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Magnetic Affinity Separation of Recombinant Fusion Proteins

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Abstract

Isolation and separation of proteins is an important procedure in all areas of biosciences and biotechnology. Currently recombinant fusion proteins are produced routinely; in order to simplify their isolation from complex samples, magnetic separation techniques can be efficiently used. This short review paper shows examples of the most important fusion affinity tags and commercially available magnetic affinity and ion exchange particles enabling efficient capture of recombinant fusion proteins. A list of realized isolation procedures documents the efficiency of magnetic separation techniques.

INTRODUCTION

Recombinant DNA techniques used for obtaining and combining genes from a variety of sources, and the possibility of expressing these genes in different host cells, enabled production of large quantities of various proteins. The production of recombinant proteins in a highly purified and well-characterized form has become an important task. To enable simple separation of the target protein from the complex biological matrices, recombinant fusion proteins are usually produced. The fusion proteins contain an appropriate affinity tag structure, usually exhibiting and enabling the following main features, namely: (a) one-step adsorption purification; (b) a minimal effect on tertiary structure and biological activity; (c) easy and specific removal to produce the native protein; (d) simple and accurate assay of the recombinant protein during purification; (e) applicability to a number of different proteins [1,2].

Many types of affinity tags have been developed and used recently. In many cases isolation of the target recombinant protein is performed using column affinity chromatography. However, magnetic batch separations, employing magnetic affinity and ion exchange particles, have been shown very useful for protein separations [3]. That's why this

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short review paper will focus especially on magnetic separation and purification of target recombinant fusion proteins using commercially available magnetic affinity particles.

Magnetic techniques for protein separation

In biochemistry and biotechnology the isolation of proteins and peptides is usually performed using variety of chromatography, electrophoretic, ultrafiltration, precipitation and other procedures. Affinity ligand techniques represent currently the most powerful tool available to the downstream processing both in term of their selectivity and recovery. The strength of column affinity chromatography has been shown in thousands of successful applications; however, this technique cannot be used for samples containing particulate material. In this case magnetic affinity, ion-exchange, hydrophobic or adsorption batch separation processes, applications of magnetically stabilized fluidized beds or magnetically modified two-phase systems have shown their usefulness [3].

The basic principle of batch magnetic separation is very simple. Magnetic carriers bearing an immobilized affinity or hydrophobic ligand or ion-exchange groups, or magnetic biopolymer particles having affinity to the isolated structure, are mixed with a sample containing target compound(s). Following an incubation period when the target compound(s) bind to the magnetic particles the whole magnetic complex is easily and rapidly removed from the sample using an appropriate magnetic separator. After washing out the contaminants, the isolated target compound(s) can be eluted and used for further work [3].

Magnetic separation techniques have several advantages in comparison with standard separation procedures. This process is usually very simple, with only a few handling steps. All the steps of the purification procedure can take place in one single test tube or another vessel. The separation process can be performed directly in crude samples containing suspended diamagnetic solid material. Due to the ferromagnetic, ferrimagnetic or superparamagnetic properties of magnetic adsorbents (and diamagnetic properties of majority of the contaminating molecules and particles present in the treated sample), they can be relatively easily and selectively removed from the sample. Magnetic separation is usually very gentle to the target proteins or peptides. Even large protein complexes that tend to be broken up by traditional column chromatography techniques may remain intact when using the very gentle magnetic separation procedure. Magnetic techniques can also be used for protein concentration from diluted solutions [3].

Detailed information about various aspects of magnetic separation of proteins can be found in recent review papers [3,4].

Examples of affinity tags

Up to now, large amount of fusion tags have been constructed. Fusion tags vary in their sizes, sources, and biochemical properties. Depending on their applications and biochemical properties, fusion tags are categorized into a number of groups (which may overlap with each other), including affinity fusion tags, fluorescence protein tags, enzyme tags, protein tags, peptide tags and epitope tags [5]. In this review only tags which can be used for magnetic separation of recombinant fusion proteins with commercially available magnetic particles will be mentioned.

Polyhistidine-tag (His-tag)

The polyhistidine tag (usually 6-His) is one of the most often used affinity tags because it enables purification of the fusion protein by an immobilized metal ion affinity chromatography (IMAC) and analogous magnetic techniques. This procedure is

based on the interaction between a transition metal ion (Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}) immobilized on a matrix and specific amino acid side chains. Histidine is the amino acid that exhibits the strongest interaction with immobilized metal ion matrices, as electron donor groups on the histidine imidazole ring readily form coordination bonds with the immobilized transition metal. Peptides containing sequences of consecutive histidine residues are efficiently retained on immobilized metal ion affinity adsorbents. Following washing of the matrix material, peptides containing polyhistidine sequences can be easily eluted by either adjusting the pH of the buffer or by adding free imidazole. An additional advantage with the small polyhistidine affinity tag is that it can easily be genetically fused to a target gene by PCR techniques [1].

