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Original Research Article

Constructing the Active Sites of an Artificial Hydrolase Using Mercaptoethanol as a Destructive Agent

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Abstract

In the present study, (*N*-acryloyl-L-cysteine-benzyl amide)$_2$ (Acryl-L-Cys-NHBn)$_2$, *N*-acryloyl-L-histidine-benzyl amide (Acryl-L-His-NHBn), and *N*-acryloyl-aspartic acid-benzyl amide (Acryl-L-Asp-NHBn) were chemo-enzymatically synthesized from Boc-L-amino acids. Then, a mixture of these acryl amino acid monomers was copolymerized to form polymers. The resulting random polymers were then activated after a mercaptoethanol-triggered reduction of disulfide linkages to thiol. The activated polymer demonstrated substrate selectivity, turnover, and rate enhancement in catalyzing the hydrolysis of the esters of Cbz-glycine. The results demonstrated that radical polymerization can facilitate the task of preparing synthetic macromolecules capable of enzyme-like catalytic turnover, substrate selectivity, and rate enhancement.

Keywords: Acryloyl-amino acids; random copolymerization; mercaptoethanol; hydrolysis; Cbz-glycine ester

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1. Introduction

Poly(amino acids) (PAAs) have been used as catalysts for asymmetric synthesis [1,2]. In parallel to the development of combinatorial catalysis technology [3,4], attempts have been made to generate multi-peptide polymers as synthetic vaccines [5-9], though to date success has been modest. Synthetic AA polymers have also been used to produce ligand-selective recognition sites, and the technique has found numerous applications in areas such as biological and pharmaceutical research, diagnostics, theranostics, therapy, and drug delivery [10-16]. In addition, PAAs have been used as tailor-made separation materials [17,18], antibody and receptor mimics in assay systems [19-22], biomimetic recognition elements in biosensors, and artificial enzymes for catalytic applications [15,23]. Over the last two decades, significant research efforts have been made to design PAAs that can mimic the catalytic activity of hydrolytic enzymes [24-29].

The Devaky group synthesized three methacrylic amino acids (metha-Asp, metha-His and metha-Ser) and copolymerized them with transition-state analogs (TSAs) to form a trifunctional enzyme mimic [30]. They then synthesized a phosphate template capable of serving as a stable transition mimic for use in molecular imprinting techniques. Our strategy differs slightly from the strategy employed by the Devaky group. We have designed protein-like side chains of catalytic polymers. Moreover, we synthesized several acrylated monomers, a process that allows for the preparation of catalytic polymers using radical polymerization methods [27,31]. Without a template, randomly

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polymerized three acryloyl-protected amino acid derivatives were executed. Given that a TSA for chemical modification would ruin the transition state, our group chose not to add a TSA to the polymerization process. This random polymerization generated non-imprinted polymers (NIPs) with a near-physiological pH. Takeuchi T. *et al.* efficiently introduced free –SO_3H through disulphide bonds followed by post-imprint treatment [32]. We then used a post-imprinting modification (PIM) method to modify the linkage of disulfide to thiol. We anticipated that having free SH in the catalytic center would be more catalytic active for the hydrolysis than free –SO_3H. By eliminating the need for a template, we eliminate both the need for an imprinting-constructed transition state and the need for PIM-based destruction of transition states. The hydrolytic process is therefore executed for a small substrate in the compact polymers.

This work aims to develop a methodology to design PAAs that can mimic the catalytic activity of hydrolytic enzymes. Specifically, we synthesized PAAs with various 3 compositions of cysteine, aspartic acid, and histidine, which are the amino acid derivatives involved in the catalytic triad of a thiol protease. We tested the ability of these polymers to hydrolyze *N*-Cbz-glycine esters as model protein substrates. The substrate specificity of such an artificial cysteine-protease was suitable mainly for hydrolyzing of a substrate with a small side chain, demonstrating a true enzyme-like catalysis with the active site using S-S bond reduction. This work enhanced the understanding of the catalyzing mechanism of hydrolytic enzymes in different systems, also helpful for the application of polymer esterification.

