Biotechnology Advances 32 (2014) 366–381

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Challenges and opportunities in the purification of recombinant tagged proteins

Ana Sofia Pina [a,b], Christopher R. Lowe [c], Ana Cecília A. Roque [a,*]

[a] REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[b] IBET—Instituto de Biologia Experimental Tecnológica, Oeiras, Portugal
[c] Institute of Biotechnology, Department of Chemical Engineering and Biotechnology, University of Cambridge, Tennis Court Road, CB2 1QT Cambridge, UK

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 4 December 2013
Accepted 8 December 2013
Available online 12 December 2013

Keywords:
Affinity tags
Fusion proteins
Recombinant proteins
Affinity ligands

ABSTRACT

The purification of recombinant proteins by affinity chromatography is one of the most efficient strategies due to the high recovery yields and purity achieved. However, this is dependent on the availability of specific affinity adsorbents for each particular target protein. The diversity of proteins to be purified augments the complexity and number of specific affinity adsorbents needed, and therefore generic platforms for the purification of recombinant proteins are appealing strategies. This justifies why genetically encoded affinity tags became so popular for recombinant protein purification, as these systems only require specific ligands for the capture of the fusion protein through a pre-defined affinity tag tail. There is a wide range of available affinity pairs "tag-ligand" combining biological or structural affinity ligands with the respective binding tags. This review gives a general overview of the well-established "tag-ligand" systems available for fusion protein purification and also explores current unconventional strategies under development.

© 2013 Elsevier Inc. All rights reserved.

Contents

1. Introduction . . . 367
2. Purification of recombinant fusion proteins by "tag-ligand" strategies . . . 367
   2.1. Biological ligands as binding partners of affinity tags . . . 367
      2.1.1. Immunoglobulin-based ligands and respective tags . . . 367
      2.1.2. Streptavidin-based ligands and respective tags . . . 371
      2.1.3. Other examples of protein/peptide affinity systems . . . 371
      2.1.4. Carbohydrates as biological ligands for tagged proteins . . . 371
   2.2. Structural ligands as binding partners of affinity tags . . . 371
      2.2.1. Metal chelators as affinity ligands for His-tagged proteins . . . 372
      2.2.2. Ion-exchange adsorbents for tagged protein purification . . . 373
      2.2.3. Covalent affinity chromatography . . . 373
3. Tags with versatile and unconventional properties and respective purification approaches . . . 373
   3.1. Solubility enhancers . . . 373
   3.2. Versatile fusion partners . . . 373
   3.3. Affinity tags for the purification of membrane proteins . . . 374
   3.4. Unconventional fusion partners . . . 374
      3.4.1. Hydrophobic tags . . . 374
      3.4.2. Oil bodies . . . 374
      3.4.3. Elastin-like polypeptides (ELPs) . . . 375
      3.4.4. Hydrophobin . . . 375
      3.4.5. γ-zein (ZERA) . . . 375
4. Alternative schemes for tag removal . . . 375
5. Concluding remarks and future trends . . . 376
Acknowledgements . . . 377
References . . . 377

* Corresponding author. Tel.: +351 212948385; fax: +351 212948550.
E-mail address: cecilia.roque@fct.unl.pt (A.C.A. Roque).

0734-9750/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.biotechadv.2013.12.001

1. Introduction

The wealth of products and methodologies for recombinant protein production and purification has increased enormously in recent years. This has contributed to the growth in the use of recombinant proteins for academic research and therapeutic and diagnostic applications as well as in industrial settings (Demain and Vaishnav, 2009; Palomares et al., 2004). The production and purification of recombinant proteins are intimately linked. The choice of host for protein production affects not only the amplification and isolation of the protein, but also the way in which the product can be subsequently purified. The advances in genetic engineering have increased the availability of large amounts of recombinant proteins produced in host cells – bacterial, mammalian, insect and yeast – and where *Escherichia coli* still represents the most widely used platform (Demain and Vaishnav, 2009).

Chromatography is a well-established platform for protein purification, as it is considered economically feasible and yields high recoveries at high purities with very few process steps (Carta and Jungbauer, 2010; Milne, 2011; Walsh, 2003; Walter and Gottschalk, 2010). In affinity chromatography, selectivity towards a specific target protein is introduced through the chemical functionalization of the solid support with desired affinity ligands, which can be divided into three main categories: biological, structural and synthetic (Fig. 1(A)) (Roque and Lowe, 2007). Synthetic affinity ligands have been developed in an attempt to overcome disadvantages of natural and structural ligands, by combining the best of two worlds: Molecular recognition features associated with high resistance to chemical and biological degradation and high scalability as well as low production costs and low toxicity (Clonis et al., 2000; Lowe, 2001; Lowe et al., 2001). These have been tailor-made for the purification of specific biomolecules as antibodies (Haigh et al., 2009; Qian et al., 2012; Roque et al., 2005) although they are not regarded as universal purification adsorbents for fusion proteins, and therefore will not be widely discussed in this review.

The diversity of proteins and their biochemical properties makes the development of universal purification and capturing strategies difficult. Most proteins of interest lack a suitable, specific and robust affinity ligand for capture on a solid matrix. Genetically encoded affinity tags are a viable and common option for the purification of recombinant proteins and also represent important tools for structural and functional proteomics initiatives. This approach requires the existence and availability of specific ligands for the capture of the fusion protein through an encoded affinity tag tail (Fig. 2), which can be denominated as affinity “tag-ligand” pairs. Currently available affinity “tag-ligand pairs” fall within one of these categories: Protein–protein, protein–small biological ligands, peptide–protein or peptide–metal chelating ligands.

