Functional dependency of structures of ionic liquids: do substituents govern the selectivity of enzymatic glycerolysis?

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The concept of regulating the preference of a reversible multi-step reaction by adjusting the substituents of ionic liquids (ILs) has been successfully exemplified with a group of tetraammonium-based ionic liquids as medium for the enzymatic glycerolysis. Simultaneous existence of long chain hydrophobic substituents and hydrophilic ethoxyl or hydroxyl moieties is found, respectively, to be essential for triglycerides (TG) dissolving and equilibrium shifting. The reactions in the ILs with cations consisting of long chain and free hydroxyl groups gave markedly higher conversion of TG and better preference to monoglyceride formation. Interestingly the predicted results from COSMO-RS (a quantum chemical model programme) achieved a good agreement with the experimental data, mapping out the specific solvation from the ILs as well as demonstrating the interaction between ILs, substrates and products being the intrinsic causes that govern reaction evolution and direct equilibrium shifting.

Introduction

As a new class of solvents, ionic liquids (ILs) possess many unique properties.[1] From the application point of view, the most attractive feature of ILs might be the tunablity of their properties to specific applications by selecting appropriate cations and anions.[2] With the judicious incorporation of some functional substituents, a broad array of so-called "task-specific ILs" could be obtained. This will definitely offer many new opportunities, and has already resulted in quite a number of interesting and promising applications.[3] However, the interplay of forces, which contributes to the properties of ILs, is so complicated that the introduction of functional groups further complicates the issue.[4] Therefore, varying the cation substituents to explore promising possibilities and understand the interaction of the moieties of ILs and solutes as well as the structural causes behind the functionalities constitutes a challenging and fascinating aspect of IL applications.[5]

Enzymatic selective esterification of polyhydric alcohols represents an important application in food and pharmaceutical processing and a promising alternative to poorly selective or impracticable chemical approaches.[6,7] Recently we have demonstrated an efficient and promising protocol for enzymatic glycerolysis of oils and fats.[7] This novel system employed a functionalized tetraammonium-based IL (CPMA·MS) to resolve the poor compatibility of glycerol and triglycerides (TG) and also to selectively shift the equilibrium for the production of monoglycerides (MG) to give a higher yield. To better understand the impact of IL-bound functional groups on the selectivity of glycerolysis and to evaluate the contributions of the appended functional groups to equilibrium shifting, herein we investigated the lipase-catalyzed glycerolysis of TG employing a group of tetraammonium-based ILs with varied anions and different cation substituents.

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Results and discussion

IL-structural dependency of reaction behaviours

In a previous publication, we assumed that the presence of an alkyl group promoted the dissolving of TG and the existence of a polyethoxyl moiety altered the reaction equilibrium.[7] To support and extend this assumption, and more importantly, to figure out to what extent these structural variations contribute to the selectivity alteration of glycerolysis, five types of tetraammonium-based ILs with varied anions and cation substituents (Ammoeng 100 named CPMA·MS in our previous work[7]) were used as a reaction medium for lipase-catalyzed glycerolysis of TG (Fig. 1).† The results of the effects of IL structure, together with chain-length of TG, substrate ratio (Gly/TG) and different enzymes, are depicted in Table 1.

With excess of glycerol (Entries 1–5), the results could be categorized into three groups: the reactions in Ammoeng 100 and 102 gave a high conversion of TG and a remarkably high yield of MG; in 111 and 112 lower conversion and different DG and MG proportions; and in 120 high TG conversion and moderately high MG. The reaction selectivity towards MG (denoted by MG/DG) is markedly high for 100 and 102 (33–38), and much lower for 112 and 120 (approx. 4) and further down to 1 for 111, which yielded almost the same amount of MG and DG. The reaction performance in Ammoeng 102 was just what was logically expected since Ammoeng 100 and 102 have similar molecular structures[7] (Fig. 1).

