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Chemoselective enzymatic preparation of *N*-hydroxyalkylacrylamides, monomers for hydrophilic polymer matrices

Eduardo M. Rustoy, Alicia Baldessari *

Departamento de Química Orgánica y UMYMFOR, Facultad de Ciencias Exacta y Naturales, Universidad de Buenos Aires, Pabellón 2, Piso 3, Ciudad Universitaria, C1428EGA, Buenos Aires, Argentina

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Abstract

A lipase-catalyzed procedure is described for the preparation of *N*-hydroxyalkylacrylamides useful among a number of electrophoretical applications such as capillary and gel electrophoresis. The *N*-hydroxyalkylacrylamides were prepared through an aminolysis reaction of alkanolamines on ethyl acrylate. The reaction was catalyzed by *Candida antarctica* lipase. The addition of radical inhibitors improved chemoselectivity and amides were obtained in high yield and purity at room temperature.

Keywords: Lipase; Substituted acrylamides; Electrophoresis; Chemoselectivity

1. Introduction

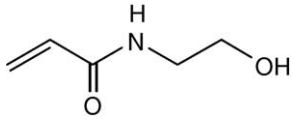
The sequence of the first human genome has been accomplished on an accelerated schedule, thanks to recent advances in the development of automated DNA sequencing technologies, in particular capillary electrophoresis (CE) [1]. This technique offers the advantages of higher speed of analysis, improved peak efficiency and the possibility of full automation. The different formats of DNA electrophoresis have a common requirement for a polymeric separation medium for DNA analysis, typically termed a "separation matrix" [2].

A range of polymer types have been used for DNA sequencing by CE, the most practically useful of which so far are linear polyacrylamide (LPA) and poly-*N*,*N*-dimethylacrylamide (PDMA).

The best sequencing performance to date is that of LPA matrices, which can produce up to 1000 bases of contiguous sequence in about 1 h [3]. Although LPA has a high sieving capacity for DNA fragments, it suffers a few drawbacks: high viscosity and electroosmotic flow (EOF) [4].

The drawbacks of LPA matrices prompted the search for other separation media. Lower viscosity polymers such a PDMA and polyvinylpyrrolidone (PVP) have been introduced for DNA sequencing in capillaries. They have also reduced EOF to negligible levels [5]. But, even while they are water-soluble, these polymers are slightly hydrophobic, and none of these matrices have demonstrated a DNA sieving capacity as high as that of LPA, for instance PDMA resolved only 600 bases in 2 h [6].

Polymer hydrophobicity has a negative impact on DNA sequencing performance [7]. Recently, a novel hydrophilic matrix poly-*N*-hydroxyethylacrylamide (PHEA) was reported for application in DNA sequencing by CE. This new matrix is prepared by free radical polymerization of *N*-hydroxyethylacrylamide **1a** (HEA) [8] (Fig. 1).



1a

Fig. 1. *N*-(2-hydroxyethyl)-acrylamide.

The chemical synthesis of HEA is not an efficient process to date. The high reactivity of the double bond on acrylate makes the selective aminolysis reaction on the ester moiety difficult, and a mixture of products is obtained.

In previous reports HEA has been prepared by reacting acryloyl chloride with the bifunctional ethanolamine in ethanol [9] or following a two phase method [10]. The more recent preparation of HEA was reported by BioWhitttaker Molecular Applications, with the trade name of Duramide [11].

* Corresponding author. Tel.: +54 11 4576 3300x262; fax: +54 11 4576 3385.
E-mail address: alib@qo.fcen.uba.ar (A. Baldessari).

