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Note

# Differential activities of a lipase and a protease toward straight- and branched-chain acyl donors in transesterification to carbohydrates in an organic medium

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Enzymatic transesterification reactions catalyzed by lipases and proteases in organic media have been extensively used for enantioselective and regioselective synthesis of organic compounds [1–15]. In particular, the potential of these enzymes as catalysts for the acylation of carbohydrates is currently of considerable interest, because reaction occurs selectively at the primary hydroxyl group of the sugar. In this decade, various reports have been published on the enzymatic transesterification of carbohydrates using activated acyl donors such as trihaloethyl esters [1–3], vinyl esters [4], acid anhydrides [5,6], and oxime esters [7]. For instance, Wang et al. reported that vinyl propionates reacted faster than vinyl acetates in the transesterification of glucose with *Candida cylindracea* lipase [4]. In the transesterification of 2-hydroxy acids with primary alcohols by the *C. cylindracea* lipase, it was also reported that the presence of bulky groups in the vicinity of the 2-hydroxy group lowered the catalytic efficiency of this enzyme [8]. However, the activities of the available enzyme preparations have not been extensively compared, particularly with regard to transesterifications in organic media.

In the present study, we reacted D-glucose with various straight- and branched-chain acyl donors in pyridine in the presence of some crude commercial lipase and protease preparations to evaluate the catalytic efficiency of those enzyme preparations for the

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synthesis of carbohydrate esters. The results obtained are discussed with respect to the interactions between the acyl donors and the substrate-binding domains of the enzymes. The steric effects of acceptor molecules in the transesterification of *p*-nitrophenyl glucosides by *Pseudomonas cepacia* lipase [16] are also discussed.

To identify enzymes with high transesterification activity, lipase and protease preparations from 16 different sources were used as catalysts for the reaction of glucose with trichloroethyl propionate, vinyl acetate, and vinyl benzoate in pyridine (Table 1). As may be seen in the table, different substrate specificities were observed among the enzymes used. When glucose was reacted with a threefold excess of trichloroethyl propionate in pyridine at 45°C for 48 h, 6-*O*-propionylglucose was obtained in a 29% yield using *Pseudomonas cepacia* lipase (LPS). Yields obtained with the other enzymes were less than 10%. On the other hand, vinyl acetate reacted with glucose to give a yield of 79% of 6-*O*-acetylglucose in the presence of LPS. Proteolytic enzymes from *Bacillus* sp. (Proleather) and papaya (papain) also exhibited higher activity toward vinyl acetate than toward trichloroethyl propionate. However, among the 16 enzymes listed in Table 1, transesterification of vinyl benzoate was observed only with Proleather.

To analyze the activities of the LPS and Proleather preparations in more detail, 23 acyl donors were reacted with glucose in the presence of these enzymes. As shown in

Table 1
Transesterification of 2,2,2-trichloroethyl propionate, vinyl acetate, and vinyl benzoate with D-glucose catalyzed by lipases and proteases [a]

| Enzyme | Origin | Yields of products (by wt., as % of wt of acceptor added) | | |
|---|---|---|---|---|
| | | 6-*O*-Propionyl-D-glucose | 6-*O*-Acetyl-D-glucose | 6-*O*-Benzoyl-D-glucose |
| Lipase A | *Aspergillus niger* | 4.2 | 0 | 0 |
| Lipase M | *Mucor javanicus* | 7.7 | 0 | 0 |
| Lipase F-AP | *Rhizopus oryzae* | 0 | 0 | 0 |
| Lipase PS[b] | *Pseudomonas cepacia* | 29 | 79 | 0 |
| Lipase AY | *Candida rugosa* | 5.0 | 4.9 | 0 |
| Pancreatin F | Hog pancreas | 2.2 | 9.9 | 0 |
| Newlase F | *Rhizopus niveus* | 0 | 0 | 0 |
| Protease M[c] | *Aspergillus oryzae* | 8.7 | 0 | 0 |
| Protease A[c] | *Aspergillus oryzae* | 0.7 | 13 | 0 |
| Protease N | *Bacillus subtilis* | 0.9 | 0 | 0 |
| Protease P | *Aspergillus melleus* | 0 | 0 | 0 |
| Protease S | *Bacillus stearothermophilus* | 0 | 4.7 | 0 |
| Proleather | *Bacillus* sp. | 5.2 | 31 | 33 |
| Papain W-40 | Carica papaya | 5.4 | 37 | 0 |
| Bromelain F | Pineapple cannery | 3.1 | 0 | 0 |
| Protease B | *Penicillium citrinum* | 2.9 | 0 | 0 |
| Control | | 0 | 0[d] | 0 |