† All ILs were procured from Solvent Innovation Gmbh, Köln, Germany (http://www.solvent-innovation.com) and used as received. Novozym 435 (*Candida antarctica* B), Lipozyme RM IM (*Rhizomucor miehei*), Lipozyme TL IM (*Thermomyces lanuginosa*) were provided by Novozymes A(S (Bagsvaerd, Denmark); and PS-D (*Pseudomonas* sp.) and lipase AK (*Pseudomonas fluorescens*) from Amano Pharmaceutical Co. Ltd (Nagoya, Japan). High-Oleic Sonnenblumenöl 90plus with over 90% triolein content was purchased from Dr Frische GmbH (Alzenau, Germany) and glycerol, tributyrin and tricaprin were from (Sigma Chemical Co. St. Louis, MO) and of 99% purity.

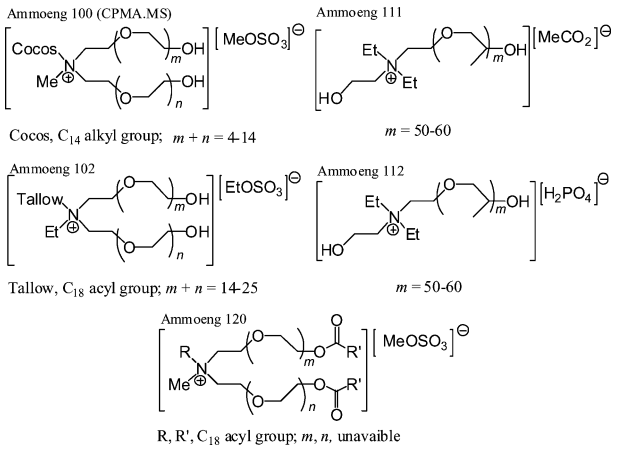

Fig. 1 Molecular structures of the tetraammonium-based ionic liquids used.

Applying this system to other TG profiles generated a similar high conversion and excellent yields of MG (Entries 1, 6 and 7), and the product selectivity varied depending on the acyl chain-lengths of TG (13–33), possibly resulting from lipase specificity to substrates and different interaction of substrates and products with ILs. In general among Entries 1, 6 and 7, no reaction yielded over 7% DG (data not shown). However, it is this small change of DG that leads to the significant change of selectivity value from 13 to 33 (MG/DG). The results demonstrate the universal applications of the protocol to different TG profiles, suggesting ILs are a predominant factor to control the reaction direction.

We tested different species of lipases in Ammoeng 102; no other lipases yielded over 10% conversion of TG after 24 h apart from Lipozyme RM IM (data not shown), which agrees with the previous observation using CAMP·MS as the medium.[7] Lipozyme RM IM gave 35.6% TG conversion of which 31.8% was MG (Entry 8). This reveals that Lipozyme RM IM exhibits a similar higher selectivity towards MG and the same shifting tendency of reaction equilibrium, which indicates a similar impact from this type of IL on different lipase-catalyzed reactions. However this does not naturally conclude that solvation can change the enzyme specificity.

The conclusion about this selectivity can be further demonstrated in an alternative way. In the test range of the ratio of Gly/TG from 5/1 to 1/2, the reaction remained at high-level conversion and yielded a higher percentage of MG. The reaction preference can be clearly evaluated with the Gly/TG ratio at 1 : 1, where the stoichiometric ratio should theoretically yield equimolar amounts of DG and MG. However, even at this ratio the yield of MG could still reach up to 80% and the selectivity up to 9. Moreover, applying excessive TG (Entry 11), a condition favouring the formation of DG, did not result in a higher yield of DG. In this entry more DG was degraded into MG and free fatty acid (14.7%), resulting in a higher yield of MG. Acyl transfer to the reactive hydroxyl group of Ammoeng 102 was also observed when less glycerol employed, however this will not lead to significantly different results or influence conclusions drawn. Anyway these findings suggested that there was a strong impetus in the system to push the reaction towards the formation of MG.

