Lipase-Catalyzed Hydrolysis: Effect of Alcohol Configuration on the Stereobias for 2-Methyloctanoic Acid

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The reactivities of 2-methyloctanoic acid esters of *rac*-2,3-isopropylidene glycerol and *rac*-glycidol have been examined with several commercial lipase preparations. The *S* configuration of the acid always reacted faster. The (*S*)-2,3-isopropylidene glycerol esters and (*R*)-glycidol esters reacted faster than did the esters of the enantiomeric alcohols. The effect of alcohol configuration on the stereobias of the lipase-catalyzed hydrolysis, however, was modest, which is consistent with a mechanism whereby the oxygenated alcohols enable binding of catalyst to substrate while offering only slight changes to the energetics of the catalytic step. The most promising candidate alcohols for resolving 2-methyloctanoic acid by hydrolysis appear to be *rac*-2,3-isopropylidene glycerol (*Candida rugosa* lipase) and (*S*)-glycidol (*Mucor miehei* lipase).

INTRODUCTION

A timely review has documented the increasing interest in using lipases (triacylglycerol hydrolases, EC 3.1.1.3) as catalysts for esterification and interesterification of fats, oils, and related compounds for both food and nonfood uses (Mukherjee, 1990). Examples include particularly those materials not readily prepared by conventional synthesis and having use as components of dietetic products, emulsifiers, cocoa butter substitutes, and wax esters (cosmetics). Additionally, lipases have been employed to produce selected flavor and fragrance esters under conditions that are very mild and thereby avoid the rearrangements to which terpenoids are sometimes subject (Gray et al., 1990; Langrand et al., 1988, 1990). Gathering fundamental information about lipase characteristics (positional selectivity, enhancement of stereochemical resolution, general rate enhancement, etc.) is critical to their industrial adoption. Pertinent to this are methods for resolving 2-methyl-substituted acids and their esters, compounds that are important naturally occurring flavor substances (Maarse and Visscher, 1987), but their reactivity with lipases is markedly reduced by the steric bulk of the substituent (Sonnet and Baillargeon, 1991).

Acyl-transfer reactions catalyzed by lipases are accelerated if the alcohol residue of the ester substrate is substituted in the β-position with an electron-withdrawing group (Zaks and Klibanov, 1988). Enol esters have been employed similarly with the additional benefit of irreversible reaction (Wang et al., 1988). The greater rate of reaction of such esters is inferred to derive from increased carbonyl polarization that favors the catalytic event, which is seen as the nucleophilic attack of a serine hydroxyl upon the carbonyl group.

Esters of 2,3-isopropylidene glycerol (solketal) and glycidol also can react faster than triglycerides of the same fatty acid (Sonnet and Antonian, 1988; Sonnet, 1991). Such alcohols are not expected to affect the carbonyl group directly and may therefore assist in the binding step of the catalytic mechanism or facilitate by additional noncompetitive associations with the catalyst. In any case, such alcohols may accelerate acyl transfer of an otherwise slowly reacting acid (such as an α- or β-branched acid) and, by virtue of alcohol configuration, may offer additional stereobias to affect resolution of that acid residue. The aim of this paper is to document these accelerations and to describe the stereobiases of reactions of 2-methyloctanoic acid esters as models to assess the effects of the configuration of the alcohols.

MATERIALS AND METHODS

The following enzyme preparations were employed (company, location, brand name, source, gift/purchase): Enzyme Development Co., New York, NY, Lipase R, *Candida rugosa*, purchase; Amano, Co., Troy, VA, GC-20, *Geotrichum candidum*, gift; Amano, Lipase P, *Pseudomonas* sp., gift; Tanabe Co., Marlborough, NJ, *Rhizopus delemar*, purchase; Gist-Brocades, Charlotte, NC, GB-S, *Mucor miehei*, gift; Amano, LPL, lipoprotein lipase, gift; Sigma Co., St. Louis, MO, porcine pancreatic lipase, purchase. These materials were employed directly; their protein content varied from 1.25 to 7.78% (Sonnet and Baillargeon, 1991). All organic solvents were of HPLC grade. *rac*-2,3-Isopropylidene glycerol (IPG) and the *S*-enantiomer were synthesized according to known procedures (Eibl, 1981), and (*R*)- and (*S*)-glycidol were purchased from Aldrich Chemical Co., Milwaukee, WI, and used directly; each is known to be 94–96% configurationally pure. All fatty acids were purchased from Aldrich; α-alkylated acids were synthesized from the acid dianion prepared in tetrahydrofuran and hexamethylphosphoramide and the appropriate alkyl iodide or bromide (Pfeffer and Silbert, 1970). (*S*)-α-Phenylethylamine was purchased from Hexcel Corp., Zeeland, MI, and was 99.4% *S*. Esters of IPG and glycidol were prepared according to standard procedures and gave satisfactory spectral data.