2. Materials and Method

2.1 General Information

2.1.1 Materials

All the reagents, including, Boc-amino acids, benzylamine, azobisisobutyronitrile (AIBN), acryloyl chloride, *N*-CBz-glycine-*p*-nitrophenyl ester, celite, papain, potassium hydroxide, sodium carbonate, sodium bicarbonate ($NaHCO_3$), *N,N'*-dicyclohexylcarbodiimide (DCC), citric acid, methanol (MeOH), ethyl acetate (EA), trifluoroacetic acid (TFA), dichloromethane (DCM), triethylamine (TEA), hydrogen chloride (HCl), dimethylformamide (DMF), hexane, tetrahydrofurane (THF), mercaptoethanol and other reagents for synthesis were of guaranteed grade (GR) chemicals and were used without further purification.

2.1.2 Instrumental analysis

The synthesized reactions were monitored by thin-layer chromatography (TLC) using pre-coated silica gel 60 glass plates containing the Fluorescence indicator green 254 nm (F254) (Merck, Darmstadt, Germany). UV light (254 nm) in combination with iodine and potassium permanganate staining solutions was used to accomplish a rigorous degree of visualization. Neutral column chromatography on silica gels with either 70–230 mesh or 230–400 mesh was used to purify the products. ^1H and ^{13}C NMR spectral data were obtained from Bruker 500 MHz spectrometer. The ^1H and ^{13}C NMR spectra were obtained using methanol-*d*4 and $CDCl_3$ as solvents for component analysis. With residual solvent resonance serving as the internal standard, all chemical shifts (δ) are reported as parts per million (ppm), coupling constants are reported as Hertz (Hz), and multiplicities are abbreviated as follows: br = broad, s = singlet, d = doublet, dd = doublet of doublet, t = triplet, and m = multiplet. For the identification of enantiomeric excesses, chiral High-Performance Liquid Chromatography (HPLC) System LC-20 Prominence with photodiode array detector SPDM20A (Shimadzu, Japan) was used. Infrared spectra were recorded on a Perkin Elmer Spectrum one FT-IR spectrometer (PerkinElmer, Shelton, CT, USA) using KBr pellets (4000 – 400 cm^{-1}). Optical rotations with a Perkin Elmer polarimeter at the indicated temperature achieved with a sodium lamp (589 nm) were measured. Elemental analysis with assistance from a device manufactured by ThermoQuest (Flash 1112EA, Italy) was conducted. Unless otherwise noted, yields refer to materials that possess chromatographical and spectrographical purity.

2.2. Formation of Acryl-L-His-NHBn

2.2.1 Enzymatic synthesis of Boc-L-His-OMe

Celite (0.2 g) and papain (0.1 g) were mixed with citric acid/ potassium hydroxide (KOH), pH of 4.2, 3 M buffer. The mixture was shaken at 37 °C for 30 min and was then added to a solution of Boc-L-His-OH (51 mg; 0.2 mmole) in 2 mL of methanol (MeOH). This new solution, after being shaken at 37 °C for 2 days, was filtered. For the elimination of MeOH, the filtered solution was evaporated until dryness was achieved. And for the extraction of MeOH from solution, 25 mL of ethyl acetate (EA) and 2 mL of 5% sodium bicarbonate ($NaHCO_3$) were used for the extraction. The extraction was carried out for 4 times. The combined organic layers were dried with magnesium sulfate, and EA was evaporated, resulting in 44 mg of the Boc-L-His-OMe product (81%).

^{1}H NMR (300 MHz, CD_3OD): δ 1.30 (9H, s), 2.89 (2H, m), 3.60 (3H, s), 4.27 (1H, s), 6.76 (1H, s), 7.49 (3H, s).
^{13}C NMR (75 MHz, CD_3OD): δ 29, 30, 53, 55, 81, 118, 136, 174.
Mass (FB^+) = 270.0 m/z; IR(KBr) 1753.46 cm^{-1} (sharp, C = O).
$[\alpha]_D^{22}$ = -27.4 (c = 0.5, MeOH). M.p. 105.4 °C.

