Solvent Polarity Influences Product Selectivity of Lipase-Mediated Esterification Reactions in Microaqueous Media

S.-J. Kuo and K.L. Parkin*

Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

ABSTRACT: Esterification reactions were evaluated by using lipases from *Rhizomucor miehei* (Lipozyme IM20) and *Pseudomonas cepacia* (PS-30) with equimolar levels (1.77 mmol) of undecanoic acid and glycerol or 1,3-propanediol (1,3-PD) or 1,2-propanediol (1,2-PD) in organic solvents of log P (partition coefficient between 1-octanol/water) values of (–0.33–4.5. Reaction yields (percentage of esterified undecanoate) with glycerol ranged from 1.4 to 72%, with greatest yields observed in solvents of log P 4.0–4.5 for Lipozyme, whereas the PS-30 lipase was similarly effective (27–38% yield) over the full range of solvent polarities. For both enzymes, as solvent apolarity increased, so did the extent of acylation of glycerol in the final product mixture. Reaction yields with 1,3-PD ranged from 8.1 to 64% for Lipozyme and from 18 to 84% for PS-30 lipase, with greatest yields observed for both enzymes in solvents of log P values in the range 1.2–5.0. For both lipases, the shift to greater solvent apolarity was accompanied by an increased molar ratio of diacylated-1,3-PD/monoacylated-1,3-PD in the product mixture. Reaction yields with 1,2-PD ranged from 2.5 to 45% for Lipozyme and from 12 to 52% for PS-30 lipase, with greatest yields observed in solvents of log P values in the ranges 1.4–1.9 and 1.4–4.5, respectively. The shift to greater solvent apolarity was accompanied by an increased molar ratio of diacylated-1,2-PD:monoacylated-1,2-PD in the product mixture, except for Lipozyme in the three most apolar solvents (log P of 3.5–4.5) in which there was a general attenuation of activity. These results suggest the existence of a solvent polarity influence on reaction product selectivity in multiproduct reactions, which can be partially explained on the basis of differential solvation and extraction properties of solvents.
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KEY WORDS: Esterification, glycerol, lipase, microaqueous media, multiproduct reactions, propanediols, reaction product selectivity, solvent polarity.

Reactions mediated by lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) in microaqueous media have been vigorously studied over the past decade (1–5). The most common foci of many efforts in this field include the choice of lipases and their native selectivity, the choice of reaction configurations as esterification, acyl-transfer (transesterification) or acyl-exchange (interesterification) processes, proper selection and control of water activity, and the choice of co-substrates among fatty acid, alcohol and ester derivatives (3–11). Perhaps one factor that has not received commensurate scrutiny over the last decade has been defining and/or refining the criteria that serve as the basis for solvent selection for microaqueous biocatalysis.

A rather dogmatic view has emerged concerning the selection of solvent for biocatalysis in organic media; this view is based on log *P* values (*P* is the partitioning coefficient between 1-octanol/water) of the solvent (12). Solvents of log *P* >4 are considered most suitable for biocatalysis, whereas those with log P values between 2 and 4 are moderately effective, and those with log *P* values <2 (the most polar solvents) are generally ineffective. Part of the basis for this general rule resides with the water-sorbing ability of the more polar solvents and the attendant competition with biocatalyst for water in the reaction mixture (12–15). For the most part, this general rule for solvent selection evolved from experiments in which apolar substrates were reacted to form products of even greater apolarity (12,14,16). Thus, the foundation for the original general rule of using log *P* values for solvent selection may be embedded in the compatibility of polarities between reaction products and the selected solvents. Recently, two studies have questioned the generality of the log *P* rule for solvent selection on the basis that neither solvent apolarity nor water-miscibility is specifically relevant to microaqueous biocatalysis (17), and that solvent selection should be done within the context of the nature (polarity) of the substrates and products of the desired reaction (18). Interestingly, the originators of the general log *P* rule suggested that proper juxtapositioning of polarities of substrate, product, solvent, and enzyme-solvent interphase was important to process optimization (12); however, this consideration has generally received little attention in studies in this area.

Interest in refining criteria for the proper selection of solvent for microaqueous biocatalysis was perhaps renewed by a theoretical analysis and prediction of reaction efficacy in various solvents, based on substrate and product partitioning, and the subsequent effect on equilibrium position of the reac-

*To whom correspondence should be addressed at the Department of Food Science, Babcock Hall, University of Wisconsin, 1605 Linden Drive, Madison, WI 53706.

tion(s) (5,19,20). The selective solvation power of solvents is a concept central to the approach of designing reaction media for extractive biocatalytic processes (21,22). As predicted, the equilibrium position or "practical equilibrium constant" of a lipase-mediated esterification reaction (yielding the apolar dodecyl dodecanoate) was inversely correlated with the solubility of water in the predominantly organic medium (14). Studies of solvent effects on lipase-mediated esterification reactions were extended to those involving free acids and glycerol and other alcohols, with the intent to evaluate the prediction of reaction equilibrium by computer modeling in two-phase systems, based on mass balances and the UNIFAC group contribution method (23–25). In these latter studies, organic solvents were used at molar levels similar to those of other reaction components, and only the organic phase was analyzed for acylglycerol esterification products. However, some clear trends were established in that there was an increasing proportion of more extensively acylated glycerol species accumulating in the organic phase at equilibrium conditions as the apolarity (log *P* values) of the added solvent was increased. Thus, it is apparent that the polarity of the reaction medium can influence the product profile or "reaction selectivity" in situations in which multiple products of differing polarities can be formed.