[a] Reaction conditions are given in the Experimental section. [b] Lipase PS is abbreviated as LPS in the text. [c] Protease M is an isozyme of Protease A. Their optimum pHs for hydrolysis are 3.0 and 7.0, respectively. [d] When vinyl acetate was reacted with glucose in pyridine in the absence of enzyme, partially acetylated derivates were formed. However, $^{13}C$ NMR analysis of the reaction products revealed that the esterification occurred nonselectively at various hydroxyl groups of the glucose.

Table 2, Proleather was active with vinyl octanoate, whose carbon number is 10, whereas LPS was active with the vinyl esters of fatty acids having carbon numbers less than 8. From this result, it is surmised that the substrate-binding pocket of Proleather is larger than that of LPS.

When LPS and Proleather were used with bulky acyl donors, marked differences were observed in their transesterification activities. The yield of 6-*O*-methacryloylglucose with Proleather was 83%, while with LPS it was 38% (Table 2). Likewise, a branched bulky acyl donor, vinyl pivalate, reacted with glucose to give a 48% yield of 6-*O*-acyl derivative in the presence of Proleather, whereas with LPS the yield was 24%. Even vinyl *o*-chlorobenzoate reacted with glucose in the presence of Proleather. From these results, we concluded that Proleather interacted more favorably with bulky hydrophobic acyl donors than LPS.

With regard to the effects of the leaving groups of acyl donors, differences between LPS and Proleather were also observed. In particular, the activity of Proleather toward trichloroethyl and trifluoroethyl acylates was very low, compared to that of LPS.

Next, the effects of acceptor-molecule configuration on the transesterification were analyzed. When vinyl acetate was reacted with *p*-nitrophenyl α-D-glucopyranoside in the presence of LPS in pyridine, *p*-nitrophenyl 6-*O*-acetyl-α-D-glucopyranoside was synthesized in a 76% yield, while the acylation of the corresponding β-anomer proceeded in 19% yield. This might be explained by differences in the steric hindrance and stereoelectronic effects of the anomeric substituents of the acceptors. However, Riva et al. [13] synthesized 6′-*O*-monobutyryl disaccharides by the reaction of trichloroethyl butyrate and various disaccharides in the presence of subtilisin in DMF, and showed that

Table 2
Activities of *P. cepacia* lipase (LPS) and *Bacillus* sp. protease (Proleather) for transesterification from various acyl donors in pyridine

| Acyl donor | Yields of 6-*O*-acyl-D-glucose (% of theory) | |
|---|---|---|
| | LPS | Proleather |
| Vinyl stearate | 0 | 0 |
| Vinyl palmitate | 0 | 0 |
| Vinyl myristate | 0 | 0 |
| Vinyl laurate | 0 | 0 |
| Vinyl decanoate | 0 | 0 |
| Vinyl octanoate | 38 | 0 |
| Vinyl hexanoate | 49 | 31 |
| Vinyl butyrate | 39 | 55 |
| Vinyl propionate | 39 | 31 |
| Vinyl acetate | 52 | 31 |
| Vinyl acrylate | 11 | 0 |
| Vinyl methacrylate | 39 | 83 |
| Vinyl sorbate | 0 | 0 |
| Vinyl crotonate | 0 | 0 |
| Vinyl pivalate | 24 | 48 |
| Vinyl trifluoroacetate | 0 | 0 |
| Vinyl chloroacetate | 0 | 0 |
| Vinyl benzoate | 0 | 21 |
| Vinyl *p-tert*-butylbenzoate | 0 | 0 |
| Vinyl *o*-chlorobenzoate | 0 | 16 |
| Vinyl cinnamate | 0 | 0 |
| 2,2,2-Trichloroethyl propionate | 29 | 5.2 |
| 2,2,2-Trifluoroethyl acrylate | 29 | 0 |

a bulky aglycon linked either $\alpha$ or $\beta$ to the acyl-accepting glycosyl unit does not substantially reduce the catalytic efficiency of subtilisin.

