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Green Chemistry

Paper

Using Imidazolium-Based Ionic Liquids as Dual Solvent-Catalysts for Sustainable Synthesis of Vitamin Esters: Inspiration from Bio- and Organo-Catalysis

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Vitamin E (VE) has significant biological activities and thus its acylation to increase its stability is of extreme interest. We developed an efficient and sustainabtle approach using imidazolium-based ionic liquids as dual solvent-catalysts for the esterification between α-tocopherol (the most active form of VE) and succinic anhydride. Although in literature it is reported that lipase can catalyze this reactIon, hereby we demonstrate that the reaction observed in DMSO and DMF is catalyzed by the histidyl residues of the protein. Histidine and analogous containing imidazole ring were tested as organocatalysts for the production of α-tocopherol succinate. In light of the imidazole organocatalysis, commercially-available 3-alkyl-1-methyl imidazolium ILs [C_nC_1Im][X^-] were investigated as dual solvent-catalysts for the esterification of α-tocopherol with succinic anhydride, and provided satisfactory yields and reaction rates. [C_5C_1Im][NO_3^-] can be recycled by water extraction, instead of organic solvents extraction to separate α-tocopherol succinate from [C_5C_1Im][NO_3^-], with an average yield of 94.1% for 4 subsequent batches, while the catalytic activity of the recycled ILs showed almost no loss after 4 batches. The developed protocol for synthesis of α-tocopherol esters and ILs recycle bears an industrial potential due to the ease of use and the efficient recycling.

Introduction

The development of sustainable synthesis of chemicals has strongly required owing to the continuously rising environmental concerns of conventional chemical approaches. The use of either biocatalysts or ionic liquids (ILs) as catalysts is widely deemed to be as the sustainable alternative way to achieve the concept of green chemistry[1]. For the cases of esters synthesis via direct esterification, several green approaches can be available both from the catalysts and green solvents, such as lipase/esterase-mediated catalysis[2], using functionalized ionic liquids as dual solvent-catalysts[1b, 1c]. Lipase/esterase-mediated esterification in non-aqueous systems possess many advantages compared to the acid/base catalysis[2], as the mild reaction conditions, high selectivity, creation of less waste, possibility of solvent-free system and reuse of biocatalyst via immobilization. On other hand, the obstacles of biocatalytic approaches especially the unsatisfactory stability of enzyme for industrial applications should be overcome[2a].

Room-temperature ionic liquids become alternative solvents and catalysts since from an environmental perspective they offers many advantages including negligible vapor pressure, designable properties, possibly simplified separation of products and potential reuse. The majority of ILs reported for esterification is imidazole or pyridine based derivatives. One typical and widely used family of this kind ILs is Brønsted acidic ionic liquids (BAILs); SO_3H-fuctionalized imidazolium-based ILs with acidic counter anion and protonated N-alkylimidazolium cation has been highlighted as dual solvent-catalysts with satisfactory conversion rates and selectivity for esterification[3], although the complicate preparation of BAILs may limit their industrial applications. The produced hydrophobic esters were immiscible with the hydrophilic ILs so that esters could be easily separated from ILs by decantation. However, considering the solubility of substrates in ILs, so far the synthesized esters were mainly the products of short/medium-chain alcohols with saturated aliphatic acid: ethyl acetate[3a], butyl acetate[4], glycerol triacetate[5] and methyl oleate [6]. Compared with ILs, most of these substrates investigated for esterification were of much lower viscosity[7].

Elegant works on esterification in ILs have been carried out because it was found that most ILs do not inactivate enzyme like polar organic solvents do[8]. In such system, ILs mostly served as the solvents while the enzyme acted as the catalysts so that structural-complex esters could be synthesized. For

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instance, a clean lipase-catalyzed process for producing flavor esters by direct esterification in switchable ILs/solid phases was described with almost 100% yield and the enzyme activity was practically unchanged during seven consecutive operation cycles[9]. However, to our knowledge, comparative study among biocatalysis and process using ILs as catalysts has not been carried out so far.