2.2.2 Enzymatic synthesis of Boc-L-His-NHBn

Celite (0.2 g) and papain (0.1 g) were mixed with citric acid/KOH, pH of 5.5, 3 M buffer. The mixture was shaken at 37 °C for 30 min and was then added to a solution of Boc-L-His-OMe (54 mg; 0.2 mmole) in 2 mL of EA. This new solution, after being shaken at 37 °C for 6 h, was filtered. The filtered solution was dried with magnesium sulfate. EA was evaporated to result in 66.5 mg of the Boc-L-His-NHBn product (96.5%).
^{1}H NMR (300 MHz, $CDCl_3$): δ 1.36 (9H, s), 3.09 (2H, d, *J* = 5.9 Hz), 4.36 (2H, s), 4.54 (1H, s), 6.84 (1H, s), 7.14~7.28 (5H, m), 7.54 (3H, s).
^{13}C NMR (75 MHz, $CDCl_3$): δ 29, 31, 44, 56, 81, 118. 128, 128, 129, 135, 136, 140, 158, 174.
Mass (FB^+) = 345.1 m/z; IR(KBr) 1664.27 cm^{-1} (sharp, secondary amide stretch)
$[\alpha]_D^{22}$ = -14.9 (c = 0.5, MeOH). M.p. 157.2 °C.

2.2.3 Synthesis of Acryl-L-His-NHBn

Boc-L-His-NHBn (50 mg; 0.145 mmole) was treated with 0.15 mL of trifluoroacetic acid (TFA) in 0.3 mL of dichloromethane (DCM) for 1 h. Evacuation of the solvent was carried out in vacuum overnight. The residue was then treated with 0.12 mL of triethylamine (TEA) in 1 mL of DCM. Acrylation was carried out through the addition of acryloyl chloride (12 μL; 0.145 mmole) at 0 °C for 1 h. Evacuation of the solvent and the residue was purified on a neutral aluminum-oxide column to obtain 13 mg of the Acryl-L-His-NHBn product (36%).
^{1}H NMR (300 MHz, CD_3OD): δ 2.94 (2H, m), 4.24 (2H, s), 4.61 (1H, s), 5.56 (1H, s), 6.14 (2H, m), 6.72 (1H, s), 7.07 ~ 7.17 (5H, m), 7.49 (1H, s).
^{13}C NMR (75 MHz, CD_3OD): δ 29, 43, 44, 54, 81, 117, 126, 127, 127, 128, 134, 135, 138, 165, 172.
Mass (FB^+) = 299.1 m/z; IR(KBr) 1563.5 cm^{-1} (sharp, secondary amide stretch).
$[\alpha]_D^{22}$ = -32.3 (c = 0.5, MeOH). M.p. 207.3 °C.

2.3. Formation of Acryl-L-Asp-NHBn

2.3.1 Enzymatic synthesis of Boc-L-Asp-NHBn

Celite (0.2 g) and papain (0.1 g) were mixed with citric acid / KOH, pH of 5.5, 3 M buffer. The mixture was shaken at 37 °C for 30 min and was then added to a solution of Boc-L-Asp-OH (350 mg; 1.5 mmole) in 20 ml of EA. After being shaken at 37 °C for 5 days, the resulting solution was washed with 1N hydrogen chloride (HCl) (150 mL) and filtered. The filtered solution was dried with magnesium sulfate. EA was evaporated, resulting in 383 mg of the Boc-L-Asp-NHBn product (79.2%).
^{1}H NMR (300 MHz, CD_3OD): δ 1.35 (9H, s), 2.7 (2H, d, *J* = 7.36 Hz), 4.32 (2H, s), 4.37 (1H, m), 6.84 (1H, s), 7.15 (5H, m).
^{13}C NMR (75 MHz, CD_3OD): δ 27, 36, 43, 51, 80, 127, 127, 128, 128, 138, 156, 172, 173.
Mass (FB^+) = 323.1 m/z; IR(KBr) 1651.7 cm^{-1} (sharp, secondary amide stretch).
$[\alpha]_D^{22}$ = -25 (c = 0.5, MeOH). M.p. 165.8 °C.