The relative content of DG (41%) and MG (24.6%) at a Gly/TG ratio of 1 : 1 in Ammoeng 120 (Entry 12) demonstrates that this type of IL was incapable of significantly steering the reaction direction, a higher MG yield was achieved even with excessive glycerol (Entry 5). Lower conversion of TG identifies the importance of the occurrence of a long chain hydrophobic moiety in the cation part of ILs (Entries 3, 4, 13 and 14), as suggested elsewhere,[7] which promotes the dissolution of TG (low solubility of TG in Ammoeng 111 and 112 was observed) as well as "immobilize" generated partial glycerides by van der Waal's interaction with long acyl chains. The selectivity difference between 111 and 112 was caused most likely from the differing contribution of their anion parts. In all, the results depicted in Table 1 clearly show that the selectivity of glycerolysis strongly depends on the molecular structures of ILs used, especially the functional substituents in the cation parts.

Table 1 Effects of the molecular structures of ILs as a reaction medium on the selectivity of lipase-catalyzed glycerolysis of triglycerides

Entry	Ionic liquid[a]	TG	Lipase	Gly/TG (mol/mol)	Conversion of TG (mol%)[b]	Yield of MG (mol%)[b]	MG/DG (mol/mol)[b]
1	100	Triolein	Novozym 435	5/1	99.07 ± 0.47	90.41 ± 1.76	33.21 ± 6.61
2	102	Triolein	Novozym 435	5/1	99.00 ± 0.49	90.25 ± 5.24	38.26 ± 6.62
3	111	Triolein	Novozym 435	5/1	42.18 ± 2.86	19.90 ± 2.45	1.02 ± 0.22
4	112	Triolein	Novozym 435	5/1	41.15 ± 0.47	31.75 ± 1.61	4.16 ± 0.29
5	120	Triolein	Novozym 435	5/1	96.61 ± 0.27	72.45 ± 1.64	3.99 ± 0.08
6	100	Tributyrin	Novozym 435	5/1	98.94 ± 0.25	93.40 ± 1.12	20.66 ± 1.37
7	100	Tricaprin	Novozym 435	5/1	98.02 ± 1.10	91.06 ± 1.21	13.09 ± 0.38
8	102	Triolein	Lipozyme RM IM (24 h)	5/1	35.55 ± 1.29	31.81 ± 0.09	8.96 ± 2.86
9	102	Triolein	Novozym 435	2/1	98.21 ± 0.35	84.41 ± 1.72	14.05 ± 1.23
10	102	Triolein	Novozym 435	1/1	97.86 ± 1.21	79.57 ± 3.92	9.00 ± 2.56
11	102	Triolein	Novozym 435	1/2	95.26 ± 0.54	70.69 ± 7.06	6.88 ± 0.23
12	120	Triolein	Novozym 435	1/1	80.49 ± 0.83	24.60 ± 1.52	0.60 ± 0.06
13	111	Triolein	Novozym 435	1/1	27.70 ± 0.16	11.46 ± 0.47	0.75 ± 0.05
14	112	Triolein	Novozym 435	1/1	47.28 ± 3.57	29.62 ± 1.59	1.80 ± 0.08

All runs were conducted employing 0.5 mmol triglyceride (TG), corresponding glycerol (Gly) with the desired Gly/TG ratio and 0.625 mmol IL (for Ammoeng 120 use 2 g) with lipase load of 100 mg with magnetic stirring at 600 rpm. All reactions were carried out at 60 ± 0.1 °C for 10 h (equilibria were essentially attained) unless indicated (24 h). Glycerol-free based area percentage was used as mass for yield and conversion calculation. TG, MG, DG and Gly represent triglyceride, monoglyceride, diglyceride and glycerol, respectively.[a] ILs 100–120 correspond to Ammoeng 100–120 in Fig. 1, respectively. [b] The values are mean ± standard deviation from duplicate determination ($P < 0.05$).

Structural reasons accounting for the specificity of reaction systems

Unlike other commonly used ILs, there exist substantial functional groups in the cations, *e.g.* hydroxyl, ether oxygen and long hydrocarbyl groups, and the cation part also represents the major mass portion of this type of ILs (Fig. 1). This might be the direct and dominant reason that governs the reaction evolution and product selectivity. Qualitatively, the occurrence of free hydroxyl, ethoxyl groups in Ammoeng 100 and 102 could serve as hydrogen bonding donors or acceptors, and the long chain alkyl or acyl groups, which have a matchable size to the acyl moiety of MG, would result in effective dispersion interactions. Both of these reasons will contribute to the "immobilization" of the generated MG and induce the reaction shifting towards the formation of MG.