It was our intent to extend the studies of solvent effects on product distribution of esterification reactions with glycerol and glycerol analogues (diols) in reaction systems in which organic solvent is clearly the dominant and dispersing phase.

MATERIALS AND METHODS

Materials. Lipases were from *Pseudomonas cepacia* (type PS-30; Amano International Enzyme Co., Troy, VA) and *Rhizomucor miehei* (Lipozyme IM20, immobilized on an ion-exchange resin; Novo-Nordisk Bioindustrials, Inc., Danbury, CT). Solvents were high performance liquid chromatography (HPLC) grade (Aldrich Chemical Co., Milwaukee, WI), and all other reagents were obtained from Sigma Chemical Co. (St. Louis, MO). Water was deionized and distilled. Moisture contents of the PS-30 lipase, Lipozyme, glycerol, 1,2-propanediol (1,2-PD) and 1,3-propanediol (1,3-P) were 1.5, 10, 0.74, 0.80 and 0.74%, respectively, as determined by Karl Fischer titration. Solvents were dried with molecular sieves (3 Å; Sigma Chemical Co.) prior to use.

Reaction mixtures. Equimolar levels (1.77 mmol) of undecanoic acid and an alcohol acceptor (glycerol, 1,2-PD or 1,3-PD) were reacted in 5 vol of solvent. First, undecanoic acid was dissolved in solvent and tempered at a reaction temperature of 35°C for PS-30 lipase and 50°C for Lipozyme. The PS-30 lipase reaction mixtures also contained 12–24 µL exogenous water (~0.5–1.0%, vol/vol). Enzyme was added at 10% (w/w, based on amount of substrate added) and reactions were initiated by the addition of polyol substrate. Reaction mixtures were allowed to incubate with magnetic stirring for 12 h, and reactivity of the entire mixture was finally quenched by the addition of 5 vol chloroform and immediate filtration over a 0.25-µm nylon membrane to remove particulate enzyme. The different temperatures used for these lipases were based on our preliminary studies and those reported earlier (7) which indicated the (near)-optimum temperatures for acyl group modification reactions in microaqueous media.

Reaction product analysis. The extent of reaction was measured by either analysis of esterified undecanoic acid by gas–liquid chromatography (GLC) and/or acylated glycerol, 1,2-PD and 1,3-PD species by HPLC by methods previously used (10,26). Esterified undecanoate was analyzed as its methyl ester derivative after treatment of the product mixture in anhydrous methanolic 0.5 N KOH for 15 min at 55°C. GLC (model GC-9AM with Chromatopac C-R3A; Shimadzu Scientific Instruments Co., Columbia, MD) analysis was achieved with a 10% SP-2330 (100/200 mesh) Chromosorb WAW column (Supelco Inc., Bellefonte, PA) and flame detection.

Analysis for differentially acylated product species was achieved with an HPLC designed for separating mono-, di-, and triacylglycerol species (MAG, DAG, and TAG, respectively) and fatty acid (26). This method required a normal silica phase column (Econosil, 250 mm × 4.6 mm, 5 µm; Alltech Associates, Deerfield, IL), and gradient elution with hexane/chloroform/acetic acid (60:40:0.2, vol/vol/vol) and hexane/chloroform/acetone (5:13:7, vol/vol/vol) solvent systems. We used model 510 pumps and Baseline 810 software (Waters Associates, New Milford, CT) with a light-scattering detector (model ELSD IIA; Varex, Rockville, MD). Results of product species distribution analysis are reported in terms of mol fractions (as %), only when all possible product species in the reaction mixture could be quantitated. Limitations that prevented this analysis in some experiments existed when at least one product was present below a threshold level for accurate quantitation, a condition sometimes dictated by a general lack of reactivity in some reaction mixtures. The results presented are representative of 2–3 individual trials in which the coefficient of variation was <10–15%.

Log P values of solvents, substrates, and products. If available, literature sources were used to assign log *P* values (12,14,23,27). In the absence of such information, log *P* values were calculated on the basis of the fragmental constant approach (28) without any attempt to correct for proximity effects of functional groups. Solvents (and their corresponding log *P* values) used for this study were acetonitrile (–0.33), acetone (–0.23), tetrahydrofuran (0.49), *t*-butanol (0.80), *t*-butyl-methyl ether (1.4), diisopropyl ether (1.9), hexane (3.5), heptane (4.0), and isooctane (4.5). Published or calculated log *P* values for reactants and products appear in Table 1.