Glutathione S-transferase-tag (GST-tag)

Glutathione S-transferase-tag belongs to macro-molecular tags, with a molecular mass of 26-kDa. Fusion proteins could be purified from crude lysate by affinity techniques using immobilized glutathione. Bound fusion proteins can be eluted with a solution of reduced glutathione under nondenaturing conditions. In the majority of cases, fusion proteins are soluble in aqueous solutions. The GST-tag can be easily detected using an enzyme assay or an immunoassay. The tag can help to protect against intracellular protease cleavage and stabilize the recombinant protein [1].

Strep-tag II

The Strep-tag II is a short peptide (8 amino acids, WSHPQFEK), which binds with high selectivity to Strep-Tactin, an engineered streptavidin. The Strep-tag II allows affinity separation on immobilized Strep-Tactin under physiological conditions, enabling native, active Strep-tagged proteins to be purified in a single step. After a short washing step, gentle elution of purified recombinant protein is performed by addition of low concentrations of biotin or desthiobiotin. Desthiobiotin is a stable, reversibly binding analog of biotin, the natural ligand of streptavidin. Proteins containing the Strep-tag II epitope can be detected with high specificity and sensitivity using Strep-tag antibodies [1].

Polyarginine-tag (Arg-tag)

The Arg-tag usually consists of five or six arginine residues. Arginine is the most basic amino acid and that's why Arg-tagged proteins can be purified by cation exchange chromatography or magnetic batch adsorption. After binding, the tagged proteins are eluted by the increase of ionic strength or change of pH [1].

Chitin-binding domain (CBD)

The chitin-binding domain from *Bacillus circulans* consists of 51 amino acids. The affinity tag is commonly available in combination with self-splicing inteins which enable to separate the target protein from the affinity tag. This tag distinguishes itself from other purification systems by its ability to purify, in a single affinity separation step, a native recombinant protein without the use of a protease. The recombinant fusion protein binds to chitin affinity adsorbent. Self-cleavage of the thioester bond, induced by thiol reagents such as 1,4-dithiothreitol or β-mercapto ethanol, releases the target protein from the tag, still bound on the chitin matrix. A high salt concentration or the use of non-ionic detergents can be employed to reduce non-specific binding, thus increasing purity. The uncleaved fusion precursor and the intein tag remain bound to the chitin resin during target protein elution and can be stripped from the resin by 1% SDS or 6 M guanidine HCl [1].

Maltose-binding protein (MBP)

The 40-kDa maltose-binding protein (MBP) is encoded by the malE gene of *E. coli* K12. Fused proteins can be purified using one-step affinity adsorption process on cross-linked amylose. Bound

fusion proteins can be eluted with 10 mM maltose in physiological buffer. MBP can increase the solubility of overexpressed fusion proteins in bacteria, especially eukaryotic proteins. The MBP-tag can be easily detected using an immunoassay. It is necessary to cleave the tag with a site-specific protease [1].

FLAG-tag

The FLAG-tag system utilizes a short, hydrophilic 8-amino-acid peptide (DYKDDDDK) that is fused to the protein of interest. The FLAG peptide binds to the Anti-FLAG monoclonal antibody. The FLAG-tag can be located at the C- or N-terminus of the protein. The purification condition of the system is non-denaturing and thus allows active fusion proteins to be purified. The complex can be dissociated by chelating agents such as EDTA or by transiently reducing the pH. A disadvantage of the system is that the monoclonal antibody purification matrix is not as stable as others separation systems. Finally, the FLAG-tag can be removed by treatment with enterokinase, which is specific for the five C-terminal amino acids of the peptide sequence [1].

SNAP-tag

SNAP-tag is a 20 kDa mutant of the DNA repair protein O^6-alkylguanine-DNA alkyltransferase that reacts specifically and rapidly with benzylguanine (BG) derivatives, leading to irreversible covalent labeling of the SNAP-tag with a synthetic probe. The rate of the reaction of SNAP-tag with BG derivatives is to a large extent independent of the nature of the synthetic probe attached to BG, permitting the labeling of SNAP fusion proteins with a wide variety of synthetic probes. SNAP-tag has no restrictions with respect to cellular localization and expression host. SNAP-tag substrates are chemically inert towards other proteins, avoiding nonspecific labeling in cellular applications. Many SNAP-tag substrates are cell permeable, permitting labeling of intracellular proteins in live cells. Magnetic particles with immobilized benzylguanine have been used for fusion proteins separation [6].

c-myc-tag

This fusion tag is composed from eleven amino acids (EQKLISEEDL) and can be detected by the murin anti-c-myc antibody 9E10. c-myc-tagged proteins can be affinity-purified using Mab 9E10 coupled to an appropriate matrix. The washing conditions are physiological followed by elution at low pH. It is a widely used detection system but is rarely applied for purifications [1].