Affinity tags display different size ranges from a single amino acid to entire proteins, and can be genetically fused to the N- or C-terminal of the target biomolecule (Arnau et al., 2006; Hedhammar et al., 2005; Waugh, 2005; Young et al., 2012). Apart from facilitating the purification process, affinity tags can also enhance protein solubility and stability, increase expression levels (Hu et al., 2001; Walls and Loughran, 2011) and allow labelling for cellular localization and imaging studies (Malhotra, 2009). An overview of the main advantages and disadvantages associated with different affinity tags has been already thoroughly discussed in the literature (Arnau et al., 2006; Hearn and Acosta, 2001; Hedhammar et al., 2005; Hu et al., 2001; Malhotra, 2009; Terpe, 2003; Walls and Loughran, 2011; Waugh, 2005; Young et al., 2012). In general, shorter affinity tags (peptides) are more attractive as they are less likely to interfere with the expression, structure and function of the target protein, and their removal can be exempt, decreasing thus the overall costs of the purification process (Hearn and Acosta, 2001; Hedhammar et al., 2005; Terpe, 2003).

This review is a comprehensive overview of the well-implemented “tag-ligand” pairs used for the purification of recombinant fusion proteins with a detailed discussion of the lesser known tags with unconventional properties currently under development.

2. Purification of recombinant fusion proteins by “tag-ligand” strategies

2.1. Biological ligands as binding partners of affinity tags

Biological ligands comprise biomolecules obtained from natural sources and from *in vitro* selection techniques. These are usually associated with high selectivity and affinity for the target, but also with high costs of production and purification, poor stabilization under SIP (sterilization-in-place) and CIP (cleaning-in-place) conditions, and potential leakage and end-product contamination (Clonis et al., 2000; Lowe, 2001; Roque and Lowe, 2006). Examples of common biological affinity ligands include immunoglobulins against a target protein (antigen), bacterial immunoglobulin-binding domains such as Staphylococcal proteins A, G and L (Björck and Kronvall, 1984; Duhamel et al., 1979; Füglistaller, 1989; Lindmark et al., 1983; Nilson et al., 1993), and natural lectins targeting glycoproteins (Vretblad, 1976). Novel biological affinity ligands can be obtained through *in vitro* selection techniques such as phage, ribosome or yeast display (Smith and Petrenko, 1997), with phage display being very popular for these purposes.

A general overview of the biological ligands and respective tags employed in the purification of tagged recombinant proteins is given in Table 1 and Fig. 1(B). Biological ligands include peptides, proteins and carbohydrates.

2.1.1. Immunoglobulin-based ligands and respective tags

Immunoglobulin-based adsorbents for affinity chromatography are usually very selective for the target proteins but the costs associated tend to be high. Also, as the interaction between ligand and tag is often strong, elution traditionally involves drastic conditions, typically extremes of pH.

The first affinity “tag-ligand” pair was based on the intrinsic selectivity and affinity between the bacterial immunoglobulin-binding domain Staphylococcal protein A (SpA) and the Fc region of mammalian IgG (Nilsson and Abrahmsén, 1990). As an example, alkaline phosphatase was fused to SpA and its purification was performed in a single step using an IgG adsorbent, with elution at acidic pH (Nilsson et al., 1985). It is known that SpA presents five homologous domains (E, D, A, B and C) and that IgG binds preferentially to the B domain (Nilsson and Abrahmsén, 1990). This domain was mutated to improve its resistance towards tag removal by chemical methods and denominated as the Z domain (Nilsson et al., 1987). The bacterial immunoglobulin-binding domain staphylococcal protein G (SpG) has been also studied as a fusion partner due to its bifunctional behaviour – SpG is composed of different domains (A, B, C and D) with affinity for both IgG and human serum albumin (HSA) (Akerström et al., 1987; Nygren et al., 1988) – allowing the purification of SpG tagged proteins through HSA and IgG affinity chromatography (Akerström et al., 1987; Hedhammar et al., 2005; Nilsson et al., 1997; Nygren et al., 1988).

Other affinity tags recognizing immunoglobulin-based adsorbents include the peptide epitopes FLAG, c-myc, T7, hemagglutinin antigen (HA) and Softags (Hedhammar et al., 2005; Young et al., 2012). The c-myc is a product of a proto-oncogene whose epitope presents high affinity for the monoclonal antibody 9E10 (Evan et al., 1985). This affinity pair has been mostly used as a tool for the detection of recombinant proteins through immunoblotting assays rather than for purification processes (Evan et al., 1985; Kipriyanov et al., 1996; Terpe, 2003). The same is observed for the affinity tags T7 and HA (Walls and Loughran, 2011; Young et al., 2012). The T7-tag is a leader peptide of phage T7 with eleven amino acids with affinity for the anti-T7 monoclonal antibody (Jarvik and Telmer, 1998; Studier and Moffatt, 1986), and the HA tag is a peptide epitope of the influenza virus hemagglutinin (Wilson et al., 1984) recognized by the monoclonal antibody 12 CA5 (Field et al., 1988; Foreman and Davis, 1994).

The FLAG tag technology is quite popular for purification purposes, and has been successfully employed on the purification of several

Download English Version:

https://daneshyari.com/en/article/10231614

Download Persian Version:

https://daneshyari.com/article/10231614

Daneshyari.com