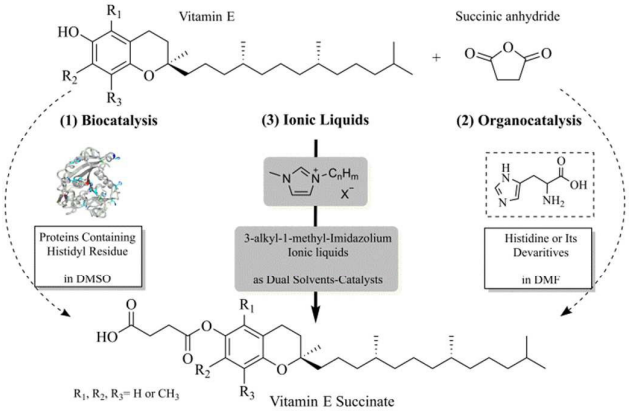
Vitamin E (VE) is a major natural antioxidant and an essential component of biological membranes. The term VE covers a group of 8 isoforms: α-, β-, γ-, δ-tocopherol and α-, β-, γ-, δ-tocotrienol. Among all these isoforms, α-tocopherol shows the highest VE activity[10]. However, VE is unstable and its antioxidant value is reduced by light, air or oxidizing chemicals. Aiming to improve the stability of VE, acetylation of VE was suggested[11]. The stable derivatives of VE, such as VE succinate, have the same biological activity as VE and highlighted as high-selective anticancer agents[10, 12], efficiently inducing the apoptosis of cancer cells via the mitochondrial route[13]. The hydrophobicity of VE due to the long alkyl chain leads to the immiscibility of VE in most hydrophilic BAILs, resulting in the low or even no conversion of VE esterification in such ILs. The esterification of vitamins is a topic that draws significant attention in the hydrolase community, due to the ability of lipases to catalyze the acylation in non-conventional media. Lipase-catalyzed synthesis of VE succinate in molecular solvents is significantly affected by the solvent due to the opposite polarity between VE and succinic anhydride: high yield was obtained in aprotic polar solvents like dimethyl sulfoxide (DMSO) and N,N-dimethyl formamide (DMF), but almost no reactions in conventional organic solvents [11, 14]. Until now, the catalytic activity in DMSO and DMF was thought by the contribution of the lipase, while any possible chemical acylation reaction was neglected and never reported in these studies although DMSO and DMF is believed with high denaturation capacity to the enzyme in most literature[15].

Herein, we firstly demonstrated whether the reaction observed in DMSO and DMF is contributed by the catalytic activity of lipase or not. We found that the undergoing cause for such reactions in DMSO is chemical catalysis of histidyl residue in protein, so that histidine and analogous containing imidazole ring were tested as organocatalysts for the production of α-tocopherol succinate. Bearing in mind of green chemistry, an efficient and sustainable approach using commercially-available 3-alkyl-1-methyl-imidazolium ionic liquids as dual solvent-catalysts was investigated. To enhance the green impact of the IL catalyzed reaction, we developed a water extraction protocol, instead of organic solvents extraction method, so that the IL can be reused. The reaction efficiency, final yield and product separation of these strategies (Scheme 1) were compared and the distinct mechanisms of histidine and imidazolium-based ILs catalysis were proposed.

Results and discussion

Plausible lipase-mediated synthesis of vitamin E succinate in DMSO

Over the past three decades, a remarkable number of works and publications concern lipase/esterase-mediated catalysis of transesterification or direct esterification since the essential paper[16] of Zaks and Klibanov on enzymatic processes in organic solvents. We initially studied the lipase-mediated esterification of α-tocopherol with fatty acids, aiming to improve α-tocopherol stability. For the esterification of α-tocopherol with succinic anhydride, solvents have profound effects on the reaction possibility and rates[17], due to the different polarity of these two substrates. When screening organic solvents, the reactions were highly solvent-dependent (Supporting Information, Table S1), as described in literature[14c]. Commonly applied organic solvents such as hexane, *tert*-amyl alcohol, *tert*-butanol and acetonitrile are not good media for these reactions whereas they were efficiently performed in DMSO and DMF.



Scheme 1. Three strategies (1) biocatalysis, (2) organocatalysis and (3) using ionic liquids as dual solvent-catalysts for the esterification of vitamin E with succinic anhydride.

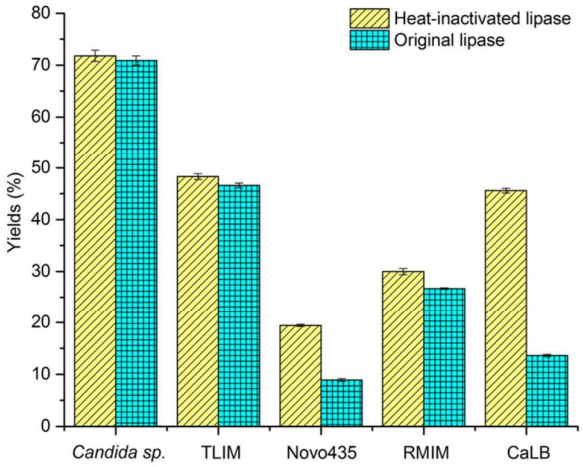


Fig. 1 Comparison of heat-inactivated (120 °C, 20 min) and original lipase catalyzing the esterification of α-tocopherol succinate in DMSO. TLIM: Lipozyme TLIM; RMIM: Lipozyme RMIM; CaLB: *Candida antarctica* Lipase B.

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Table 1 Yields of several proteins catalyzing the esterification of α-tocopherol succinate

Catalysts	Yields (%)	Percentage of histidine residue[a] (%)
Protamine	0	0.0
Pepsin	0	0.3
Glucose oxidase	61.9 ±0.5	2.7
Catalase	65.8 ±0.8	2.8
BSA	70.0 ±0.6	2.8

[a] the values were calculated from the sequence analysis in NCBI (http://www.ncbi.nlm.nih.gov/protein/).