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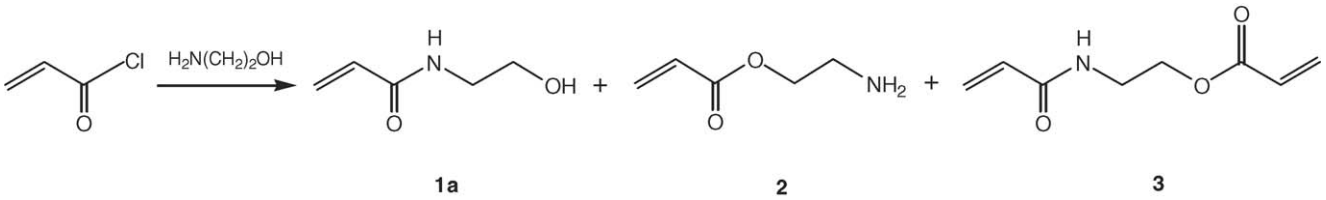
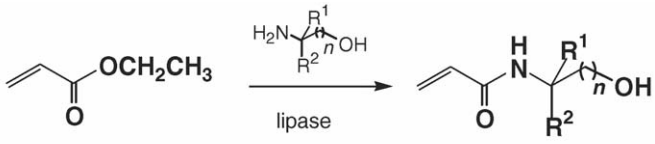


Scheme 1.



1a: $R^1 = R^2$: H, n = 1; **1b:** $R^1 = R^2$: H, n = 2; **1c:** $R^1 = R^2$: H, n = 3; **1d:** $R^1 = R^2$: CH_3, n = 1

Scheme 2.

None of these strategies let to obtain HEA in high yield and purity. While amidation of the amine group on ethanolamine is the desired reaction, esterification of the hydroxyl group on ethanolamine also takes place. Therefore, in addition to the desired product HEA **1a**, there are two possible by products, aminoethyl-acrylate **2** and acrylamidoethylacrylate **3** (Scheme 1).

Hence, in most cases the product has to be purified by silica gel chromatography, which is impractical to produce large quantities. Another drawback to the purification step is that a considerable amount of product is lost because of the polymerization of HEA monomer which happens during the process.

The application of lipases to catalyze amide bond formation is an interesting alternative to conventional methods using proteases because lipases can act in low hydrated organic solvents [12] showing no or very low amidase activity. Amidation reactions catalyzed by lipases have been extensively applied by Gotor and co-workers [13–19] and Sheldon and co-workers [20–25].

Following our work on lipase-catalyzed aminolysis of esters [26,27], we wish to report an effective and convenient enzymatic way to synthesize HEA and related hydroxyalkylacrylamides (Scheme 2).

2. Experimental

2.1. Analysis and materials

For TLC, Merck silica gel 60F 254 aluminum sheets (0.2 mm thickness) were used. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS as internal standard using a Bruker AM-500 spectrometer. Chemical shifts are reported in δ units relative to tetramethylsilane (TMS) set at 0 δ, and coupling constants are given in Hz. EI-MS were obtained at 70 eV using a TRIO-2 VG Masslab Shimadzu QP-5000 and a Finnigan TSQ70 instrument mass spectrometers.

All solvents and reagents were reagent grade and used without purification. Lipase from *Candida rugosa* (CRL) (905 U/mg solid), and type II crude from porcine pancreas (PPL) (190 U/mg protein) were purchased from Sigma Chemical Co.; *Candida antarctica* lipase A (CAL A): Chirazyme L-5, c.-f. lyo (400 U/g) was purchased from Roche Diagnostics GmbH; *Candida antarctica* lipase B (CAL B): Novozym 435 (7400 PLU/g) and Lipozyme RM 1M (LIP) (7800 U/g) were generous gifts of Novozymes Latinoamerica Ltda and Novozymes A/S. All enzymes were used "straight from the bottle". Ethyl acrylate, ethanolamine, 3-amino-1-propanol and 4-amino-1-butanol; cyclohexene, *p*-benzoquinone, *N*-phenyl-2-naphtylamine and 2,6-di-*tert*-butyl-4-methylphenol (BHT) were purchased from Sigma–Aldrich de Argentina S.A., 2-amino-2-methyl-1-propanol was kindly provided by San Antonio-Pride S.A.

2.2. Enzymatic reactions

2.2.1. N-(2-Hydroxyethyl)-acrylamide (*1a*)

CAL B (1 g) was added to a solution of BHT (3%, w/w, ester) in diisopropylether (60 ml). After addition of ethyl acrylate (0.5 ml, 5 mmol) and ethanolamine (300 μl, 5 mmol), the suspension was shaken (200 rpm) at 30 °C and the progress of the reaction was monitored by TLC. After 24 h, the enzyme was filtered off and washed with acetone (2 × 15 ml). The solvent was evaporated affording a colorless oily residue. Yield: 485 mg (93%).