2.3.2 Synthesis of Acryl-L-Asp-NHBn

Boc-L-Asp-NHBn (161.5 mg; 0.5 mmole) was treated with 1 mL of TFA in 2 mL of DCM for 1 h. Evacuation of the solvent was carried out in vacuum overnight. The residue was then treated with 0.12 mL of TEA in 1 mL of DCM. Acrylation was carried out through the addition of acryloyl chloride (40 μL; 0.5 mmole) at 0 °C for 1 h. Evacuation of the solvent and the residue was purified on a neutral aluminum-oxide column to obtain 13 mg of the Acryl-L-Asp-NHBn product (96%).
^{1}H NMR (300 MHz, CD_3OD): δ 2.67 (2H, dd, *J* = 5 Hz, *J* = 2 Hz), 2.81 (2H, dd, *J* = 5 Hz, *J* = 2 Hz), 4.32 (2H, s), 5.61 (2H, dd, *J* = 3 Hz, *J* = 1 Hz), 6.19 (2H, m), 7.16 (5H, m).
^{13}C NMR (75 MHz, CD_3OD): δ 35, 43, 50, 126, 127, 127, 128, 130, 138, 167, 172, 172.
Mass (FB^+) = 277.1 m/z; IR(KBr) 1615.1 cm^{-1} (sharp, secondary amide stretch).
$[\alpha]_D^{22}$ = -10 (c = 0.5, MeOH). M.p. 153.2 °C.

2.4. Formation of (Acryl-L-Cys-NHBn)$_2$

2.4.1 Synthesis of (Boc-L-Cys-NHBn)$_2$

To a 250-ml reaction vessel, *N,N'*-bis-Boc-L-Cys-OH (1.1 g; 2.5 mmole) was dissolved in 75 mL of DCM. The reaction mixture was chilled in an ice bath. *N,N'*-dicyclo-hexylcarbodiimide (DCC) (1.55 g; 7.5 mmole) was added in portions. Then, benzylamine in dimethylformamide (DMF) (0.82 mL; 7.5 mmole) was added slowly stepwise. After the addition, the ice bath was removed so that the reaction mixture could be stirred overnight at room temperature (rt). The resulting solution was washed 3 times with 100 mL of 1 N HCl and then with 100 mL of 5% $NaHCO_3$. The organic layer was dried with magnesium sulfate. DCM was evaporated, resulting in a reduced volume of 15 mL, which permitted the formation of precipitate at 5 °C. The filtrate was evaporated to result in 44 mg of the (Boc-L-Cys-NHBn)$_2$ product (76%).
^{1}H NMR (300 MHz, CD_3SOCD_3): δ 1.38 (18H, s), 2.84 (2H, t, *J* = 4.3 Hz), 3.06 (2H, dd, *J* = 4.3 Hz, *J* = 1.4 Hz), 4.26 (6H, m), 5.56 (2H, d, *J* = 2.6 Hz), 7.13 (2H, d, *J* = 2.67 Hz), 7.24 (8H, m), 8.4 (2H, s).

Mass (FB^+) = 619.3 m/z; IR(KBr) 1658.75 cm^{-1} (sharp, secondary amide stretch).
$[\alpha]_D^{22}$ = -9 (c = 0.5, MeOH). M.p. 146.6 °C.