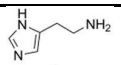
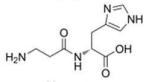
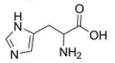
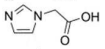
Over the last years growing efforts on the improvement of the enzymatic processes in anhydrous DMSO and DMF via protein engineering, immobilization and computational chemistry were documented. It was reported that the lipases' activities in neat DMSO can be maintained and even promoted through lipase modification[14a, 18] or immobilization[14b, 19]. However, DMSO and DMF, which are known as universal solvents with high denaturation capacity[15a], not only strip essential water molecules from the enzyme, but also dissociate the enzyme tertiary structure leading to enzyme unfolding and subsequent deactivation[15b, 15c]. This conventional wisdom still holds owing to our unexpected results: several heat-inactivated (120 °C, 20 min) lipases from different sources can perform the esterification of α-tocopherol with succinic anhydride in neat DMSO, as shown in Fig. 1. More interestingly, heat inactivated proteins and enzymes of other classes, like glucose oxidase and catalase can perform the reaction (Table 1). Surprisingly, some of the heat-denatured samples exhibited higher activity than the native enzymes. It is expected that the unfolding, as result of the heating process, renders the histidine residues of the protein core accessible, while in the folded protein only the histidine residues on the surface of enzyme were available for the catalysis.

Subsequently, we investigated the amino acid sequences and found that the activity of the heat inactivated protein was in line with the histidine content (Table 1); no reaction was observed for protamine, a protein without histidine residues. At the same time, enzymes that are not reported to catalyze esterification reactions, such as glucose oxidase, where quite efficient on this specific reaction, due to their histidine content. In summary, these results indicated that the underlying cause for esterification of α-tocopherol esters in DMSO is not a lipase-catalyzed reaction, but an organocatalysis.

Organosynthesis of vitamin E succinate

Enzymatic transformations generally have a chemical catalysis counterpart, but amino acids as the building blocks composing the protein, rarely fulfill their chemical counterpart roles. Typically, amino acids exhibit their catalytic potency after proper folding of the polypeptide chain, mostly due to the specific environment that the neighboring residues are producing. For instance, hydrolases perform their activities via a catalytic triad usually formed by serine, histidine and aspartate/glutamate residues[20]. The results of the previous paragraph gave an insight that the histidine can be an effective esterification catalyst for the synthesis of vitamin esters.

Table 2. Histidine derivatives/analogues tested as catalysts for esterification of α-tocopherol with succinic anhydride

Entry	Compound	Initial rate[a] (10^{-2}/min)	Yield after 24h[b] (%)
1		10.8 ±0.2	98.1 ±1.3 [c]
2		4.4 ±0.1	97.2 ±1.5 [c]
3		3.1 ±0.2	91.7 ±1.9 [c]
4		1.1 ±0.1	89.1 ±0.6
5		0.73 ±0.06	86.9 ±0.8
6[d]		0.20 ±0.02	45.7 ±0.6
7		0.25 ±0.03	45.3 ±0.4
8[d]		0.17 ±0.01	39.8 ±0.4
9[d]		0.11 ±0.01	28.5 ±0.3
10		n.d.[e]	n.d.[e]
11	Xanthine; Adenine, Thymine, Uracil, Guanine	n.d.[e]	n.d.[e]

[a] initial rate refers to moles of product formatted per moles of catalyst per minute; [b] Conditions: histidine derivatives (30 %, mol of α-tocopherol) in 1 mL DMF containing 0.2 M α-tocopherol and 0.8 M succinic anhydride was incubated at 800 rpm, 50 °C in dark and under nitrogen atmosphere for 24 h and yield was determined through HPLC analysis, using an external standard; [c] Yield at 9 h; [d] these catalysts are slightly soluble in DMF; [e] not detectable.

Reacting L-histidine (30 %, mol towards α-tocopherol) in 1 mL DMF containing 0.2 M α-tocopherol and 0.8 M succinic anhydride at 50 °C for 24 h led to 40% yield of α-tocopherol succinate (Table 2, entry **8**). This result was quite remarkable since histidine is slightly soluble in neat DMF (up to about 0.06 M histidine). The efficiency of histidine-catalyzed esterification in aprotic polar solvents followed the order: DMSO > DMF > dimethylacetamide > tetrahydro-1,3-dimethyl-2(1H)pyrimidine > 1,3-dimethyl-2- imidazolidinone. It needs to be stated that the medium of histidine-mediated esterification gradually became homogeneous in anhydrous DMSO, indicating a ring-open reaction of succinic anhydride with histidine. This was not observed in DMF, thus this solvent is expected to be more suitable, regarding the efficiency while minimizing the ring opening of the anhydride.

We also compared commercially available histidine analogues and derivatives under the standard conditions with 30 % mol (towards α-tocopherol) of catalyst (Table 2).

