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REVIEW

Exploiting the interactions between poly-histidine fusion tags and immobilized metal ions

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Abstract Immobilized metal affinity chromatography (IMAC) of proteins containing poly-histidine fusion tags is an efficient research tool for purifying recombinant proteins from crude cellular feedstocks at laboratory scale. Nevertheless, to achieve successful purification of large amounts of the target protein for critical therapeutic applications that demand the precise removal of fusion tags, it is important to also take into consideration issues such as protein quality, efficiency, cost effectiveness, and optimal affinity tag choice and design. Despite the many considerations described in this article, it is expected that enhanced selectivity, the primary consideration in the field of protein separation, will continue to see the use of IMAC in solving new purification challenges. In addition, the platform nature of this technology makes it an ideal choice in purifying proteins with unknown properties. Finally, the unique interaction between immobilized metal ions and poly-histidine fusion tag has enabled new developments in the areas of biosensor, immunoassay, and other analytical technologies.

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Introduction of immobilized metal affinity chromatography

Porath et al. (1975) first introduced Immobilized Metal Affinity Chromatography (IMAC) for the fractionation of serum proteins. Ever since, IMAC has become one of the most powerful tools in the field of separation science. IMAC is based on the interaction between transitional metal ions, such as Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, or Fe^{3+}, immobilized on a matrix and specific amino acid side chains in proteins. Transition metal ions can be immobilized on an agarose or silica gel support derivatized by ligands such as iminodiacetic acid (IDA), *N*,*N*,*N*′-tris-(carboxymethyl)-ethylenediamine or nitrilotriacetic acid (NTA) groups as shown by Fig. 1. The main amino acid in proteins that interacts with the immobilized transitional metal ions is histidine. This is due to the fact that the electron donor groups on the histidine imidazole ring form coordination bonds with the immobilized transition metal. The metal chelate ligands have the advantage of being more resistant to physical, chemical or microbiological degradation than other highly selective ligands such as immunoglobulin-binding proteins, which facilitates the use and storage of the immobilized metal affinity adsorbents.

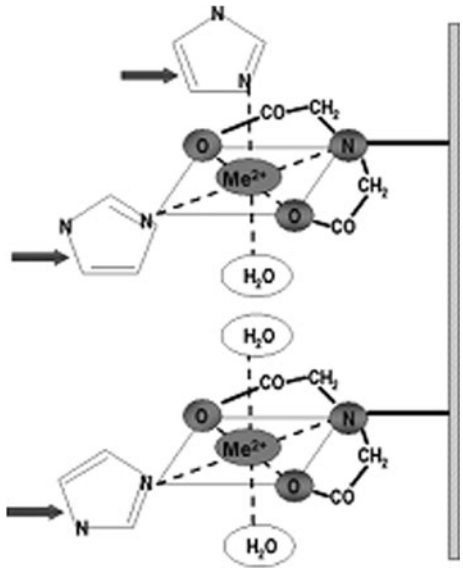

Fig. 1 Adsorption of imidazole groups to immobilized metal ions. In this illustration, structures of octahedral complexes are formed around metal ion, Me^{2+}, bound to the imino-diacetic acid chelating groups immobilized to a surface. *Arrows* point to imidazoles from side chains of histidines (Gaberc-Porekar and Menart 2005)

Coupling IMAC with poly-histidine tag

The fast-developing field of proteomics has promoted the use of recombinant proteins containing a poly-histidine fusion partner to expedite the isolation of the target compound (Bolanos-Garcia and Davies 2006). These fusion tags can be attached to a wide range of different proteins. In addition, the method of tagging enables simple and accurate assay of the recombinant protein during purification. The use of poly-histidine fusion tags was first developed by Hochuli et al. (1988) and has indeed become the most widely used type of affinity fusion tag. Poly-histidine fusion tags range from 2 to 10 residues, with hexa-histidine tags containing six consecutive sequences of histidine residues, having an overall size of 0.84 kDa, being the most popular (Terpe 2003). Peptides containing sequences of consecutive histidine residues are found to be particularly effectively bound to IMAC adsorbents. After washing of the loaded IMAC adsorbent to remove non-specifically adsorbed proteins, these peptides can be easily eluted by either lowering of the pH or by increasing concentrations of imidazole in the irrigating buffers. Following elution, the target protein can exhibit purity higher than 95% (Terpe 2003).

Antibody fragments, labile enzymes, viruses, and membrane-associated targets present a unique set of challenges to the purification researcher that can often be solved by the use of the ubiquitous poly-histidine fusion tag that has gained popularity in purification and structural studies over the last two decades. For instance, attaching a poly-histidine fusion tag to proteins through genetic engineering has proven successful for the IMAC purification of recombinant antibody-like structures (Bannister et al. 2006), complex virus-like particles (Hu et al. 1999), protein complexes (Kubota et al. 2010), and viral vectors for gene therapy (Hu et al. 2003).

