Trends in Analytical Chemistry 65 (2015) 73–82

Contents lists available at ScienceDirect

Trends in Analytical Chemistry

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

Analytical applications of affibodies

Celine I.L. Justino [a,b,*], Armando C. Duarte [a], Teresa A.P. Rocha-Santos [a]

[a] Department of Chemistry & CESAM, University of Aveiro, Campus de Santiago, 3810-193 Aveiro, Portugal
[b] ISEIT/Viseu, Instituto Piaget, Estrada do Alto do Gaio, Galifonge, Lordosa, Viseu 3515-776, Portugal

ARTICLE INFO

Keywords:
Affibody
Affinity
Bioassay
Biological receptor
Biosensor
Diagnostics
Molecular imaging
Phage-display technology
Protein scaffold
Therapeutics

ABSTRACT

Affibodies are a new class of engineered affinity proteins widely used in imaging, diagnostics and therapeutics, due to their improved properties, such as small size, robustness, high stability, and high imaging contrast compared to the best known affinity molecules (i.e., antibodies). Affibodies can also be used as biological receptors in bioassays and their incorporation in biosensors constitutes a research field of high potential at its very beginning. This review provides an up-to-date overview of the analytical applications of affibodies, and their isolation and characteristics. We also address trends in the future outlook on using affibodies in research.

Contents

1. Introduction

The detection and the determination of specific proteins in living organisms allow the measurement of some physiological conditions, thus becoming an important tool in clinical diagnostics. The affibody molecules constitute a new class of engineered affinity proteins, which have considerable affinity for and specificity to any target protein or peptide after their isolation.

The three-helical scaffold domain of affibodies has been suggested as being responsible for the high molecular affinity, which is crucial to the various applications associated with such molecular structures. For example, therapeutics, *in vitro* and *in vivo* diagnostics, and biotechnological applications, such as incorporation in microarrays and affinity chromatography columns, have been associated with affibody molecules [1].

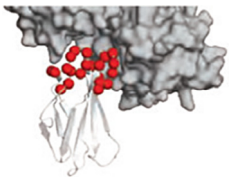
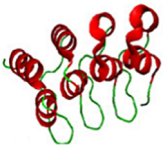
Unlike antibodies, affibody molecules are composed of alpha helices and lack the disulfide bonds enabling intracellular applications. Until now, antibodies were the best-known affinity proteins successfully used in applications of science (e.g., biotechnological and medical applications), due to the high affinity associated with the immune system. However, some limitations could be identified in antibodies, since only a small part of the molecule is used in recognizing antigens.

Engineered affinity proteins have progressively replaced antibodies, which are large, bivalent, multi-domain proteins, dependent on disulfide bonds, leading to poor thermal stability and expensive manufacture [1]. However, antibodies are known for their strong binding and high selectivity. Thus, the affibodies have been

* Corresponding author. Tel.: +351 232 910 100; Fax: +351 232 910 183.
E-mail address: celinejustino@ua.pt (C.I.L. Justino).

http://dx.doi.org/10.1016/j.trac.2014.10.014
0165-9936/

Table 1
Characteristics and properties of affinity proteins

Affinity proteins	Characteristics / properties	Figure / Copyright
Monobodies or adnectins	- β-sandwich structure connected by six loops - single-domain structure - 94 aa - absence of disulfide bonds - absence of cysteines - affinity approximately of 1 pM comparable to that of antibodies	 Monobody in interaction with maltose (the higher molecule); the circles are the binding sites. {Reprinted from [6], ©2012, with permission from Elsevier}.
Anticalins	- stable β-barrel structure with four loops - 170 aa with a fold normally stabilized with disulfide bonds	 {Reprinted from [3], with kind permission from Springer Science and Business Media}.
Affibodies	- three helical structure where 13 aa positions on helices one and two are randomized to create diversity - 58 aa - single domain structure - absence of cysteines - structural and thermal stability	
DARPins (i.e., designed ankyrin repeat proteins)	- three repeated β-turns and 33 aa in each ankyrin proteins - absence of cysteines	
Nanobodies	- derived from the single variable domain of camelidae antibody (absence of light chains) - 7-β sheets forming a sandwich structure - properties comparable to antibody but with low immunogenicity	

considered new, viable alternative affinity proteins, belonging to the class of scaffold proteins, imitating monoclonal antibodies, but with improved properties. Such scaffold proteins are robust supporting structures with a spatially defined surface area, where amino acids can be modified [2]. Besides that, scaffold proteins are powerful for targeting various cell-signal receptors and cancer-related molecules {i.e., they can be used for the development of *in vivo* molecular probes, as reviewed by Miao et al. [3]}.

Affibody molecules directed to antigens associated with tumors have been studied most [e.g., to human epidermal growth factor receptor 2 (HER2), a cancer-specific cell-surface receptor and best studied as an imaging agent]. In this field, Baum et al. [4] synthesized the first affibody molecule to be administered to humans with recurrent breast cancer, reporting the first clinical data on tumor imaging using HER2-binding affibody molecules.

Recently, clinical trials of specific affibodies investigated distribution, dosimetry, and efficacy for determining HER2 status in metastatic breast cancer, through imaging technologies based on emission tomography [5]. Sörensen et al. [5] found that the affibody complex was well tolerated without drug-related adverse agents in the detection of several lesions considered likely to be metastases, thus allowing the selection of patients who might benefit from HER2-targeted therapies, so improving utility and cost effectiveness.

Affibodies are engineered proteins of great interest in life sciences, since therapeutic, diagnostic imaging, and biotechnological applications have been developed, as reported in this review paper. In the past decade, in addition to the affibodies, other affinity proteins, such as adnectins, anticalins, and DARPins were synthesized but there is little information about their analytical applications, as they are mainly directed to diagnostic imaging or therapeutical concerns due to their general high affinity and specificity to therapeutically relevant targets.

Table 1 compares the structural characteristics and the properties of such affinity proteins, according to recent literature [1,3,6–8]. Table 1 includes illustrations of some affinity proteins in order to compare their molecular structures.

The main objective of this review paper is to identify the main analytical applications commonly associated with the affibody molecules, giving special attention to the future outlook for applications of affibodies in various areas that are just beginning (i.e., incorporation of affibodies in bioassays and biosensors). We also address the isolation and the characteristics of affibodies.

2. Isolation and characteristics of engineered affibody molecules

2.1. Isolation of affibody molecules

The isolation of affibody molecules is based on non-immunoglobulin scaffolds using synthetic combinatorial libraries and selection systems, mainly by phage-display technology. The selection of affibodies from the combinatorial libraries is derived from the alpha-helical receptor domain of protein A (Z-domain) of *Staphylococcus aureus* [9]. This Z-domain is used as a template scaffold for production of affibodies. The libraries are obtained by combinatorial genetic randomization of 13 amino-acid surface-located