There is no free hydroxyl group in Ammoeng 120 but the carboxyl oxygen can still contribute to hydrogen bonding. Therefore, Ammoeng 120 could somewhat reduce the activity coefficient of MG but not so significantly as Ammoeng 100 and 102 can do. Using the same hypothesis, it is natural to raise the question: why the existence of free hydroxyl and ether oxygen groups in Ammoeng 111 and 112 did not lead to higher yield of MG? A reasonable explanation could be that the ether oxygen group is embedded in an alkyl chain and partly blocked by the adjacent methyl group (Fig. 1). Furthermore the free hydroxyl group is also thermodynamically unfavourable for MG to access for H-bonding, because of steric hindrance and the high probability of the free hydroxyl group to bind substantially to ether oxygens of adjacent molecules. Likewise, this O-interrupted long alkyl chain structure will not facilitate the approaching of triglyceride molecules, which leads to a lower solubility of TG in Ammoeng 111 and 112 as experimentally observed.

Thermodynamic map revealing solvation of ILs and intermolecular actions in reaction systems

The dictum *like dissolves like* neatly sums up the multitude of factors that account for the ability of a molecular solvent to dissolve a given substrate. However, concisely summarizing the solvation behaviour of ILs is impossible, since ionic liquids are among the most complex solvents.[4] Recently a modified chemical quantum model—"Conductor-like Screening Model-for Real Solvents" (COSMO-RS) was developed as an efficient predicative method for the thermodynamic properties of solutes and solvents.[8] Based on quantum chemical calculation, COSMO-RS could present a unique 3D polarization density distribution function on the surface of a molecule X, denoting molecular interactions (so called σ-profile).[8] σ-Profiles give an interesting and detailed quantitative and qualitative description of polarity and H-bonding feature of solutes; and σ-potentials ($\mu(\sigma)$) provide a quantitative and integral description of solvent behaviour regarding electrostatics, H-bonding and hydrophilicity. Since these interactions constitute the dominant factors among solute-IL systems,[4] COSMO-RS was therefore adopted in this work to evaluate the results.

As reported elsewhere,[8] in σ-profiles the peaks' occurrence and area percentage (intensity) below −0.01 e Å^2 or greater than 0.01 e Å^{-2} represent the possibility and capacity of a molecule as a H-bonding donor or acceptor, respectively. As shown in Fig. 2a, for 1-monoolein the peaks around −0.017, 0.014 and 0.009 e Å^{-2} correspond to hydroxyl hydrogens and oxygens of