GFP-tag (Green fluorescent protein-tag)

Fluorescent proteins such as green fluorescent protein (GFP) can be fused to the protein of interest as a marker for gene expression and protein localization. Applications of fluorescent proteins include visualization and determination of cellular protein localization, identification of protein interactions, and determination of the subunit structure of protein complexes. In addition, the fusion proteins can be separated using particles with immobilized specific antibody [5].

Magnetic separation of recombinant fusion proteins with commercially available magnetic particles

Commercially available magnetic particles for the separation of recombinant fusion proteins can be obtained from a variety of companies. Table 1 shows typical examples of such materials; the list is not complete and new magnetic particles can be offered in the near future. In most cases magnetic microparticles (with the diameters between 1 and 75 μm) are produced, which can be easily magnetically separated using standard magnetic separators for microtubes, standard test tubes or microtitration plates. Alternatively, robotic systems can be used for manipulation of large amount of samples. Small amount of magnetic affinity particles belongs to the

Table 1. Examples of commercially available magnetic particles for the affinity purification of recombinant fusion proteins.

Recombinant tag	Diameter (μm)	Particles composition	Immobilized affinity ligand	Commercial product	Producer/distributor
Arg-tag	1	Magnetic polystyrene microparticles	-	Dynabeads SCX (cation exchanger)	Invitrogen, USA
CBD (Chitin binding domain)	50-70	Magnetic chitin	-	Chitin Magnetic Beads	New England Biolabs, USA
c-myc-tag	0.05	Superparamagnetic Microbeads	Mouse IgG1	μMACS Anti-c-myc Microbeads	Miltenyi Biotec, Germany
FLAG	0.25	Dextran-magnetite	Monoclonal antibody	Anti FLAG M1	Gentaur, Belgium
GST (glutathione S-transferase)	10-25	Crosslinked Agarose 4B with magnetite	Reduced glutathione	GST•Bind Magnetic Agarose Resin	ELPIS-Biotech, Korea
	20-75	Magnetic agarose particles	Reduced glutathione	GST Magbeads	GenScript Corporation, USA
	20-75	Magnetic agarose particles	Mouse Anti-GST mAb	Mouse Anti-GST mAb Magbeads	GenScript Corporation, USA
	1-10	Crosslinked agarose with iron oxide	Reduced glutathione	MagneGST Glutathione Particles	Promega Corporation, USA
	0.05	Superparamagnetic Microbeads	Mouse IgG1	μMACS Anti-GST Microbeads	Miltenyi Biotec, Germany
	1	Maghemite - silica microparticles	Glutathione	SiMAG-Glutathione	Chemicell, Germany
His (6xHis)	10-25	Crosslinked Agarose 4B with magnetite	Iminodiacetic acid (IDA), Ni^{2+} charged	His•Bind Magnetic Agarose Resin	ELPIS-Biotech, Korea
	20-70	Magnetic agarose	Nitrilotriacetic acid (NTA), Ni^{2+} charged	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany
	1	Maghemite-silica	Iminodiacetic acid (IDA), Ni^{2+} charged	SiMAG-IDA/Nickel	chemicell, Germany
	20-70	Magnetic agarose	Nitrilotriacetic acid (NTA), Ni^{2+} charged	PerfectPro Ni-NTA Magbeads	5 Prime GmbH, Germany
	0.05	Superparamagnetic MicroBeads	Mouse IgG2b	μMACS Anti-His Microbeads	Miltenyi Biotec, Germany
	20-75	Magnetic agarose	Quadridentate chelate, Ni^{2+} charged	HIS-Select Magnetic Agarose Beads	Sigma, USA
	2-14	Magnetic silica microparticles	Nitrilotriacetic acid (NTA), Ni^{2+} charged	Magne-His Ni-Particles	Promega, USA
	1.5	Magnetic agarose microparticles	Anti-His monoclonal antibody	THE Anti-His mAb Magbeads	GenScript Corporation, USA
MBP (Maltose binding protein)	10-25	Cross-linked Agarose 4B with magnetite	Amylose	MBP•Bind Magnetic Agarose Resin	ELPIS-Biotech, Korea
	10	Superparamagnetic microparticles	Amylose	Amylose Magnetic Beads	New England Biolabs, USA
	1	Superparamagnetic microparticles	Monoclonal anti-MBP	Anti-MBP Magnetic Beads	New England Biolabs, USA
SNAP	10	Magnetic cellulose	Benzylguanine	SNAP-Capture Magnetic Beads	New England Biolabs, USA
Strep-tag II		Magnetic agarose	Strep-Tactin	Strep-Tactin Magnetic Beads	Qiagen, Germany
	1	Magnetic silica	Strep-Tactin	MagStrep Beads	IBA BioTAGnology, USA

group of magnetic nanoparticles, and specialized equipment (such as High gradient magnetic separators) has to be used for their separation from the suspension [3].