^{1}H NMR ($CDCl_3$): 6.31 (dd, J = 16.9 Hz, J = 1.4 Hz, 1-H), 6.19 (br, 1H, NH), 6.11 (dd, J = 16.9 Hz, J = 10.3 Hz, 2-H), 5.68 (dd, J = 10.3 Hz, J = 1.4 Hz, 3-H), 3.78 (dd, 2H, J = 5.8 Hz, J = 5.2 Hz, –CH_2–OH), 3.52 (m, 2H, –CH_2–NH–).
^{13}C NMR ($CDCl_3$): 173.64 (C=O), 130.48 (CH_2=CH–CO–), 126.94 (*CH*–CO), 62.43 (–CH_2–OH), 42.5 (–CH_2–NH–).
EI-MS (*m*/*z*): 115 (M^+) (7), 97 (14), 84 (38), 74 (20), 56 (42), 42 (100).

*2.2.2. N-(3-Hydroxypropyl)-acrylamide (**1b**)*

As described for **1a**, but using 3-amino-1-propanol (350 μl, 5 mmol) as alcanolamine: 515 mg (89%).

^{1}H NMR ($CDCl_3$): 6.39 (br, 1H, NH), 6.29 (dd, J = 17.0 Hz, J = 1.4 Hz, 1-H), 6.13 (dd, J = 17.0 Hz, J = 10.3 Hz, 2-H), 5.66 (dd, J = 10.3 Hz, J = 1.4 Hz, 3-H), 3.66 (t, 2H, J = 5.5 Hz –CH_2–OH), 3.50 (t, 2H, J = 5.5 Hz, –CH_2–NH–), 1.73 (m, 2H, –CH_2–).
^{13}C NMR ($CDCl_3$): 169.73 (C=O), 130.40 (*CH_2*=CH–CO–), 126.90 (*CH*–CO), 59.37 (–CH_2–OH), 36.42 (–CH_2–NH–), 32.12 (–CH_2–).
EI-MS (*m*/*z*): 129 (M^+) (5), 112 (3), 100 (7), 84 (20), 74 (21), 55 (49), 44 (100).

*2.2.3. N-(4-Hydroxybutyl)-acrylamide (**1c**)*

As described for **1a**, but using 4-amino-1-butanol (450 μl, 5 mmol) as alkanolamine: 605 mg (94%).

^{1}H NMR ($CDCl_3$): 6.27 (dd, J = 17.1 Hz, J = 1.5 Hz, 1-H), 6.08 (dd, J = 17.1 Hz, J = 10.3 Hz, 2-H), 5.82 (br, 1H, NH), 5.64 (dd, J = 10.3 Hz, J = 1.5 Hz, 3-H), 3.70 (t, 2H, J = 5.9 Hz –CH_2–OH), 3.50 (t, 2H, J = 5.9 Hz, –CH_2–NH–), 1.65 (m, 4H, –CH_2–).
^{13}C NMR ($CDCl_3$): 169.50 (C=O), 130.90 (*CH_2*=CH–CO–), 126.45 (*CH*–CO), 62.40 (CH_2–OH), 39.64 (–CH_2–NH–), 32.12 (HO–CH_2–CH_2–), 26.23 (NH–CH_2–CH_2–).
EI-MS (*m*/*z*): 143 (M^+) (6), 84 (68), 70 (22), 55 (100), 42 (77).

*2.2.4. N-(2-Amino-2-methyl-1-propyl)-acrylamide (**1d**)*

As described for **1a**, but using 2-amino-2-methyl-1-propanol (450 μl, 5 mmol) as alkanolamine: 685 mg (96%).