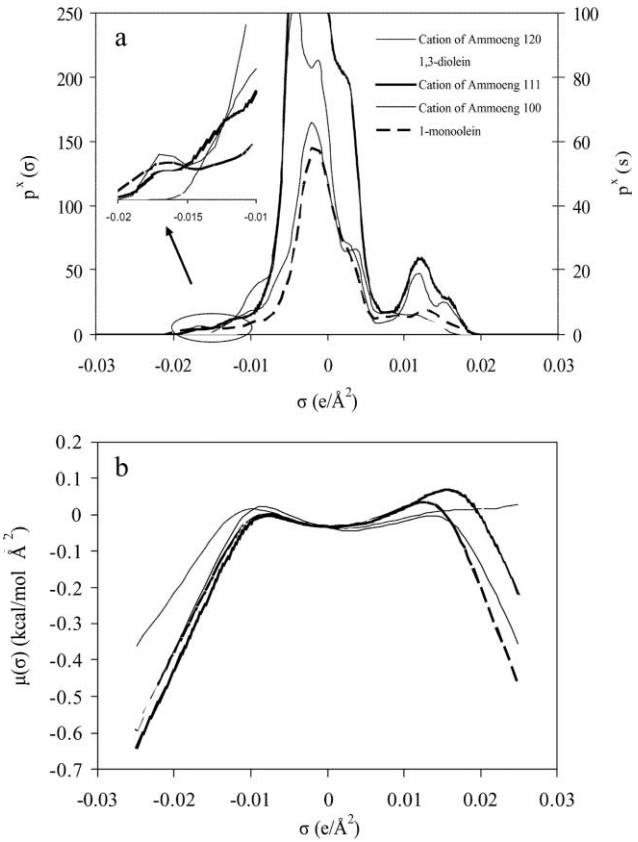

Fig. 2 (a) σ-Profiles of 1-monoolein, 1,3-diolein and cations of Ammoeng 100, 111 and 120; (b) σ-potentials of 1-monoolein, 1,3-diolein and cations of Ammoeng 100, 111 and 120 at 298.15 K. In Fig. 2a, σ-profiles of 1-monoolein and the cation of Ammoeng 100 are defined in the secondary axis.

glycerol backbone, as well as carbonyl oxygen, respectively. The peaks at −0.003 and 0.003 e Å^{-2} (around zero) from hydrocarbyl hydrogens and carbons indicate the occurrence of hydrophobic group. While 1,3-diolein gives relatively lower peaks below −0.01 or greater 0.01 e Å^{-2}, indicating a relatively lower amount of surface to act as a hydrogen-bonding donor or acceptor.

The σ-profile of the cation of CPMA·MS is more like 1-monoolein, having obvious peaks at −0.017, 0.013 and 0.016 corresponding to hydroxyl hydrogens, hydroxyl oxygens and polyethoxyl oxygens, respectively (Fig. 2a). The peaks at −0.005, −0.002 and 0.004 could be assigned to alkanyl hydrogen, alkyl carbons and polyethoxyl carbons. The σ-profile similarity of 1-monoolein and CPMA arises from the structural similarity: both having two free hydroxyl groups contributing to intermolecular hydrogen bonding and hydrophobic hydrocarbyl groups. This is also reflected in σ-potentials (Fig. 2b). The σ-potentials of 1-monoolein and CPMA are pretty similar and symmetric, of which the negative values around zero area indicated a thermodynamically favourable interaction with the hydrophobic surface. On both negative and positive sides the σ-potential quickly becomes strongly negative suggesting strong hydrogen bonding capacity as either donor or acceptor.

Contrarily 1,3-diolein gave positive σ-potential on negative and positive sides, suggesting that this is thermodynamically unfavourable as a hydrogen bonding donor and acceptor due

to steric hindrance from two acyl groups. For Ammoeng 120 the situation is different. On the negative side the σ-potential is comparably higher and very slowly become negative, suggesting that this is thermodynamically unfavourable as a H-bonding acceptor. On the positive side the σ-potential increases steadily, indicating no hydrogen bonding donor capacity. The similarity of the σ-profile and σ-potential of 1,3-diolein and cation of Ammoeng 120 accounts for the reaction preference to DG in Ammoeng 120 (Entry 12). The cation of Ammoeng 111, having a big peak at 0.013 e $Å^{-2}$, indicates that it can be a good H-bonding acceptor; while area percentage at −0.017 e $Å^{-2}$ is very small, suggesting it not a good H-bonding donor, which is also reflected as a higher σ-potential on the positive side (Fig. 2b).

The selectivity phenomenon in Table 1 can be further confirmed by the calculation of activity coefficients. As discussed elsewhere,[7] for a reversible reaction at a given temperature the equilibrium constant of activity coefficients ($K_{eq,\gamma}$) governs the shift of equilibrium and the reaction selectivity. For a specific reaction system, $K_{eq,\gamma}$ depends, to a large extent, on the solvent nature and the interaction of reactants, products and solvents. The COSMO-RS model allows one to calculate activity coefficients based on quantum chemical calculation. To quantitatively assess the specific contribution of ILs to equilibrium shifting, we calculated the infinite dilution ($\ln \gamma^{inf}$) of 1-monoolein, 1,3-diolein and triolein in CPMA.MS to be −0.154, 1.924 and 6.304, respectively. These data can help explain why the equilibrium strongly shifted to the formation of MG.