Different types of materials can be used for magnetic particles production, including biopolymers (agarose, dextran, cellulose, chitin), synthetic polymers (polystyrene) and inorganic matrices (silica), to which appropriate affinity ligands are

immobilized. In specific cases the microbead material itself serves as an affinity ligand (e.g., magnetic chitin microparticles for the affinity separation of recombinant fusion proteins with chitin binding domain).

For many magnetic affinity adsorbents the adsorption capacity for the target fusion proteins is given in the corresponding manuals and technical information. In many cases the declared capacity is around 5 to 10 mg of target fusion protein per 1 ml of the sedimented magnetic adsorbent; however, for some adsorbents (e.g., chitin magnetic beads or SNAP-Capture Magnetic Beads) the capacity is around 0.4-0.5 mg/ml.

Table 2 shows a few typical examples of application of magnetic affinity adsorbents for one-step separation of target recombinant fusion proteins. Only application of commercially available magnetic affinity adsorbents is given; of course, in many cases laboratory prepared magnetic particles were used successfully.

Table 2. Examples of recombinant fusion proteins purified with commercially available magnetic affinity particles.

Protein	Fusion tag	Source (native protein)	Expressed in	Magnetic adsorbent	Producer/distributor	Further details	Reference
Cytoplasmic proteins	His	*Shewanella oneidensis*	*Escherichia coli*	MagneHis Ni-Particles	Promega, USA	Automated protein purification	[7]
DST (drought and salt tolerance) transcription factor	MBP	Rice	*Escherichia coli*	Anti-MBP Magnetic Beads	New England Biolabs, USA	Effect on salt and drought tolerance	[8]
Endo-β-1,4-xylanases	His	*Fusarium graminearum*	*Escherichia coli*	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Study of enzymes inhibition	[9]
Endo-1,4-xylanase I	His	*Aspergillus niger*	*Escherichia coli*	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Functional display of endoxylanase on the surface of phage M13	[10]
Endo-1,4-xylanase A	His	*Bacillus subtilis*	*Escherichia coli*	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Functional display of endoxylanase on the surface of phage M13	[10]
Erythrocyte binding antigen-181 (4.1 R binding region)	His	*Plasmodium falciparum*	*Escherichia coli*	HIS-Select Magnetic Agarose Beads	Sigma, USA	Study of molecular interactions	[11]
Esterase	His	*Pseudomonas fluorescens*	*Escherichia coli*	MagneHis Ni particles	Promega, USA	Automated protein purification	[12]
Human peptidases	His	Human	Insect cell lines	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Study of biological function	[13]
M18 aspartyl aminopeptidase	His	*Plasmodium falciparum*	*Escherichia coli*	His-Select Magnetic Agarose Beads	Sigma, USA	Study of interactions with erythrocyte membrane proteins	[14]
Lysophospho-lipase I	His	Rat	*Escherichia coli*	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Enzyme characterization	[15]
Rsc8p	Strep	*Saccharomyces cerevisiae*	*Saccharomyces*	Strep-Tactin Magnetic Beads	Qiagen, Germany	Study of interaction with Htl1p	[16]
Single chain Fv antibody fragments	His	Mice	*Escherichia coli*	Ni-NTA Magnetic Agarose Beads	Qiagen, Germany	Interaction with human cell surface antigen CD13	[17]
Structural domains of the human erythrocyte membrane protein 4.1	GST	Human	*Escherichia coli*	MagneGST Glutathione Particles	Promega, USA	Study of molecular interactions	[11]

CONCLUSIONS

The growth in the use of recombinant proteins has increased greatly in recent years. The advantage of using fusion proteins to facilitate purification and detection of the recombinant proteins is widely recognized. This short review summarizes the basic information about the application of commercially available magnetic affinity and ion exchange particles for this purpose. Currently separations in small scale prevail, anyway, it can be expected that further development will be also focused on larger-scale (industrial) processes. Of course, low cost magnetic affinity adsorbents have to be available to enable further application of this progressive technology.

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