2.4.2 Formation of (Acryl-L-Cys-NHBn)$_2$

(Boc-L-Cys-NHBn)$_2$ (110 mg; 0.178 mmole) was treated with 0.25 mL of TFA in 0.5 mL of DCM for 4 h. Evacuation of the solvent was carried out in vacuum overnight. The residue was then treated with 0.2 mL of TEA in 1.5 mL of DCM. Acrylation was carried out through the addition of acryloyl chloride (20 µL; 0.25 mmole) at 0 °C for 1 h. Evacuation of the solvent and the residue was purified on a neutral aluminum-oxide column to obtain crude product. Crystallization was carried out with both hexane and EA to obtain 33.7 mg of the pure (Acryl-L-Cys-NHBn)$_2$ product (36%).
^{1}H NMR (300 MHz, CD_3SOCD_3): δ 2.92 (2H, dd, *J* = 4.5 Hz, *J* = 2.8 Hz), 3.14 (2H, dd, *J* = 4.5 Hz, *J* = 1.8 Hz), 4.28 (4H, m), 4.70 (2H, m), 5.62 (2H, d, *J* = 3.4 Hz), 6.08 (2H, d, *J* = 5.5 Hz), 6.28 (2H, dd, *J* = 5.5 Hz, *J* = 3.4 Hz), 7.26 (10H, s), 8.51 (2H, d, *J* = 2.8 Hz), 8.65 (2H, s).
Mass (FB^+) = 527.36 m/z; IR(KBr) 1626.42 cm^{-1} (sharp, secondary amide stretch).
$[\alpha]_D^{22}$ = -34.3 (c = 0.5, MeOH). M.p. 181.6 °C.

2.5. Random Copolymerization of Acryl-L-Asp-NHBn and Acryl-L-His-NHBn with (Acryl-L-Cys-NHBn)$_2$

Acryl-L-Asp-NHBn, Acryl-L-His-NHBn, and (Acryl-L-Cys-NHBn)$_2$ (molar ratio 2:2:1) were added to a 20-mL reaction vessel, were mixed, and were then dissolved in both tetrahydrofuran (THF) (5 mL) and MeOH (1 mL). Azobisisobutyronitrile (AIBN) (6 mg) was added to the solution, which was then heated to 80 °C under nitrogen for 8 h. The catalyst was removed by filtration, and the filtrate was evaporated to obtain a white solid compound (48.5 mg, 50%).

2.6. Activation of the Random Polymer

20 mg of the above solid was activated with 2 mL of mercaptoethanol. The activation was performed for an hour so that the solution would become homogeneous. To induce precipitation, 6 mL of ether was added. The resulting precipitate was washed with ether again 4 times to obtain 18 mg of white powder.

2.7. Random-polymer Hydrolysis of Ester in a Homogeneous Solution

16.5 mg of *N*-Cbz-glycine-*p*-nitrophenyl ester was dissolved in 5 mL of acetonitrile. The aliquot of the above solution (0.8 mL) was mixed with 0.1 M, pH of 7, Na_2HPO_4 buffer (0.2 mL) containing 3 mg of the polymer. The reaction was performed at 37 °C, with the polymer serving as a homogeneous solution. The formula to calculate the percentage yield was defined as follows:

$$Yield\ (\%) = \frac{weight\ of\ experimental\ ester\ (\mathrm{mg})}{weight\ of\ theoretical\ ester\ (\mathrm{mg})} \times 100 \quad (1)$$

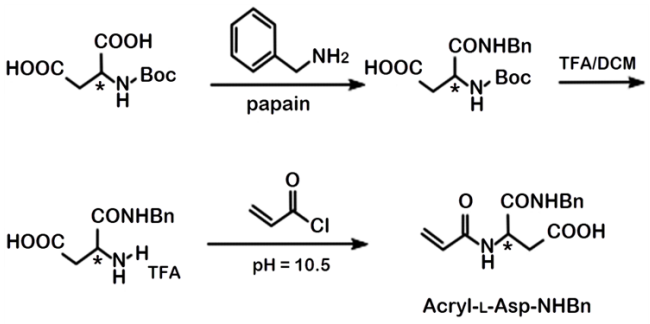
2.8. Random-polymer Hydrolysis of Ester in a Biphasic System

16.5 mg of *N*-Cbz-glycine-*p*-nitrophenyl ester was dissolved in 5 mL EA. The aliquot of the above solution (0.8 mL) was mixed with 0.1 M, pH of 7, TEA/phosphate buffer (0.2 mL) containing 3 mg of the polymer. The reaction was performed at 37 °C in a biphasic system. The formula to calculate the percentage yield was defined in Equation (1).