To summarize, the bulk or chain length of the acyl moiety and the nature of the leaving group affect the transesterification catalyzed by Proleather. The first of these influences may be ascribed to the strong van der Waals forces between the side chain and the amino acid group of the substrate-binding domain. Furthermore, the configuration of the acceptor molecule also affected the yield of transesterification products in the reaction catalyzed by LPS. The analysis of substrate specificities toward different acyl donor and acceptor molecules should contribute not only to synthetic organic chemistry but also to the further understanding of the enzymology of serine esterases.

## 1. Experimental

*General methods.*—Lipases and proteases were obtained from Amano Pharmaceutical Co. Ltd. Pyridine was used without further purification aside from drying with potassium hydroxide. Acyl donors except for 2,2,2-trichloroethyl propionate were obtained from Tokyo Kasei Co. Ltd. 2,2,2-Trichloroethyl propionate was synthesized from 2,2,2-trichloroethanol and propionyl chloride in pyridine. Purification of the activated ester was carried out by fractional vacuum evaporation [$^1$H NMR ($CDCl_3$): $\delta$ 1.21 (t, 3 H, $CH_3CH_2COO$), 2.48 (q, 2 H, $CH_3CH_2COO$), and 4.74 (s, 2 H, $CH_2CCl_3$)]. Glucose and *p*-nitrophenyl glucosides were obtained from Nacalai Tesque Co. Ltd. TLC was carried out on precoated silica gel 60 $F_{254}$ plates from Merck, with a developing system consisting of 7:1:2 *i*-PrOH–EtOH–$H_2O$. NMR spectra were recorded at 18°C on a Varian XL-200 spectrometer ($^1$H at 200 MHz, $^{13}$C at 50.3 MHz). Chemical shifts were determined relative to the internal standards sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate ($\delta_H$ 0.00), tetramethylsilane ($\delta_H$ and $\delta_C$ 0.00), and dioxane ($\delta_C$ 67.40). Spectra were recorded in $D_2O$ except where otherwise noted. Signal assignments were based on the NMR shift parameters described by Yoshimoto et al. [17]. Pyridylamination was carried out as described in Kondo et al. [18]. HPLC of the pyridylaminated sugars was done on an Asahikasei NH2P-50 column (4.6 mm × 250 mm) in a Waters 600 system using aqueous 80% acetonitrile as a mobile phase at a flow rate of 1 mL/min.

*Enzymatic synthesis of 6-O-acyl glucoses.*—D-Glucose (60 mg) was reacted with acyl donors (1 mmol) in the presence of lipase or protease (100 mg) in pyridine (1 mL) at 45°C for 48 h. The enzymes used were crude, having hydrolyzing activities for their corresponding substrates of more than 5500 U/g. After reaction the enzyme was inactivated by heating in a boiling water bath for 10 min. The reaction products were separated by silica-gel column chromatography with 100:20:1 EtOAc–MeOH–$H_2O$ as eluent, and identified by NMR spectroscopy. The yield of acylated sugar was determined by HPLC after pyridylamination.

The following acyl derivatives were characterized. The crystalline products were largely in the $\alpha$-anomeric form; where no mp is given the product was a syrup. Optical rotations were determined on solutions at mutarotational equilibrium.

*6-O-Acetyl-D-glucose.* mp 137–140°C; $[\alpha]_D^{20}$ +50.5° (*c* 1.0, $H_2O$, equil.); lit. mp

143°C, $[\alpha]_D^{30}$ + 50.6° (*c* 0.7, $H_2O$, equil.) [1]; the $^1H$ and $^{13}C$ NMR data were in accord with those in the literature [19]. Anal. Calcd for $C_8H_{14}O_7$: C, 43.24; H, 6.35. Found: C, 43.12; H, 6.33.