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Histamine (Entry **1**) exhibited high initial rate and yield; this could be due to the better solubility in DMF, owing to the absence of the carboxylic group. Although the final product (α-tocopherol succinate) was obtained and confirmed by NMR, we believed that exposure of histamine to succinic anhydride in DMF will form the terminal N-succinoyl derivative as intermediate. It was reported that succinoylhistamine was formed with a yield of 90% at room temperature within 1.5 h in DMF and a white precipitate formed[21]. However, in our cases, no white precipitate was observed at 50 °C, probably due to the further usage of the succinic moiety for the acylation. The possibly formed N-succinoyl derivative arise the question: what is the actual acylation catalyst? We assumed that both the imidazole ring and the amine group are essential for the efficient catalysis to occur.

Modification of side chains of the imidazole ring (Entries **1-5** and **7-10**) resulted in the change of catalyst solubility in the organic solvents, but also in its reactivity: the presence of a carboxylic group led to the decrease of solubility (Entry **6**, **8** and **9**), while the incorporation of a thiol group diminished any activity. Interestingly, neither purines nor pyrimidines (Entry **11**) exhibited any catalysis, although they possess a similar structure as imidazole ring. Different kinds of anhydrides and fatty acids were tested as acyl donor: only anhydrides can be activated in DMSO or DMF and thus act as the acyl donor, but none of fatty acids (data not shown). Reactions of vitamin A and vitamin C with succinic anhydride (Scheme S1) were further carried out to extend the applications of histidine-catalyzed esterification. Although that histidine was able to perform the esterification of both vitamins, no regioselectivity was observed in the case of vitamin C (Fig. S1).

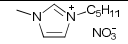
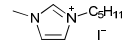
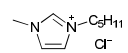
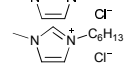
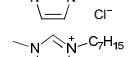
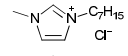
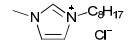
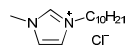
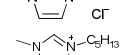
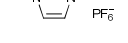
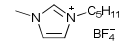
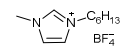
Although the use of histidine derivatives/analogues as catalysts for these acylation reactions brings several benefits related to their simplicity, low price and availability, the purification of the produced ester for organocatlysis is really difficult via an organic solvent extraction with from DMSO or DMF. The recycle of chemical catalysts is not easy but except for immobilization of the catalysts.

Imidazolium-based ionic liquids as dual solvent-catalysts for synthesis of vitamin E succinate

The application of ILs as both solvent and catalysts for the esterification of α-tocopherol with succinic anhydride become an alternative sustainable approach since enzymatic process are not available for these substrates as discussed before. Considering the importance of ILs properties *e.g.* dissolving ability, viscosity, etc. for the possibility and efficiency of α-tocopherol succinate synthesis, an appropriate choice of the anion and the cation of ILs is necessary. BAILs containing an alkane-sulfonic acid group attached to an imidazole or pyridinium cation and bearing acid counteranions are of special importance as the most used ILs for the esterification process because they possess simultaneously the proton acidity and the characteristic properties of an ionic liquid[3a, 22].

However, in light of organocatalysis using imidazole as catalyst, imidazolium based ILs without any modifications as shown in Table 3 were selected in our work to avoid the complicate preparation of functionalized BAILs. This simple ILs family was previously reported mostly as the solvents for enzymatic catalysis and already commercially available[1b, 23]. The high viscosity of ILs limiting the mass transfer rate would be the first challenge to the use of the ILs during the synthesis of α-tocopherol succinate; the resulted ester with a free carboxylic group due to the use of anhydride may become miscible with ILs, which would increase the difficulty of the product separation and ILs recycle. Herein, we screened several imidazolium based ILs and developed an easy way to reuse the ILs via extraction using water.

Table 3 Imidazolium based ionic liquids as dual solvent-catalysts for esterification of vitamin E with succinate

Entry	ILs	Name	Initial rate [a] (10^{-2}/min)	Yield after 1.5 h [b] (%)
12		$[C_5C_1Im][NO_3^-]$	0.69 ±0.12	96.2 ±1.6
13		$[C_5C_1Im][I^-]$	0.56 ±0.15	89.8 ±2.1
14		$[C_5C_1Im][Cl^-]$	0.17 ±0.03	44.7 ±1.6
15		$[C_6C_1Im][Cl^-]$	0.33 ±0.06	50.8 ±1.5
16		$[C_7C_1Im][Cl^-]$	0.62 ±0.05	57.7 ±2.1
17		$[C_8C_1Im][Cl^-]$	0.05 ±0.01	33.8 ±0.9
18		$[C_{10}C_1Im][Cl^-]$	0.20 ±0.01	54.6 ±1.2
19		$[C_5C_1Im][PF_6^-]$	0.10 ±0.01	28.9 ±0.9
		$[C_5C_1Im][BF_4^-]$		
		$[C_5C_1Im][BF_4^-]$		
20		$[C_2C_1Im][NTF_2^-]$	n.d. [c]	n.d. [c]
		$[C_3NC_1Im][NTF_2^-]$		

[a] initial rate refers to moles of product formatted per moles of catalyst per minute; [b] Conditions: 4 mM ILs, 0.5 mM α-tocopherol and 1 mM succinic anhydride was incubated at 800 rpm, 50 °C in dark and under nitrogen atmosphere for 3 h and yield was determined through HPLC analysis, using an external standard; [c] not detectable.