Obviously, the significantly high activity coefficient of TG will promote conversion of TG. The higher activity coefficient of 1,3-diolein and the considerable low activity coefficient of MG are likely also to move the reaction to MG production (Entries 1, 6 and 7). According to the COSMO-RS estimation, the $K_{eq,\gamma}$ of glycerolysis in *tert*-butyl alcohol (1.875×10^{-3}) (one of the most efficient normal solvents for glycerolysis) is 2.74 times bigger than $K_{eq,\gamma}$ in the CPMA.MS system (6.85×10^{-4}), which means that, theoretically, the equilibrium constant of mole fraction in CPMA.MS ($K_{eq,x}$) could be about 2.74 higher than in *tert*-butyl alcohol, corresponding to a higher yield of MG in CPMA·MS.

In Ammoeng 102 the situation is similar, while in Ammoeng 120, the infinite dilution activity coefficients ($\ln \gamma^{inf}$) of 1,3-diolein (0.576) and triolein (3.238) decreased, in accordance with the experimentally decreased conversion of TG and increased yield of DG (Entry 12). Interestingly Ammoeng 120 gave a comparable $K_{eq,\gamma}$ value (2.11×10^{-3}) to *tert*-butyl alcohol so that a similar reaction behaviour (reaction evolution, equilibrium and selectivity) was also observed (data not shown). Significantly low $\ln \gamma^{inf}$ values of triolein (0.6–0.8) and 1,3-diolein (around 0) in both Ammoeng 111 and 112, corresponding to their lower reactive activities, constitute possibly the leading reason for lower conversion of TG. The selectivity difference between Ammoeng 111 and 112 resulted more likely from contribution of the respective anions. As revealed by the COSMO-RS calculation, TG and DG in Ammoeng 112 have higher $\ln \gamma^{inf}$, suggesting that the effects from anions are not negligible and also a synergistic action of cations and anions. Furthermore, the similar TG conversions also support the assumption that the cation part plays an important role. In brief, the reaction behaviour of glycerolysis in this group of tetraammonium-based ILs, to a certain extent, depends on the structural specificities of the ILs. The high preference for the formation of MG results from their structural similarity of those ILs to MG as described by COSMO-RS qualitatively and quantitatively.

Conclusions

This work presents an interesting and vivid example of applying the concept of shifting a multi-step reversible reaction to the desired direction or to give improved product selectivity. This could be implemented by deliberately designing the reaction medium through judicious selection of IL cation substituents. A vivid picture representing function–structure relationships has been described by COSMO-RS qualitatively and quantitatively. This work also demonstrates structural characteristics of a promising group of ILs for MG production with industrial potential.[7] This study, accordingly, is believed to be helpful for the better understanding of intrinsic reasons of selectivity change associated with solvent structure. This work is also believed to be of instructive value for IL structure screening and optimization to create an efficient reaction system with desired product formation.

Experimental

Typical experimental procedure for glycerolysis

Lipase-catalyzed glycerolysis of TG and sample analysis were performed as before.[7] Typically, 0.5 mmol triolein and 5 × 0.5 mmol glycerol were mixed with 0.625 mmol Ammoeng 100, 102, 111 and 112 (for Ammoeng 120 use 2 g) in a 25 mL jacketed vial by magnetic stirring. The reaction was initiated by the addition of 100 mg Novozym 435 (*Candida antarctica* lipase B) and conducted at the desired temperature controlled by the circulated water. The evolution of the reaction was monitored by sample withdrawal and TLC-FID analysis (Iatroscan MK-6s, Bechenheim, Germany) after dissolving the sample in chloroform : methanol (2 : 1 v/v). All reactions were performed in duplicate. The developing solvents for TLC-FID consist of n-hexane, diethyl ether and acetic acid (45:25:2 v/v/v). Area percentage on a glycerol-free basis was used as weight for the calculation of conversion of oil and yield of glycerides.

Model processing

Generation of molecular COSMO files was implemented on Turbomole 5.8 and thermodynamic calculations were carried out on CosmothermX_2.1 (COSMO*logic* GmbH & Co KG, Leverkusen, Germany). Cavity radius (Å) used is the optimized data: C (2.00), H (1.30), O (1.72), N (1.83), S (2.16) and P (2.106).

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