RESULTS AND DISCUSSION

Esterification of undecanoic acid and glycerol. The extent of Lipozyme-mediated acylation of glycerol increased with increasing apolarity of the solvent used as dispersing phase (Fig. 1). The greatest reaction yield of 72% undecanoate esterification was recorded in isooctane, and this trend in reac-

TABLE 1
Log *P* Values of Substrates and Products for Reaction Mixtures[a]

Substrate		Product	
Glycerol	−1.6	Monoundecanoylglycerol	2.5
1,2-Propanediol	−1.3	Diundecanoylglycerol	8.2
1,3-Propanediol	−1.3	Triundecanoylglycerol	13.7
Undecanoic acid	4.5	Monoundecanoylpropanediol	4.3
		Diundecanoylpropanediol	9.9

[a]Log *P* values for all alcohol substrates were taken from Reference 27. Log *P* values for all other components were calculated as described in the Materials and Methods section.

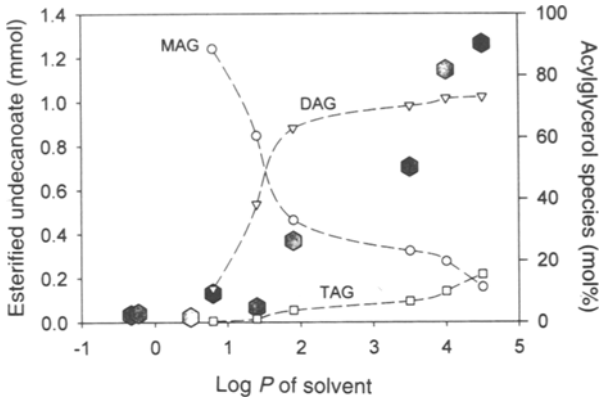

FIG. 1. Effect of solvent log *P* value on esterification reactions with undecanoic acid and glycerol as mediated by Lipozyme. Hexagonal symbols represent mmol esterified undecanoate (1.77 mmol total in reaction mixture), where gray symbols indicate that dual-liquid phases existed, and the black symbol indicates that a single-liquid phase existed. Small open symbols represent the mol% of each acylglycerol species present (monoacylglycerol = MAG, diacylglycerol = DAG, and triacylglycerol = TAG).

tivity conformed to the general rule for solvent suitability in supporting microaqueous biocatalysis (12,14). It was difficult to conclude what effect, if any, phase behavior had in these reaction mixtures, because only *t*-butanol promoted a single- liquid phase. Addition of exogenous water (at ~0.5%) in reaction mixtures containing polar solvents (log $P < 2$) had little influence on the relationship between esterification activity and solvent log *P* value (data not shown), indicating that activity was probably not attenuated in the more polar solvent systems because of stripping of moisture from the enzyme. Another profound effect of solvent was that the more polar solvents favored partially acylated glycerol species as products, whereas the more apolar solvents favored a more complete acylation of glycerol. One interpretation of this trend is that the favored product species are those with polarities similar to that of the dispersing phase and is related to the selective solvation power of the solvent. On the other hand, the general restriction in enzyme activity in the more polar solvents may also be accountable for the accumulation of MAG species as products, as predicted from the reaction stoichiometry of moles esterified undecanoate:total glycerol of 1:26 to 1:74 in solvents of log $P \leq 1.4$.

The PS-30 lipase was a similarly effective mediator of esterification reactions in solvents that encompassed nearly the full range of log *P* values used in this study (Fig. 2). However, to express enzyme activity in two of the more polar solvents (acetone and tetrahydrofuran), supplementation of the reaction medium with an additional ~0.5% water was necessary, and this can be explained in terms of counteracting the water-stripping tendency of these solvents (15). Specific solvent effects on enzyme activity may account for the inability to express enzyme activity in acetonitrile and *t*-butanol, even when supplemental moisture was added. In any case, for the PS-30 lipase, similar extents of undecanoate esterification (27–38%) were achieved in solvents that ranged in log *P* valve from –0.23 to 4.5, allowing for a more valid comparison of any reaction product selectivity exhibited among these solvents than was possible for the Lipozyme-mediated reaction (Fig. 1). Again, the most profound effect of solvent on PS-30 lipase activity was that the more polar solvents favored accumulation of the more polar products (MAG), whereas the more apolar solvents favored the accumulation of the more apolar products (DAG and then, TAG) (Fig. 2). Thus, it was evident that reaction product distribution or selectivity was related to polarity of the solvent used as the dispersing phase. Even though the stoichiometry of the initial reactants was 1:1, after a 12-h incubation, the molar ratio of reacted undecanoic acid:reacted glycerol ranged from 1.09:1 in acetone to 2.20:1 in isooctane. This difference in product profile was achieved despite a corresponding and similar reaction stoichiometry of esterified undecanoate:total glycerol of 1:2.62 and 1:3.21, respectively.

The ability to form TAG in these reaction mixtures is probably attributable to acyl-migration, especially for Lipozyme, which shows rather strict *sn*-1,3-specificity (29). Acyl-migration may be enzyme-mediated in microaqueous media (30) and has been held accountable for the synthesis of TAG by esterification reactions with Lipozyme (31).