Initial rates and yields of the selected imidazolium based ILs for the synthesis of α-tocopherol succinate is shown in Table 3. Investigation of ILs anions (Entry **12-14**, **19** and **20**) on the efficiency of α-tocopherol esterification showed that the most effective nucleophilic anion was NO_3^- among all the studied anions although these anions may slightly change the ILs viscosity. α-Tocopherol was found immiscible with the hydrophilic ILs containing BF_4^- and NTF_2^-, resulting in no reactivity. It is worthy pointing that the initial rate depends on the substrates' concentration: when millimole ratio of α-tocopherol: Succinic anhydride: ILs was 4: 8: 4, the initial rate when using $[C_5C_1Im][NO_3^-]$ reached 2.86×10^{-2} min^{-1} (See supporting information Table S1, Entry **S5**), which is

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comparable with the values of organocatalysts. However, respecting to the productivity, the approach using ILs as dual solvent-catalysts showed higher efficiency both than biocatalysis and organocatalysis.

The change of substituted alkyl group on imidazole ring of cations mainly resulted in the change of ILs viscosity: previously study reported that an increase in the van der Waals forces primarily contributed to the increase in viscosity of ILs[24]. In agreement with this statement, in the 3-alkyl-1-methylimidazolium [PF_6^-] series, viscosity increases as the number of carbon atoms in the linear alkyl group is increased[25]. However, in our work, 3-alkyl-1-methylimidazolium [Cl^-] series showed more complicated behavior, resulting in the disordered reactivity: there was no linear relationship between reactivity and the substituted number of carbon atoms, while [C_7C_1Im][Cl^-] showed the best reactivity for α-tocopherol esterification.

The reuse of ILs is necessary due to its relative high cost and so far several ILs have been demonstrated with feasible recyclability merely via decantation of esters[3c, 5, 26] because hydrophobic esters are mostly immiscible with the used ILs. When using [C_5C_1Im][NO_3^-] as dual solvent-catalyst for α-tocopherol esterification in its first batch, the reaction mixture tends to spontaneously separate into two layers, and the recycled yield of ILs was around 72.5±1.8%; unfortunately and surprisingly, in the second batch, homogenous mixture was observed even after centrifugation under 10,000 g for 30 min. 1-alkoxymethylimidazolium lactates was synthesized and lactate could serve as the anion[27]. Herein, we assumed that the resulted free carboxylic group of α-tocopherol succinate owing to the use of succinic anhydride may act as anion to coordinating with imidazolium cations. Consequently, this ionic liquid became miscible with the reactants.

Hence, extraction of products by solvents became an alternative choice. Firstly, we used organic solvents, like 10 volumes ethyl acetate or diethyl ether to extract VE and α-tocopherol succinate from the IL while succinic anhydride was firstly removed by centrifugation because it became insoluble in ILs with the decrease of temperature. Although high isolated yield with 92.1±1.2% of α-tocopherol succinate could be obtained, the extraction by organic solvents is not compatible to the concept of green chemistry. Water as solvent is strong environmentally favorable with respect to safety, cost and sustainability. To our delight, [C_5C_1Im][NO_3^-] is totally miscible with water while the other reactants are not. After the removal of succinic anhydride by centrifugation, addition of 2-3 times volume water into the reaction mixture recycled almost 95.2±2.1% ionic liquid after removing water via evaporation under 80 °C and reduced pressure (around 100 Pa).

After the removal of water, the reusability of [C_5C_1Im][NO_3^-] ionic liquid was investigated. As summarized in Table 4, about 94 ± 2 % is the recovery of the IL after all batches tested. Initial rate of α-tocopherol converted into α-tocopherol succinate for 4 batches was almost similar but yield was slightly decreased due to the loss of ionic liquid in each batch. Meanwhile, the recovered [C_5C_1Im][NO_3^-] ionic liquid showed no structural difference to the original one from the comparison of ^1H and ^{13}C NMR test in DMSO-*d6* (SI, Fig. S4). Thus, the developed protocol for synthesis of α-tocopherol esters and ILs recycle is practicable and applicable.

In order to highlight the applicability of the developed process, we investigated the synthesis of several vitamin esters with different acyl donors in [C5C1Im][NO_3^-]. Vitamin A, C and E could be easily modified by esterification with several acyl donors; however, it is clear that the formation as an anhydride is a pre-requirement for the success of the transformation (Table S3). However, it needs to be mention that in the case of the vitamin C, where multiple hydroxyl groups are available, no regioselectivity was observed.