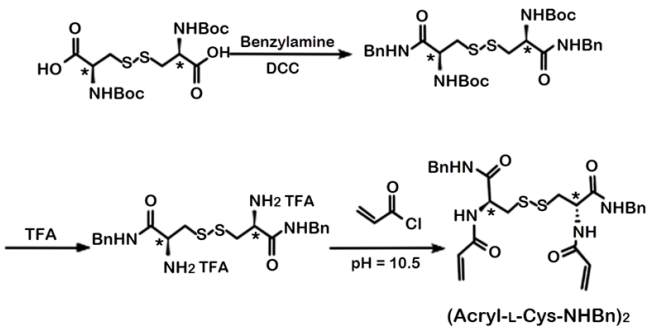
3. Results and Discussion

3.1. Concept of Functionality-chain Monomers

As shown in Schemes 1, 2, and 3, three acryl amino acid derivatives were prepared. For the preparation of *N*-acryloyl-L-aspartic acid-benzyl amide (Acryl-L-Asp-NHBn), a three-step synthesis process involving *N*-Boc-L-aspartic acid (Asp) was prepared. Papain-catalyzed regioselective amidation [33] proceeded smoothly and provided α-carboxylic benzyl amides of *N*-Boc-L-Asp in an 81.5% yield. The Boc protecting group was then quantitatively removed via TFA. Using the Schotten-Baumann reaction, a final acrylation was established in a 90 % yield [34-36].



Scheme 1. Synthesis of *N*-acryloyl-L-aspartic acid-benzyl amide (Acryl-L-Asp-NHBn).



Scheme 2. Synthesis of (*N*-acryloyl-L-cysteine-benzyl amide)$_2$ (Acryl-L-Cys-NHBn)$_2$.

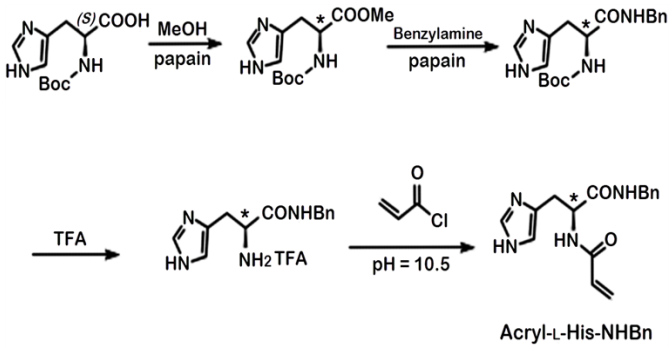
(*N*-acryloyl-L-cysteine-benzyl amide)$_2$ (Acryl-L-Cys-NHBn)$_2$ was synthesized in three steps starting with (Boc-L-Cys)$_2$. Upon treatment with DCC [37,38] in the presence of benzylamine in DMF at room temperature (rt), the yield of bisbenzylamide (Boc-L-Cys-NHBn)$_2$ was 80%. The cleavage of the Boc protecting group with TFA in DCM at rt resulted in a quantitative yield. Our acrylation of the resulting diamine with acryloyl chloride and TEA at 0 °C afforded (Acryl-L-Cys-NHBn)$_2$ for a yield of 50%.

Using papain as a catalyst in MeOH, the carboxylic group of Boc-L-His-OH was enzymatically esterified (87%) [39]. Papain-catalyzed coupling, with benzylamine serving as the nucleophilic catalyst, yielded Boc-L-His-NHBn (> 99%). A TFA deblocking solution removed the deprotection of a Boc-protected amine (100%). Subsequent acrylation [7] was performed at pH of 10.5 in an ice bath to form Acryl-L-His-NHBn (94%).

3.2. Self-assembly of Random Copolymers

Figure 1a illustrates the production of catalytic polymers with amino acid residues. Specifically, preparation and evaluation of an (Acryl-L-Cys-NHBn)$_2$, Acryl-L-His-NHBn and Acryl-L-Asp-NHBn copolymer were studied. By taking advantage of unusual side chain reactivity, post-imprinting modification can be utilized to access the linkage of disulfide to thiol. These amino acids form the triad of a thiol protease. We anticipated that, upon the formation of benzyl amide at the carboxylic end of these amino acids, there would be a relatively neutral and hydrophobic environment, which would stem from both stacking interactions and van der Waals interactions.