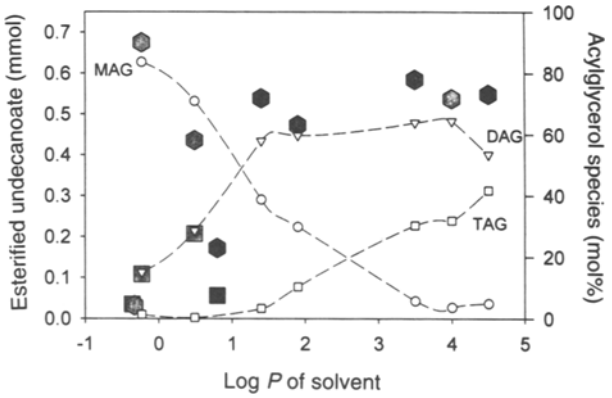

FIG. 2. Effect of solvent log *P* value on esterification reactions with undecanoic acid and glycerol as mediated by PS-30 lipase. Large gray and black symbols represent mmol esterified undecanoate (1.77 mmol total in reaction mixture) where dual- and single-liquid phases existed, respectively. Water added to reaction mixture was ~0.5% (vol/vol) for hexagonal symbols at log *P* values >1 and for closed square symbols. Water added to reaction mixture was ~1% (vol/vol) for hexagonal symbols at log *P* values <1. The rest of the legend is the same as for Fig. 1, and mol% MAG, DAG, and TAG are for conditions represented by the hexagonal symbols.

Esterification of undecanoic acid and 1,3-propanediol (1,3-PD). Solvents of log *P* value >1 were similarly capable of supporting Lipozyme-mediated esterification reactions with 1,3-PD as alcohol acceptor (Fig. 3). Reactivity appeared to be independent of phase behavior of the reaction mixtures, and supplemental moisture did not improve reactivity in solvents with log *P* values <1. Generally, there was an increase in proportion of the more apolar, diacylated(DA)-1,3-PD product, compared with the more polar monoacylated(MA)-1,3-PD product formed as solvent apolarity (log *P* value) increased. This was especially apparent in solvents of log $P > 1$ in which similar extents of reaction took place, indicating that reaction product selectivity was at least partially controlled by solvent polarity.

Results similar to those with Lipozyme were obtained when the reaction was mediated by the PS-30 lipase (Fig. 4), except that the latter lipase was more capable of reaction over the full range of solvents/polarities evaluated. Again, phase behavior appeared to have little impact on reaction efficacy, and the most profound solvent effect was that, as solvent apolarity increased, so did the proportion of the more apolar product (DA-1,3-PD) in the reaction mixture.

Esterification of undecanoic acid and 1,2-propanediol (1,2-PD). Esterification reactions, mediated by Lipozyme when 1,2-PD was the alcohol acceptor, proceeded to the greatest extent in solvents of intermediate log *P* values of 1–2 (Fig. 5). The most supportive solvents in this reaction configuration promoted both single- and multi-liquid phases, indicating that phase behavior may not be the dominant factor to control reactivity. For the solvent systems encompassing the lower range of log *P* values (–0.33–1.9), as solvent apolarity increased, so did the proportion of the most apolar product species (DA-1,2-PD) yielded in the reaction mixture. The formation of DA-1,2-PD by Lipozyme can be explained, in part,

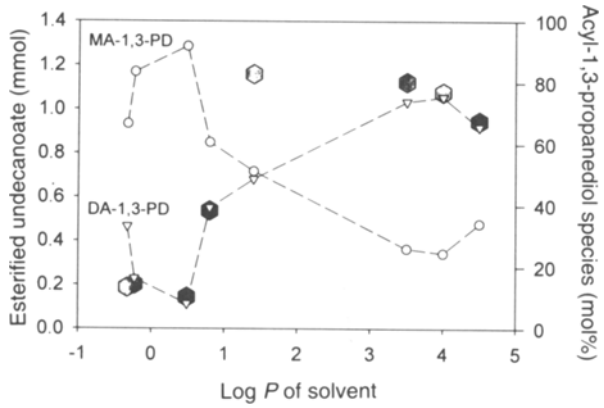

FIG. 3. Effect of solvent log *P* value on esterification reactions with undecanoic acid and 1,3-propanediol (1,3-PD) as mediated by Lipozyme. Hexagonal symbols represent mmol esterified undecanoate (1.77 mmol total in reaction mixture), where gray symbols indicate that dual-liquid phases existed, and the black symbols indicate that a single-liquid phase existed. Small open symbols represent the mol% of each acyl-1,3-PD species present (monoacyl-1,3-PD = MA-1,3-PD, diacyl-1,3-PD = DA-1,3-PD).

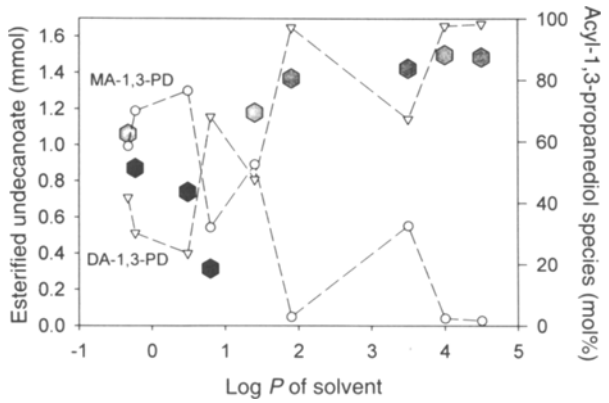

FIG. 4. Effect of solvent log *P* value on esterification reactions with undecanoic acid and 1,3-propanediol (1,3-PD) as mediated by PS-30 lipase. Legend is the same as for Figure 3.

