

Unexpected reversal of the regioselectivity in *Thermomyces lanuginosus* lipase-catalyzed acylation of floxuridine

Ning Li · Min-Hua Zong · Ding Ma

Received: 12 December 2008 / Revised: 19 March 2009 / Accepted: 20 March 2009 / Published online: 11 April 2009
© Springer Science+Business Media B.V. 2009

Abstract Unexpected inversion of the 3':5'-regioselectivity was observed in the enzymatic methacryloylation, crotonylation and cinnamoylation of floxuridine (1.5:1, 2.3:1 and 4.4:1, respectively), where *Thermomyces lanuginosus* lipase preferentially catalyzed the acylation of 3'-hydroxyl rather than that of 5'-hydroxyl group. The possible reason might be the presence of a remote interaction between the unsaturated bond in the acyl group and the aromatic ring of amino acid residue Trp89 in the lid of the lipase.

Keywords Enzymatic acylation · Floxuridine · Nucleoside · Reversal of regioselectivity · *Thermomyces lanuginosus* lipase

Electronic supplementary material The online version of this article (doi:10.1007/s10529-009-0001-x) contains supplementary material, which is available to authorized users.

N. Li · M.-H. Zong (✉)
Lab of Applied Biocatalysis, College of Light Industry and Food Sciences, South China University of Technology, 510640 Guangzhou, China
e-mail: btmhzong@scut.edu.cn

N. Li · D. Ma (✉)
State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 116023 Dalian, China
e-mail: dma@dicp.ac.cn

Introduction

Floxuridine (FUdR), a fluorinated uridine, is extensively used an anticancer agent. Fatty acid esters of FUdR have higher oral bioavailability and better biological activities than the parent drug (Landowski et al. 2005). Therefore, it is desirable to synthesize new ester derivatives, such as crotonate, cinnamate, methacrylate, and pivalate, that might be potentially good pro-drugs. Many biological active compounds contain these groups. For example, the anti-tumor agents, COTC [2-crotonyloxymethyl-(4R,5R,6R)-4,5,6-trihydroxy-2-cyclohexenone] (Aghil et al. 1992) and COMC (2-crotonyloxymethyl-2-cyclohexenone) (Takeuchi et al. 1975), both contain crotonyl moieties. The cinnamoyl group is present in 5-caffeoylquinic acid, which has a broad spectrum of biological activities (Kroon and Williamson 1999). Additionally, methacrylates of FUdR are polymerizable pro-drug monomers.

Regioselective acylation is one of the important approaches for the enzymological protection of hydroxyl and amino groups (Li et al. 2008a). *Thermomyces lanuginosus* lipase (TLL) is a glycosylated hydrolase with a molecular weight of 30 kDa and an optimum pH of 11–12 (Neves Petersen et al. 2001). The lipase has a unique lid which lies over the catalytic triad and shields the active site from the external environment. Although enzymatic regioselective acylation of nucleosides has been well-established (Ferrero and Gotor 2000; Li et al. 2007, 2008b), there have been few reports regarding TLL-catalyzed acylation of

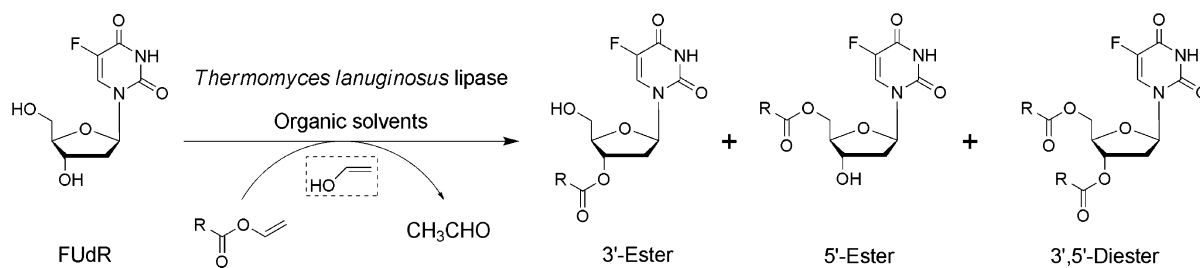


Fig. 1 Regioselective acylation of floxuridine (FUdR) catalyzed by *Thermomyces lanuginosus* lipase

nucleosides (Wang et al. 2007). In most cases, TLL exhibited preferential regioselectivity toward the primary hydroxyl over the secondary ones (Ferrer et al. 2005; Wang et al. 2007). In this work, we report unexpected inversion of the regioselectivity of the lipase in the acylation of FUdR, where the lipase favored the acylation of the secondary hydroxyl over that of the primary one (Fig. 1).

Materials and methods

Materials

Floxuridine (FUdR) was purchased from Shanghai Hanhong Co. Ltd., China. *Thermomyces lanuginosus* lipase (lipozyme TL IM) was from Novozymes Co. Ltd., China. The specific esterification activity of *Thermomyces lanuginosus* lipase (433 U/g) was assayed as described previously (Li et al. 2007). Vinyl crotonate and vinyl methacrylate were from Sigma-Aldrich, USA. Vinyl butyrate, vinyl cinnamate, vinyl pivalate and vinyl 2-ethylhexanoate were from TCI, Japan. Vinyl oleate was synthesized by ourselves (see in Supplementary Information). All other chemicals were from commercial sources and of the highest purity available.