^{1}H NMR ($CDCl_3$): 6.26 (dd, J = 16.9 Hz, J = 1.6 Hz, 1-H), 6.08 (dd, J = 16.9 Hz, J = 10.8 Hz, 2-H), 5.64 (dd, J = 10.8 Hz, J = 1.6 Hz, 3-H), 3.61 (s, 2H –CH_2OH), 1.33 (s, 6H, $(CH_3)_2$C–).
^{13}C NMR ($CDCl_3$): 168.60 (C=O), 131.00 (*CH_2*=CH–CO–), 127.20 (*CH*–CO), 70.03 (CH_2–OH), 42.54 (–CNH–), 25.33 ((CH_3)$_2$–C–).
EI-MS (*m*/*z*): 143 (M^+) (1), 84 (13), 72 (42), 58 (100), 41 (53).

3. Results and discussion

3.1. Optimization of the reaction conditions

Five commercial lipases were tested in the enzymatic aminolysis of ethyl acrylate by ethanolamine: porcine pancreas lipase (PPL), lipozyme (LIP), immobilized *Mucor miehei* lipase, immobilized *Candida antarctica* lipases A and B (CAL A and CAL B) and *Candida rugosa* lipase (CRL) (Table 1). None of the lipases afforded satisfactory results in the aminolysis reaction working at room temperature. In every case a mixture of starting materials, Michael adducts **4** and **5** and polymeric material were obtained (Scheme 3).

Table 1
Enzyme catalyzed preparation of *N*-(2-hydroxyethyl)-acrylamide **1a**[a]

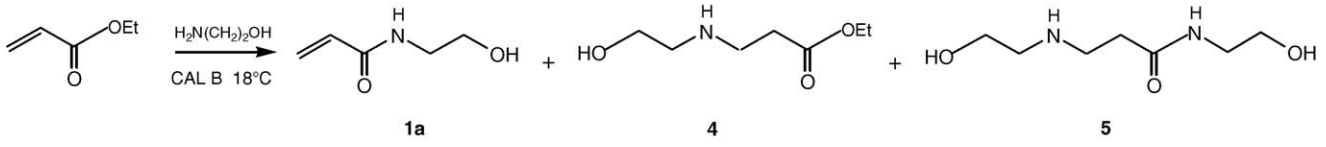
Lipase	% Yield **1a**	
	18 °C[b,c]	30 °C[b,d]
None	n.d.	n.d.
Porcine pancreatic lipase (PPL)	n.d.	26
Lipozyme (LIP)	n.d.	37
Candida rugosa lipase (CRL)	n.d.	38
Candida antarctica lipase A (CAL A)	7	44
Candida antarctica lipase B (CAL B)	28 (55[c])	93

n.d.: not detected.
[a] Only **1a** yield is reported.
[b] Temperature.
[c] Reactions were performed under standard conditions, time: 24 h, but without radical inhibitor.
[d] *p*-Benzoquinone added as radical inhibitor.

CAL B gave the best results and allowed us to isolate a small amount of product **1a** (6%). Hence, we decided to explore the reaction at lower temperature to eliminate, or at least decrease, the proportion of Michael adducts and polymer. We carried out the enzymatic aminolysis at 10, 15 and 18 °C. No reaction products were detected working at 10 and 15 °C. But at 18 °C, we could isolate **1a** in 28% yield using CAL B as biocatalyst. The rest of the lipases did not react or gave the product in lower yield than CAL B. As it was not possible to avoid the formation of Michael adducts **4** and **5** in these conditions, we added radical inhibitors to the reaction system and performed the reaction at 30 °C.

In accordance with results previously reported by Gotor and co-workers [28] CRL did not catalyze the reaction of ethyl acrylate and ethanolamine, without radical inhibitors. Other lipases such as PPL, LIP and CAL A showed similar results. However, by addition of *p*-benzoquinone, the activity displayed by the lipases was variable and CAL B satisfactorily catalyzed the formation of **1a** in almost quantitative yield. Under these conditions, the amide was the only product obtained, showing that CAL B worked in a highly chemoselective way.

In order to study the influence of radical inhibitor on the reaction, we tested the aminolysis with other inhibitors, such as cyclohexene, *N*-phenyl-2-naphtylamine and 2,6-di-*tert*-butyl-4-methylphenol (BHT). The last one (BHT) gave the same result as *p*-benzoquinone (93%). *N*-Phenyl-2-naphtylamine and cyclohexene afforded **1a** in 92 and 83% yield, respectively.



