Biotechnology Advances 34 (2016) 77–87

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Developments in the production of mucosal antibodies in plants

Nikolay Vasilev [a], C. Mark Smales [b], Stefan Schillberg [a], Rainer Fischer [a,c], Andreas Schiermeyer [a,*]

[a] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Department of Plant Biotechnology, Forckenbeckstrasse 6, 52074 Aachen, Germany
[b] School of Biosciences, University of Kent, CT2 7NJ Kent, UK
[c] RWTH Aachen University, Institute for Molecular Biotechnology, Worringerweg 1, 52074 Aachen, Germany

ARTICLE INFO

Article history:
Received 2 September 2015
Received in revised form 17 November 2015
Accepted 24 November 2015
Available online 25 November 2015

Keywords:
Biopharmaceuticals
Immunoglobulin A
Immunoglobulin M
Plant glycoengineering
Plant-made recombinant proteins
Secretory antibodies
Transgenic plants

ABSTRACT

Recombinant mucosal antibodies represent attractive target molecules for the development of next generation biopharmaceuticals for passive immunization against various infectious diseases and treatment of patients suffering from mucosal antibody deficiencies. As these polymeric antibodies require complex post-translational modifications and correct subunit assembly, they are considered as difficult-to-produce recombinant proteins. Beside the traditional, mammalian-based production platforms, plants are emerging as alternative expression hosts for this type of complex macromolecule. Plant cells are able to produce high-quality mucosal antibodies as shown by the successful expression of the secretory immunoglobulins A (IgA) and M (IgM) in various antibody formats in different plant species including tobacco and its close relative *Nicotiana benthamiana*, maize, tomato and *Arabidopsis thaliana*. Importantly for biotherapeutic application, transgenic plants are capable of synthesizing functional IgA and IgM molecules with biological activity and safety profiles comparable with their native mammalian counterparts. This article reviews the structure and function of mucosal IgA and IgM antibodies and summarizes the current knowledge of their production and processing in plant host systems. Specific emphasis is given to consideration of intracellular transport processes as these affect assembly of the mature immunoglobulins, their secretion rates, proteolysis/degradation and glycosylation patterns. Furthermore, this review provides an outline of glycoengineering efforts that have been undertaken so far to produce antibodies with homogenous human-like glycan decoration. We believe that the continued development of our understanding of the plant cellular machinery related to the heterologous expression of immunoglobulins will further improve the production levels, quality and control of post-translational modifications that are 'human-like' from plant systems and enhance the prospects for the regulatory approval of such molecules leading to the commercial exploitation of plant-derived mucosal antibodies.

Contents

Abbreviations: Ara, arabinose; CDR, complementary-determining region; CHO cells, Chinese hamster ovary cells; CMP-Neu5Ac, cytidine 5′-monophosphate *N*-acetylneuraminic acid; CRISPR/Cas9, clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR-associated protein 9 (Cas9) system; DC, dendritic cell; ΔXT/FT, a mutant plant line lacking β1,2-xylosyltransferases and α1,3-fucosyltransferases; dIgA, dimeric IgA; ER, endoplasmic reticulum; ETEC, enterotoxigenic *Escherichia coli*; Fab′, fragment antigen-binding; Fc region, fragment crystallizable region; FcRL, Fc receptor-like proteins; FR1, framework region 1; fwt, fresh weight; Gal, galactose; Gb3, globotriaosylceramide receptor; GlcNAc, *N*-acetylglucosamine; GnTII, *N*-acetylglucosaminyltransferase II; GS, *N*-glycosylation site; HC, heavy chain; HRGP, hydroxyproline-rich glycoproteins; Hyp, hydroxyproline; Ig, immunoglobulin; IgAN, IgA nephropathy; IL, interleukin; J-chain, joining chain; LC, light chain; MALDI MS, matrix-assisted laser desorption/ionization mass spectrometry; pIgR, polymeric immunoglobulin receptor; Pro, proline; PSV, protein storage vacuoles; PTMs, post-translational modifications; SC, secretory component; sIgA, secretory immunoglobulin A; sIgM, secretory immunoglobulin M; SIGNR1 receptor, specific intracellular adhesion molecule-grabbing non-integrin receptor; Stx1, Shiga toxin 1; TALENs, transcription activator-like endonucleases; Thr, threonine; TNF, tumor necrosis factors; TSP, total soluble protein; VHH, variable domains of heavy chain-only antibodies; ZFNs, zinc-finger nucleases.

* Corresponding author at: Fraunhofer IME, Forckenbeckstrasse 6, 52074 Aachen, Germany.
E-mail address: andreas.schiermeyer@ime.fraunhofer.de (A. Schiermeyer).

http://dx.doi.org/10.1016/j.biotechadv.2015.11.002
0734-9750/

1. Introduction

Plant expression systems are emerging as an attractive platform for the production of recombinant pharmaceutical proteins including enzymes, vaccines and antibodies (Fischer et al. 2013; Fischer et al. 2015; Schiermeyer and Schillberg 2012). The cost and difficulties of manufacturing some of the new biotherapeutic molecules in development are among the main driving forces for the increased acceptance of transgenic plants as production hosts of valuable and complex therapeutic proteins (Schiermeyer and Schillberg 2010). This is largely due to the fact that plants can be cultivated at large scale and low cost in the field or in the greenhouse. Although here we review the use of plant systems for the production of IgA and IgM molecules, plant cell culture systems are also now well established whereby cells can be cultured in simple, chemically defined media at a scale to produce recombinant material as an alternative to whole plant systems (Schillberg et al. 2013).