Proposed catalytic mechanism

Lipase-mediated esterification occurs via the formation of a tetrahedral acyl-enzyme intermediate, which undergoes a nucleophilic attack from the second substrate, leading to the product formation and release of the product, regenerating

Table 4 Reusability of [C_5C_1Im][NO_3^-] ionic liquid for esterification of vitamin E with succinate

Runs	Initial rate (10^{-2}/min)	Yield after 1.5 h (%)	Recycled yield of ionic liquid (%)
1	0.69 ±0.12	96.2 ±1.6	95.2 ±2.1
2	0.67 ±0.08	95.8 ±1.2	96.3 ±1.8
3	0.66 ±0.09	93.8 ±2.3	92.8 ±1.5
4	0.66 ±0.05	91.5 ±1.0	93.5 ±0.8

the free enzyme[28]. In the catalytic triad of lipases, the basic nitrogen of histidine abstracts a proton from serine to activate it as nucleophile. As discussed before, the approach using lipase as catalyst for the esterification of vitamin E with succinic anhydride was not contributed by the lipase but the imidazole ring of histidyl residue in proteins. Thus, here we discuss the mechanism based on imidazole catalysis.

Imidazole is known as an ester hydrolysis catalyst[29]. Imidazole carbamates and ureas acting as catalysts and substrates mediated the chemoselective esterification and amidation of carboxylic acids in acetonitrile[30]. Peptides or polymers containing histidine residues were reported to have the hydrolytic activity on *p*-nitrophenyl esters[31] and the activity on Michael additions[32], aldol reactions[33], oligomerisation[34], etc. However, the proposed mechanisms for these catalysts were remarkable different from the one of the lipase-catalyzed reaction. Imidazole carbamates catalyzed esterification was supported to a mechanism involving an activated ester intermediate[30b].

Being cognizant of the early reported works[30b, 31a, 35], we currently propose this histidine-mediated esterification via acyl-imidazole intermediate (Scheme 2). The imidazole moiety is expected to be partly deprotonated in DMSO or DMF since the p*K*a of imidazole in DMSO is 18.6[36]. As a result of the participation of the electron pair of the amide nitrogen in the

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π-electron system of the ring, tautomeric forms in five-membered N-heterocycles happen[37], leading to the possibility that nucleophilic attack can be alternatively accomplished by one of the electronegative nitrogen atoms in the imidazole ring. The first step of the substitution pathway, involving nucleophilic attack by the imidazole ring on the anhydride carbonyl and the leaving of the other carbonyl group forms the acyl-imidazole intermediate. The subsequent transition begins with a S_N2 nucleophilic attack on the carbonyl by the hydroxyl group of α-tocopherol and ends with the formation of final product through expelling the catalyst. The amine group of histamine may be properly coordinating the acyl-imidazole intermediate, resulting in the significant increase of reaction rate.

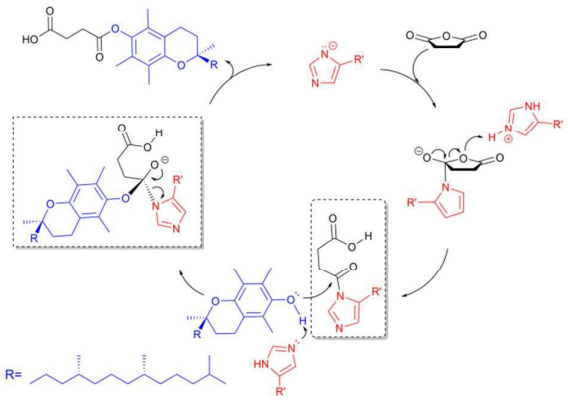
Understanding the mechanism of catalysis by ILs at the molecular level is crucial for the rational design of ILs due to the impossibly experimental study even a small fraction of the potential cations-anions combinations[38], but the mechanism of nucleophilic substitution by ILs seems more complicate. It was recognized that C2 of 1,3-alkyl-imidazolium anions is positively charged due to the electron deficit in the C=N bond whereas the other carbons are practically neutral[38-39]. This resulting acidity of C2 hydrogen atom is the key to understanding the mechanism of ILs catalysis.

As shown in scheme 3, the imidazolium ILs initiates the esterification by donating the C2 proton to the oxygen atom of anhydride, inducing the electrophilic activation of anhydride carbonyl. The subsequent necleophilic attack on carbonyl following a S_N1 mechanism by the alcohol was proposed for ILs catalyzed synthesis of biodiesel[40] or lipophilic esters[41] in previous literatures, in which the nucleophilic role of anions was neglected. Chakraborti *et al.*[42] proposed an "electrophile nucleophile dual activation" role of the $[C_4C_1Im][CH_3COO^-]$ in catalyzing *O-tert*-butoxycarbonylation of 2-naphthol. They concluded that counteranions also involved in the cooperative hydrogen bonds and charge-charge interactions with both substrates. Welton *et al.*[43] proposed that the hydrogen bond basicity of the ILs, controlled by the anions, is the dominant factor in determining the esterification rate. In view of these crucial issues and considering the weak necleophilic attack ability of phenol hydroxyl group of α-tocopherol due to the shielding effect of two neighbor methyl groups, we promote that the anions in ILs performs the necleophilic attack on anhydride carbonyl in the first step, forming an anion-anhydride intermediate after the leaving of the other carbonyl group, and then the second step following a S_N2 mechanism starts with a nucleophilic attack on the carbonyl by the hydroxyl group of α-tocopherol and ends with the formation of final product through expelling the anion.