IMAC in conjunction with poly-histidine fusion tags can be used as a highly selective primary capture step as target proteins fused with poly-histidine fusion tag can be purified without much prior knowledge of the target protein and consequently, a platform process can be developed that can be employed for a wide range of targets. Besides aiding purification, poly-histidine fusion tags can help increase protein yield (Sun et al. 2005) and prevent proteolysis (Tang et al. 1997). In one example, incorporation of a poly-histidine tag helped to improve rates of cell growth and protein expression levels in cells producing recombinant protein products because of the hydrophilic nature of a poly-histidine tag (Svensson et al. 2006). In fact, with minimum optimization of the poly-histidine tag sequence, most IMAC purification processes are often associated with high yield, fast development time and low costs (Lichty et al. 2005). Likewise, a major advantage of using IMAC as a primary capture tool for protein isolation is the ability to reduce a multistep process to a single unit operation that provides highly pure, good quality end-products. The reduction in the number of processing steps not only decreases process time and costs such as the associated cleanroom residence time for therapeutic production, but also decreases the likelihood of potential protein degradation as a result of prolonged processing times, hence ensuring better product quality. Furthermore, the scalability of the technology makes it an ideal option for large-scale manufacturing.

Other advantages of IMAC include the stability of the metal chelates over a wide range of buffer conditions and temperatures, high metal ion loadings,

and high protein loading capacities (Arnold 1991). With further developments that have increased IMAC column binding capacities and dynamics, IMAC can perform equally well, if not better, than other commonly used affinity chromatographic methods such as immunoglobulin-binding protein chromatography. Besides being cheap and robust, metal chelate ligands can complex a variety of metal ions, thus it is possible to tailor adsorption characteristics to the protein of choice. For instance, SlyD, a major host cell contaminant protein in *E. coli* often co-purifies with the heterologously-expressed hexa-histidine tagged proteins when using nickel-based IMAC (Bolanos-Garcia and Davies 2006). Wulfing et al. (1994), on the other hand, found that SlyD has a markedly reduced affinity for Co^{2+} than Ni^{2+} and the alternative use of the former immobilized ion provides an answer to the problem of SlyD co-purification.

Challenges in the use of IMAC and poly-histidine tag for purification

The addition of poly-histidine fusion tags can sometimes lead to undesirable protein aggregation and insoluble protein expression (Woestenenk et al. 2004). By manipulations of the temperature during expression and dilution refolding, this solubility problem may be able to be solved (Bird et al. 2002). Nevertheless, the length, composition and location of the poly-histidine tag can necessitate additional optimization depending upon the amino acid sequence of the native protein (Chant et al. 2005; Gaberc-Porekar et al. 1999). For instance, the fusion tag can be attached to the *N*-terminus, *C*-terminus or both termini of virtually any protein in order to facilitate its purification by IMAC. The decision to which end the poly-histidine tag is fused depends primarily on the characteristics of the particular protein and the techniques selected to cleave the tag. Some poly-histidine tag termini become buried inside the protein core and others have a significant effect on the protein function. In such cases it is necessary to try attaching the tag to the opposite terminus. Nevertheless, it is generally difficult to predict if there is a negative influence of the tag and which terminal is likely to be least problematic for a given protein (Goel et al. 2000; Lee and Altenberg 2003; Mason et al. 2002). Consequently it is best to perform functional characterization of both *C*-terminal and *N*-terminal tagged protein variants. One investigation suggests that the location of the hexa-histidine tag on the protein *N*-terminus or *C*-terminus has an influence on the binding strength of some proteins to IMAC adsorbents and a tag on both ends of the protein always increases binding strength (Amersham Biosciences 2002). On the other hand, Tsai et al. (2006) concluded that although the capacity and binding affinity of IMAC adsorbents for fusion proteins having more than one affinity tag can, in general, be predicted depending on the size of the protein and the number of affinity tags, the structure-specific interactions between protein molecules and the surface accessibility and orientation of the affinity tags on the protein surface also need to be considered.

Similarly, it is often difficult to exclude the possibility that the affinity tag may interfere with protein activity and function (Wu and Filutowicz 1999), and especially when used in applications such as therapeutic manufacturing, the tag fused to the target protein must be subsequently removed (Kenig et al. 2006; Rowland et al. 2005). Some researchers suggest that the histidine tag does not need to be removed for use in structural determination (Carson et al. 2007). Likewise, Case and Mukhopadhyay (2007) compared two variants of a recombinant human cytosolic phosphoenolpyruvate carboxykinase, one with a His10-tag and one without a poly-histidine tag; both exhibited similar kinetic properties. However, other reports suggest altered biological or physicochemical properties of the histidine tagged proteins as compared to their native counterparts (Arnau et al. 2006; Gaberc-Porekar and Menar 2005). Introducing a poly-histidine tag can adversely affect the biochemical properties (Horchani et al. 2009), alter the binding characteristics (Gaberc-Porekar et al. 1999), change protein structure conformation (Chant et al. 2005), prompt protein oligomerization (Amor-Mahjoub et al. 2006), and thus render the purified target unsuitable for use in X-ray crystallography or other characterization studies (Renzi et al. 2006). Consequently, an important part of the design of a fusion tag is the choice of the method for removing the tag after purification. Ideally, molecular constructs contain a specific cleavage sequence included to ensure that selective cleavage can be achieved. The removal method

usually then involves the use of a protease, to cleave a specific peptide linker between the tag and the target protein. However, the insertion of a cleavage site in a fusion tag can sometimes have a negative impact on the solubility of the expressed proteins (Kurz et al. 2006), and this is an issue that deserves careful consideration. Some of the commonly used cleavage enzymes include the tobacco etch virus (TEV) protease, thrombin (factor IIa, fIIa), enterokinase (EK), and factor Xa (fXa), which all belong to the protease class of endopeptidases. Some of these enzymes have been genetically modified to improve their specificity and stability such as AcTEV from invitrogen and ProTEV from promega.

