FEBS Letters 346 (1994) 123–126

FEBS 13928

Minireview

Biosynthetic lipid-tagging of antibodies

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Received 18 March 1994

Abstract

Chemical conjugation of fatty acids to antibodies generates lipid-modified molecules which have found use in the targeting of liposome-mediated drug delivery and in liposome-based immunoassays. Alternatively, bacterial expression of antibodies as single-chain Fv fragments fused to lipoprotein signal peptide and N-terminal sequence leads to in vivo enzymatic addition of a single glycerolipid group at the N-terminus of the molecule. This lipid-modification converts the antibody from a soluble protein into a functional membrane-bound molecule. These biosynthetically lipid-tagged antibodies may prove useful for immobilization of antibodies to membranes in various biotechnological applications.

Key words: Immunoliposome; Antibody engineering; *E. coli* lipoprotein; Lipid modification

1. Introduction

In several biotechnological applications, e.g. in the targeting of liposomal drug delivery [1–4], in liposome-based immunoassays [5] and in the design of biomimetic sensors [6,7] lipid-modified antibody molecules have been immobilized on planar and liposomal phospholipid membranes. Chemical in vitro conjugation to fatty acids or other lipid moieties is used to convert normally soluble antibodies into a form which can be inserted into the membrane. In addition to endowing antibodies with membrane anchors, chemical lipid modification of proteins has been used to increase immunogenicity of peptides and proteins in the design of improved vaccines [8–10]. Furthermore, 'hydrophobization' by chemical conjugation to lipids has been used to increase the membrane permeation [11] and stability [12] of proteins. The chemical derivatizations most commonly involve direct coupling of fatty acyl groups to appropriately exposed sulphhydryl and amino groups in the protein molecule [13,14]. Subjecting antibodies and other polypeptides to this treatment may lead to loss or decrease in the activity of the labeled molecule due to the denaturation or through indirect effects the fatty acid moieties impart on the function of the molecule. In chemical coupling procedures the product often shows heterogeneity in terms of number and location of lipid moieties. Biotin–avidin technology has been used to link biotinylated antibodies to biotinylated liposomes via an avidin (or streptavidin) linker [15]. The use of this approach, however, also requires chemical derivatization of the antibody (i.e. biotinylation) and the presence of an additional protein layer of (strept)avidin may limit the general usefulness of this approach.

Despite the above-mentioned limitations of the chemical lipid-modification, the ability of membrane-anchored antibodies to provide liposomes with binding specificity for delivery of liposome-encased drugs, DNA and imaging agents and for the use of immobilized liposomes in diagnostic applications has been demonstrated [1–5]. Here we discuss the biochemical background and future prospects for an improved methodology for the production of lipid-tagged antibodies by use of genetic engineering.

2. In vivo lipid-tagging of bacterial lipoproteins

Bacterial lipoproteins are a diverse group of proteins which share in common a particular lipid modification at the N-terminal cysteine residue [16]. The covalently linked lipid moiety consists of two fatty acids linked by ester bonds and one fatty acid linked by an amide bond to glycerylcysteinyl [17] (see Fig. 1b). This lipid group is very hydrophobic and anchors the protein to the membrane [18,19]. The details of the biosynthesis of lipoproteins have been elucidated with the prototype lipoprotein, the lpp lipoprotein of *Escherichia coli* [18–21]. Lpp is first synthesized as a signal peptide-containing precursor to which a diacylglyceride is added at the cysteine residue which later will become the N-terminus of the mature lipoprotein. The signal peptide is cleaved off by a lipoprotein-specific signal peptidase whereafter the newly exposed amino group is acylated and the mature Lpp is translocated to the outer membrane [20]. The molecular determinants responsible for the lipid modification and targeting of Lpp have been localized to the signal peptide and a short amino-terminal stretch of Lpp

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SSDI 0014-5793(94)00313-K

[18]. This was demonstrated by the lipid modification, correct processing and transport to the outer membrane of a hybrid protein, lipo-β-lactamase containing the signal peptide and nine N-terminal amino acids of the mature Lpp polypeptide fused to a soluble periplasmic protein, β-lactamase [18]. Importantly, the amino-terminal sequence of lipo-β-lactamase features no obvious hydrophobic character and it appears that the lipid moiety alone tethers the hybrid protein to the inner leaflet of the outer membrane [18,22].

