Immobilization of a Neutral Protease in Magnetic Particles Using Direct Binding Procedure

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Immobilization of a Neutral Protease in Magnetic Particles Using Direct Binding Procedure

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Abstract. In a recent study we have found that Bovine serum albumine (BSA) can be bound to freshly precipitated magnetic particles directly by a novel procedure. In this procedure BSA linked covalently to the -OH group of Fe_3O_4 using Carbodiimide. The binding was confirmed by FTIR spectra and electron microscopy. We have also immobilized several enzymes which have biomedical application using this procedure and confirmed the binding by the above mentioned method. In the present experiment we have immobilized Dispase, a neutral protease using the direct binding procedure. We have found that the protein was bound to the extend of 90% using the dye binding procedure. We have measured the activity of this enzyme by its proteolytic property and showed that it retained 80% of its activity after immobilization.

1. Introduction

During the past decade it was demonstrated that magnetic particles coated with a suitable stabilizer can not only serve as an efficient and advantageous diagnostic agent in the field of medicine but are also useful in the field of biotechnology. For example Lauva et al [1] used heparin - stabilized colloidal magnetite for binding cells from whole blood, dextran - coated magnetite was used by Rusetski and Ruuge as a drug carrier [2] while Witherspoon et al [3] used silane - coated ferrite particles for radioimmuno assay.
The aim of this work was to show the possibility of immobilization of dispase (neutral protease) to freshly precipitated magnetic particles directly by covalent binding in the presence of carbodiimide. This method of binding can also be adopted for cells, antibodies , enzymes and proteins.

2. Materials and experimental methods

The magnetic paricles Fe_3O_4 used in the experiment were made by addition of an ammonium hydroxide solution to a solution containing 2 moles of ferric ($FeCl_3.6H_2O$) and 1 mole of ferrous ($FeSO_4.7H_2O$) salts. Then impurity ions such as chlorides and sulphates were removed by washing several times with distilled water. The average particle size diameter was determined from observation under electron microscopy (Fig.1a) and was found to be 10nm.
The reagents used for the immobilization such as dispase (neutral protease), carbodiimide and brilliant blue G dye (for estimation of the proteins from unreacted fraction) were obtained from the SIGMA chemical company. Stock solution of dispase in phosphate buffer (pH = 6.3) and carbodiimide were stored at $4°C$. The reaction mixture contained dispase, magnetic particles and carbodiimide in phosphate buffer in the ratio of 1:2:2. It was shaken for a period of 24hrs at room temperature. After 24hrs samples were placed on the top of a bar magnet where sedimentation of magnetic particles occured within 2 minutes. The reacted as well as unreacted fraction of proteins were examined by two independent methods, e.g. by FTIR and by dye binding method. The magnetically sedimented fraction was washed and decanted several times with distilled water and acetone. In order to assess the binding of magnetic particles to protein FTIR was used (Fig.2b). The percentage of bound proteins was estimated by determining the amount of protein in unreacted fraction using dye binding procedure.

3. Results and discussion

By comparing figures (Fig.1a, Fig.1b) for reacted fraction of magnetic particles from electron microscope one can see that there exists a layer on the surface of the particles which shows the coating of protein molecules on the particles.

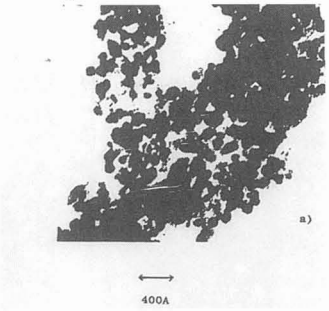

Figure 1a: Electron micrograph of magnetic particles

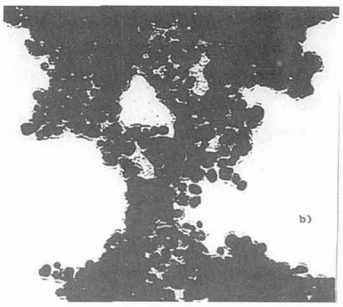

Figure 1b: Electron micrograph of protein bound magnetic particles

This was also confirmed by FTIR spectra of the protein (Fig.2a) and of the reacted fraction of particles (Fig.2b). The characteristic bands of protein at $1648cm^{-1}$ and $1540cm^{-1}$ were also observed in the reacted fraction of the particles.

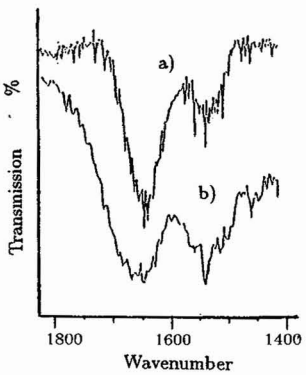

Figure 2: FTIR Spectrum of (a) protein and (b) protein bound magnetic particles

Results obtained from dye binding procedure showed that more than 90% of added dispase was bound to magnetic particles. Activity of this dispase was also measured and it was found more than 80% of the added activity of dispase was retained.

The obtained results clearly show that it is possible to bind proteins on magnetic particles in the presence of carbodiimide without the aid of primary coating. Binding is due to the presence of hydroxyl groups on the surface of freshly prepared magnetite. The usefulness of the present method is mainly in biomedical applications especially in enzyme targeting in different diseases and applications in biotechnology field in drug targeting such as targeting streptokinnase to solubilize blood clots in coronary arterial diseases [4].

Acknowledgment

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