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Enzymatic aminolysis of lactones in aqueous miniemulsion: Catalysis through a novel pathway

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ABSTRACT

Lipase-catalyzed aminolysis of lactones under aqueous conditions usually leads to the hydrolysis of the ester bond with only a minor content of the corresponding amide. However, the aminolysis of pentadecanolide (PD) and hexadecanolide (HD), respectively, with oleylamine (OA) in aqueous miniemulsion under optimized conditions (temperature, concentration of enzyme, reaction time) yields >90% amide. Kinetic investigations performed with OA and PD reveal that the lipase catalyzes a novel reaction pathway, i.e., the hydrolysis of a lactone followed by the amidation requiring 30 min and 8 days reaction time, respectively. The demands of a high amount of lipase as well as the long reaction time are caused by the low reactivity of the carboxylic group and the formation of salt with the amine. Similar reactions were performed with PD and other amines such as dodecyl, decyl, octyl, benzyl and hexyl amine resulting the analogous amide compounds.

1. Introduction

The aminolysis of carboxylic acids with amines is an important but arduous reaction for the formation of amides, because, upon mixing an amine with a carboxylic acid, an acid–base reaction occurs first to form a stable salt, which makes it only possible to perform the condensation reaction at high temperature (160–180 °C). Therefore, activated carboxy components as acyl halides, acyl azides, acylimidazoles, anhydrides, esters, etc., are generally used for the formation of the amide bond. However, these reactions are often accompanied by low yields, racemisation, degradation, difficult purification, etc. [1].

Aminolysis of lactones as one of the aforementioned examples is a highly fascinating reaction in modern organic synthesis to produce amide bonds, although it generally requires still harsh reaction conditions such as high temperature or strong alkali-metal catalysts [2,3]. Furthermore, an excess of amine is normally used for the quantitative conversion and to ensure a sufficiently high reaction rate, making the direct aminolysis not practicable especially when the amines are not readily accessible [4,5]. A number of methods have been reported in the literature for facilitating the reaction of smaller ring size lactones (4–6 membered rings) with amines. The Weinreb reagents [6,7] coming from the reaction of trimethylaluminium with an amine, or the use of 2-hydroxypyridine [8] have been considered as being the most popular ones. Me_2AlCl-HN(OMe)Me [9] and $LiNTf_2$ [10] as efficient amidating agents have also been reported in the literature.

In the last two decades, enzyme-catalyzed organic reactions have provided a great impetus to organic synthesis [11]. Enzymes, especially lipases are known for their relatively low cost and great tolerance towards a wide range of substrates [12]. Lipases are generally used for the preparation and resolution of chiral alcohols, esters, carboxylic acids and lactones through the corresponding hydrolysis and transesterification reactions as well as the synthesis of polyesters [13–17]. However, the amidation reaction has been relatively less studied [18–21].

The use of lipases to produce amide bonds was reported several years ago, and the enzymatic aminolysis reaction was applied to the synthesis of peptides [22]. Thereafter, several articles were published on lipase-catalyzed aminolysis and ammonolysis reactions for the preparation of different amides and for the resolution of esters, amines and aminoalcohols [18]. All these reactions were performed in organic solvents such as hexane, toluene, isopropyl alcohol methyl tert-butyl ether, etc. Lipase-catalyzed amidation in bulk (without a solvent) by reacting the racemic amine with aliphatic acids at 90 °C was reported by Prasad et al. [23]. The use of

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lipases in organic solvent with ammonia as nucleophile is described for the preparation of primary fatty amides [24,25].

There are only a few examples for enzymatic reactions where the substrate is present in heterophase like lipase catalysis for synthesizing polyesters [26], optically active amino acids [27], and monoesters [28] in miniemulsion. It shall be emphasized that the lipase-catalyzed condensation reactions for synthesizing monoesters from various linear carboxylic acids and ω-phenyl labeled primary alcohols were performed in aqueous miniemulsion, i.e., in the presence of large amounts of water. The generated byproduct water during these condensation reactions at the interface is effectively expelled from the hydrophobic miniemulsion droplets (considered as separate nanoreactors), which contain the reactants, into the continuous aqueous phase of the miniemulsion. The advantage of using miniemulsions is that stable, small, and narrowly distributed nanodroplets (30–500 nm) are formed [28] which provide a large (and stable) interfacial area for the lipase to catalyze the reaction. Moreover, the miniemulsion processes with enzyme catalysis allows the usage of relatively low amount of surfactants (∼5 wt% with respect to dispersed phase) compared to the microemulsion process (∼200 wt% with respect to dispersed phase), which makes easy the product purification [28–30]. Additionally, by considering the challenges in developing green chemical technology, the employment of water in miniemulsion as a continuous phase and enzyme as a catalyst for performing organic reactions, would be highly appreciated.