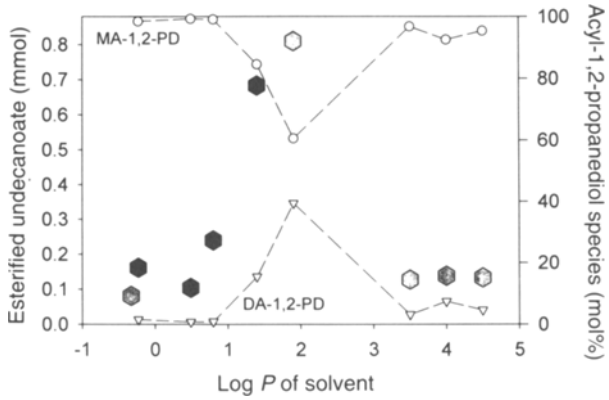

FIG. 5. Effect of solvent log *P* value on esterification reactions with undecanoic acid and 1,2-propanediol (1,2-PD) as mediated by Lipozyme. Legend is the same as for Figure 3, except that the small open symbols represent the mol% of each acyl-1,2-PD species present (monoacyl-1,2-PD = MA-1,2-PD, diacyl-1,2-PD = DA-1,2-PD).

by acyl-migration of the 1-acyl-PD species, allowing esterification of a second undecanoate residue (30,31). In the most apolar solvents used, this trend was not continued, and instead there was an increase in proportion of the more polar (MA-1,2-PD) product species in the reaction mixture. This apparent anomaly may be caused by the general restriction in enzyme activity because the ratio of esterified undecanoate:total glycerol ranged from 1:13 to 1:16, a stoichiometry that favors monoacylation in these apolar solvents.

The attenuation of reactivity in the three most apolar solvents can be accounted for by two factors. The first is the *sn*-1,3-regioselectivity of Lipozyme (7,29), which would restrict the direct enzymic production of DA-1,2-PD. The second is that the direct product of enzymic reaction, 1-acyl-PD species, is a relatively polar product, whose accumulation would not be easily accommodated by apolar solvents because of limited solvation capacity.

Reactivity of the PS-30 lipase with 1,2-PD as alcohol acceptor was best achieved in solvents of log P values >1, and phase behavior appeared to have little influence on reactivity (Fig. 6). The most profound effect of solvent was that a greater proportion of DA-1,2-PD was formed as solvent apolarity increased throughout the range of solvents evaluated. The formation of both MA- and DA-1,2-PD species by the PS-30 lipase can be attributed to a reported random selectivity, which allows direct esterification of the *sn*-2 site (32,33). However, acyl-migration from the *sn*-1 to *sn*-2 site may also be important in these reaction systems, because a recent report indicates a strong preference of PS-30 lipase for hydrolysis at *sn*-1,3 sites (34), and this selectivity may become more pronounced in microaqueous media where enzyme plasticity and reactivity with sterically hindered substrates (such as 2° alcohol residues) are often reduced (8,35,36).

General discussion. These results of lipase-mediated esterification processes involving glycerol and diol analogues show that the choice of solvent to serve as the dominant reaction medium had a profound effect on "reaction product selectivity." Second, depending on what degree of acylation of the polyols is desired, the most suitable solvent was not always the most apolar or characterized by a log *P* value of >2–4. Generally, the predominant product formed in the multiproduct reaction mixtures was the one at an end of the spectrum of polarity similar to that of the solvent used to conduct the reaction (see Table 1 for calculated log *P* values of reaction products). Monoacylated products were favored in polar media, whereas fully acylated products were favored in apolar media. The only exception noted was for the Lipozyme reaction with 1,2-PD in apolar media, in which *sn*-1,3-selectivity, coupled with a general restriction in enzyme reactivity (and possibly, acyl migration), may account for the relative lack of fully acylated products. In this case, overall reactivity was optimum in solvents of intermediate log *P* values of 1–2, an observation clearly not in accord with the general rule for solvent selection (12–16), and one we have recorded previously with other reaction systems (18).

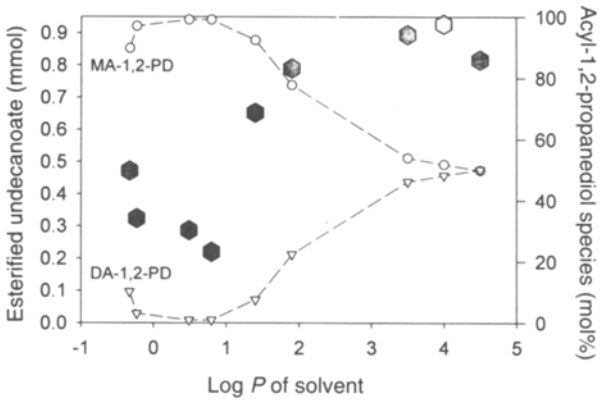

FIG. 6. Effect of solvent log *P* value on esterification reactions with undecanoic acid and 1,2-propanediol (1,2-PD) as mediated by PS-30 lipase. Legend is the same as for Figure 5.

