdalena, M. Ferrero, R. J. Kazlauskas, V. Gotor, *ChemBioChem* **2005**, *6*, 1381–1390.

[13] J. Pleiss, M. Fischer, R. D. Schmid, *Chem. Phys. Lip.* **1998**, *93*, 67–80.

[14] O. Kirk, F. Bjoerkling, S. E. Godtfredsen, T. O. Larsen, *Biocatalysis* **1992**, *6*, 127–134.

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