Scheme 3.

Table 2
Solvent effect in the preparation of *N*-(2-hydroxyethyl)-acrylamide **1a**[a]

Solvent (%)	Yield
None	0
Hexane	0
Acetonitrile	20
Acetone	15
Tetrahydrofuran	30
Dioxane	28
Diisopropylether	93

[a] Reactions were performed under standard conditions, time: 24 h.

The enzymatic reaction of ethyl acrylate with ethanolamine was carried out at different ester and alkanolamine concentrations. The best yield was obtained at an alkanolamine/ester molar ratio equal to 1 and at 0.7 M ester concentration. The ratio enzyme/substrate was also studied for CAL B, varying the ratio from 0.1 to 5. The best results were achieved with E/S = 2.

In Table 2 are given the results obtained by varying the solvent in the enzymatic reaction. Attempts to carry out the reaction in a solvent free system or by using a non polar solvent such as hexane were unsuccessful, recovering the starting materials in both cases. Completely water miscible solvents such as acetone, acetonitrile, tetrahydrofuran and dioxane gave also poor results, obtaining **1a** in yields between 15 and 30%. The influence of diisopropylether was remarkable, since it allowed to improve the product yield to 93% when it was used as solvent in the enzymatic aminolysis.

Concerning to the enzyme re-use possibilities, we observed a loss of only 18% in yield when recovered enzyme was used in eight consecutive reactions with fresh substrate, ethanolamine and inhibitor.

3.2. Application of the enzymatic procedure to several alkanolamines

We also applied the enzymatic procedure to the preparation of hydroxyalkylacrylamides from ethyl acrylate and several alkanolamines. The enzymatic aminolysis with higher length chain alkanolamines afforded the products **1b** and **1c** in high yield (89 and 94%, respectively) and the same chemoselectivity. Only the amino groups of the corresponding alkanolamine reacted giving only the corresponding hydroxyalkylacrylamide free of the aminoester isomer.

Considering the excellent properties exhibited by poly-*N*-hydroxyethylacrylamide as a polymer matrix for DNA sequencing, it would be interesting to test the performance in capillary electrophoresis of the polymers synthesized from these two new products (**1b** and **1c**). Moreover, due to their hydrophilic nature, these polymers could exhibit new application possibilities.

We also performed the reaction using a branched alkanolamine as aminolysis reagent: 2-amino-2-methyl-1-propanol. The efficiency and selective behaviour of the lipase were also kept with this substrate. The two methyl groups as substituents in the carbon next to the amino group did not preclude the amide formation and **1d** was obtained in excellent yield (94%). The *N*-(2-Amino-2-methyl-1-propyl)-acrylamide (**1d**) is precursor of 2-acrylamido-2-methylpropansulfonic acid (well-known as AMPS). AMPS is the raw material for the preparation of a variety of copolymeric hydrogels used in crude oil production, for precipitation and recovery of some substances as flocculants and coagulants, in production of synthetic fibers to impart them antistatic properties, etc. [29,30]. Current synthesis of AMPS involves the reaction of isobutene, sulfuric acid and acrylonitrile, through a process which requires very low temperature (−7 °C) and toxic and irritating raw materials [31]. The biocatalytic approach offers an alternative to its synthesis from the hydroxyalkylacrylamide **1d** which was prepared following a simple and mild procedure.

4. Conclusion

An efficient procedure for the enzymatic preparation of *N*-hydroxyalkylacrylamides from ethyl acrylate and several alkanolamines has been developed. This procedure provides a simple and mild alternative method for the synthesis of substituted acrylamides proceeding from linear alkanolamines of variable chain length and also a branched one. The most interesting products are *N*-(2-hydroxxyethyl)-acrylamide (HEA), the monomer used in the synthesis of polymeric matrices with application in capillary electrophoresis and *N*-(2-Amino-2-methyl-1-propyl)-acrylamide, precursor of AMPS. Following a biocatalytic approach, all the products were obtained in high yield and purity and under environmentally friendly reaction conditions.

Acknowledgements

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