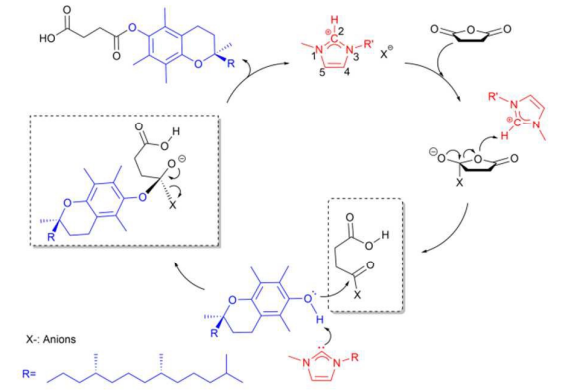
Experimental

General aspects

Vitamin E containing 96% α-tocopherol and succinic anhydride with a purity of 99% were obtained from Aladdin Industrial Inc. (Shanghai, China). Lipase formulation from *Candida sp.* was produced by Beijing CTA New Century Biotechnology Co., Ltd. Lipases including Lipozyme TLIM, Lipozyme RMIM, *Candida antarctica* lipase B were purchased from Novozymes®. The other proteins were commercially available. All 3-alkyl-1-methyl-imidazolium ionic liquids with a purity of 99% were purchased from the center for Green Chemistry and Catalysis, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences (Lanzhou, China). Methanol of chromatographic grade was purchased from Sigma-Aldrich. The other solvents and salts of analytical grade were obtained from Beijing Chemical Factory.



Scheme 2. Proposed acyl-imidazole mechanism of the histidine-catalyzed esterification of vitamin E with succinic anhydride.



Scheme 3. Proposed mechanism for esterification of α-tocopherol with succinic anhydride using 3-alkyl-1-methylimidazolium ionic liquids as dual solvent-catalysts.

Esterification reactions

For biocatalysis, 40 mg protein was added into 1 mL DMSO containing 0.2 M α-tocopherol and 0.8 M succinic anhydride at 50 °C for 24 h; for organocatlysis, reacting L-histidine or its analogues (30 %, mol towards α-tocopherol) in 1 mL DMF containing 0.2 M α-tocopherol and 0.8 M succinic anhydride was carried out at 50 °C for 24 h; for experiments using ILs as dual solvent-catalysts, a standard mixture containing 0.5 mmol α-tocopherol, 1 mmol succinic anhydride and 4 mM ionic liquids was used and the reactions were carried out at 50 °C

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for 3 h. All reactions were investigated in dark and under nitrogen atmosphere. Samples were obtained at scheduler time and yield was determined through HPLC analysis (Thermo Scientific Dionex Ultimate 3000 equipped with an AcclaimTM C18 column), using an external standard. Initial rate refers to moles of product formatted per moles of catalyst per minute when yield was under 20%. α-Tocopherol succinate yield was calculated from the ratio between observed and theoretical production of α-tocopherol succinate.

Recyclability of ionic liquids

The usability of biocatalysts and organocatalysts were not investigated due to the difficulty of the catalysts recycle. Considering the relative high cost of ILs, the recyclability study of ILs is important and necessary; however, decantation of products for recycling ILs was not available for our selected ILs. To our delight, $[C_5C_1Im][NO_3^-]$ is totally miscible with water while the reactants are not. After the removal of succinic anhydride by centrifugation at room temperature, the reaction mixture was strongly mixed after the addition of 2-3 times volume deionized water. After removing water via evaporation under 80 °C and reduced pressure (around 100 Pa), the reusability of $[C_5C_1Im][NO_3^-]$ ionic liquid was investigated under the standard procedure.

Purification

Purification of vitamin E succinate for biocatalysis and organocatalysi approaches was proceeded as following**:** (a) 1 mL of the reaction medium was added to 2 mL diethyl ether and incubated overnight under –18 °C; (b) after centrifugation at 8000 ×g for 2 min, the organic phase was removed and added to 1.5 mL deionized water, and then mixed by vortex for 1 min; (c) after centrifugation at 8000 ×g for 2 min, the organic phase was carefully transferred into a new tube and then dried with Na_2SO_4; (d) after the removal of solvent in a reduced-pressure rotary evaporator, 400 μL *n*-hexane were added to dissolve the unreacted α-tocopherol further purification of the final isolated ester because α-tocopherol succinate is not soluble in *n*-hexane; (e) Centrifuging at 8000 ×g for 2 min again, the liquid was discarded and after dried by nitrogen, white powder was obtained for further characterization.