General procedure for the enzymatic reaction

In a typical experiment, the reaction was initiated by adding TLL (60 U) to 2 ml anhydrous tetrahydrofuran containing FUdR (20 mM), vinyl ester (120 mM) at 200 rpm and 40°C. Aliquots were withdrawn at intervals and diluted 25-fold with corresponding mobile phase prior to HPLC analysis.

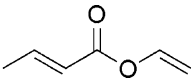
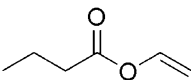
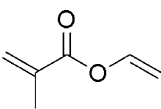
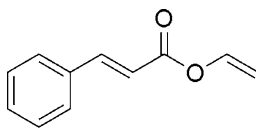
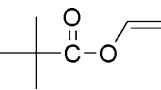
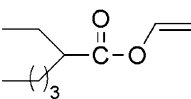
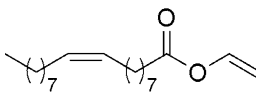
Results and discussion

Solvent engineering is a simple and efficient approach for the manipulation of the selectivity in non-aqueous biocatalysis (Rubio et al. 1991). For example, regioselectivity can be inverted in the acylation of 6-*O*-trityl- β -D-glycopyranosides via solvent engineering (MacManus and Vulfson 1997). Herein, the reversal of the regioselectivity in TLL-catalyzed acylation of FUdR was observed.

The TLL-catalyzed acylation of FUdR was conducted with seven acyl donors (Table 1). The reaction rate of crotonylation (entry 1) was much lower than that of the butanoylation (entry 2) (3.9 vs. 47.5 mM/h). This could be attributed to the resonance effect of the C–C double bond present in the crotonyl moiety. This would lead to the increment of the electron density of the carbon of the carbonyl group, thus making the formation of the tetrahedral intermediate more difficult. Likewise, low initial reaction rates were observed for methacryloylation and cinnamoylation (6.7 and 5.3 mM/h, respectively, entries 3 and 4). Nevertheless, the reaction rate of oleoylation was only slightly affected by the double bond (45.8 mM/h, entry 7) as it is far away from the carbonyl group. When there exists an α -substituent in the acyl donors (pivaloyl and 2-ethylhexanoyl), the initial acylation rates were even lower (entries 5 and 6), owing to the steric effect. Unexpectedly, although the steric hindrance of methacryloyl group seems to be more severe than that of crotonyl group due to the presence of α -methyl, the initial methacryloylation rate was approximately twice that of the crotonylation (6.7 vs. 3.9 mM/h, entries 1 and 3).

TLL has excellent regioselectivities toward the 5'-hydroxyl group in the acylation of 5-fluorouridine (Wang et al. 2007). Although the enzyme catalyzed

Table 1 Effect of acyl donors on the enzymatic acylation

Entry	Acyl donor	V_0 (mM/h)	C (%)	5' (%)	3' (%)	3',5' (%)	3':5'-Regio-selectivity ^a
1		3.9	95	24	67	9	2.3:1
2		47.5	99	62	17	21	1:2.2
3		6.7	95	32	54	14	1.5:1
4		5.3	98	13	80	7	4.4:1
5		0.9	42	59	41	0	1:1.4
6		0.3	41	84	16	0	1:5.3
7		45.8	99	75	19	6	1:3.2

Conditions: 20 mM FUDR, 120 mM vinyl ester, 60 U TLL (433 U/g), 2 ml anhydrous THF at 40°C, 200 rpm

^a The selectivity was defined as the molar ratio of the 3'- and 5'-esters. 3',5'-Diester included both a 3'- and a 5'-acylation, so the amount of diester was added to both of the 3'- and 5'-esters to estimate the selectivity

the acylation of 5'-hydroxyl preferentially in the butanoylation, pivaloylation and 2-ethylhexanoylation, the 3':5'-selectivities were still low to moderate (1:2.2, 1:1.4 and 1:5.3, respectively, entries 2, 5 and 6). This suggests that the difference is marginal between the activation energy of 3'-acylation and that of 5'-acylation. Interestingly, reversal of the regioselectivity was observed in the crotonylation, methacryloylation and cinnamoylation (2.3:1, 1.5:1 and 4.4:1,

respectively, entries 1, 3 and 4), where the secondary hydroxyl group was acylated preferentially.