Infrared data were obtained with a Perkin-Elmer Model 1310 spectrophotometer using 3% solutions in CCl_4. NMR spectra (^1H and ^{13}C) were obtained in $CDCl_3$ solution using a JEOL JNM-GX400FT-NMR spectrometer. Gas chromatography (GLC) was performed with a Chrompack Model 438A instrument using a SPB-1 capillary column (30 m × 0.25 mm i.d.) and helium carrier (50:1 split ratio). Free fatty acid titrations were performed with a Radiometer instrument comprised of an ABU-80 Autoburette module operated in "end point" or "pH stat" mode as appropriate.

Initial Rate Assay. Weighed amounts of commercial lipases were allowed to react in an emulsion created by brief (15 s) sonication of 1–2 mmol of substrate in 5.0 mL of 10% gum arabic. Enzyme solutions were prepared in diluted water; the conditions chosen were those that optimized activity on olive oil. Free fatty acid was measured by titration with 0.100 N NaOH using pH stat mode at pH 7.0. Amounts of enzyme and substrate were adjusted so as to allow a value for maximum initial velocity to be determined with each substrate. Reaction progress in each case did not exceed 2%. Initial rate assays provide reproducible data by which comparisons of enzymatic activity on a given substrate may be made.

Analysis of Stereochemistry. Reactions of 2-methyloctanoic esters were interrupted and worked up to separate acidic from

Table I. Initial Velocities of Lipase-Catalyzed Hydrolysis of Octanoic (O) and 2-Methyloctanoic (MO) Acid Esters Relative to That of Olive Oil[a]

	alcohol				
	CH_3CH_2OH	*rac*-IPG		*rac*-glycidol	
enzyme	O	O	MO	O	MO
C. rugosa	0.07	3.65	0.46	0.73	0.19
G. candidum	0.05	1.29	0.32	1.34	0.07
Pseudomonas sp.	0.13	1.69	0.02	14.7	0.47
R. delemar	0.15	1.14	0.10	8.8	1.77
M. miehei	0.17	0.79	0.16	6.2	0.65
LPL[b]	0.12	2.07	0.06	10.2	0.36
PPL[c]	0.13	0.88	0.23	9.6	0.02

[a] Actual values for olive oil [enzyme: μmol FFA, min^{-1}, (mg of powder)$^{-1}$] *C. rugosa*, 2.6; *G. candidum*; 4.1; *P.* sp., 6.4; *R. delemar*, 11.3; *M. miehei*, 11.0; LPL, 440; PPL, 0.56. [b] LPL, lipoprotein lipase. [c] PPL, porcine pancreatic lipase (see Materials and Methods).

neutral materials (cold 1.25 N NaOH). The organic acid was recovered by acidification of the aqueous phase (cold 2 N HCl) and extraction with ether. The acid was then converted to an amide via its acid chloride using (*S*)-α-phenylethylamine for GLC analysis of the resulting diastereomers (Sonnet, 1982). The unreacted ester was (1) reduced with lithium aluminum hydride, and (2) the resulting 2-methyl-1-octanol was washed free of IPG or glycidol, (3) oxidized to carboxylic acids with CrO_3 in acetone at 0–5 °C, and (4) similarly converted to diastereomeric amides.