Acryl-L-Asp-NHBn (110 mg), Acryl-L-His-NHBn (120 mg), and (Acryl-L-Cys-NHBn)$_2$ (105 mg) were mixed with the radical initiator, AIBN (6 mg), in MeOH and THF. Radical polymerization was carried out at 50 °C (24 h). A copolymerization process involving acryl (functional monomers) were chemically modified by the post-imprinting treatment. The resulting inactivated polymers were activated with mercaptoethanol. As shown in Figure 1b, the disulfide groups on the side-chain were selectively transformed into catalytic residues (the free SH), resulting in a space at the catalytic center [32]. This process completed the formation of the desired catalytic site while efficiently transforming the original residue.



Scheme 3. Synthesis of N-acryloyl-L-aspartic acid-benzyl amide (Acryl-L-His-NHBn).

3.3. Catalytic Systems for Ester Hydrolysis

Processing both inactivated polymers and activated polymers, we turned our attention to the hydrolytic activity associated with *N*-Cbz-glycine esters. For reference, blank polymers were synthesized in the absence of monomers under similar experimental conditions. Hydrolysis was carried out in either a homogeneous solution or a biphasic system. To quantitatively analyze the above result, we measured the hydrolyzed reaction yield and plotted that yield as a function of hydrolysis duration in Figure 2. It is also noteworthy that only the activated polymers exhibited higher yields of *N*-Cbz-glycine than blank polymers or the inactivated polymers in homogenous solution. In contrast, regarding the specific performance of activated polymers in biphasic systems, excellent enzymatic activity toward hydrolysis of alkyl esters achieves 20.1 % conversion after 180 min (unfortunately, as we added organic solvent to

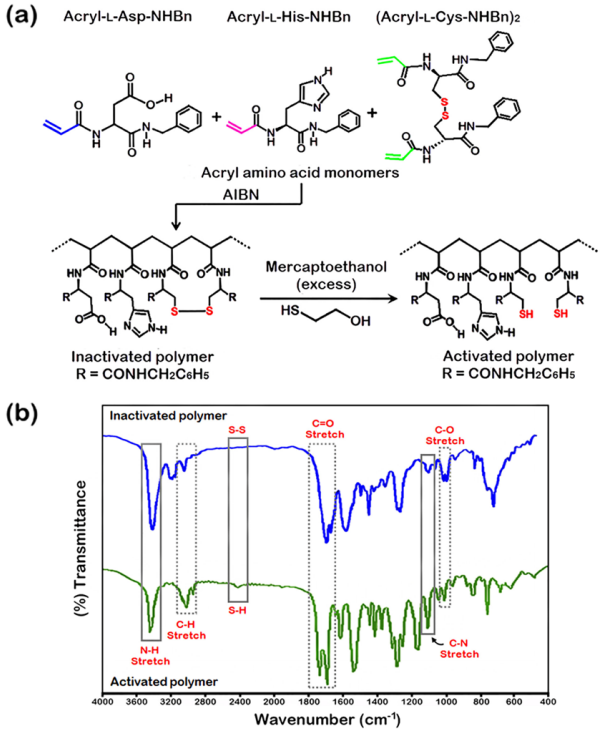


Figure 1. a) The scheme illustrated the prospective excess mercaptoethanol synthesis of activated polymer. (b) FTIR spectra of inactivated polymer and activated polymer.

the homogeneous solution, salt was precipitated). This fact has revealed the opportunities for activated polymer-enzymatic catalysis in multiphasic systems with added benefits in downstream bioprocessing.

3.4. Artificial Hydrolase-catalyzed Hydrolysis of Esters

As shown in Table 1, the catalytic results of the blank polymers, the inactivated polymers, and the activated polymers were compared. The results of our ester hydrolysis involving *N*-Cbz-glycine methyl ester of biphasic system and *N*-Cbz-glycine-*p*-nitrophenyl ester of homogeneous solution were presented. The hydrolysis involving the activated polymer resulted in higher yields than did the hydrolysis involving no catalyst blank polymers and the hydrolysis involving the inactivated polymers.