Two molecules whose expression has been investigated in plant systems and that have potential commercial biopharmaceutical applications are IgA and IgM antibodies. IgA and IgM are considered difficult-to-produce glycoproteins as recombinants (as opposed to IgG molecules where technology for their expression is well established), because they require complex post-translational modifications (PTM) and subunit assembly.

IgA and IgM belong to the group of multimeric antibodies. IgA is the most abundant antibody class in humans in terms of the biosynthesis rate. The estimated biosynthetic rate of IgA is 66 mg/kg body weight per day, compared with 34 mg/kg/day and 7.9 mg/kg/day for IgG and IgM, respectively (Manz et al. 2005). However, IgG is the predominant class in serum, making up to 85% of total serum immunoglobulins, followed by monomeric IgA constituting 7–15% of the total and (mainly) pentameric IgM which constitutes approximately 5% of the total immunoglobulins (Manz et al. 2005).

The high cumulative biosynthetic rates of IgA and IgM are explained by the large surface of mucosae where both antibody classes dominate. The mucosal surface comprises a vast area of approximately 400 m^2 (compared with 1.8 m^2 for skin) and represents the major site of attack by invading pathogens (Childers et al. 1989; Woof and Kerr 2006). While the mucosal linings producing IgAs and IgMs provide a physical barrier against infection, additional protection is provided by the mucosal immune system. IgA and IgM play an essential role in the first-line defense at the mucosal surfaces of the gastrointestinal, uro-genital and respiratory tracts and also in the fluids of tears, saliva and milk (Bakema and van Egmond 2011b; Norderhaug et al. 1999). The growing knowledge around the previously neglected polymeric IgA and IgM antibodies as potential biotherapeutics has opened up the possibility of developing these for applications such as mucosal vaccination, treatment of congenital disorders in the mucosal defense and design of a next generation of improved immunotherapeutics (Chintalacharuvu and Morrison 1999; Corthésy 2002; Corthésy 2003; Longet et al. 2013).

Recombinant IgAs have now been produced successfully in several expression platforms including mammalian, plant and insect cells and transgenic animals (Yoo et al. 2007). The yields from these systems remain low (mg's/L), largely due to their complex assembly and PTM requirements. Plants have been considered as an economical and safe system for production of secretory antibodies due to their scale-up potential and the lack of contaminating mammalian viruses or prions (Chargelegue et al. 2004; Wycoff 2005). An IgM antibody was recently produced for the first time in plants, 20 years after the successful expression of sIgA antibodies in transgenic plants (Loos et al. 2014; Ma et al. 1994). Here, we summarize the advancements in the expression of mucosal antibodies by plant-based systems over this period of time. Specifically, various characteristics of IgA and IgM molecules and their heterogeneous expression are reviewed here, with a particular focus on the expression and assembly, biological activity, intracellular trafficking and glycosylation of both these mucosal antibodies in plants.

2. IgA and IgM antibodies

2.1. Structure and classification of IgA and IgM antibodies

The basic monomer units of IgA (~160 kDa) and IgM (~180 kDa), in common with antibodies from other classes of immunoglobulins (Igs), consist of two paired heavy chains (α- and μ-chain for IgA and IgM, respectively) and two light (κ- or λ-) chains, each linked to one heavy chain (Fig. 1). The nature of these linkages is discussed in more detail below. The monomeric structures are arranged into two Fab regions (responsible for antigen recognition) and one Fc region, which mediates interactions with receptors and effector molecules (Woof and Russell 2011). The Fab arms are associated through a hinge region with the Fc region in IgA and IgM (Fig. 1A). IgA exists predominantly in serum as a monomer, whereas at the mucosal surfaces IgA is present mainly as dimeric (Fig. 1B) or polymeric macromolecule forms (Fig. 1C). IgM exists in pentameric or hexameric forms (Fig. 1D) in the secretions and blood circulation and in a monomeric form (Fig. 1A) as antigen receptor on B-lymphocytes (Reth 1992; Woof and Kerr 2006; Woof and Mestecky 2005).

Each IgA α-chain consists of four domains (starting from the N-terminus: Vα (variable domain), Cα1, Cα2 and Cα3 (constant domains)) while the IgM μ-chain comprises five domains (from N-terminus: Vμ (variable domain), Cμ1, Cμ2, Cμ3, and Cμ4 (constant domains)); each light chain (LC) contains two domains, the VL (variable light) and CL (constant light) domains (Arnold et al. 2005; Woof and Russell 2011). There are two subclasses of IgA in humans, IgA1 and IgA2, which differ in respect to the hinge region that separates the Cα1 and Cα2 domains and the glycosylation patterns. IgA1 possesses a hinge region which is 13 amino acids longer than that found in IgA2 molecules and this hinge insertion provides a flexible stretch and thus the potential for interactions with more distant antigens (Woof and Kerr 2006). The hinge region is rich in amino acid Pro, Ser and Thr residues, resulting in the decoration of this region with three to five, or occasionally six, *O*-linked oligosaccharides in IgA1 molecules (Tarelli et al. 2004). IgA1 predominates in human serum and airways, while IgA2 is