For the approach using ILs as dual solvent-catalysts, white solid was obtained after the removal of anhydride and the recycle of ILs. 500 μL *n*-hexane were added to dissolve the unreacted α-tocopherol further improving the purity of final ester.

Conclusions

Comparative study among bio-, organocatalysis and using ILs as dual solvent-catalysts for the direct esterification of vitamin E with succinic anhydride was carried out. Each approach possesses its own advantages and disadvantages. It is demonstrated that lipase-catalyzed esterification in DMSO or DMF was not attributed to the catalytic activity of lipase, but the chemical catalysis of the histidyl residue in protein. Although the use of histidine derivatives/analogues as catalysts for these acylation reactions brings several benefits related to their simplicity, low price and availability, the purification of the produced ester for organocatlysis is really difficult via an extraction with organic solvent from DMSO or DMF. The recycle of chemical catalysts is not easy but it can become even more appealing by immobilizing the catalyst to be able to reuse it, or even use cheap sources of histidine, such as commercial soymeal.

Bearing in mind of green chemistry and in light of the imidazole organocatalysis, we developed an efficient and sustainable strategy using 3-alkyl-1-methylimidazolium ILs as dual solvent-catalysts for the synthesis of α-tocopherol succinate, with satisfactory yields and reaction rates. $[C_5C_1Im][NO_3^-]$ ionic liquid can be recycled by water extraction with an average regeneration yield of 94.1%. Initial rate of α-tocopherol converted into α-tocopherol succinate for 4 batches was almost similar but yield was slightly decreased due to the small amount loss of ionic liquid in each batch, indicating that the catalytic activity of recycled ILs was unaffected. The developed protocol for synthesis of VE esters and ILs recycle is practicable and applicable.

Although imidazole ring is one of the main structural part composing histidine and cations of imidazolium ILs, the catalytic mechanism significantly differs from histidine-catalyzed to imidazolium ILs catalyzed esterification. We proposed an acyl-imidazole intermediate for histidine catalyzed esterification, whereas for ILs catalyzed esterification, the role of anions of imidazolium ILs was promoted as performing the nucleophilic attack while the esterification was initiated by donating the proton of C2 on imidazole ring to the oxygen atom of anhydride.

Moreover, we have highlighted that the lipase-catalyzed esterification reactions taking place in aprotic polar solvents should be treated with caution, as the histidines of the protein (not necessarily the one of the active site) could perform the reaction as chemical catalysts. Thus, we would suggest that in such works, the imidazole concentration after purification of lipases via His-Tag should be titrated, in order to be able to exclude the potential of chemical catalysis via the imidazole used for the elution of the protein.

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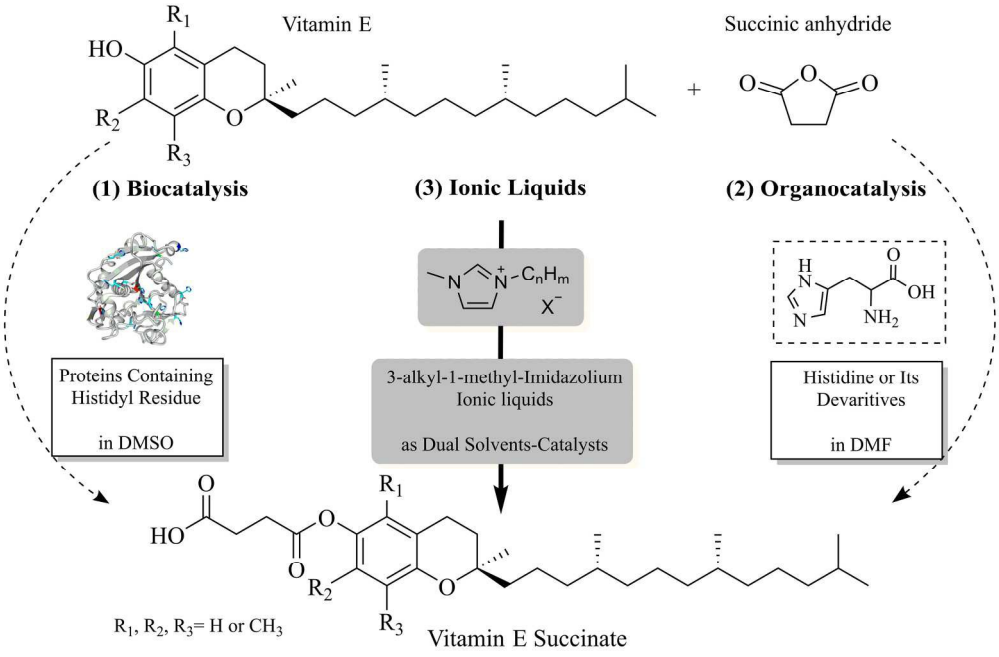

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