Ideally, cleavage should be highly specific in order to avoid reducing protein activity and function. Non-specific cleavage can sometimes be resolved by using a lower enzyme to fusion-protein ratio, optimizing the temperature, pH, salt concentration, and the duration of cleavage reaction (Jenny et al. 2003). Most commercially available cleavage enzymes need to recognize a specific amino acid sequence for reaction to occur. For instance, the most common sequence recognized by thrombin is LVPRG or LVPRGS, for enterokinase the sequence is DDDDK, and for factor Xa, it is usually IEGR (Jenny et al. 2003). Nevertheless, cleavage by thrombin, enterokinase or factor Xa often results in unspecific internal cleavage, which is highly undesirable (Jenny et al. 2003; Choi et al. 2001; Ko et al. 1993). Guan and Dixon (1991) found that by inserting five glycine residues between the thrombin cleavage sequence and the *N*-terminal tag, the unspecific internal cleavage decreased. Similarly, TEV recognizes the sequence ENLYFQG or ENLYFQS (Carrington and Dougherty, 1988). Nevertheless, at the completion of the cleavage reaction by TEV, a G or S residue remains on the *N*-terminal end of the target protein (Nallamsetty et al. 2004).

In some cases, the problem of unspecific internal cleavage stems from steric hindrance arising from the target protein structure that prevents the protease from access to the intended cleavage site (Kim et al. 2007; Shahravan et al. 2008). Certain concentrations of denaturant were added in an attempt to improve the specificity and yield of the cleavage reactions (Shahravan et al. 2008). The addition of 1–4 M urea successfully improved the cleavage specificity and yield by making the protein molecules assume a more open structure. Nevertheless, to make this a general approach to improve protease specificity and yield applicable to other proteins, the tolerance of the chosen protease and target protein to denaturant concentration is important. For example, urea at 5 mM began to inhibit enterokinase activity (Shahravan et al. 2008).

Enabling the wider use of IMAC and poly-histidine tags for purification

As mentioned earlier, major issues that arise when using histidine tag approaches to protein purification include the precision and efficiency required to remove the fusion tag at the end of the purification process. Some additional limitations concerning the removal of fusion tags are non-specific cleavages yielding truncated forms of the target protein, addition of extra amino acids, partial removal of the tag, and the presence of contaminating proteases and cleaved peptides contaminating the target protein. Indeed, cleavage by endopeptidases has not been observed to be very specific and was suspected to generate complex mixtures of variants (Gaberc-Porekar and Menart 2005). Furthermore, the use of endopeptidases is costly and, as scales increase, the cost could become prohibitive. In order to address these problems, the selective enzymatic removal of tags using exopeptidases has been studied. Such strategies mainly utilize dipeptide aminopeptidase I (DAPase) to successively cleave the *N*-terminal fusion tag until a dipeptide stop point in the sequence is encountered (Arnau et al. 2006). The precise removal of poly-histidine tags to yield the target protein without any additional residues has been widely proven and further variants of this strategy have been engineered to work with a diversity of sequences (Block et al. 2008; Kenig et al. 2006). The use of exopeptidases provides a convenient solution to the occurrence of non-specific cleavage within the target protein encountered with endopeptidases, avoiding unnecessary destruction of the protein product. In addition, the enzyme-to-substrate ratio required for the use of exopeptidases in comparison to conventional endopeptidase systems is much lower, making the overall process more cost-effective (Arnau et al. 2006).

However, any protease added to achieve the processing of poly-histidine tags must be removed

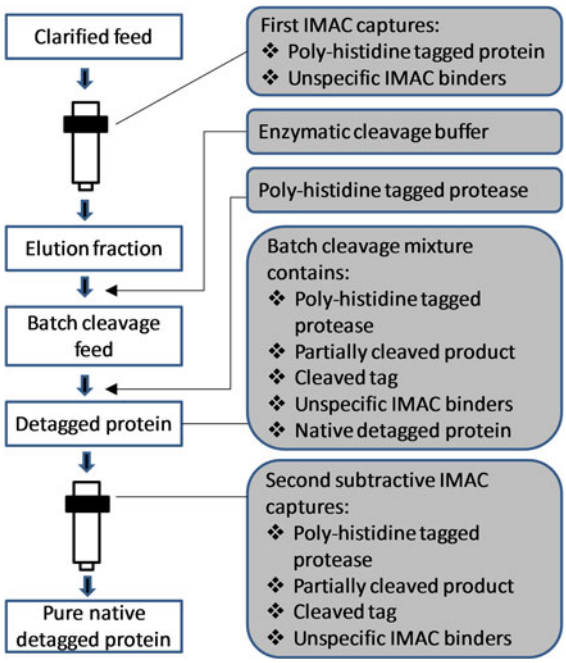

Fig. 2 Typical two-step IMAC purification process involving the use of poly-histidine tagged recombinant proteases

at the end of the cleavage reactions due to the immunogenic nature of these enzymes and other problems arising from their presence contaminating the target protein. In most cases, this involves an additional chromatography step as shown by the downstream process in Fig. 2. The first IMAC column produces purified proteins containing fusion tags and following batch enzymatic digestion, the mixture containing native de-tagged protein and contaminants arising from tagged processing are then separated using a second IMAC column. The latter process is feasible as the proteases used are often genetically-modified such that they themselves contain a hexa-histidine fusion tag, thus allowing the clearance of the enzymes by the second subtractive IMAC column step. Alternatively, it is also possible to immobilize non-tagged proteases onto solid supports allowing their separation from the desired product by simple filtration or centrifugation. Similarly, non-tagged thrombin, fXa, and EK, which are often produced directly from natural sources, can be removed by affinity chromatography using adsorbents such as benzamidine-agarose.