To the extent that previous studies are relevant, our results with glycerol are consistent with earlier findings. Previous studies (23–25) on esterification reactions of decanoic acid and glycerol also found that MAG was the most favored product in the presence of polar organic solvents, whereas DAG and TAG product species were more favored in the presence of apolar organic solvents. What distinguishes our studies from these previous ones is that different biocatalysts were used [(*Candida rugosa* and *Chromobacterium viscosum* lipases in (23–25)], and more importantly, we used an excess of solvent to exploit any influence of solvent on reaction product selectivity more fully, whereas the former studies used limited quantities (roughly equimolar to substrates) of solvent. In addition, the former studies analyzed the acylglycerol components in the separated organic phase after reaction equilibrium, whereas we measured these same components in the entire reaction mixture after the reaction was terminated.

There has been even less previous work on propanediols for esterification reactions as influenced by solvent selection. Our results are consistent with the findings regarding esterification of decanoic acid with 1,3-PD in that as solvent apolarity increased, so did the molar ratio of DA-1,3-PD/MA-1,3-PD (23). Solvents over a range of log *P* values of 0.85–3.5 were similarly supportive of Lipozyme esterification of ethylene glycol and vinyl laurate, but no details were provided on co-substrate ratios and the distribution of products as mono- and diesters (37). Esterification of eicosapentaenoic acid and 1,2-PD (1:4.5 molar ratio), mediated by Lipozyme, yielded more monoester product in a more polar 1:9 *t*-butanol:hexane mixture (calculated log *P* of 3.23) than in hexane alone (log *P* of 3.5) (11). In contrast, another study reported that the esterification yield of stearic acid as propylene glycol monostearate with Lipozyme was maximum at about 60–70% in solvents of log *P* values ranging from 2.9 to 4.5 (over the range of 2.0–4.5) (38). However, the stearic acid:1,2-PD molar ratio used (38) was 1:10, a stoichiometry favoring the formation of the monoester product more so than in the comparative study (11). Furthermore, when these same authors (38) evaluated three model TAG as substrates for acyl-transfer reactions at an acyl group:1,2-PD molar ratio of 1:3.33, whenever more than 10% acyl-transfer occurred, a greater mol% of diester was accumulated in hexane (log *P* of 3.5) than in the more polar solvent, toluene (log *P* of 2.5).

The progress and selective nature of product accumulation in these esterification or acyl-transfer reactions in which multiple products can be formed can be explained in terms of solvation and extraction effects of solvent, especially in dual liquid phase media (19,21,22). In polar solvents, as polar products are formed, they are easily solvated by the solvent, which serves as a repository for the polar product. In contrast, in apolar solvents, the initial formation of a polar product would not be easily sorbed by the solvent, and the product would be subject to further reaction (acylation), if permitted, until a product of considerable apolarity was formed (such as DAG or TAG) that was easily dissolved or extracted by the dispersing solvent. Thus, the net effect of solvent is to affect selec-

tively the equilibrium of one of the competing steps in a manner that drives the reaction to the formation of one of many possible products.

One factor important to microaqueous biocatalysis in general is phase behavior of reaction media, where it is often considered that single-liquid phases (*viz.*, homogeneous catalysis) are generally more benign to enzymes (because of less interfacial inactivation) and pose less restraints on mass-transfer or diffusional processes than do multiple-liquid phase systems (heterogeneous catalysis) (2,39). We found little evidence to suggest that either single- or multiple-liquid phase mixtures were more efficient for the lipase reactions studied here. This may be founded on the well-established property of lipases to act efficiently (exclusively?) at interfaces, making the need for single-liquid phases less obvious when lipases are used. In fact, one could argue that multiple-liquid phase systems may add another dimension to reaction control by taking advantage of differences in selective solvation and extraction power of the multiple phases. However, one factor that was important for promoting activity in some of the two-phase mixtures was the order of addition of substrates. When polyol substrate was the last reactant added, clumping and an attendant reduction in enzyme activity were minimized, an observation made in a previous study (40). This consideration was particularly important in reproducing the behavior of PS-30 lipase in reaction with 1,2-PD (Fig. 6). When the enzyme or fatty acid substrate was the last reagent added, PS-30 lipase behaved like Lipozyme (Fig. 5), presumably because of enzyme aggregation and a general restriction in activity, in the most apolar solvents in which two-phase mixtures resulted.

Another set of observations from this study may reflect the nature of enzyme-substrate recognition and selectivity. For Lipozyme, the greatest reactivity, as measured by yield of esterified undecanoate, was recorded with glycerol (72%) and 1,3-PD (66%), whereas 1,2-PD was less reactive (45% yield). This appears to reflect the rather strict *sn*-1,3-regioselectivity of this enzyme (29). However, the presence of the *sn*-2 alcohol functional group does not necessarily impair activity (*cf.*, glycerol and 1,3-PD) as long as both primary alcohol/ester groups are present (cf., glycerol and 1,2-PD). For the PS-30 lipase, the greatest reaction yield was observed for 1,3-PD (84%), whereas both other alcohol acceptors were less reactive (glycerol, 38%; 1,2-PD, 62%). Even though PS-30 lipase is generally regarded as exhibiting random specificity, the preference or selectivity of this enzyme for the primary sites (34) may be reflected by our finding that it had greater reactivity with the diol devoid of the *sn*-2 alcohol/ester group.