*6-O-Propionyl-D-glucose.* $[\alpha]_D^{20}$ + 50.7° (*c* 1.4, $H_2O$, equil.); $^1H$ NMR: δ 5.21 (d, $J_{1,2}$ 3.6 Hz, H-1α), 4.65 (d, $J_{1,2}$ 7.9 Hz, H-1β), 3.21–4.46 (m, 6 H, H-2–6), 2.44 (q, 2 H, $CH_3CH_2$), and 1.12 (t, 3 H, $CH_3CH_2$); $^{13}C$ NMR: δ 178.21 (C=O), 92.97 (C-1α), 72.24 (C-2α), 73.42 (C-3α), 70.43 (C-4α), 69.99 (C-5α), 64.16 (C-6α), 96.84 (C-1β), 74.85 (C-2β), 76.37 (C-3β), 70.43 (C-4β), 74.25 (C-5β), 64.31 (C-6β), 28.01 ($CH_3CH_2$), and 9.21 ($CH_3CH_2$). Anal. Calcd for $C_9H_{16}O_7$: C, 45.76; H, 6.83. Found: C, 45.82; H, 6.81.

*6-O-Butyryl-D-glucose.* mp 113–118°C; $[\alpha]_D^{20}$ +50.5° (*c* 2.0, $H_2O$, equil.); lit. mp 116–117°C, $[\alpha]_D^{30}$ + 45.3° (*c* 1, $H_2O$) [1]; the $^1H$ and $^{13}C$ NMR data were in accord with those in the literature [20]. Anal. Calcd for $C_{10}H_{18}O_7$: C, 48.00; H, 7.25. Found: C, 47.91; H, 7.24.

*6-O-Hexanoyl-D-glucose.* mp 130–133°C; $[\alpha]_D^{20}$ + 44.2° (*c* 2.7, $H_2O$, equil.); $^1H$ NMR: δ 5.20 (d, $J_{1,2}$ 3.6 Hz, H-1α), 4.63 (d, $J_{1,2}$ 7.8 Hz, H-1β), 3.21–4.45 (m, 6 H, H-2-6), 2.40 [t, 2 H, $CH_3(CH_3)_3CH_2$], 1.61 [m, 2 H, $CH_3(CH_2)_2CH_2CH_2$], 1.30 [m, 4 H, $CH_3(CH_2)_2CH_2CH_2$], and 0.88 (t, 3 H, $CH_3$); $^{13}C$ NMR: δ 176.41 (C=O), 92.92 (C-1α), 72.33 (C-2α), 73.49 (C-3α), 70.63 (C-4α), 70.01 (C-5α), 64.23 (C-6α), 96.85 (C-1β), 74.87 (C-2β), 76.43 (C-3β), 70.66 (C-4β), 74.34 (C-5β), 64.39 (C-6β), 39.58 [$CH_3(CH_2)_3CH_2$], 34.45 [$CH_3(CH_2)_2CH_2CH_2$], 31.74 [$CH_3CH_2CH_2(CH_2)_2$], 22.86 [$CH_3CH_2(CH_2)_3$], and 14.31 ($CH_3$). Anal. Calcd for $C_{12}H_{22}O_7$: C, 51.61; H, 8.00. Found: C, 51.69; H, 8.04.

*6-O-Methacryloyl-D-glucose.* $[\alpha]_D^{20}$ + 41.0° (*c* 4.0, $H_2O$, equil.); $^1H$ NMR: δ 6.16, 5.74 (s, 2 H, $CH_2=CCH_3$), 5.23 (d, $J_{1,2}$ 3.5 Hz, H-1α), 4.68 (d, $J_{1,2}$ 7.9 Hz, H-1β), 3.24–4.53 (m, 6 H, H-2-6), and 1.94 (s, 3 H, $CH_2=CCH_3$); $^{13}C$ NMR: δ 170.12 (C=O), 136.38 ($CH_2=CCH_3$), 128.06 ($CH_2=CCH_3$), 92.96 (C-1α), 72.28 (C-2α), 73.44 (C-3α), 70.42 (C-4α), 70.05 (C-5α), 64.44 (C-6α), 96.85 (C-1β), 74.87 (C-2β), 76.37 (C-3β), 70.50 (C-4β), 74.26 (C-5β), 64.44 (C-6β), and 18.25 ($CH_2=CCH_3$). Anal. calcd for $C_{10}H_{16}O_7$: C, 48.39; H, 6.50. Found: C, 48.32; H, 6.54.