In this paper, we are reporting aminolysis reactions of lactones (PD and HD) with a series of amines in aqueous miniemulsion at ambient reaction conditions using lipases as bio-catalysts. The synthesized amide products are entirely new to the chemical world and might be potentially employed, e.g., as plasticizers and linker chains for gene delivery applications [31,32]. Kinetic investigations were performed between PD with OA to picturize the reaction pathway. The analytical characterization was performed by ^{1}H NMR spectroscopy and dynamic light scattering (DLS).

2. Experimental

2.1. Materials

Dodecylamine, decylamine, octylamine, and hexadecane were purchased from Aldrich. 16-Hexadecanolide (HD) and 15-hydroxypentadecanoic acid (15-HPD) were supplied by Alfa Aesar. 15-Pentadecanolide (PD) was procured from Fluka. Oleylamine (OA) and hexylamine were obtained from Acros. Benzylamine was purchased from Merck. Lutensol AT50 [poly(ethylene oxide)-hexadecylether] was kindly donated by BASF. The following enzymes were used: chirazyme L-5 (candida antarctica) (Roche), lipase PS (*Pseudomonas cepacia*), lipase from candida rugosa and Novozyme 435 (candida antarctica) (all from Amano), Lipase from pig pancreas and Lipase from Pseudomonas fluorescens (Fluka), Esterase 009 (recombinant aspergillus oryzae) (Jülich Chiral Solutions GmbH). All chemicals were used without further purification. Demineralized water was used throughout the experiments.

2.2. General procedure for the enzymatic aminolysis/hydrolysis of lactone in miniemulsion

2.5 mmol of a lactone (PD or HD) and 2.5 mmol of an amine, 50 mg of hexadecane and 5 g of a 1 wt% Lutensol AT50 solution in water were stirred for 1 h at 60 °C for creating emulsions. The miniemulsion was prepared by ultrasonicating the mixture during 120 s at 90% amplitude using Branson sonifier W450D, ½ in. tip. From a suspension of 50 mg of lipase PS from *P. cepacia* in 2.5 g of surfactant solution was given a certain volume (for details see below) to the miniemulsion resulting in 6 mg lipase/mL of the total emulsion. The mixture was stirred at 60 °C for 24 h. After 1 day, the sample was freeze dried for 1–2 days. ^{1}H NMR spectroscopy of the product was performed to confirm the formation of the product.

2.3. Simultaneous enzymatic amidation of PD and 15-hydroxypentadecanaoic acid (15-HPD) by OA in miniemulsion

A mixture of 3 mmol of PD, 2 mmol of 15-HPD and 5 mmol of oleylamine, 50 mg of hexadecane and 5 g of a 1 wt% Lutensol AT50 solution in water was stirred for 1 h at 40 °C. Then, the miniemulsion was prepared using the procedure as discussed in the previous section. A suspension of 500 mg of lipase PS from *P. cepacia* in 2.5 g of surfactant solution was given to the miniemulsion and stirred at 40 °C for 8 days.

2.4. Enzymatic amidation of 15-hydroxypentadecanaoic acid (15-HPD) by OA in miniemulsion

A mixture of 2.5 mmol of 15-HPD and 2.5 mmol of oleylamine, 50 mg hexadecane, and 5 g of a 1 wt% Lutensol AT50 solution in water was stirred for 0.5 h at 90 °C. Then, the miniemulsion was prepared by ultrasonicating the mixture for 2 min (30 s pulse with 10 s pause) at 90% amplitude using Branson sonifier W450D, ½ in. tip. During ultrasonication, a heated oil bath at 90 °C was used instead of ice bath to stop solidification of the reaction mixture. 500 mg of lipase PS from *P. cepacia* is dispersed in 2.5 g of surfactant solution and given to the miniemulsion and stirred at 40 °C for 48 h. Two more similar miniemulsions were prepared with and without enzyme, respectively. Directly after preparation they were freeze dried for 48 h. From the three reaction mixtures, samples were taken after 12, 24, and 48 h and analyzed by ^{1}H NMR.

2.5. Analytical techniques

Particle sizes were measured in dilute dispersions by means of dynamic light scattering using a Nicomp particle sizer (model 370, PSS Santa Barbara, CA, USA) at a fixed scattering angle of 90°.