Interestingly, the sorting of Lpp to the outer membrane (as opposed to inner membrane) is strongly affected by the identity of the second amino acid residue, next to lipid-modified cysteine as introduction of negatively-charged aspartate (but not of any other amino acid) leads to inner membrane localization [19].

3. Genetically lipid-modified antibodies

The deciphering of the molecular details of bacterial lipoprotein processing has set the stage for the rational design and bacterial expression of lipid-tagged proteins of biotechnological interest. To date, single-chain antibodies [23] and peptide antigens [24] have been produced using this approach. In principle any soluble protein which can be expressed as functional in *E. coli* can be produced as a lipoprotein fusion. Minimally these fusion proteins contain the lipoprotein signal peptide and only a few amino acids derived from lpp or other bacterial lipoprotein (see Fig. 1). The product will carry an amino-terminal cysteine residue decorated by the glycerolipid moiety, which anchors the protein to the bacterial membrane in situ and facilitates the insertion of the purified product to reconstituted liposomal or planar phospholipid membranes.

Recently, we demonstrated functional expression in *E. coli* of an anti-2-phenyloxazolone antibody single-chain Fv fragment as a lipoprotein fusion [23]. This hybrid antibody displayed all major features of a membrane protein: it was cell-bound and co-purified with the outer membrane fraction, was released into solution only in the presence of detergents, partitioned into the Triton X-114 detergent phase in temperature-induced phase separation, and spontaneously and quantitatively associated with liposomes upon removal of detergent [23]. These features of the lipid-tagged antibody were in striking contrast to those of its soluble counterpart which was secreted into the periplasm by using the *pelB* signal peptide [23,25]. The different behaviour of the two antibody molecules was assigned to the presence of the covalent lipid modification, demonstrated by metabolic incorporation of radiolabeled palmitic acid into the lipoprotein fusion antibody [23]. In terms of hapten (2-phenyloxazolone)-binding activity and specificity the two antibodies showed no differences. Importantly, the lipid-tagged

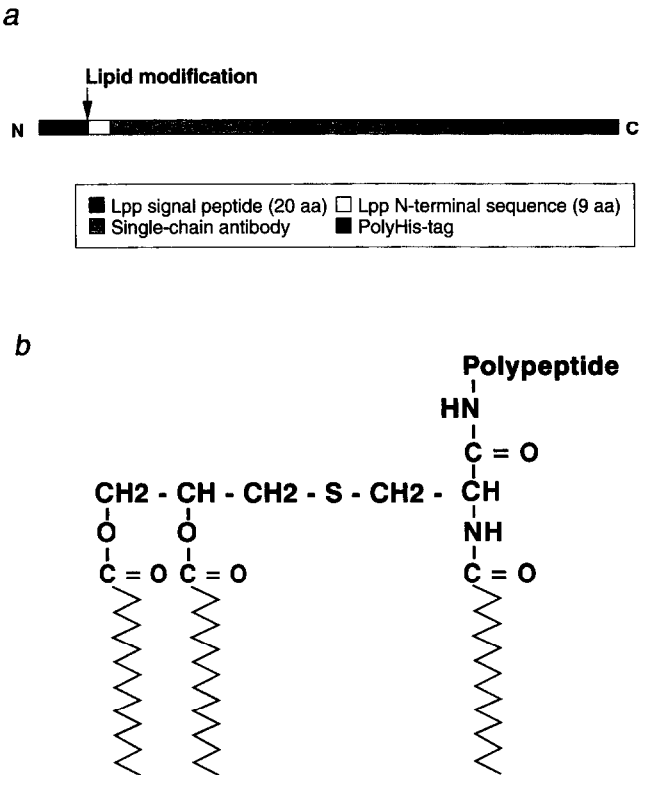

Fig. 1. Outline of the lipoprotein fusion antibody (a) and of the N-terminal covalent lipid modification (b).

antibody was active both in the detergent-solubilized state and in sonicated bacterial membrane vesicles.