Cleavage efficiency should also be high as this directly affects the extent of recovery of the native target protein (Kenig et al. 2006). The extent of recovery of multimeric proteins can be significantly lower than that of monomeric proteins as the cleavage efficiency achieved with the former proteins has an increased influence on the overall recovery. This is because only one residual partially cleaved subunit is needed to result in adsorption of an entire multimeric protein on the second subtractive IMAC column during the contaminant clearance step shown in Fig. 2 (Schafer et al. 2002).

An apparent inefficiency observed in most downstream processes is the large number of sequential unit operations involved, resulting in poor overall process economics. It is highly desirable to combine the multiple steps involved in the processing of tagged proteins, especially the steps associated with tag removal, and hence intensify multiple unit operations to give a more streamlined process. As an illustration, the potential of adopting on-column detagging strategies is attractive as these strategies are often advantageous due to their ease of integration with purification operations, which leads to improved process economics and yield by reducing the number of separate downstream unit operations. After the capture step from the crude feedstock, the on-column cleavage of the tag from the tagged protein was performed by dispensing the solution of cleavage enzyme into the chromatography column and allowing the cleavage reaction to proceed. In most cases, on-column cleavage strategies have been designed for integration with endopeptidase systems (Bhikhabhai et al. 2005; Dian et al. 2002; Hefti et al. 2001). Nevertheless, on-column cleavage with tagged exopeptidases was also found to be feasible, although some inherent process complexities do exist (Kuo and Chase 2010). The poly-histidine-tagged exopeptidase cannot normally gain access to the *N*-terminal end of the fusion tag on the target protein as both entities are tightly bound to the surface of the IMAC adsorbent. However, as shown in Fig. 3, the introduction of imidazole into the buffer in which the cleavage reaction takes place can induce a dynamic binding situation for the poly-histidine tagged species. The consequent on–off binding characteristics of the poly-histidine tagged target protein then enable the exopeptidase to diffuse between the surface of the IMAC adsorbent and the fusion tag on the target protein, thus allowing the exopeptidase to bind to the *N*-terminal end of the fusion tag and thus allow the

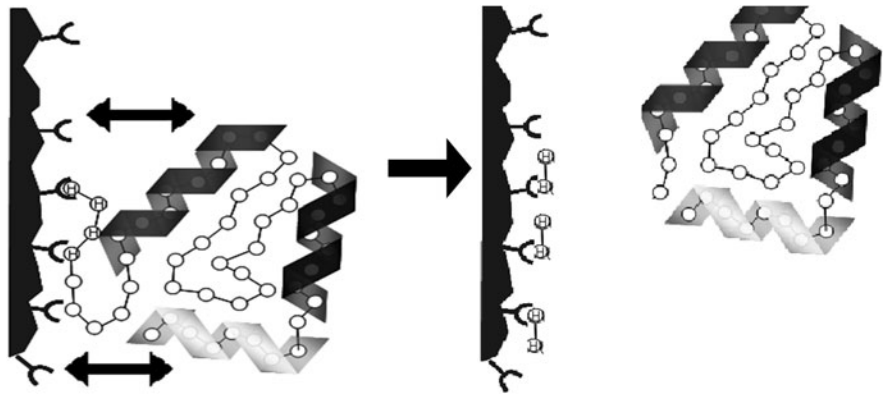

Fig. 3 Schematic of on-column exopeptidase cleavage mechanism: introduction of imidazole induced a dynamic binding situation for the poly-histidine tagged species, which enabled the diffusion of exopeptidase to the *N*-terminal of the target fusion tag, leading to successful cleavage reactions (Kuo and Chase 2010). The cleaved dipeptides then remain bound on the IMAC column, allowing the recovery of the native detagged protein

cleavage reaction to proceed. After incubation in the column, the cleaved, untagged protein can then be recovered simply by washing the column, followed by elution of the cleaved dipeptides and any remaining un-cleaved proteins. This procedure avoids the need for additional steps to ensure separation of tag fragments and detagging enzymes. A further advantage is the ability to recover the protein in a suitable buffer that facilitates the subsequent downstream step. This can reduce the processing time as well as maintain product quality for proteins that are less stable to extensive pH titration and concentration/diafiltration conditions. Likewise, the ability to recover the product in buffers containing no imidazole has also proved to be beneficial as the imidazole normally added to elute poly-histidine tagged proteins from IMAC can affect the subsequent analyses by NMR, interfere in studies involving crystallography as well as increase the likelihood of protein aggregation (Hefti et al. 2001).