As expected, the hydrolyzing of *N*-Cbz-glycine methyl ester and *N*-Cbz-glycine-*p*-nitrophenyl ester led to nucleophilic attacks targeting SH to the carbonyl center. These attacks then led to the formation of a tetrahedral carbon atom, which could be mimicked by a natural hydrolytic process. The tetra-coordinated SH atom of the polymers enhanced both the specificity of hydrolysis and the rate of catalysis. Although, as a leaving group, *p*-nitrophenol is better than MeOH, *N*-Cbz-glycine methyl ester is better than *N*-Cbz-glycine-*p*-nitrophenyl ester in the role of substrate for our artificial cysteine-protease. The catalytic rate of the activated polymers promoted the efficient hydrolysis of *N*-Cbz-glycine methyl ester.

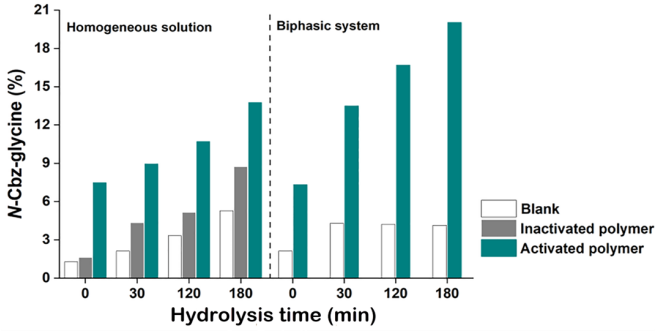


Figure 2. The yield of N-Cbz-glycine-p-nitrophenyl ester reaction without or with catalytic polymers in homogeneous solution and biphasic system, respectively.

4. Conclusion

The results presented in the present study demonstrate that radical polymerization can facilitate the task of preparing synthetic macromolecules capable of enzyme-like catalytic turnover, substrate selectivity, and rate enhancement. Although we used no template for this process, the original disulfide groups that were within the PAA and that were covalently bound to the polymer matrix were reorganized to the thiol groups (i.e., the catalytically active groups). The space, thus generated, was enough to adapt to a small substrate, such as *N*-Cbz-glycine methyl ester. This is not regular for biological enzymes [40]. Our work in the present study demonstrates a true enzyme-like catalysis with the active site using S-S bond reduction. Because they are simple to prepare, mechanically attractive, thermally sound, chemically stable, and not dependent on TSAs, catalytic polymers-acting as "artificial hydrolase"-constitute compelling alternatives to their biological counterparts. Finally, the technique that we have presented herein offers considerable potential for the development of artificial receptors, artificial vaccines, and catalysts with functionalities seldom or never used in biology.

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CRedit Author Statement

Author Contributions: Conceptualization, D.-F. Tai; Methodology, S.-C. Wang, L. Chen and V. Angamuthu; formal analysis, D.-F. Tai and C.-Y. Lin; data curation, S.-C. Wang and C.-Y. Lin; writing—original draft preparation, D.-F. Tai and V. Angamuthu; writing—review and editing, D.-F. Tai and C.-Y. Lin. All authors have read and agreed to the published version of the manuscript.

Table 1. Hydrolysis of the ester of Cbz-glycine without or with catalytic polymer.

Yield of Cbz-glycine (%)	Blank	Inactivated polymers	Activated polymers
N-Cbz-glycine-*p*-nitrophenyl ester[a]	5.3	8.7	13.8
N-Cbz-glycine methyl ester[b]	4.3	8.3	20.1
N-Cbz-glycyl glycine	0	0	0

[a]N-Cbz-glycine-p-nitrophenyl ester (3.3 mg) in acetonitrile (0.8 mL) and 0.1 M, pH of 7, phosphate buffer solution (0.2 mL) was mixed with 3 mg of polymer at rt for 180 mins.
[b]N-Cbz-glycine methyl ester (15 µL) was added to a 0.1 M, pH of 7, phosphate buffer solution (1 mL), followed by 3 mg of polymer at rt for 180 mins.

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