Selectivity of *Candida antarctica* B Lipase toward Fatty Acid and (Iso)propanol Substrates in Esterification Reactions in Organic Media

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Fatty acid (FA) selectivity of immobilized *Candida antarctica* B lipase was assessed as influenced by various cosubstrate systems for ester synthesis. Reaction mixtures contained a homologous series of even-chain *n*-acyl donor (C_{4-16}) substrates (FA or their methyl esters, FAME) and a single alcohol cosubstrate (propanol, 2-propanol, or their acetate derivatives) in hexane. Multiple FA optima were often observed, with preferences for C_6 (or C_4) followed by C_{14} and sometimes C_{10}. The degree of selectivity among acyl donors was modest (up to 1.28−2.60, based on ratios of selectivity constants) and was dependent on the choice of cosubstrate system. Acyl group selectivity ranged up to 1.31−1.36 for [FA + alcohol], 1.48−2.60 for [FAME + alcohol], 1.30−1.72 for [FA + alcohol acetate], and 1.28−1.88 [FAME + alcohol acetate] reaction systems. General shifts in selectivity were observed between short-chain (C_{4-8}) and long-chain (C_{10-16}) FA as groups with propanol cosubstrate, whereas shifts in reaction selectivity were observed toward specific FA(s) for 2-propanol cosubstrate. Selectivity among a series of alcohol cosubstrates ranged up to 13-fold in esterification reactions with C_6 FA.

Keywords: *Lipase; anhydrous; selectivity; ester modification; Candida antarctica*

INTRODUCTION

The current prospectus for using lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) to transform basal lipid resources into value-added and functional derivatives is founded on the various types of reaction selectivity exhibited by lipases (Jensen et al., 1990; Villeneuve and Foglia, 1997). Thus, it stands to reason that the greatest exploitation of the synthetic potential of lipases will be conferred when a full understanding of enzyme selectivity and the factors that influence it are elucidated. This view finds support from the vast literature available on lipase-mediated reactions designed for enantiomeric synthesis/resolution or to prepare synthons or pharmaceuticals (Kazlauskas, 1994; Alcántara et al., 1998; Anderson et al., 1998; Ohtani et al., 1998).

The use of lipases to transform basal lipid resources (primarily triacylglycerols) in the food and applied sciences has been largely confined to studies in which only "coarse" features (such as regioselectivity) of lipase selectivity have been considered. These studies have demonstrated the general utility of using lipases to prepare a variety of types of lipid derivatives (Vulfson, 1993; McNeill and Sonnet, 1995), and two commercially developed processes exploit lipase regioselectivity in the preparation of cocoa butter substitutes and lipids for use in infant formula (Haumann, 1997; Gunstone, 1998). However, it seems evident that finer control of reaction selectivity will be a requirement to prepare "structured" lipids, which can be defined as "triglycerides whose fatty acid composition and location on the glycerol backbone have been predetermined by unequivocal synthetic routes" (Villeneuve and Foglia, 1997). The prospect of preparing structured lipids has emerged as a primary motivation to continue exploring lipases as synthetic tools for food lipid modification (Akoh, 1995), and the estimated global market for structured lipids to deliver essential fatty acids alone is U.S. $10 billion (Siguel, 1996).

Several reaction parameters can influence lipase reaction and product selectivities, including water activity (Halling, 1994), immobilization matrix (Adlercreutz, 1991), and the nature of the organic medium (Kuo and Parkin, 1996). Although there are several choices of cosubstrate systems to achieve the same net modification (through esterification, acidolysis, alcoholysis, or interesterification reactions), to our knowledge this parameter has not been systematically studied except for a parallel study on *Rhizomucor miehei* lipase in our laboratory (Arsan and Parkin, 2000). This paper was intended to evaluate the patterns of fatty acid selectivity of *Candida antarctica* B lipase, as influenced by cosubstrate systems and primary and secondary alcohol acceptor groups, principally using (iso)propanol to model reactivity of the *sn*-glycerol backbone.