Finally, while some general patents regarding the use of poly-histidine fusion tags have expired, there may still be licensing issues associated with the commercial use of fusion tag sequences containing at least two adjacent histidine residues such as the use of the 6 × His tag developed by F. Hoffmann-La Roche Ltd. Another potential difficulty is that when used for the production of pharmaceuticals, Ni^{2+} commonly used in IMAC would be considered a hazard if immobilized nickel ions were leached from the IMAC adsorbent during processing as nickel may produce an allergic reaction. Nevertheless, IMAC using ligands that demonstrate very low levels of leaching of chelated ions such as the tetradentate ligand NTA can be a technology compatible with the requirements of a medicinal manufacture (Block et al. 2008).

Other applications of immobilized metal affinity or poly-histidine tag

Besides exploiting the interaction between metal ions and poly-histidine tags for protein purification, several other applications employ the specific interactions of immobilized metal ions and poly-histidine tags. For example, poly-histidine fusion tags with high affinity to metal ions are also useful tools to immobilize and stabilize peptides or proteins on surfaces (Noji et al. 1997). Proteins fused with poly-histidine tags can become specifically orientated when bound to surfaces derivatized with immobilized nickel with high binding energy and good stability (Johnson and Martin 2005). In the fabrication of biosensors, appropriate orientation of the immobilized immunoreagents is a key step. Vallina-Garcia et al. (2007) have demonstrated the possibilities of electrochemical immunosensing through the orientation of poly-histidine tagged, anti-pneumolysin antibody fragments by nickel-histidine complexation or histidine-anti-histidine-antibody interactions. In both cases, gold films were chosen as the electrode material as they were considered to be ideal solid surface for protein immobilization and electrochemical transduction.

Such electrochemical methods of detection have the advantages of sensitivity, miniaturization potential, and simplicity.

It is also possible to assemble quantum dot bio-conjugates by conjugating proteins to quantum dots via a poly-histidine tag (Goldman et al. 2005). Quantum dots are luminescent semiconductor nanocrystals that have been actively studied due to their potential in overcoming the drawbacks of traditional organic fluorophores. The conjugation of quantum dots to poly-histidine tagged proteins is based on the coordination of histidine residues to zinc metal ions on the surface of cadmium selenide/zinc sulphide nanocrystals. Again, such an approach to conjugation can be generalized to a wide range of proteins through the poly-histidine fusion tag technology. Consequently, adoption of this procedure opens up the prospect of forming quantum dot bio-conjugates for applications such as imaging, immunoassay, and other diagnostics.

The ability of metal ions to chelate with terminal poly-histidine tags in recombinant proteins can be extended to the fabrication of affinity membranes (Ke et al. 2010). Compared to packed-bed columns typically used in protein purification, affinity membranes provide benefits such as no intra-particle diffusion, short axial-diffusion paths, low pressure drops, and no possibility of bed compaction. Alternatively, for analytical applications, Ahmed et al. (2006) coated chitosan, which has nickel-binding properties, onto the surface of polycarbonate track-etched membranes. Recombinant proteins fused with poly-histidine tags were then immobilized to the membrane surface by binding to Ni^{2+} ions adsorbed to the chitosan. The ability of the metal-immobilized proteins to capture their specific antibody was then compared with those immobilized directly onto the membranes using glutaraldehyde reaction chemistry. The histidine-metal immobilization method provided enhanced control of protein orientation and maintained good bioactivity which led to higher capacity, affinity, and sensitivity. Such affinity membranes with high selectivities can increase the sensitivity of multi-membrane blotting methodologies, such as layered expression scanning (LES) for high-throughput analysis of proteomic profiles, as well as conventional blotting when only trace amounts of the target protein is present (Ahmed et al. 2006).

A poly-histidine tag can also be used as an epitope tag for antibody binding. An epitope tag is a short stretch of amino acids that is a part of a molecule, usually a protein, that can be specifically bound by an antibody. Use of a poly-histidine epitope can facilitate the visualization of the target molecule via a gel, Western blot or an immunofluorescence label. Monoclonal and polyclonal antibodies have been raised against poly-histidine fusion tags by immunizing mice or rabbits with poly-histidine tagged fusion proteins (Noedl et al. 2005). Consequently, by adding an epitope tag onto any target protein, a universal method for following the given protein can be established by using a generic assay for detecting the known molecular handle that has been added. Indeed, Huang et al. (2007) studied the binding kinetics between hexa-histidine-tagged insulin-like growth factor-I (IGF-I) and anti-hexa-histidine antibody using surface plasmon resonance (SPR). They concluded that by using an enzyme-labelled anti-hexa-histidine antibody as a tracer, a competitive sandwich ELISA has the potential to be used as a generic immunoassay for the detection of a wide range of polypeptides or proteins tagged with a hexa-histidine sequence.

Finally, IMAC ligands have been used in protein chips for the enrichment of proteins analyzed by surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI–TOF-MS) (Kong et al. 2006). Also as an enrichment method for mass spectrometry, Jalili et al. (2007) derivatized phosphorylated peptides with a poly-histidine tag and later enriched the tagged peptides on a nano-scale IMAC column. The subsequent cleavage of the enriched tagged peptides with factor Xa to release the target peptides imparted even higher selectivity to the process. The eluted peptides were then mapped using mass spectrometry. This approach to the enrichment of phosphopeptides at low concentrations produced better results than those achieved by other, more common enrichment methods. Similarly, Kaur-Atwal et al. (2007) described the potential of interfacing capillary IMAC with mass spectrometric detection. The direct combination of these techniques allowed the integration of on-line isolation of histidine-containing peptides and mass spectrometric detection in a high-throughput manner. IMAC beads with diameters of 20 μm were packed into a 250 μm diameter fused silica column and the resulting capillary IMAC column was used successfully in isolating the histidine-containing peptides in order to simplify complex peptide mass spectrometric analysis.