^{1}H NMR spectroscopy was performed on a Bruker DRX400 using either deuterated trifluoroacetic acid (TFA-d_1) or deuterated tetrachloroethane ($C_2D_2Cl_4$) as the solvent. The spectra were referenced to the solvent signal for $C_2D_2Cl_4$ (δ = 6.00 ppm) while in the case of TFA-d_1 as solvent the spectra were referenced to the signal of the CH_2CH_2O protons (δ = 3.94 ppm) of the surfactant Lutensol AT50 to avoid any trouble with the sensitivity of the signal of the solvent toward traces of water. ^{1}H NMR analysis in $C_2D_2Cl_4$ was performed at 80 °C, where the signals corresponding to polyester (4.15 ppm) and unreacted lactones (4.08 ppm) can be differentiated. However, ^{1}H NMR analysis of any synthesized sample in both of these solvents yields similar results in respect to the calculation of product conversion.

3. Results and discussion

3.1. Hydrolysis and aminolysis of lactone

In a first set of experiments, the lipase-catalyzed aminolysis between two hydrophobic components, PD and OA, was performed at 60 °C for 24 h. For the stabilization of the miniemulsions a non-ionic surfactant (Lutensol AT50) was chosen to prevent any eventual denaturing of the enzyme and thus deactivation by the interaction with a charged surfactant. The amount of surfactant was always 16 mg/mmol dispersed phase corresponding to 1 wt% with respect to the continuous aqueous phase.

The reaction might lead to both, the hydrolysis product, i.e., 15-HPD and the corresponding amide product, i.e., N-oleyl-15-

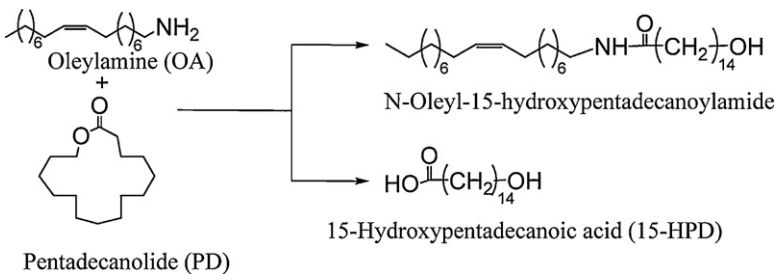

Fig. 1. Enzymatic hydrolysis and aminolysis of PD with OA.

hydroxypentadecanoylamide (Fig. 1). ^{1}H NMR analysis (performed in TFA-d_1) reveals that 15-HPD is the major component from the enzymatic reaction in miniemulsion. However, the signals with relatively low intensity at 3.61 (–CO–NH–CH_2–) and 2.77 ppm (–CH_2–CO–NH–) indicate the formation of the amide product N-oleyl-15-hydroxypentadecanoylamide as well. From the intensity ratio of the signals corresponding to amide (3.61 or 2.77 ppm) and 15-HPD/ester (2.53 and 2.46 ppm), the composition of hydrolysis and aminolysis products was calculated to be 89 and 7%, respectively. The signal at 4.26 ppm (–CO–O–CH_2–) is caused by the unreacted PD and/or polyester (4%), which cannot be differentiated further by ^{1}H NMR analysis performed in TFA-d_1.

Increasing the lactone ring size from 15 (PD) to 16 (HD) in a corresponding aminolysis reaction with OA does not make any change on the conversion of hydrolysis and amide products as expected. Thus, in those and further experiments the lactones PD and HD are exchangeable. ^{1}H NMR analysis of this product shows 90% hydrolysis product [16-hydroxyhexadecanoic acid (16-HHD)] with 6% amide product (N-oleyl-16-hydroxyhexadecanoylamide). To ensure that those reactions are solely promoted by the action of the enzyme experiments without enzyme were performed showing neither hydrolysis nor aminolysis of PD and HD.

The performed aminolysis reactions yield only a small amount of amide products. Instead, the enzyme is hydrolyzing the lactones efficiently and thus producing the corresponding hydrolysis products (15-HPD and 16-HHD) predominantly. In order to investigate the influence of several reaction parameters on the composition of the product mixture and especially to promote the formation of amide, the temperature, the origin and concentration of the lipases, the pH, and the reaction time were varied. The same lactones were employed as above (HD and OA). The percentage of hydrolysis and aminolysis products was estimated from ^{1}H NMR spectroscopy.

3.1.1. Effect of temperature

Enzymes are highly temperature sensitive and they are decidedly active only at a narrow temperature range. It also depends on the substrate and therefore the optimum temperatures must be individually determined for each system. So, the aminolysis reactions were performed at different temperatures (25, 40, 50, 60, 70 and 80 °C). In the special case of 25 °C reaction temperature and HD as lactone, the reaction mixture was stirred for 1 h at 40 °C before ultrasonication due to the melting of HD only at ~36 °C. The percentage of the amide in the resulting product mixture is given in Fig. 2. It increases until 40 °C (from 8% to 14%) and at 80 °C a decrease (4%) is observed. Therefore, the optimum temperature for lipase-catalyzed lactone aminolysis in aqueous miniemulsion was determined to be 40 °C which was chosen in the following experiments. ^{1}H NMR spectroscopy reveals that small amounts of residual lactone (1–4%) and polyester (1–4%) are present in the product. At 80 °C, it is observed that HD had been completely consumed.