*6-O-Benzoyl-D-glucose.* $[\alpha]_D^{20}$ + 42.4° (*c* 0.8, $H_2O$, equil.); lit. $[\alpha]_D^{21}$ + 45° ($H_2O$, equil.) [21]; a crystalline preparation was described in ref. [21], and in a more recent paper [22]. Our $^1H$ NMR data were in accord with those in the literature [23]. $^{13}C$ NMR: δ 169.06 (C=O), 134.70 (Ph C-4), 130.33 (Ph C-3,5), 129.75 (Ph C-1), 129.49 (Ph C-2,6), 93.03 (C-1α), 72.34 (C-2α), 73.53 (C-3α), 70.58 (C-4α), 70.20 (C-5α), 64.77 (C-6α), 96.95 (C-1β), 74.96 (C-2β), 76.48 (C-3β), 70.68 (C-4β), 74.44 (C-5β), and 64.77 (C-6β). Anal. Calcd for $C_{13}H_{16}O_7$: C, 54.93; H, 5.67. Found: C, 54.89; H, 5.63.

*Enzymatic synthesis of* p-*nitrophenyl 6-O-acetyl-D-glucopyranosides.*—*p*-Nitrophenyl α- and β-D-glucopyranosides (60 mg) were reacted with vinyl acetate in pyridine (1 mL) at 45°C for 24 h, and purified by silica-gel column chromatography using 7:1 $CHCl_3$–MeOH as eluent. The following acyl derivatives were characterized.

p-*Nitrophenyl 6-O-acetyl-α-D-glucopyranoside.* $[\alpha]_D^{20}$ + 156° (*c* 0.5, pyridine); NMR (1:1 $CD_3OD$–$D_2O$): $\delta_H$ 8.22 (d, 2 H, Ph H-3,5), 7.29 (d, 2 H, Ph H-2,6), 5.75 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1α), 3.34–3.99 (m, 4 H, H-2-5), and 2.00 (s, 3 H, $CH_3$); $\delta_C$ 173.48 (C=O), 162.35 (Ph C-1), 143.02 (Ph C-4), 126.34 (Ph C-3,5), 117.47 (Ph C-2,6), 97.72

(C-1), 71.88 (C-2), 73.82 (C-3), 70.51 (C-4), 71.55 (C-5), 64.09 (C-6), 20.54 ($CH_3$). Anal. Calcd for $C_{14}H_{17}O_9N$: C, 48.98; H, 4.99. Found: C, 49.08; H, 5.02.

p-*Nitrophenyl 6-O-acetyl-β-D-glucopyranoside*. $[\alpha]_D^{20}$ −82.7° (*c* 0.2, pyridine); lit. $[\alpha]_D^{22}$ −106° (*c* 0.2, MeOH) [24]; a crystalline preparation is described in this reference; NMR (7:1 $CDCl_3$–$CD_3OD$): $\delta_H$ 8.20 (d, 2 H, Ph H-3,5), 7.13 (d, 2 H, Ph H-2,6), 5.00 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1β), 3.37–4.46 (m, 4 H, H-2-5), and 2.10 (s, 3 H, $CH_3$); $\delta_C$ 171.62 (C=O), 162.18 (Ph C-1), 142.77 (Ph C-4), 125.80 (Ph C-3,5), 116.60 (Ph C-2,6), 100.23 (C-1), 73.03 (C-2), 76.43 (C-3), 69.88 (C-4), 74.35 (C-5), 63.68 (C-6), and 20.85 ($CH_3$). Anal. Calcd for $C_{14}H_{17}O_9N$: C, 48.98; H, 4.99. Found: C, 49.00; H, 4.97.

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