Conclusions

The technology of exploiting the interactions between poly-histidine fusion tags and immobilized metal ions has proved to be a successful and generic procedure for the isolation of antibody fragments, membrane proteins, and fragile enzymes that are otherwise difficult to purify. Incorporating a fusion tag on the target protein is beneficial as the protein purification method can be chosen without much prior knowledge of the target protein. Such flexibility is also a preferred quality of any downstream purification technology as multiproduct processing facilities are most commonly sought-after today. Furthermore, advances in bio-separation demand more cost-effective methods that have the potential of meeting the most stringent product requirements. For instance, lowering the cost of protein therapeutics has become more important as healthcare funders are increasingly reluctant to pay for expensive biotechnological pharmaceuticals (Aggarwal 2007). In addition, in order to design and manufacture protein therapeutics for sale as bio-generics, new technologies are needed to control production costs and thus achieve lowered pricing. Reducing the costs of manufacture can also result in these therapeutics becoming accessible to third-world nations.

Despite the many advantages of using IMAC and poly-histidine tag in fulfilling these promises, there remain several obstacles that need to be overcome prior to the wider adoption of this technology. The challenge of removing the fusion tag post-purification is an important issue as well as the potential validation steps to show that tags are removed correctly and remnants are not present in the final product. This review has discussed various approaches to overcome these shortcomings. Finally, the ability to prepare a wide range of recombinant proteins fused with poly-histidine tags and to capture these proteins in a highly specific manner are key advantages of the technology, making it generic and highly valuable to any analysis based on the binding of biomarker molecules.

References

Aggarwal S (2007) What's fueling the biotech engine? Nat Biotechnol 25:1097–1104

Ahmed SR, Kelly AB, Barbari TA (2006) Controlling the orientation of immobilized proteins on an affinity membrane through chelation of a histidine tag to a chitosan-Ni^{2+} surface. J Membr Sci 280:553–559

Amersham Biosciences (2002) Purification of (His)6-tagged proteins using HiTrap Chelating HP columns charged with different metal ions. Application Note 18-1145-18 AA: 1–8

Amor-Mahjoub M, Suppini JP, Gomez-Vrielyunck N, Ladjimi M (2006) The effect of the hexahistidine-tag in the oligomerization of HSC70 constructs. J Chromatogr B 844: 328–334

Arnau J, Lauritzen C, Pedersen J (2006) Cloning strategy, production and purification of proteins with exopeptidase-cleavable His-tags. Nat Protoc 1:2326–2333

Arnold FH (1991) Metal-affinity separations—a new dimension in protein processing. Biotechnology 9:151–156

Bannister D, Wilson A, Prowse L, Walsh M, Holgate R, Jermutus L, Wilkinson T (2006) Parallel, high-throughput purification of recombinant antibodies for in vivo cell assays. Biotechnol Bioeng 94:931–937

Bhikhabhai R, Sjoberg A, Hedkvist L, Galin M, Lijedahl P, Frigard T, Pettersson N, Nilsson M, Sigrell-Simon JA, Markeland-Johansson C (2005) Production of milligram quantities of affinity tagged-proteins using automated multistep chromatographic purification. J Chromatogr A 1080:83–92

Bird GH, Lajmi AR, Shin JA (2002) Manipulation of temperature to improve solubility of hydrophobic proteins and cocrystallization with matrix for analysis by MALDI-TOF mass spectrometry. Anal Chem 74:219–225

Block H, Kubicek J, Labahn J, Roth U, Schafer F (2008) Production and comprehensive quality control of recombinant human interleukin-1 beta: a case study for a process development strategy. Protein Express Purif 57:244–254

Bolanos-Garcia VM, Davies OR (2006) Structural analysis and classification of native proteins from *E coli* commonly co-purified by immobilized metal affinity chromatography. BBA Gen Subjects 1760:1304–1313

Bresolin IT, Borsoi-Ribeiro M, Tamashiro WM, Augusto EF, Vijayalakshmi MA, Bueno SM (2010) Evaluation of immobilized metal-ion affinity chromatography (IMAC) as a technique for IgG(1) monoclonal antibodies purification: the effect of chelating ligand and support. Appl Biochem Biotechnol 160:2148–2165

Carrington JC, Dougherty WG (1988) A viral cleavage site cassette—identification of amino-acid sequences required for tobacco etch virus polyprotein processing. P Natl Acad Sci USA 85:3391–3395

Carson M, Johnson DH, McDonald H, Brouillette C, DeLucas LJ (2007) His-tag impact on structure. Acta Crystallogr D 63:295–301

Case CL, Mukhopadhyay B (2007) Kinetic characterization of recombinant human cytosolic phosphoenolpyruvate carboxykinase with and without a His(10)-tag. BBA Gen Subjects 1770:1576–1584

Chant A, Kraemer-Pecore CM, Watkin R, Kneale GG (2005) Attachment of a histidine tag to the minimal zinc finger protein of the *Aspergillus nidulans* gene regulatory protein AreA causes a conformational change at the DNA-binding site. Protein Express Purif 39:152–159