MATERIALS AND METHODS

Materials. Immobilized *C. antarctica* lipase (triacylglycerol acylhydrolase, EC 3.1.1.3) fraction B (Chirazyme L-2) was supplied by Roche Diagnostics/Boehringer Mannheim Corp. Indianapolis, IN). *n*-Propanol, propyl acetate, isopropyl acetate, cyclohexanol, and 0.5 M sodium methoxide, of the highest grade available, were obtained from Aldrich Inc. (Milwaukee, WI). 2-Propanol, amyl and isobutyl alcohols, and anhydrous forms of sodium sulfate, sodium phosphate and sodium

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Table 1. Factors Expected To Influence Acyl Donor Selectivity for Various Cosubstrate Systems for Ester Modification Reactions[a]

		factors influencing acyl group reaction selectivity for the half-reaction[b] of	
reaction type	cosubstrates	acyl donor + enz → acyl−enz + *X*	acyl−enz + Nu: → acyl−Nu + enz
A. esterification	FA Alc	FA (X = H_2O)	acyl−enz, Alc
B. alcoholysis	FAME Alc	FA, ME (X = CH_3OH)	acyl−enz, Alc, CH_3OH
C. acidolysis	FA AlcAcet	FA (X = H_2O, CH_3COO^-)	acyl−enz, Alc, [Alc]
D. acyl exchange	FAME AlcAcet	FA, ME (X = CH_3OH, CH_3COO^-)	acyl−enz, Alc, CH_3OH, [Alc]

[a] Abbreviations used: enz, enzyme; Nu:, nucleophile; acyl−Nu, new ester formed; [X, leaving group(s) for reactions forming acyl−enz]; Alc, alcohol; [Alc], alcohol concentration; AlcAcet, alcohol acetate; FA, fatty acid; FAME, fatty acid methyl ester. [b] Lipase-mediated ester modification reactions are known to conform to Ping-Pong Bi-Bi kinetics, reflecting a mechanism comprising of two half-reactions, each involving a tetrahedral intermediate (Zaks and Klibanov, 1985; Chulalakananukul et al., 1990; Rizzi et al., 1992; Ramamurthi and McCurdy, 1994; Kazlauskas, 1994).

carbonate were products of Mallinckrodt Chemicals (Paris, KY). Sodium phosphate dibasic dihydrate and sodium carbonate monohydrate were obtained from Fluka Chemie AG (Buchs, Switzerland). Fatty acid propyl ester (FAPE) standards were obtained from PolyScience (Niles, IL). Potassium carbonate and phenol were obtained from Baker Inc. (Phillipsburg, NJ). All other reagents were obtained from Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Chicago, IL).

Reaction Mixtures. Reaction mixture design was based on a competitive assay system (Deleuze et al., 1987; Berger and Schneider, 1991; Chang et al., 1999). Seven *n*-fatty acids (FA) (saturated fatty acids/acyl groups are designated C_X, where *X* is the number of *n*-acyl carbons and where $X = 4-16$), or their methyl ester derivatives (FAME), were included in reaction mixtures at 50 mM each in the presence of 0.4 M of the selected alcohol cosubstrate (propanol or 2-propanol, or their acetate derivatives). Hexane was used as the continuous phase (total volume of 20 mL), and the reaction mixture also contained the internal standard, limonene. Reaction mixtures were pre-equilibrated, initiated by the addition of enzyme (70 or 105 mg when FA and FAME substrates were used, respectively), and incubated at 40 °C with rotary agitation as described elsewhere (Arsan and Parkin, 2000).

When selectivity among a series of alcohols was evaluated, hexanoic acid (C_6) was the sole acyl donor used at 2.1 M in the presence of 0.05 M of each alcohol used. The level of enzyme used was 105 mg, and other protocols used for these reactions mixtures were identical to those described in the preceding paragraph.

Water activity (a_w) was controlled by the use of salt hydrates (Halling, 1992); 0.5 g of each Na_2HPO_4 (anhydrous) and $Na_2HPO_4 \cdot (2H_2O)$ was used to achieve an a_w of 0.21 for reactions with *C. antarctica* lipase (this a_w value was determined in preliminary studies to be near optimally supportive of enzyme activity).

Sampling and Analysis. For the FA-based cosubstrate systems, 0.50 mL of reaction mixture was removed at selected intervals for up to 60 min and combined with 0.50 mL of 2.1 M potassium carbonate. The mixture was vigorously agitated by hand, and phase separation was allowed to take place prior to analysis of the hexane phase for ester products of the reaction. Phase separation allowed for removal of unreacted FA from the ester products prior to analysis of the latter.

For the FAME-based cosubstrate systems, small volume (<0.1 mL) subsamples of the hexane phase were removed at selected intervals of up to 120 min and directly subjected to analysis for ester products as outlined in the next paragraph. The original FAME substrates could be resolved from the corresponding FA (iso)propyl ester products of the reaction, obviating the need for intermediate steps of sample preparation.