There was a greater tendency of Lipozyme to yield more fully acylated products in the most apolar media evaluated with glycerol than with 1,2-PD as the alcohol acceptor. Assuming that a major route of esterification at the *sn*-2 site is by acyl-migration from the *sn*-1(3) site, this may be explained by the greater opportunity for acyl migration to occur with glycerol (two primary sites) relative to 1,2-PD. We do not expect that differences in electron withdrawing/donating properties between the $-CH_2OH$ and $-CH_3$ groups at the *sn*-3 site would sufficiently alter the nucleophilicity of the *sn*-2 oxygen to affect rates of acyl-migration. However, to our knowledge, no study has directly examined the comparative kinetics of acyl-migration among related polyols.

In conclusion, there may be advantages in revisiting the criteria used for solvent selection for biocatalysis on microaqueous media. Solvent selection may be fundamental to control of reaction selectivity in rather simple reaction systems. This dimension of control is not possible in traditional aqueous reactions, and solvent effects on reaction selectivity have been recognized as important for enantiomeric synthesis (20,41). Solvent selection may not always be best guided by the simple log $P > 2$–4 rule but should be viewed in the context of the progress and dynamics of the reaction, the nature (polarity) of the substrates, intermediates and terminal products, and which product is the target of synthesis. Other considerations, depending on the specific enzyme used, include phase behavior of the reaction medium as well as toxicity of solvent to the enzyme.

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REFERENCES

1. Klibanov, A.M., Enzymes That Work in Organic Solvents, *Chemtech. 16*:354–359 (1986).
2. Dordick, J.S., Enzymatic Catalysis in Monophasic Organic Solvents, *Enzyme Microb. Technol. 11*:194–211 (1989).
3. Macrae, A.R., Lipase-Catalyzed Interesterification of Oils and Fats, *J. Am. Oil Chem. Soc. 60*:291–294 (1991).
4. Vulfson, E.N., Enzymatic Synthesis of Food Ingredients in Low-Water Media, *Trends Food Sci. Technol. 4*:209–215 (1993).
5. Bell, G., P.J. Halling, B.D. Moore, J. Partridge, and D.G. Rees, Biocatalyst Behaviour in Low-Water Systems, *Trends Biotechnol. 13*:468–473 (1995).
6. Valivety, R.H., P.J. Halling, and A.R. Macrae, Reaction Rate with Suspended Lipase Catalyst Shows Similar Dependence on Water Activity in Different Organic Solvents, *Biochim. Biophys. Acta 1118*:218–222 (1992).
7. Li, Z.-Y., and O.P. Ward, Lipase-Catalyzed Esterification of Glycerol and n-3 Polyunsaturated Fatty Acid Concentrate in Organic Solvent, *J. Am. Oil Chem. Soc. 70*:745–748 (1993).
8. Santaniello, E., P. Ferraboschi, and P. Grisenti, Lipase-Catalyzed Transesterification in Organic Solvents: Applications to the Preparation of Enantiomerically Pure Compounds, *Enzyme Microb. Technol. 15*:367–382 (1993).
9. Kuo, S.-J., and K.L. Parkin, Substrate Preferences for Lipase-Mediated Acyl-Exchange Reactions with Butteroil are Concentration-Dependent, *J. Am. Oil Chem. Soc. 70*:393–399 (1993).
10. Kuo, S.-J., and K.L. Parkin, Acetylacylglycerol Formation by Lipase in Microaqueous Milieu: Effects of Acetyl Group Donor and Environmental Factors, *J. Agric. Food Chem. 43*:1775–1783 (1995).