RESULTS AND DISCUSSION

Initial velocity measurements were made for the lipase-catalyzed hydrolysis of esters of octanoic acid and 2-methyloctanoic acid with ethanol, *rac*-2,3-isopropylidene glycerol (IPG), and *rac*-glycidol using several commercial lipase preparations. The rates are compared to the rate of reaction of olive oil (Table I), a triglyceride that seems to be uniformly accepted as a good substrate by all of the triacylglycerol hydrolases. The slower rate of hydrolysis of esters of aliphatic alcohols, such as ethanol, has been observed routinely. By contrast, most of the lipases catalyzed faster reactions with the IPG octanoate than with ethyl octanoate. *C. rugosa* lipase accelerated the hydrolysis of IPG octanoate by a factor of 3.6 over olive oil, while reaction of the IPG ester of 2-methyloctanoic acid was elevated to nearly half the rate of olive oil. Many of the lipases evaluated produced notably faster reactions with the glycidic esters; glycidol octanoate reacted almost 15 times as fast as did triolein with lipase from *Pseudomonas*. Interestingly, rate enhancement for the unbranched acid ester was not necessarily parallel to that for the α-branched acid. Lipase of *R. delemar*, which enhanced the rate of hydrolysis of glycidol octanoate over olive oil by a factor of 8.8, also boosted reaction of the corresponding 2-methyl ester over olive oil by a factor of 2. In other words, it was possible to increase the reactivity of a sterically hindered acid residue over that of the unbranched acid by selecting a different alcohol. For comparison, the β,β,β-trifluoroethyl ester of 2-methyloctanoic acid was also exposed to lipases, and the rates of hydrolysis were quite similar to those of the glycidic esters (enzyme, trifluoroethyl ester/glycidic ester: Amano LPL, 1.0; GB-S 1.2, *R. delemar* 0.26; *Pseudomonas*, 0.83).

Table II. Reactivity of (*S*)-IPG and *rac*-2-Methyl Octanoate Ester with Several Lipases[a]

enzyme	eeP(*S*)[b]	eeS(*R*)[b]	*C*[c]	E_R[d]
C. rugosa	0.84	0.84	0.50	30
Pseudomonas sp.	0.86	0.31	0.26	22
	0.68	0.59	0.46	9.9
R. delemar	0.74	0.34	0.32	8.6
M. miehei	0.44	0.48	0.52	4.1
LPL	0.92	0.45	0.33	35
	0.54	0.70	0.57	6.5
PPL	0.88	0.40	0.32	18

[a] Reactions conducted at pH 7.0 (see Materials and Methods). [b] Enantiomeric excess (ee) = mol % of enantiomer 1 − mol % of enantiomer 2. [c] Fraction conversion (*C*) = ee (starting material)/[ee (starting material) + ee (product)]. [d] Enantiomeric ratio (E_R) = ln (1 − *C*)(1 − ee)/ln (1 − *C*)(1 + ee), where ee of starting material is used.

The (*S*)-IPG has been noted to react faster than its enantiomer in lipase-catalyzed hydrolysis (Ladner and Whitesides, 1984) and esterifications (Sonnet et al., 1986) and is readily available synthetically (Eibl, 1981). Reactions of the (*S*)-IPG ester of *rac*-2-methyloctanoic acid with several lipases are shown in Figure 1 and Table II. Evidently a high initial enantiomeric ratio, E_R, may be achieved, but the value one calculates declines during the course of the reaction. This phenomenon has been discussed as part of a probing treatment of the subject of biocatalytic resolution (Chen and Sih, 1989) and may be circumvented in several ways, one of which is to conduct the resolution as an esterification reaction instead. Unfortunately, those alcohols that facilitate hydrolysis including IPG and glycidol have not been useful as nucleophiles for the acylated enzyme, and thus the advantage of speed is lost. The lipase of *Pseudomonas* did catalyze esterification of 2-methyloctanoic acid with 1-propanol in hexane with (*S*)-acid reacting faster (E_R = 40) (Sonnet, 1991), but the reaction took several days. *C. rugosa* lipase, by contrast, catalyzed the hydrolysis of the (*S*)-IPG ester to 50% conversion within 6 h, producing enantiomeric excesses of 84% in product acid and recovered ester. The ester of *rac*-IPG was slightly slower in reaction (the relative rate for enantiomeric IPG esters of normal fatty acids is ca. 1–4), but the stereobias was essentially the same. Thus, one can perform a useful resolution of 2-methyloctanoic acid employing an IPG ester as the facilitator, with *C. rugosa* lipase the most useful catalyst of those evaluated. The stereobias (*C. rugosa*) was also high for the (*S*)-IPG esters of 2-methylhexanoic and 2-methyldecanoic acids, indicating some generality for the reaction, but discrimination between the fatty acid stereoisomers declined for the 2-methylbutanoic acid ester and was very low for the 2-ethyl- and 2-*n*-propyloctanoic acid esters. It should also be noted that studies have been reported in which acidolysis of 2-methyloctanoic acid esters has been used for attempted resolution (Engel, 1992).