The structure of TLL-inhibitor complex indicates that the acyl chain interacted closely with amino acid residue Trp89 in the lid of the lipase (Lawson et al. 1994). In addition, Martinelle et al. (1996) demonstrated that Trp89 played an important role in the acyl chain-length specificity of the lipase through site-directed mutagenesis. Hence, a remote interaction

might occur between the double bond of the acyl chain and the aromatic ring of Trp89 in the conformation of 3'-acylation transition state. This remote interaction might contribute to the reversal of the regioselectivity in the crotonylation, methacryloylation and cinnamoylation. Compared to that in the crotonylation, the higher regioselectivity might be ascribed to the stronger interaction in the cinnamoylation due to the bigger conjugate system in the cinnamoyl group (2.3:1 vs. 4.4:1, entries 1 and 4). Likewise, Botta et al. (2002) attributed the high regioselectivity toward the C-2 side-chain hydroxyl group in *Mucor miehei* lipase-catalyzed acylation of resorcin[4]arenes to a favorable π -interaction between the phenyl of the substrate and the aromatic ring of Trp88 in the lid of the enzyme. The preferential acylation position remained 5'-hydroxyl in the enzymatic oleoylation, in spite of the presence of the C=C double bond in the oleoyl moiety (1:3.2, entry 7). This is not surprising in that the double bond is too far away from the carbonyl group to form a remote interaction with Trp89 in the lid of the lipase.

Conclusion

Regioselectivity in TLL-mediated acylation of FUDR could be reversed and the secondary hydroxyl was preferentially acylated with the acyl donors bearing a conjugate double bond. This might be attributed to the presence of a remote interaction between the unsaturated bond in the acyl group and the aromatic ring of amino acid residue Trp89 in the lid of the lipase. This study would provide some novel and interesting information about the interactions between the substrates and the enzyme. Doubtlessly, further work is necessary to get an in-depth understanding of their interactions.

Acknowledgements We wish to thank Ms. Xiu-Mei Liu for the help on NMR analysis. This research was financially supported by the National Natural Science Foundation of China (Grant Nos. 20676043 and 20603036), Science and Technology Project of Guangdong Province (Grant No. 2006A10602003), Science and Technology Project of Guangzhou city (Grant No. 2007Z3-E4101), the Open Project Program of the State Key Laboratory of Catalysis, Dalian Institute of Chemical Physics, Chinese Academy of Sciences (Grant No. N-06-06).

References

- Aghil O, Bibby MC, Carrington SJ et al (1992) Synthesis and cytotoxicity of shikimate analogues. Structure: activity studies based on 1-crotonyloxymethyl-3R,4R,5R-trihydroxycyclohex-2-enone. *Anticancer Drug Des* 7:67–82
- Botta B, Zappia G, Tafi A et al (2002) Lipase-catalyzed regioselective acylation of resorcin[4]arenes. *J Mol Catal B* 16:241–247
- Ferrer M, Soliveri J, Plou FJ et al (2005) Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme Microb Technol* 36:391–398
- Ferrero M, Gotor V (2000) Biocatalytic selective modifications of conventional nucleosides, carbocyclic nucleosides, and C-nucleosides. *Chem Rev* 100:4319–4347
- Kroon PA, Williamson G (1999) Hydroxycinnamates in plants and food: current and future perspectives. *J Sci Food Agric* 79:355–361
- Landowski CP, Song XQ, Lorenzi PL et al (2005) Floxuridine amino acid ester prodrugs: enhancing Caco-2 permeability and resistance to glycosidic bond metabolism. *Pharm Res* 22:1510–1518
- Lawson DM, Brzozowski AM, Rety S et al (1994) Probing the nature of substrate binding in *Humicola lanuginosa* lipase through X-ray crystallography and intuitive modelling. *Protein Eng* 7:543–550
- Li N, Zong MH, Liu XM et al (2007) Regioselective synthesis of 3'-O-caproyl-floxuridine catalyzed by *Pseudomonas cepacia* lipase. *J Mol Catal B* 47:6–12
- Li N, Ma D, Zong MH (2008a) Enhancing the activity and regioselectivity of lipases for 3'-benzoylation of floxuridine and its analogs by using ionic liquid-containing systems. *J Biotechnol* 133:103–109
- Li N, Zong MH, Ma D (2008b) Regioselective acylation of nucleosides catalyzed by *Candida antarctica* lipase B: enzyme substrate recognition. *Eur J Org Chem* 2008: 5375–5378
- MacManus DA, Vulfson EN (1997) Reversal of regioselectivity in the enzymatic acylation of secondary hydroxyl groups mediated by organic solvents. *Enzyme Microb Technol* 20:225–228
- Martinelle M, Holmquist M, Clausen IG et al (1996) The role of Glu87 and Trp89 in the lid of *Humicola lanuginosa* lipase. *Protein Eng* 9:519–524
- Neves Petersen MT, Fojan P, Petersen SB (2001) How do lipases and esterases work: the electrostatic contribution. *J Biotechnol* 85:115–147
- Rubio E, Fernandez-Mayorales A, Klivanov AM (1991) Effect of the solvent on enzyme regioselectivity. *J Am Chem Soc* 113:695–696
- Takeuchi T, Chimura H, Hamada M et al (1975) A glyoxalase I inhibitor of a new structural type produced by *Streptomyces*. *J Antibiot* 28:737–742
- Wang H, Zong MH, Wu H et al (2007) Novel and highly regioselective route for synthesis of 5-fluorouridine lipophilic ester derivatives by lipozyme TL IM. *J Biotechnol* 129:689–695