Analysis for ester products formed by reaction mixtures was achieved by gas chromatography (model 6890) using an HP-5 capillary column (Hewlett-Packard Co., Rockville, MD), as previously described (Chang et al., 1999; Arsan and Parkin, 2000). Results represent the mean α values ± SD from two to three experiments, each of which had at least duplicate samples. In some cases, rather large SD values were noted, and these values represented the general variation in the experimental data sets relative to the FA species taken as the reference (C_6). However, the order of preference in reactivity of FA donors was very consistent among related experiments, and ordinal rankings of FA selectivity on the basis of mean α values present an accurate picture of reaction selectivity.

Quantitative Analysis of Selectivity. The determination of relative selectivity within a series of either acyl donor or alcohol substrates was achieved using a multisubstrate competitive assay approach (Deleuze et al., 1987; Berger and Schneider, 1991), as employed in our laboratory (Chang et al., 1999; Arsan and Parkin, 2000). This approach is based on the relationship between reaction velocities (*v*) for competing substrates A and B

$$v_A/v_B = \alpha\,[A]/[B] \qquad \text{where } \alpha = (V_A/K_A)/(V_B/K_B)$$

where *V* and *K* represent maximal velocity and the Michaelis constant, respectively. The integrated form of this relationship yields a linear (log−log) plot, and relative α values (proportional to *V*/*K*, which is referred to as the specificity constant; Fersht, 1985) are determined from slopes of the plots for each competing substrate. Hexanoic acid (C_6) was used as the reference acyl donor, and the corresponding α value of 1.0 was assigned. In cases when initial reaction velocities were estimated, only the first 10−20 min of progress curves (confirmed to be the linear portion) were evaluated, and rates are reported only for the fastest reacting or reference acyl donor or alcohol cosubstrate as indicated (reported rates do not represent a composite reaction rate).

RESULTS AND DISCUSSION

General Considerations. The differences between the cosubstrate systems evaluated in this study are shown in Table 1. Any differences in FA selectivity observed among various cosubstrates systems may be attributable to the methyl ester versus free acid functional group of the acyl donor or methanol or acetate liberated by the forward reaction. Because the putative alcohol reactant for all reactions is free (iso)propanol, there is a prerequisite step of (iso)propyl acetate hydrolysis in systems C and D to provide the nucleophile required for formation of the new FA (iso)propanol ester; thus, differences in steady-state levels of alcohol cosubstrate(s) will also exist among these reaction systems.

Reaction rates are also expected to be different because of differences in nucleophile (and steady-state levels), steric effects of cosubstrates, and favorability of the leaving group in these reaction systems. Propanol and 2-propanol were selected as alcohol acceptors to evaluate selectivity patterns conferred by primary and secondary alcohol functional groups.

Reactions with Propanol and Propyl Acetate. Reactions between FA and propanol were most selective for FA chain lengths of C_6 and C_{14} (Figure 1a). Even

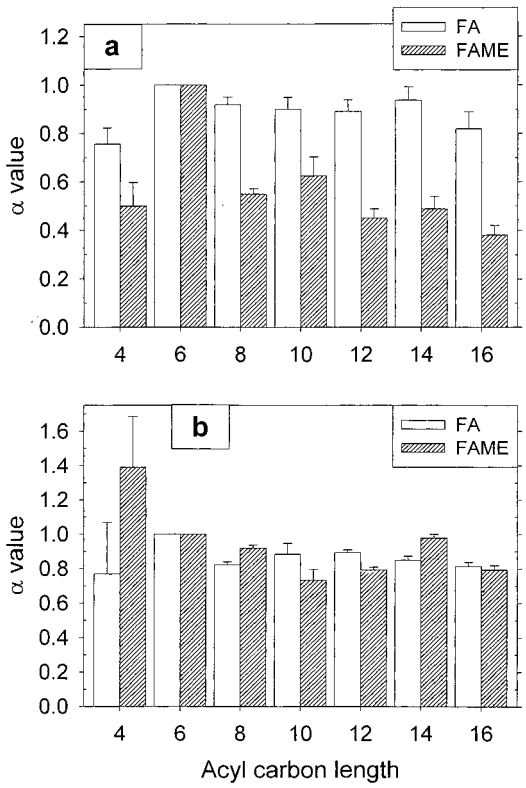

Figure 1. Relative α values for *n*-acyl donors of various chain lengths in reactions with propanol and propyl acetate. Reactions with propanol (a) and propyl acetate (b) transformed 30−70% acyl donor substrates over 40−120 min.