Companion evaluations were conducted using glycidol esters of *rac*-2-methyloctanoic acid. Initial velocity mea-

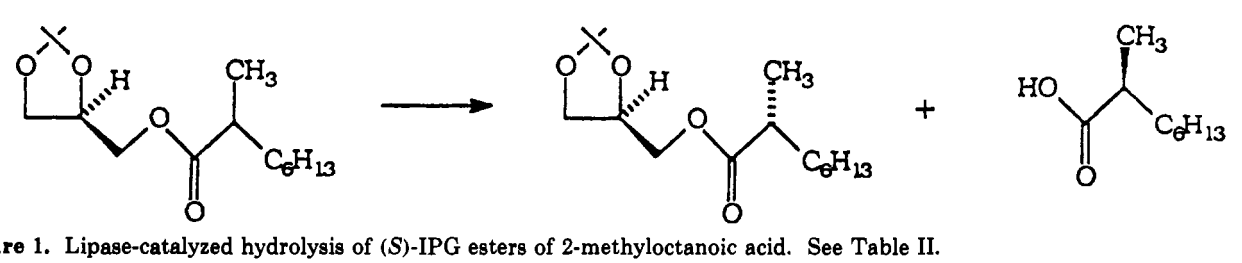

Figure 1. Lipase-catalyzed hydrolysis of (*S*)-IPG esters of 2-methyloctanoic acid. See Table II.

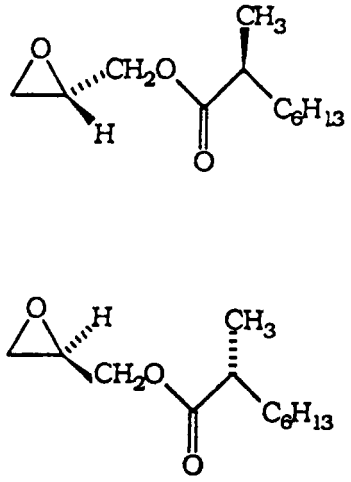

Figure 2. Fastest reacting stereoisomer in lipase-catalyzed hydrolysis of the (*R*)-and (*S*)-glycidol esters is the (*R*)-glycidol-(*S*)-acid stereoisomer (upper structure), while the enantiomeric (*S*, *R*) stereoisomer was slowest. See Table III.

Table III. Relative Reactivity of Stereoisomers of Glycidol 2-Methyloctanoate Esters with Several Lipases[a]

enzyme	*RS*	*RR*	*SS*	*SR*
C. rugosa	1.00	0.09	0.36	0.02
Pseudomonas sp.	1.00	0.14	0.22	0.03
R. delemar	1.00	0.10	0.16	<0.01
M. miehei	1.00	0.09	0.22	<0.01
LPL	1.00	0.13	0.09	<0.01

[a] See Figure 1. Reactions were conducted at pH 7.0 (see Materials and Methods). Designators are given for (*R*)- or (*S*)-glycidol first and for (*R*)- or (*S*)-2-methyloctanoic acid second. The designator for glycidol, rather than for its ester, is employed throughout to avoid the confusion that might result, since the convention for designating configuration for these alcohols results in a change upon esterification.

surements of the esters of (*R*)- and (*S*)-glycidol combined with measurements of enantiomeric ratios of each diastereomer pair at about 40–50% conversion allowed determination of the relative reactivity of each stereoisomer (Figure 2; Table III). For each lipase examined, the most reative stereoisomer was formed from (*R*)-glycidol and (*S*)-2-methyloctanoic acid. Indeed, (*R*)-glycidol esters are generally more susceptible to lipase-catalyzed hydrolysis (Ladner and Whitesides, 1984). In each diastereomer examined, the (*S*)-acid residue reacted faster, and the less reactive diastereomer [the pair formed from (*S*)-glycidol] was somewhat more stereoselective. The most promising combination for resolving 2-methyloctanoic acid involved *M. miehei* lipase-catalyzed hydrolysis of the (*S*)-glycidol ester (Table III, *SS*:*SR* = 22). Again, the effect of the alcohol configuration on the stereoselectivity of the reaction toward the acid residue was modest. This is consistent with a view that the alcohols enable the binding of catalyst to substrate perhaps through nonbonded interaction involving the ether oxygen(s), while offering only slight changes to the energetics of the catalytic step. As noted above with the IPG esters, glycidyl esters of octanoic acid substituted in the α-position with groups larger than methyl reacted with much lower enantiomeric ratios.

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