3.1.2. Effect of enzyme concentration

In order to investigate the influence of enzyme concentration, different amounts of lipase were employed (50, 125, 350, 500, and 1000 mg) representing a concentration of 6, 14, 40, 67 and 134 mg mL^{-1} of the miniemulsion and a percentage of 4, 9, 26, 38, and 76 wt% with respect to the dispersed phase, respectively. The other reaction conditions were maintained as given in the experimental section except for the reaction temperature (40 °C), because of the observed highest activity of lipase (see above).

Fig. 3 shows that the percentage of amide steeply increases with the amount of lipase. At maximum, 70% aminolysis product was observed while using 1000 mg of lipase. It is also observed that the polyester formation increases with the amount of enzyme from 0% to 8%.

3.1.3. Influence of pH

The presence of the amine causes a relatively high pH (10.2) of the miniemulsion revealing that significant parts of the amine molecules are protonated, hampering their nucleophilic attack of the enzyme activated monomer [33]. This might be a reason why

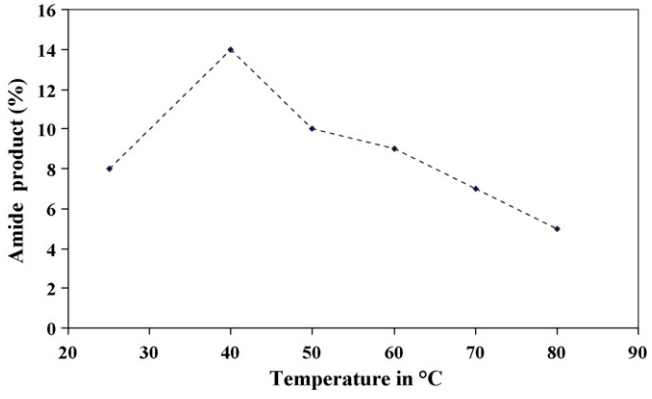

Fig. 2. Effect of temperature on lipase's catalytic activity for the aminolysis of HD in miniemulsion.

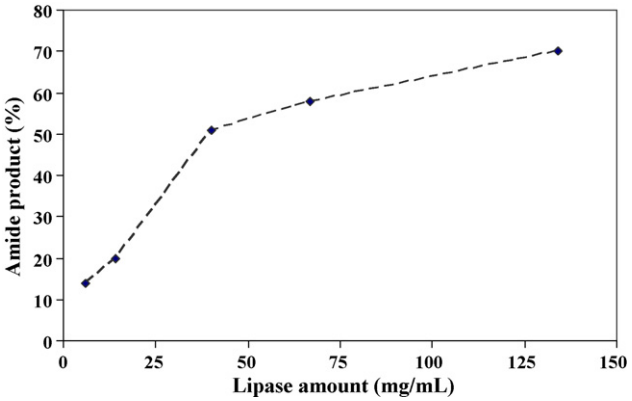

Fig. 3. Lipase concentration on aminolysis of HD in miniemulsion.

the enzyme is hydrolyzing the lactones predominantly (see above). Hence, we expected that particular attention has to be paid to the pH of the reaction medium and to its control. From the different employed buffer systems (K_2CO_3/$KHCO_3$, K_2HPO_4/KH_2PO_4, and CH_3COOK/CH_3COOH) containing 1 wt% surfactant with a pH at 4, 7, and 10, respectively, only the acetate buffered miniemulsion was stable (pH 10). Except for the optimum reaction temperature (40 °C, see above), the other conditions were as described in the experimental part. The reaction led to the formation of 8% amide product showing that apparently the pH plays only a minor role with respect to increasing content of amide.

3.1.4. Influence of the type of lipase

So far, we have used PS lipase from *P. cepacia*. There are many lipases from different micro-organisms commercially available. To validate the activity of other lipases on the aminolysis of HD in aqueous miniemulsion, different lipases were used (Table 1). The other reaction conditions were maintained as described in the experimental section except for the optimum reaction temperature (40 °C, see above).

Interestingly, all the employed enzymes are catalyzing the aminolysis of lactones and yield the amide product. Comparing the same weight concentration of the lipases, Lipase PS from *P. cepacia* produces the maximum (14%) amide product with 77% hydrolysis product (16-HHD). Novozyme 435 yields mainly 16-HHD with 9% amide. Chirazyme L-5, Lipase from candida rugosa, Lipase from pig pancreas and esterase make 8–12% amide product, however these enzymes do not yield the hydrolysis product. Lipase from pseudomonas fluorescens also predominantly (60%) yields 16-HHD with 9% amide product. Thus, among the enzymes used, Lipase PS yields the highest amount of amide product from lactone aminolysis whereas the highest overall enzymatic activity is presented by Novozyme 435 with only 1% unreacted lactone. The drawback of the high activity is the formation of 15% polyester in the case of Novozyme 435 compared to only 3% for Lipase PS. The other enzymes do not produce polyesters under the performed reaction conditions.

