Synthesis, characterization and properties of a magnetically separable and reusable bovine α-chymotrypsin

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Introduction

The ability of enzymes to catalyze chemical reactions of biological importance or industrial interest with unmatched efficiency and selectivity is well known. Being highly selective, productive, and environmentally friendly, biocatalysis is in harmony with the principles of Green Chemistry[1]. The practical application of immobilized enzyme allows enzyme recovery, reuse in batch and continuous flow systems and can be easily separated. Moreover, the immobilization of these biomolecules may improve some of their properties[2] in solution. This is particularly important for proteases, which can undergo autolysis. Owing to their large specific surface area (area/volume), magnetic nanoparticles are even more suitable for enzyme immobilization[3]. Superparamagnetic nanoparticles can be manipulated by an applied magnetic field, but do not aggregate in its absence[4]. In this study, α–chymotrypsin (αCT) was immobilized on silica coated magnetite (Fe_3O_4@silica) and the resulting Fe_3O_4@silica-αCT was characterized in amidase activity, chemical and thermal stabilities, possibility of recovery and reuse.

Results and Discussion

The synthetic work comprised the functionalization of silica-coated magnetite NPs with amino groups using APTES, activation of the functionalized NPs with glutaraldehyde and enzyme immobilization. Acid hydrolysis of the solid obtained followed by amino acid analysis of the hydrolyzate indicated αCT immobilization yield of 22 %.

Despite less active (2.6 times) than free αCT, Fe_3O_4@silica-αCT remains as such, as well as physically identical, for about 6 months when stored in suspension at room temperature (Fig 1).

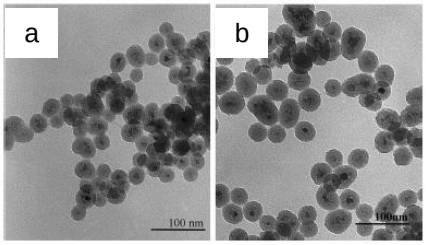

Fig 1 TEM images of (a) fresh Fe_3O_4@silica-αCT and (b) Fe_3O_4@silica-αCT after 103 days of storage[5].

Fig 2 shows its amidase activity against Bz-DL-Tyr-pNA along the storage time. Fe_3O_4@silica-αCT stored in suspension at room temperature kept the activity after 10 cycles of reutilization, indicating an advantage of this type of immobilization.

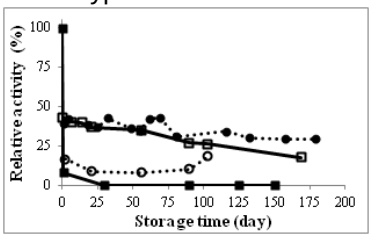

Fig 2 Stabilities of free αCT and Fe_3O_4@silica-αCT stored at different conditions. αCT at room temperature, ■; Fe_3O_4@silica-αCT in suspension at room temperature, □; Fe_3O_4@silica-αCT in suspension at 4 °C,●; Fe_3O_4@silica-αCT dry at 4 °C, ○. The relative activity (%) represents the ratio of residual activity/initial activity of αCT[5].

The immobilized enzyme displayed higher thermal stability since, after 1 h at 60°C, the free enzyme was completely inactivated, while the magnetically separable enzyme retained ca. 30 % of its initial activity. Determination of K_{cat}, K_m and K_{cat}/K_m (Tab 1) showed that no significant change was observed in the K_m, whereas the K_{cat} for the immobilized αCT dropped (25-fold).

Tab 1 Kinetics parameters for αCT and Fe_3O_4@silica-αCT[5].

	K_m (mol L^{-1})	K_{cat} (min^{-1})	$K_{cat}.K_m^{-1}$ (min^{-1} L mol^{-1})
Free aCT	$4.0x10^{-4}$±(0.5)	$2.21x10^{3}$±(0.09)	$5.5x10^{6}$±(0.9)
Fe_3O_4@silica-αCT	$5.3x10^{-4}$±(0.7)	$0.089x10^{3}$±(0.005)	$0.17x10^{6}$±(0.03)

Conclusion

The novelties of the present study are: i) bovine αCT can be easily immobilized on Fe_3O_4@silica; the resulting Fe_3O_4@silica-αCT is more stable in solution, easy to recover from the reaction media, and reusable; ii) due to such improved properties, Fe_3O_4@silica-αCT can be used in enzyme-catalyzed peptide synthesis[5] and has the potential to be useful in proteomic studies.

Acknowledgments

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