Choi SI, Song HW, Moon JW, Seong BL (2001) Recombinant enterokinase light chain with affinity tag: expression from

Saccharomyces cerevisiae and its utilities in fusion protein technology. Biotechnol Bioeng 75:718–724

Dian C, Eshaghi S, Urbig T, McSweeney S, Heijbel A, Salbert G, Birse D (2002) Strategies for the purification and on-column cleavage of glutathione-*S*-transferase fusion target proteins. J Chromatogr B 769:133–144

Gaberc-Porekar V, Menart V (2005) Potential for using histidine tags in purification of proteins at large scale. Chem Eng Technol 28:1306–1314

Gaberc-Porekar V, Menart V, Jevsevar S, Vidensek A, Stalc A (1999) Histidines in affinity tags and surface clusters for immobilized metal-ion affinity chromatography of trimeric tumor necrosis factor alpha. J Chromatogr A 852:117–128

Goel A, Colcher D, Koo JS, Booth BJM, Pavlinkova G, Batra SK (2000) Relative position of the hexahistidine tag effects binding properties of a tumor-associated single-chain Fv construct. BBA Gen Subjects 1523:13–20

Goldman ER, Medintz IL, Hayhurst A, Anderson GP, Mauro JM, Iverson BL, Georgiou G, Mattoussi H (2005) Self-assembled luminescent CdSe-ZnS quantum dot bioconjugates prepared using engineered poly-histidine terminated proteins. Anal Chim Acta 534:63–67

Guan KL, Dixon JE (1991) Eukaryotic proteins expressed in *Escherichia coli*—an improved thrombin cleavage and purification procedure of fusion proteins with glutathione-S-transferase. Anal Biochem 192:262–267

Hefti MH, Van Vugt-Van der Toorn CJG, Dixon R, Vervoort J (2001) A novel purification method for histidine-tagged proteins containing a thrombin cleavage site. Anal Biochem 295:180–185

Hochuli E, Bannwarth W, Dobeli H, Gentz R, Stuber D (1988) Genetic approach to facilitate purification of recombinant proteins with a novel metal chelate adsorbent. Biotechnology 6:1321–1325

Horchani H, Ouertani S, Gargouri Y, Sayari A (2009) The *N*-terminal His-tag and the recombination process affect the biochemical properties of *Staphylococcus aureus* lipase produced in *Escherichia coli*. J Mol Catal B Enzyme 61:194–201

Hu YC, Bentley WE, Edwards GH, Vakharia VN (1999) Chimeric infectious bursal disease virus-like particles expressed in insect cells and purified by immobilized metal affinity chromatography. Biotechnol Bioeng 63:721–729

Hu YC, Tsai CT, Chung YC, Lu JT, Hsu JTA (2003) Generation of chimeric baculovirus with histidine-tags displayed on the envelope and its purification using immobilized metal affinity chromatography. Enzyme Microb Technol 33:445–452

Huang Y, Shi RN, Zhong XF, Wang D, Zhao MP, Li YZ (2007) Enzyme-linked immunosorbent assays for insulin-like growth factor-I using six-histidine tag fused proteins. Anal Chim Acta 596:116–123

Jalili PR, Sharma D, Ball HL (2007) Enhancement of ionization efficiency and selective enrichment of phosphorylated peptides from complex protein mixtures using a reversible poly-histidine tag. J Am Soc Mass Spectrom 18:1007–1017

Jenny JR, Mann KG, Lundblad RL (2003) A critical review of the methods for cleavage of fusion proteins with thrombin and factor Xa. Protein Express Purif 31:1–11

Johnson DL, Martin LL (2005) Controlling protein orientation at interfaces using histidine tags: an alternative to Ni/NTA. J Am Chem Soc 127:2018–2019

Kaur-Atwal G, Weston DJ, Green PS, Crosland S, Bonner PLR, Creaser CS (2007) On-line capillary column immobilised metal affinity chromatography/electrospray ionisation mass spectrometry for the selective analysis of histidine-containing peptides. J Chromatogr B 857:240–245

Ke YM, Chen CI, Kao PM, Chen HB, Huang HC, Yao CJ, Liu YC (2010) Preparation of the immobilized metal affinity membrane with high amount of metal ions and protein adsorption efficiencies. Process Biochem 45:500–506

Kenig M, Peternel S, Gaberc-Porekar V, Menart V (2006) Influence of the protein oligomericity on final yield after affinity tag removal in purification of recombinant proteins. J Chromatogr A 1101:293–306

Kim SW, Kim JB, Lee WS, Jung WH, Ryu JM, Jang HW, Jo YB, Jung JK, Kim JH (2007) Enhanced protease cleavage efficiency on the glucagon-fused interleukin-2 by the addition of synthetic oligopeptides. Protein Express Purif 55:159–165

Ko YH, Thomas PJ, Delannoy MR, Pedersen PL (1993) The cystic fibrosis transmembrane conductance regulator: Overexpression, purification, and characterization of wild type and delta F508 mutant forms of the first nucleotide binding fold in fusion with the maltose-binding protein. J Biol Chem 268:24330–24338