3.1.5. Influence of reaction time

To investigate the effect of reaction time on the formation of amide in the miniemulsion, the reaction at 40 °C was prolonged to 8 days. After 8 days reaction time no liquid/liquid phase separation was observed but a kind of solidification took place in combination with an increase of the particle size from 250 nm before the reaction to 350 nm after 8 days. The polydispersity also increased during this reaction. The product analysis by ^{1}H NMR shows the formation of 30% amide product with 70% 16-HHD, which clearly indicates a strong increase of the percentage of amide with reaction time.

3.1.6. Combination of different reaction conditions

Among the investigated reaction parameters described above, the reaction temperature, the concentration of the enzyme, and the reaction time were observed to highly influence the content of the amide in the product mixture. Other reaction conditions such as pH and type of lipase do not seem to have a significant influence on the aminolysis of lactones in aqueous miniemulsion. Thus, in a concluding experiment with the optimum conditions, the combination of 500 mg Lipase PS, 40 °C reaction temperature, and 8 days reaction time for the aminolysis of HD led to the almost quantitative formation of amide with 94%.

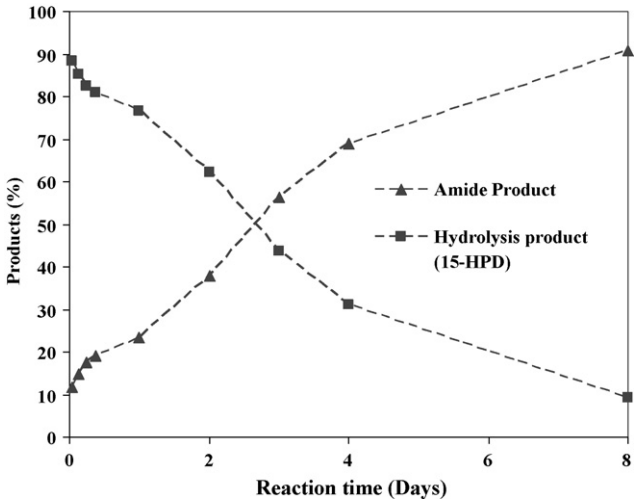

Fig. 4. Kinetics of lipase-catalyzed amidation of the hydrolysis product (15-HPD) by OA.

3.2. Kinetics of aminolysis of lactones

The results presented above show that the application of enzyme enhances product formation significantly, i.e., hydrolysis and aminolysis of lactones. Interestingly, a shorter reaction time (24 h) and lower amount of enzyme (7 mg/mL of miniemulsion), rather promotes the hydrolysis whereas a longer reaction time (8 days) and high amount of lipase (67 mg/mL of miniemulsion) yields a higher amount of amide. In order to get a quantitative insight into this behavior, kinetics of the aminolysis between OA and PD were performed. Samples were taken from the reaction mixture and freeze dried during the course of the reaction at different times, e.g., at 1, 3, 6, and 9 h as well as at 1, 2, 3, 4, and 8 days.

3.2.1. OA and PD

After 1 h reaction time, the amide and hydrolysis products were calculated to be 12 and 88%, respectively (Fig. 4). Upon increasing reaction time to 24 h, the amide product is observed to increase (23%) with a decreasing fraction of the hydrolysis product to 77%. Increasing the reaction time further to 2, 3, and 4 days, a similar trend could be observed, i.e., the percentage of the amide increases (38–69%) with a corresponding decrease of the hydrolysis product (62–31%). After 8 days reaction time, 91% amide product were formed along with 9% of 15-HPD. In summary, the performed kinetic investigation reveals that the lipase first hydrolyses the lactone and the ω-hydroxycarboxylic acid is amidated by the amine. This two-step mechanism of the formation of amide from a lactone

Table 1
Aminolysis of lactone (HD) using lipases from different origin.

Lipase	Amide (%)	Hydrolysis (%)	Polyester (%)	Unreacted lactone (%)
Lipase PS from *Pseudomonas cepacia*	14	77	3	3
Novozyme 435	9	75	15	1
Chirazyme L-5	12	–	–	88
Lipase from candida rugosa	12	–	–	88
Lipase from Pseudomonas fluorescens	9	60	–	31
Lipase from pig pancreas	8	–	–	92
Esterase	8	–	–	92

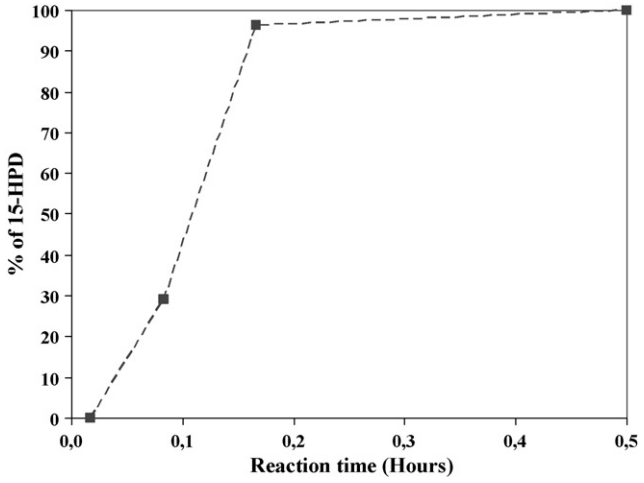

Fig. 5. Kinetics of lipase-catalyzed hydrolysis of PD.

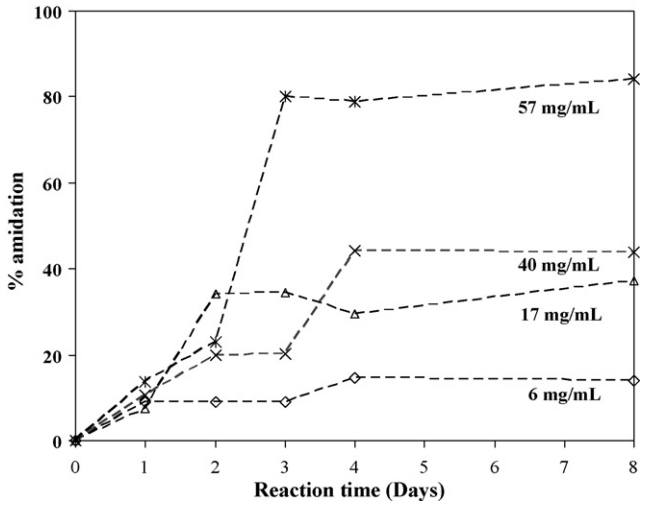

Fig. 7. Influence of amount of lipase and reaction time on producing amide product.

and an amine by the action of an enzyme under aqueous conditions can be considered as a novel reaction pathway.

In a next step, it is interesting to know the preferred reaction at shorter reaction times, i.e., hydrolysis or aminolysis of PD. Therefore, a kinetic investigation at shorter reaction time, e.g., 1, 5, 10 and 30 min was performed. During these shorter reaction times, enzymatic aminolysis between PD and OA (the mixture is a liquid at room temperature) was observed after freeze drying. Hence, a small amount of the reaction mixture (~100 mg) was removed and dissolved directly in TFA-d1 and analyzed by ^{1}H NMR (Fig. 5). After 1 min reaction time, no products were formed. However, a further increase of the reaction time to 5, 10 and 30 min, the conversion of hydrolysis product (15-HPD) increased to 29, 96 and 100%, respectively. After 30 min, the enzymatic reaction starts solely between 15-HPD and OA. Therefore, the observed amide product is exclusively from the enzymatic amidation reaction between 15-HPD and OA.

3.2.2. PD, 15-HPD and OA

To confirm the above observation, i.e., amidation of carboxylic acid, a further kinetic study was performed between OA (5 mmol) and a mixture of PD (3 mmol) and 15-HPD (2 mmol), the hydrolysis product of PD, for 8 days (Fig. 6). At the end of the reaction time, 93% amide product is observed and the investigation shows a very clear evidence of the amidation of carboxylic acid (overlay of ^{1}H NMR spectra is given in supporting information).

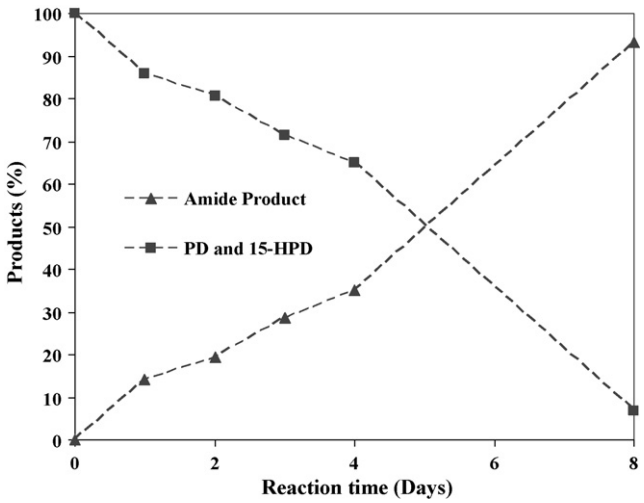

Fig. 6. Kinetics of lipase-catalyzed amidation of PD and 15-HPD.