Table 2. Initial Reaction Velocities for Reactions Mediated by *C. antarctica* Lipase B

reaction system	acyl donor	alcohol cosubstrate	initial velocity[a] (μmol mg^{-1} min^{-1})
A. esterification	FA series	propanol	0.267 (100)[b]
		2-propanol	0.175 (100)
B. alcoholysis	FAME series	propanol	0.159 (60)
		2-propanol	0.109 (62)
C. acidolysis	FA series	propyl acetate	0.135 (51)
		isopropyl acetate	0.040 (23)
D. acyl exchange	FAME series	propyl acetate	0.275 (103)
		isopropyl acetate	0.133 (76)
E. esterification	hexanoic acid (C_6)	alcohol series	0.055

[a] Initial (linear) rates are reported for fastest reacting acyl donor, for benzyl alcohol, and for reaction systems A−D and E, respectively. [b] Normalized (percent) reaction rates are provided in parentheses in each column for each alcohol cosubstrate, where 100 is the normalized rate for reaction system A.

though the error bars imply little difference among $C_{6/12/14/16}$, C_6 was preferred over C_{14}, and C_{14} was preferred over C_{12} and C_{16} in *all* experimental replicates. Thus, the magnitude of the error bars relates primarily to the variation in α values between the reference FA (C_6) and this group (C_{12-16}) of acyl donors as a whole. Generally, there was only a modest degree of selectivity (ratios of α values for any two acyl donors were ≤1.31) within this series of FA substrates in esterification reactions with propanol, consistent with previous findings for this enzyme (Kirk et al., 1992; Chang et al., 1999). The initial reaction rates for this reaction system were among the fastest observed (Table 2) and may not represent maximal rates possible because the levels of alcohol used may be sufficient to confer some degree of substrate inhibition (Kuo and Parkin, 1993; Ramamurthi and McCurdy, 1994; Martinelle and Hult, 1995).

When FAME substrates were used in acyl-transfer reactions with propanol, a greater degree of selectivity (ratios of α values for any two acyl donors were ≤2.60) was observed compared to the series of FA acyl donors. Multiple chain length optima were apparent for FAME (relative to FA) substrates, with which selectivity toward C_6 was enhanced, and C_{10} and C_{14} constituted secondary optima. Despite the variance in α value determinations, the ordinal rankings among FAME substrates appearing in Figure 1a were again observed in all experimental replicates, where C_{10} was preferred over C_{14} and C_{14} was preferred over both C_{12} and C_{16}.

The fact that FA and FAME donor systems conferred different patterns and degrees of acyl selectivity implies a role for the methoxy group in FAME acyl donors in modulating enzyme−acyl group interaction. A secondary influence on selectivity may reside with a limited degree of reverse reaction between acyl−enzyme intermediate and methanol that could take place in the FAME/propanol reaction system. Different alcohols may exhibit different selectivities for acyl−enzyme intermediates (Alcántara et al., 1998), and the selectivity of the reverse reaction will undoubtedly impact the selectivity observed for the forward/net reaction. In addition, a change in the nature of the acyl donor itself (as from FA to FAME) independent of the leaving group may have a residual effect on the enzyme in a manner that influences selectivity, as has been observed in essentially irreversible esterification reactions (Stokes and Oehlschlager, 1987; Anthosen and Hoff, 1998).

Analogous reactions using propyl acetate as alcohol acceptor were evaluated as acidolysis reactions with FA and acyl-exchange reactions with FAME (Figure 1b). Reaction selectivity with the [FA + propyl acetate] cosubstrate system was similar to that observed for reactions between FA and propanol (Figure 1a). This was expected because the acyl donor and alcohol nucleophile were identical between these two reaction systems (Table 1). C_6 was the preferred FA substrate for reactions with propyl acetate, and there was only modest selectivity (ratios of α values for any two acyl donors were ≤1.38) among members of this group of acyl donors (Figure 1b).