Kong FB, White CN, Xiao XY, Feng YJ, Xu CJ, He DC, Zhang Z, Yu YH (2006) Using proteomic approaches to identify new biomarkers for detection and monitoring of ovarian cancer. Gynecol Oncol 100:247–253

Kubota H, Sakurai I, Katayama K, Mizusawa N, Ohashi S, Kobayashi M, Zhang PP, Aro EM, Wada H (2010) Purification and characterization of photosystem I complex from *Synechocystis* sp. PCC 6803 by expressing histidine-tagged subunits. BBA Bioenergetics 1797:98–105

Kuo WHK, Chase HA (2010) Process intensification for the removal of poly-histidine fusion tags from recombinant proteins by an exopeptidase. Biotechnol Prog 26:142–149

Kurz M, Cowieson NP, Robin G, Hume DA, Martin JL, Kobe B, Listwan P (2006) Incorporating a TEV cleavage site reduces the solubility of nine recombinant mouse proteins. Protein Express Purif 50:68–73

Lee SH, Altenberg GA (2003) Expression of functional multidrug-resistance protein 1 in *Saccharomyces cereviside*: effects of *N*- and *C*-terminal affinity tags. Biochem Biophys Res Co 306:644–649

Lichty JJ, Malecki JL, Agnew HD, Michelson-Horowitz DJ, Tan S (2005) Comparison of affinity tags for protein purification. Protein Express Purif 41:98–105

Mason AB, He QY, Halbrooks PJ, Everse SJ, Gumerov DR, Kaltashov IA, Smith VC, Hewitt J, MacGillivray RTA (2002) Differential effect of a His tag at the *N*- and *C*-termini: functional studies with recombinant human serum transferrin. Biochem US 41:9448–9454

Nallamsetty S, Kapust RB, Tozser J, Cherry S, Tropea JE, Copeland TD, Waugh DS (2004) Efficient site-specific processing of fusion proteins by tobacco vein mottling virus protease in vivo and in vitro. Protein Express Purif 38:108–115

Noedl H, Bronnert J, Yingyuen K, Herwig BA, Attlmayr B, Kollaritsch H, Fukuda M (2005) Simple histidine-rich protein 2 double-site sandwich enzyme-linked immunosorbent assay for use in malaria drug sensitivity testing. Antimicrob Agents 49:3575–3577

Noji H, Yasuda R, Yoshida M, Kinosita K (1997) Direct observation of the rotation of F-1-ATPase. Nature 386:299–302

Porath J, Carlsson J, Olsson I, Belfrage G (1975) Metal chelate affinity chromatography, a new approach to protein fractionation. Nature 258:598–599

Renzi F, Panetta G, Vallone B, Brunori M, Arceci M, Bozzoni I, Laneve P, Caffarelli E (2006) Large-scale purification and crystallization of the endoribonuclease XendoU: troubleshooting with His-tagged proteins. Acta Crystallogr F 62:298–301

Rowland SS, Mayner RL, Barker L (2005) Advancing TB vaccines to phase I clinical trials in the US: regulatory/manufacturing/licensing issues. Tuberculosis (Edinb) 85:39–46

Shahravan SH, Qu XL, Chan IS, Shin JA (2008) Enhancing the specificity of the enterokinase cleavage reaction to promote efficient cleavage of a fusion tag. Protein Express Purif 59:314–319

Sun QM, Chen LL, Cao L, Fang L, Chen C, Hua ZC (2005) An improved strategy for high-level production of human vasostatin120–180. Biotechnol Prog 21:1048–1052

Svensson J, Andersson C, Reseland JE, Lyngstadaas P, Bulow L (2006) Histidine tag fusion increases expression levels of active recombinant amelogenin in *Escherichia coli*. Protein Express Purif 48:134–141

Tan LH, Kim DS, Yoo IK, Choe WS (2007) Harnessing metal ion affinity for the purification of plasmid DNA. Chem Eng Sci 62:5809–5820

Tang W, Sun ZY, Pannell R, Gurewich V, Liu JN (1997) An efficient system for production of recombinant urokinase-type plasminogen activator. Protein Express Purif 11:279–283

Terpe K (2003) Overview of tag protein fusions: from molecular and biochemical fundamentals to commercial systems. Appl Microbiol Biotechnol 60:523–533

Tsai SY, Lin SC, Suen SY, Hsu WH (2006) Effect of number of poly(His) tags on the adsorption of engineered proteins on immobilized metal affinity chromatography adsorbents. Process Biochem 41:2058–2067

Vallina-Garcia R, Garcia-Suarez MD, Fernandez-Abedul MT, Mendez FJ, Costa-Garcia A (2007) Oriented immobilisation of anti-pneumolysin fab through a histidine tag for electrochemical immunosensors. Biosens Bioelectron 23:210–217

Woestenenk EA, Hammarstrom M, van den Berg S, Hard T, Berglund H (2004) His tag effect on solubility of human proteins produced in *Escherichia coli*: a comparison between four expression vectors. J Struct Funct Genom 5:217–229

Wu J, Filutowicz M (1999) Hexahistidine (His6)-tag dependent protein dimerization: a cautionary tale. Acta Biochim Pol 46:591–599

Wulfing C, Lombardero J, Pluckthun A (1994) An *Escherichia coli* protein consisting of a domain homologous to fk506-binding proteins (fkbp) and a new metal-binding motif. J Biol Chem 269:2895–2901