Whereas crude membrane-bound and solubilized material may be used for preliminary testing, detailed experiments and immunoliposome applications would need highly purified material. To facilitate rapid purification of the lipoprotein fusion antibodies we engineered six histidines to the C-terminus to serve as an affinity tag in immobilized metal chelation chromatography (Fig. 1a). Immunoliposomes prepared from the purified antibody and phospholipids display hapten-binding activity as shown by ELISA and by surface plasmon resonance measurements (Fig. 2) (M.-L.L., K. Alfthan and K.K., manuscript in preparation). In the latter method, association and dissociation of sample molecules in a layer of dextran-immobilized ligand give rise to changes in the refractive index, which is monitored in real time [26]. Immunoliposomes display markedly slower dissociation from the immobilized hapten than the soluble antibody due to multivalent binding as there are many antibody molecules per liposome (Fig. 2, M.-L.L. and K.K., unpublished). This finding underscores the potential of immunoliposomes to significantly strengthen the binding between the antibody and the antigen.

Francisco and coworkers recently demonstrated that a fusion construct which contains the lipoprotein signal sequence and nine N-terminal amino acids followed by

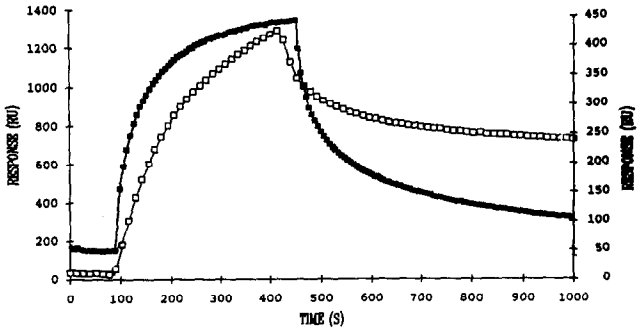

Fig. 2. Binding (association and dissociation) of immunoliposomes (open squares) and the soluble antibody (solid squares) to immobilized hapten as measured by surface plamon resonance (BIAcore, Pharmacia Biosensors AB). Note the different scales in the Y-axes for the immunoliposomes (left) and for the free antibody (right).

amino acids 45–159 of the outer membrane protein OmpA can be used to express single-chain antibodies and other proteins on the bacterial cell surface [22,27]. The lipoprotein part targets the protein to the outer membrane whereas the transmembrane OmpA part is used to obtain the correct topological orientation of the protein on the outer surface [22,27]. While this approach may prove useful in the screening of surface displayed antibodies and in catalytic and other biotechnological applications (e.g. as whole-cell absorbents) [28], the potential of these transmembrane hybrid antibodies for immunoliposome-based applications has not been evaluated.

4. Future prospects

In comparison with the available chemical conjugation methods for the production of lipid-modified proteins, bacterial expression as a lipoprotein fusion has several advantages. The product is labeled stoichiometrically and at a defined location. The single glycerolipid moiety provides sufficient hydrophobicity to anchor the lipoprotein fusion protein to phospholipid membrane in a stable and oriented manner. Use of bacterial expression may prove more economical in the scaled-up production of lipid-tagged molecules and may provide more flexibility as further modifications can be engineered relatively easily into the molecule. In addition, the recent progress in the surface display technology has dramatically simplified the isolation of antibodies with desired properties [29]. The conversion of the isolated antibody fragments into a lipid-tagged form would then require only the transfer of the antibody coding cassette from the phage display vector into an expression vector which harbours the lipoprotein elements.

Apart from the use of lipid-modified antibodies in immunoliposome technology, biosynthetic lipid-tagging may provide new tools for two-dimensional crystallization of proteins into lipid membranes for structural analysis [30] and for the development of biosensors [6,7,31]. In this respect, the presence of a single lipid anchor in a defined location in lipoprotein fusion polypeptides in contrast to chemically modified molecules is significant as it may facilitate oriented, high-density immobilization to lipid bilayers.

The biosynthetic lipid-tagging concept as based on bacterial lipoprotein modification might be extended to eukaryotic expression systems by taking advantage of the structural determinants responsible for the endogenously operating eukaryotic lipid modifications, in particular the C-terminal glycosyl-phosphatidylinositol anchor [32]. This would facilitate the use of eukaryotic expression systems to produce more complex and glycosylated polypeptides like whole antibodies in a lipid-tagged form.

In conclusion, bacterial expression of antibodies and other polypeptides as lipoprotein fusions results in the formation of a lipid tag at the amino terminus. This tag can be used to immobilize the functional polypeptide to phospholipid membranes for diverse biotechnological applications.

Acknowledgements: We thank Kaija Alfthan for help and advice in surface plasmon resonance measurements. Financial support from the Technology Development Center of Finland (TEKES) is gratefully acknowledged.

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