Reactions between FAME and propyl acetate shifted chain length selectivity toward C_4 and C_{14} (Figure 1b; C_{14} was preferred over C_{12} and C_{16} in all experimental replicates), relative to the multiple optima of C_6 and $C_{10/14}$ observed for reactions between FAME and propanol (Figure 1a). The degree of selectivity was less for the [FAME + propyl acetate] system (ratios of α values were ≤1.88 compared to ≤2.60 for the [FAME + propanol] system). However, the shift in chain length optima toward C_4 for the [FAME + propyl acetate] system was unexpected on the basis that both the acyl donor and alcohol nucleophile cosubstrates were identical for these reaction systems (Table 1). An effect of accumulating acetate (MacFarlane et al., 1991) and butanoate (Hoff et al., 1996) on enantioselectivity of lipase-mediated reactions was previously noted for acyl-transfer reactions.

Initial reaction velocities for FAME substrates were only 60% those of the FA substrates using propanol as cosubstrate (Table 2), consistent with previous results with similar reaction systems (MacFarlane et al., 1991). At least two factors can account for this, and they

include the greater steric constraints imposed by the FAME substrates and the leaving group ("X" in Table 1) being more favorable in esterification than acyl-transfer reactions with the substrates selected in this study, as predicted from p*K* values of water and methanol (Serjeant and Dempsey, 1979). The initial velocity of reactions between FA and propyl acetate was 51% of those observed for FA and propanol (Table 2). This can be accounted for on the basis of a reduced nucleophile steady-state concentration in the former system, as propyl acetate must first be hydrolyzed to yield free propanol, and acetate is not very reactive with this enzyme (Kirk et al., 1992; Patkar et al., 1998).

Initial reaction rates between FAME and propyl acetate rivaled those between FA and propanol (Table 2). This was unexpected as the latter reaction is represented by the more favorable leaving group and greater steady-state nucleophile levels. The fact that reactions between FAME and propyl acetate were as fast as they were may indicate a balance between the two respective initial reactions to yield the acyl−enzyme and nucleophile. This view is further supported by comparison to reaction rates between FA and propyl acetate wherein steady-state levels of acyl−enzyme may dominate, leaving reduced levels of free enzyme available for transforming propyl acetate into nucleophile. A balance of acyl−enzyme pools from two acyl-donor substrates was previously suggested as being most conducive to acyl-exchange reactions (Kuo and Parkin, 1995). Comparison of reaction rates between reaction systems A or B with those of system D also indicates that the levels of free propanol used caused substrate (alcohol) inhibition of the reaction, as suggested earlier.

Reactions with 2-Propanol and Isopropyl Acetate as Alcohol Acceptors. Acyl group selectivity in reactions between FA and 2-propanol yielded acyl chain length optima of C_6 and C_{14} (Figure 2a), similar to results obtained with propanol (Figure 1a). Generally, there was only a small degree of selectivity (ratios of α values for any two acyl donors were ≤1.36) within this series of FA substrates in esterification reactions with 2-propanol (Figure 2a), similar to reactions between FA and propanol (Figure 1a). When FAME substrates were used in alcoholysis reactions with 2-propanol, multiple chain length optima were observed, where C_6/C_{10} were primary optima and C_{14} constituted a secondary optimum (Figure 2a). Selectivity remained modest as ratios of α values for any two acyl donors were ≤1.48. Despite the degree of error in α value determination, a preference for C_6 and C_{10} over C_{14} was observed in all experimental replicates. Furthermore, the ordinal patterns of FAME selectivity were the same for reactions with both propanol (Figure 1a) and 2-propanol (Figure 2a). However, one difference between these alcohol cosubstrates was that upon switching from FA to FAME donors, the degree of reaction selectivity (based on range of α values) was enhanced when propanol but not 2-propanol was used as alcohol cosubstrate.

Reactions with FA or FAME acyl donors were conducted with isopropyl acetate as the alcohol cosubstrate (Figure 2b). The pattern of selectivity with the FA series of substrates in reactions with isopropyl acetate was similar to that observed for reactions between FA and 2-propanol (Figure 2a), as expected (Table 1), and the dual chain length optima for C_6 and C_{14} were identical. However, there was a general tendency for reactions with isopropyl acetate to be less selective for shorter chain length FA (C_4/C_8) than were reactions with 2-propanol, despite C_6 still being the preferred acyl donor. Reactions between FAME acyl donors and isopropyl acetate shifted chain length selectivity toward C_4 and C_{14} (Figure 2b; C_{14} was preferred over C_{12} and C_{16} in all experimental replicates), relative to the multiple optima of C_6, C_{10}, and C_{14} observed with 2-propanol as alcohol cosubstrate (Figure 2a). This same trend in changes in selectivity patterns was observed for analogous reactions with FAME and propanol/propyl acetate cosubstrate systems (Figure 1a,b).

