Biotechnology Advances 28 (2010) 293–300

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Use of oil bodies and oleosins in recombinant protein production and other biotechnological applications

S.C. Bhatla *, V. Kaushik, M.K. