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Synthesis of primary amides by lipase-catalyzed amidation of carboxylic acids with ammonium salts in an organic solvent

Mike J. J. Litjens, Adrie J. J. Straathof,* Jaap A. Jongejan and Joseph J. Heijnen

Delft University of Technology, Kluyver Laboratory for Biotechnology, Julianalaan 67, 2628 BC Delft, The Netherlands. E-mail: straathof@stm.tudelft.nl

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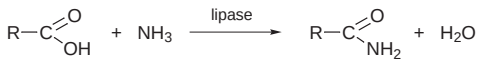
The synthesis of butyramide and oleamide, by *Candida antarctica* lipase B-catalyzed amidation of the carboxylic acids, in an organic solvent with ammonium bicarbonate or ammonium carbamate as a source of ammonia results in good yields, making prior activation of the acids unnecessary.

Primary amides are important derivatives of several types of carboxylic acids, such as fatty acids and amino acids. In addition, enantioselective amidation of chiral acids may be used as an alternative to esterification in kinetic resolution processes. However, direct reaction of carboxylic acids with ammonia requires extreme conditions (200 °C, 7 bar anhydrous ammonia).[1] This may lead to the formation of byproducts in the production of heat sensitive amides such as oleamide. A selective reaction under mild conditions is therefore desirable.

For amidation of aliphatic acids with ammonia, lipases have been considered as the catalyst, but they have been rejected because the formation of a carboxylate anion would lead to precipitation of the ammonium carboxylate rather than formation of the acyl–enzyme complex and subsequent reaction with ammonia.[2,3] For this reason amides are usually synthesized from a neutral and activated form of the acid, such as the acid chloride (which takes away the need for a catalyst) or an ester. For example, De Zoete *et al.*[2] developed a one-pot procedure for the esterification and subsequent ammoniolysis using *Candida antarctica* lipase B (CALB) to catalyze both steps. In many cases it would be attractive if such an activation step could be avoided.

A recent thermodynamic study on the hydrolysis and ammoniolysis of the ester butyl butyrate indicates that the unactivated butyric acid should react efficiently with ammonia under mild conditions in anhydrous methyl isobutyl ketone (MIBK).[4] We decided to verify these thermodynamic predictions experimentally.

Here we demonstrate that primary amides can indeed be formed in good yields under mild reaction conditions, by direct amidation of the carboxylic acids (Scheme 1). The amidation of butyric acid to form butyramide and water serves as a model reaction, using CALB as the biocatalyst. The reaction is performed in MIBK at 25 °C, and ammonium salts that partly dissolve during the course of the reaction are used as a convenient source of ammonia. The experiments were carried out in 33 ml closed glass vessels containing 25 ml dry MIBK with various butyric acid concentrations (concentrations are mentioned in Fig. 1). Immobilized CALB (15 mg) (Novozym 435, a kind gift of NOVO Nordisk, with a catalytic activity of approximately 11000 PLU g^{-1} preparation) and solid ammonium bicarbonate or ammonium carbamate (*ca.* 2.5 mmol) were added, and the suspension was stirred. Samples were taken through a septum to prevent the escape of gas during sampling, and were immediately centrifuged and analyzed for butyric acid and butyramide by GC.



Scheme 1

The experimental results are very encouraging, yields at equilibrium for the amide (after 17 days) were found to be 80–90% when using ammonium bicarbonate and 90–100% when using ammonium carbamate as the source of ammonia (Fig. 1). In addition the model predictions agree reasonably well with the experimental results. No butyramide was formed in the absence of the enzyme, thus the amidation is enzyme-catalyzed. Fig. 1 shows that the amidation proceeds better with ammonium carbamate than with ammonium bicarbonate as the source of ammonia. Model calculations show that this results from the combined effect of a higher ammonia concentration and a lower water concentration. Ammonium bicarbonate dissolves as one equivalent of ammonia, water, and carbon dioxide, whereas ammonium carbamate dissolves as two equivalents of ammonia and one equivalent of carbon dioxide. It is obvious that an increased concentration of ammonia improves the yield at equilibrium, and that an increased concentration of water, which is a coproduct of the amidation, decreases the yield at equilibrium.

With ammonium carbamate as the source of ammonia, the yields obtained in the present experiments approach the optimized yields of the two-step process by De Zoete *et al.*[2] for the synthesis of octanamide and oleamide from the carboxylic acids *via* the esters. The yield of our direct enzymatic amidation may be improved by increasing the ammonia concentration. According to the model calculations[4] the ammonia concentration is below 30 mmol l^{-1} under the experimental conditions employed. Up to 230 mmol l^{-1} of ammonia may be used by saturating the reaction medium with gaseous ammonia.[4] The removal of reaction water, for example by molecular sieves, may further improve the yield.

To estimate the productivity of CALB in our one-step procedure as compared to the two-step procedure by De Zoete *et al.*,[2] an experiment was performed with oleic acid as the substrate. The experimental conditions were similar to those mentioned for butyric acid, except that the temperature was

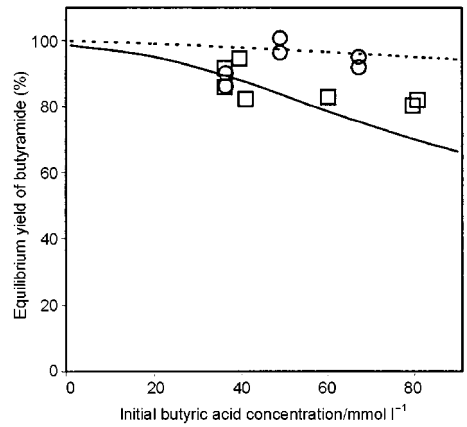


Fig. 1 Yields of butyramide at equilibrium for various initial butyric acid concentrations with solid ammonium bicarbonate (□, —) or solid ammonium carbamate (○, ···) as the source of ammonia. Markers are measured data, lines are predictions using the model presented in ref. 4.

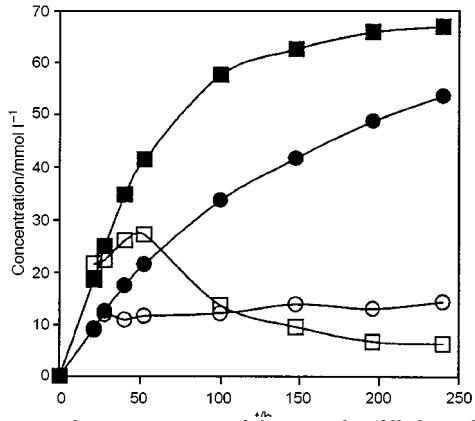


Fig. 2 Measured concentrations of butyramide (filled symbols) and dissolved butyric acid (empty symbols) for a CALB-catalyzed amidation of 70 mmol l^{-1} butyric acid in MIBK. In experiment A (□), 115 mmol l^{-1} ammonia is released gradually by dissolving ammonium carbamate. In experiment B (○), the same amount of ammonia is added in one portion.

35 °C and the enzyme concentration was 1 g l^{-1}. With 1.3 mmol ammonium carbamate as the source of ammonia, 48 mmol l^{-1} oleic acid was converted into oleamide in 95% yield in 4 days. This corresponds to a productivity of 11 mmol oleamide per gram of immobilized enzyme per day, which is more than twice the productivity calculated from the experiment by De Zoete *et al.*,[2] which was performed at 60 °C. Immobilized CALB is highly stable even at 60–80 °C.[5]

Thus, the direct amidation of aliphatic carboxylic acids with ammonia proceeds well, despite the commonly held belief that such a reaction might not be feasible. For this reason we expect that the acid is present mainly in its undissociated form, and not as the unreactive carboxylate. The ammonia concentrations that result from the dissolution of the ammonium salts are apparently sufficient for amidation of the acid, but are not so high that they cause precipitation of the acid as its ammonium salt. Model calculations[4] for butyric acid concentrations of 70 mmol l^{-1} in MIBK predict that ammonia concentrations of 115 mmol l^{-1} cause an equilibrium situation where most of the butyric acid is precipitated, and therefore not available to the enzyme. However, if the ammonia concentration is kept below 30 mmol l^{-1}, then the dissolved butyric acid concentration is much higher. For this reason it is important to add the necessary amount of ammonia gradually, for instance by adding it as slowly dissolving ammonium carbamate. The influence of a gradual addition of ammonia on the reaction rate is demonstrated by the experiments shown in Fig. 2. In a similar experimental setup as described above, 140 mmol l^{-1} butyric acid was dissolved in dry MIBK at 25 °C. In experiment A, 10 ml of this solution was diluted with 10 ml dry MIBK, and 115 mmol l^{-1} ammonia was added as 1.15 mmol solid ammonium carbamate. In experiment B 10 ml of the butyric acid solution was diluted with 10 ml MIBK that previously had been saturated with ammonia, thereby adding the same total amount of ammonia as in experiment A. Immobilized CALB (24 mg) was added to both experiments. Fig. 2 shows that the gradual addition of ammonia through dissolving ammonium carbamate (experiment A) results in higher dissolved butyric acid concentrations and therefore higher amidation rates than the instantaneous addition of the same amount of ammonia (experiment B). In this example the rate of amidation is increased by a decreased ammonia concentration.

In a recent publication Cerovsky and Kula[6] report that low yields (between 0.5 and 38%) of dipeptide amides have been obtained in the protease-catalyzed amidation of the C-terminal carboxylic group of dipeptides. The reactions were performed in MeCN with dissolved ammonium bicarbonate as the source of ammonia. This shows that the reaction described by us is also applicable to other compounds. However, the low solubility of the dipeptides in the organic medium was thought to limit the yield. For substrates of CALB such as aliphatic acids we do not expect any such limitations.

In conclusion we have demonstrated that the direct enzymatic amidation of butyric acid and oleic acid with ammonia is a feasible reaction. Using a widely applicable, commercially available and robust lipase preparation, high yields are achieved under mild reaction conditions. We intend to optimize this amidation reaction and apply it to other carboxylic acids.

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