11. Liu, K.-J., and J.-F. Shaw, Synthesis of Propylene Glycol Monoesters of Docosahexaenoic Acid and Eicosapentaenoic Acid by Lipase-Catalyzed Esterification in Organic Solvents, *J. Am. Oil Chem. Soc. 72*:1271–1274 (1995).
12. Laane, C., S. Boeren, K. Vos, and C. Veeger, Rules for Optimization of Biocatalysis in Organic Solvents, *Biotechnol. Bioeng. 30*:81–87 (1987).
13. Zaks, A., and A.M. Klibanov, The Effect of Water on Enzyme Action in Organic Media, *J. Biol. Chem. 263*:8017–8021 (1988).
14. Valivety, R.H., G.A. Johnson, C.J. Suckling, and P.J. Halling, Solvent Effects on Biocatalysis in Organic Systems: Equilibrium Position and Rates of Lipase Catalyzed Esterification, *Biotechnol. Bioeng. 38*:1137–1143 (1991).
15. Gorman, L.A., and J.S. Dordick, Organic Solvents Strip Water Off Enzymes, *Ibid. 39*:392–397 (1992).
16. Brink, L.E.S., and J. Tramper, Optimization of Organic Solvent in Multiphase Biocatalysis, *Ibid. 27*:1258–1269 (1985).
17. Narayan, V.S., and A.M. Klibanov, Are Water-immiscibility and Apolarity of the Solvent Relevant to Enzyme Efficiency? *Ibid. 41*:390–393 (1993).
18. Yang, B., S.-J. Kuo, P. Hariyadi, and K.L. Parkin, Solvent Suitability for Lipase-Mediated Acyl-Transfer and Esterification Reactions in Microaqueous Milieu Is Related to Substrate and Product Polarities, *Enzyme Microb. Technol. 16*:577–583 (1994).
19. Halling, P.J., Solvent Selection for Biocatalysis in Mainly Organic Systems: Predictions of Effects on Equilibrium Position, *Biotechnol. Bioeng. 35*:691–701 (1990).
20. Carrea, G., G. Ottolina, and S. Riva, Role of Solvents in the Control of Enzyme Selectivity in Organic Media, *Trends Biotechnol. 13*:63–70 (1995).
21. Eggers, D.K., H.W. Blanch, and J.M. Prausnitz, Extractive Catalysis: Solvent Effects on Equilibria of Enzymatic Reactions in Two-Phase Systems, *Enzyme Microb. Technol. 11*:84–89 (1989).
22. Bruce, L.J., and A.J. Daugulis, Solvent Selection Strategies for Extractive Biocatalysis, *Biotechnol. Prog. 7*:116–124 (1991).
23. Janssen, A.E.M., N.W. Boer, and K. Van't Riet, Solvent Effects on the Equilibrium Position of Lipase-Catalyzed Esterification of Decanoic Acid and Various Alcohols, *Biocatalysis 8*:133–153 (1993).
24. Janssen, A.E.M., A. Van der Padt, and K. Van't Riet, Solvent Effects on Lipase-Catalyzed Esterification of Glycerol and Fatty Acids, *Biotechnol. Bioeng. 42*:953–962 (1993).
25. Janssen, A.E.M., A. Van der Padt, H.M. Van Sonsbeek, and K. Van't Riet, The Effect of Organic Solvents on the Equilibrium Position of Acylglycerol Synthesis, *Ibid. 41*:95–103 (1993).
26. Yang, B., and J. Chen, Analysis of Neutral Lipids and Glycerolysis Products from Olive Oil by Liquid Chromatography, *J. Am. Oil Chem. Soc. 68*:980–982 (1991).
27. Reslow, M., P. Aldercreutz, and B. Mattiasson, Modification of the Microenvironment of Enzymes in Organic Solvents. Substitution of Water by Polar Solvents, *Biocatalysis 6*:307–318 (1992).
28. Rekker, R.F., and R. Mannhold, *Calculation of Drug Lipophilicity: The Hydrophobic Fragmental Constant Approach*, VCH, New York, 1992, p. 112.
29. Matori, M., T. Asahara, and Y. Ota, Positional Specificity of Microbial Lipases, *J. Ferment. Bioeng. 5*:397–398 (1991).
30. Heisler, A., C. Rabiller, and L. Hublin, Lipase Catalyzed Isomerization of 1,2-(2,3)-Diglyceride into 1,3-Diglyceride. The Crucial Role of Water, *Biotechnol. Lett. 13*:327–332 (1991).
31. Ergan, F., and Trani, M., Effect of Lipase Specificity on Triglyceride Synthesis, *Ibid. 13*:19–24 (1991).
32. Sonnet, P.E., T. Foglia, and S.H. Feairheller, Fatty Acid Selectivity of Lipases: Erucic Acid from Rapeseed Oil, *Ibid. 70*:387–391 (1993).
33. McNeill, G.P., and P.E. Sonnet, Isolation of Erucic Acid from Rapeseed Oil by Lipase-Catalyzed Hydrolysis, *Ibid. 72*:213–218 (1995).
34. Ota, Y., Y. Itabashi, and M. Hasuo, Measurement of Positional Specificity Index of Microbial Lipases by Chiral Phase High-Pressure Liquid Chromatography, *Biosci. Biotech. Biochem. 60*:145–146 (1996).
35. Zaks, A., and A.M. Klibanov, Enzymatic Catalysis in Organic Media at 100°C, *Science 224*:1249–1251 (1984).
36. Nishio, T., M. Kamimura, M. Murata, Y. Terao, and K. Achiwa, Enzymatic Transesterification with the Lipase from *Pseudomonas fragi* 22.39B in a Non-Aqueous Reaction System, *J. Biochem. 104*:681–682 (1988).
37. Berger, M., K. Laumen, and M.P. Schneider, Lipase-Catalyzed Esterification of Hydrophilic Diols in Organic Solvents, *Biotechnol. Lett. 14*:553–558 (1992).
38. Shaw, J.-F., and S. Lo, Production of Propylene Glycol Fatty Acid Monoesters by Lipase-Catalyzed Reactions in Organic Solvents, *J. Am. Oil Chem. Soc. 71*:715–719 (1994).
39. Schneider, L.V., A Three-Dimensional Solubility Parameter Approach to Nonaqueous Enzymology, *Biotechnol. Bioeng. 37*:627–638 (1991).
40. Stevenson, D.E., R.A. Stanley, and R.H. Furnaux, Glycerolysis of Tallow with Immobilized Lipase, *Biotechnol. Lett. 15*:1043–1048 (1993).
41. Westcott, C.R., and A.M. Klibanov, The Solvent Dependence of Enzyme Specificity, *Biochim. Biophys. Acta 1206*:1–9 (1994).

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