The investigations above have shown that high amounts of enzyme and long reaction times are required to form the desired amides. The question arises if the same effect can be obtained by the combination of lower reaction times with higher enzyme concentrations or by longer reaction times with lower amounts of enzyme. To check this out, we again carried out kinetic investigations on aminolysis reactions between OA and PD with four different amounts of enzyme for 8 days reaction time (Fig. 7). After 24 h reaction time, all these reactions yield ~95% conversion with a predominant amount of 15-HPD. Then, the percentage of the amide product is observed to be increased with reaction time until ~4 days and reaches a plateau for the reactions performed with 40 and 57 mg/mL of lipase whereas the plateau reaches more quickly, i.e., after 2 days for the reactions executed with lower amounts of enzyme (6 and 17 mg/mL). At the end of the 8 days reaction time, 6, 17, 40, and 57 mg/mL of lipase produce 14, 37, 44, and 79% amide products, respectively. We have already seen in the previous section that 67 mg/mL of lipase gives 91% amide product after 8 days reaction time. All these results are clearly indicating that, for producing amide, both a high amount of enzyme and longer reaction times are essential.

To confirm the true reactants (15-HPD and oleylamine) in the above performed reactions and to investigate the effect of lyophilization, a few more control experiments were carried out. Here, the reactants 15-HPD and oleylamine were employed. The miniemulsions were prepared by stirring the reaction mixture at 90 °C for 0.5 h, followed by ultrasonication under heating in an oil bath to 90 °C. Although the prepared miniemulsions were stable, they showed relatively higher average droplet sizes by DLS (1.4 μm).

Then, the following three experiments were performed for 48 h: (i) enzymatic reactions between 15-HPD and oleylamine during lyophilization which was started directly after preparation of the miniemulsion, (ii) same experimental set-up but without enzyme, and (iii) enzymatic reaction between 15-HPD and oleylamine in miniemulsion with enzyme stirring at 40 °C.

^{1}H NMR analyses indicate that there is only 3% amide bond formation during lyophilization after 48 h, without enzyme even no amidation at all can be detected. At the same time, the reaction running in miniemulsion shows 4, 12, and 22% amide bond formation after 12, 24, and 48 h, respectively. These control experiments make it very clear that the true reactants are hydroxy acid and amine. In addition, it shows that the formation of significant amounts of

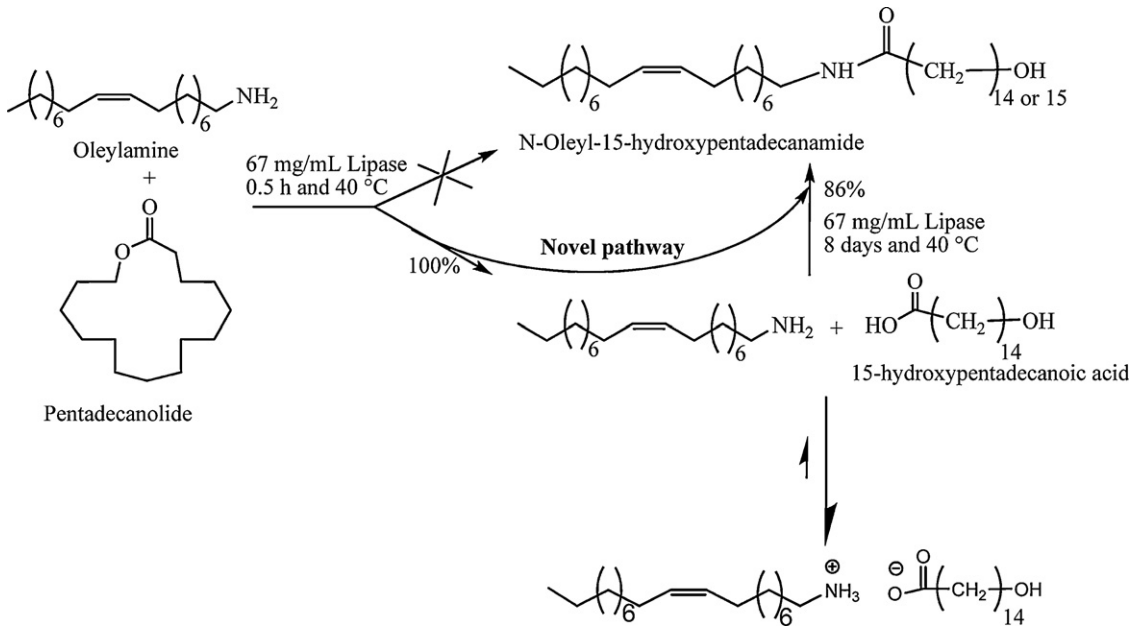

Fig. 8. Novel reaction pathway for aminolysis of a lactone demonstrated by ^{1}H NMR analysis. Conversions are obtained from kinetic study between OA and PD.

amide bonds occurs only at miniemulsion reaction conditions with enzyme and not under lyophilization conditions.