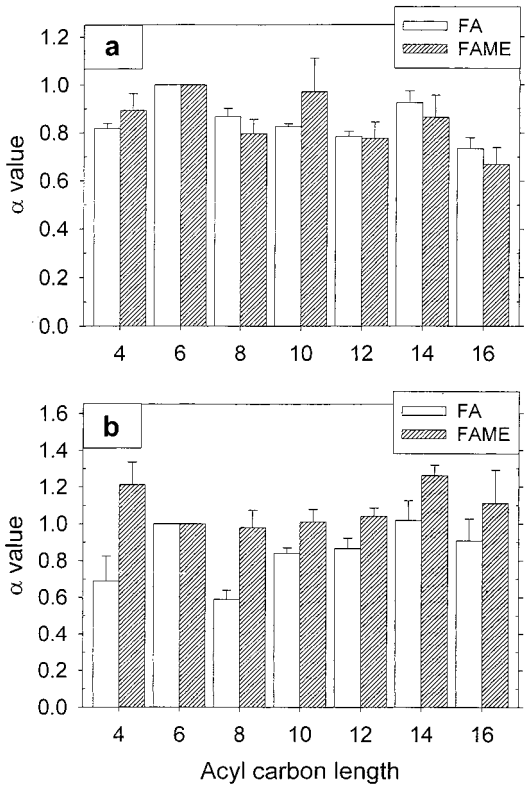

Figure 2. Relative α values for *n*-acyl donors of various chain lengths in reactions with 2-propanol and isopropyl acetate. Reactions with 2-propanol (a) and isopropyl acetate (b) transformed 8−70% acyl donor substrates over 40−120 min.

Acyl donor selectivity was enhanced in reactions between FA and isopropyl acetate compared to reactions between FA and 2-propanol (ratios of α values for any two acyl donors were ≤1.72 compared to ≤1.36) (Figure 2a,b). However, a similar enhancement of degree of selectivity did not occur when isopropyl acetate was substituted for 2-propanol in reactions with FAME cosubstrates (maximum ratio of α values for any two acyl donors was reduced to ≤1.28 from ≤1.48). This effect must be attributable to the accumulation of acetate, although the levels of acetate accumulation in this reaction system were the lowest of all those examined based on initial rates (Table 2).

Initial reaction rates for the various reaction systems employing 2-propanol as alcohol cosubstrate generally followed the same trends observed for those with propanol (Table 2). The only exceptions were that comparatively lower rates were observed with systems employing isopropyl acetate as alcohol cosubstrate. Isopropyl acetate is likely to be a less efficient substrate than propyl acetate, such that steady-state levels of nucleophile in reactions systems employing isopropyl acetate would be the lowest of all reaction systems evaluated.

Comparison of Reaction Selectivity between Propanol and 2-Propanol. Among the reaction systems employing FA and FAME as acyl donors with free alcohol cosubstrates, only the one employing FAME and propanol was different in acyl selectivity, and it was most selective for C_6 relative to the other reaction systems (Figures 1 and 2). The lack of a similar pattern of effect for the reactions of 2-propanol with FA and FAME makes it difficult to associate the basis for this effect on selectivity with either the alcohol or acyl group structure. In reaction systems employing the acetate ester forms of both alcohol cosubstrates, reactions with FAME were more selective for C_4 over those with FA acyl donors, reflecting a general shift toward short-chain-length FA.

An analysis for an overall shift in chain length selectivity was based on the calculation of reaction selectivity toward short-chain (C_{4-8}) FA as a group by taking the ratio of ($\Sigma\alpha_{C4-8}/\Sigma\alpha_{C4-16}$), with the balance of activity directed at long-chain (C_{10-16}) FA as a group [as in Arsan and Parkin (2000)]. Reactions with 2-propanol and isopropyl acetate were similar in that they exhibited reaction selectivity toward the short-chain group of acyl donors to degrees of 38−45% for FA and 42−45% for FAME reaction systems. On the other hand, reactions with propanol and propyl acetate exhibited reaction selectivity toward short-chain acyl donors to degrees of 43% for FA and 50−51% for FAME reaction systems. Thus, the distinction between reactions involving propanol and 2-propanol as alcohol cosubstrates was that with propanol a general shift *between* short and long acyl chain length selectivity was noted in some reaction configurations, whereas with 2-propanol more specific shifts in selectivity toward certain acyl donors *within* the short- or long-chain groups were observed.

The reactivity of branched-chain six-carbon FA was evaluated in esterification reactions with propanol and 2-propanol to probe for steric constraints. Both 2- and 3-methylpentanoic acid were insufficiently reactive (<1% conversion), and 4-methylpentanoic acid was 30% (with propanol) and 18% (with 2-propanol) as reactive as C_6 FA on the basis of α value determinations. Thus, steric constraints about the α- and *β*-carbons of FA substrates appear to prohibit reactivity.

Evaluation of Reactions with Other Alcohol Cosubstrates. Esterification reactions with hexanoic acid (C_6) as the sole acyl donor were most selective for *n*- and *iso*-alkyl primary four-carbon alcohols evaluated (Figure 3). Among all of the reactive alcohols tested, selectivity varied as much as 13-fold (on the basis of ratios of α values for any two alcohols) for reactions with C_6 FA. Only trace reactivity was noted for the secondary alcohols [phenol, cyclohexanol, and (−)menthol], yielding corresponding α values of ≈0.0 (not shown). Although *C. antarctica* B lipase is a non-regiospecific lipase (Anderson et al., 1998) and is reactive with secondary alcohols (Heldt-Hansen et al., 1989; Ohtani et al., 1998; Rotticci et al., 1998), reaction rates with primary alcohols are markedly faster (de Goede et al., 1994; From et al., 1997; Chang et al., 1999), and the time frame of reactions in our studies did not permit sufficient reactivity with secondary alcohols to be observed for the analysis of selectivity. As alkyl chain length was extended, a minimum in reactivity was noted for *n*-hexyl alcohol, whereas "bulkier" alcohols, such as isobutyl and benzyl alcohols, were better substrates by comparison. However, steric constraints to reactivity of

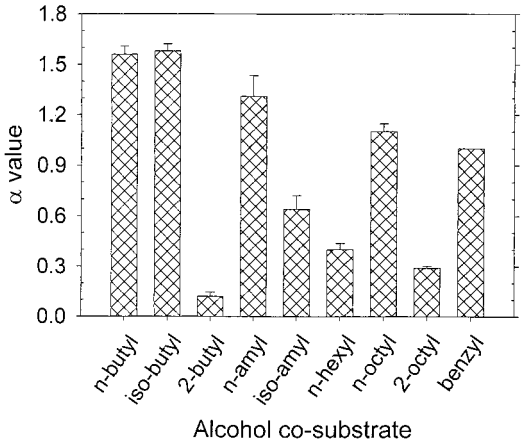

Figure 3. Relative α values for different alcohol cosubstrates in esterification reactions with hexanoic acid. Reactions transformed 20−80% acyl donor substrates over 60 min.

alcohols appeared to exist for methyl branching, especially at the α- and *γ*-carbons (compare *n*-butanol/2-butanol and *n*-amyl/isoamyl alcohols, respectively), but not at the *β*-carbon (compare *n*-butanol/isobutanol).

A previous study on esterification reaction selectivity of *C. antarctica* B lipase toward *n*-aliphatic primary alcohols with C_{12} FA cosubstrate noted that *n*-hexyl alcohol was nearly as reactive as *n*-butyl and more reactive than *n*-octyl alcohols (Patkar et al., 1998). However, the difference in acyl donor (C_6 compared to C_{12} FA) used between the present and previous studies may be a basis for the differences in alcohol selectivity observed.

Conclusions. The specific cosubstrate system used to facilitate ester synthesis and modification processes with *C. antarctica* B lipase had an influence on acyl group selectivity. Both the degree of selectivity and the acyl chain length optima were dependent on whether FA or their FAME derivatives were used as acyl donors. The choice of free alcohols or their acetate ester derivatives as cosubstrates also had an impact on reaction selectivity, most likely attributable to alterations in steady-state nucelophile levels and acetate accumulation.

Although some changes in selectivity constants and acyl chain length optima with a change in cosubstrate system were limited in some cases (ratios of α values ≤1.36), in other cases the changes were sufficient (ratios of α values ≤1.72−2.60) to impact the ability to design and execute lipid biotransformations intended to yield structured or stereospecific acylglycerol products. The choice of cosubstrate system and an understanding its role in influencing reaction selectivity should be considered as another dimension of control for lipid modification processes.

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