Ester aminolysis by lipase is a common reaction but amidation of carboxylic acids in the presence of high amounts of water at ambient reaction conditions can be regarded as a novel chemical process. In order to successfully perform this reaction, a high amount of enzyme and comparably long reaction times are required. Other chemical transformations involving esters under similar conditions in miniemulsion [28] need relatively lower amounts of enzyme (50 mg lipase) and shorter reaction times (24 h reaction time). A closer look to the aminolysis reaction between PD and OA in heterophase shall shine light on this phenomenological finding. As already described above even with the highest amount of enzyme (67 mg/mL), quantitative conversion of the hydrolysis product was observed even after 30 min. As soon as the hydrolysis product is formed in miniemulsion, the acid–base reaction will lead to salt formation, which cannot be converted to the amide product under the reaction conditions. Only heating the sample under a high vacuum would successfully form the amide. However, under the given conditions small amounts of amine and acid are present in the equilibrium state [1] (see Fig. 8) and therefore are available for further amidation, respectively. Here, the lipase is acting efficiently to convert these reactants (15-HPD and OA) to the amide product. This is the reason why this reaction demands a high amount of enzyme and reaction time.

3.3. Amidation using other amines

Similar reactions of PD with other amines with varying alkyl chain length were also carried out using lipase as a catalyst. For this reactions, dodecyl, decyl, octyl, hexyl, and benzyl amines were used and the optimized reaction conditions such as 40 °C, 67 mg/mL of lipase PS amino, and 8 days reaction time were employed (Table 2). Here, it is interesting to note that the alkyl chain length of amine is important for the lipase-catalyzed amidation. If the length of the carbon chain is larger than 7, lipase shows the highest activity and produces ~85% of the corresponding amide. For the hexylamine, the lipase exhibits the lowest activity with respect to the formation of the amide (18%). Benzylamine containing 7 carbon atoms offers an intermediate activity with 58% amide in the final product

Table 2
Amidation using other amines.

Reactants	Product name	% of amide product
Dodecylamine and PD	N-dodecyl-15-hydroxypentadecanoylamide	83
Decylamine and PD	N-decyl-15-hydroxypentadecanoylamide	88
Octylamine and PD	N-octyl-15-hydroxypentadecanoylamide	91
Hexylamine and PD	N-hexyl-15-hydroxypentadecanoylamide	18
Benzylamine and PD	N-benzyl-15-hydroxypentadecanoylamide	58

mixture. It is well established that the enzymes are highly substrate specific. We assume that a sufficiently large hydrophobic moiety in the molecules is required (≥8 carbon atoms), so that the amine can access the lipase's catalytic active site which only opens toward the hydrophobic phase [12].

4. Conclusions

Lipase-catalyzed aminolysis of PD and HD by OA in aqueous miniemulsion yields at moderate enzyme amounts at 60 °C after 24 h mainly (93%) hydrolysis products with relatively small amount of corresponding amide products (7%). The reactions yield no products in the absence of enzyme. To increase the conversion of amide product, reaction conditions such as temperature, enzyme amount, type of lipases, pH, and reaction time were varied to check any increment on the amide product formation. From these optimizations, it was observed that temperature, lipase amount and reaction time have a large influence on the increase of the amide portion in the product mixture. Excitingly, it was possible to increase the amide fraction to >90% using the optimized reaction conditions such as 40 °C, 67 mg/mL lipase PS, and 8 days reaction time.

Kinetic investigations performed between PD and OA reveal that the lipase is hydrolyzing the PD within 30 min reaction time to produce the quantitative amount of 15-HPD, which is further amidated by amine. This novel reaction pathway was confirmed by another kinetic study performed between PD, 15-HPD, and OA.

Furthermore, kinetic examinations between OA and PD with different amounts of enzyme clearly indicate that the higher reaction time and higher amount of lipase are necessary to quantitatively produce the amide. This is due to the barrier, i.e., salt formation in the reaction pathway.

Analogous reactions of PD with other amines such as dodecyl, decyl, hexyl and benzyl amines were also carried out using lipase as a catalyst to produce the resultant amide product. If the length of the carbon chain exceeds 7, the lipase shows the highest activity and produces ~85% corresponding amide and the aromatic benzylamine, which possesses 7 carbon atoms, demonstrates intermediate activity with 58% amide product. For the hexylamine, lipase shows a lowest activity in the amidation (18%).

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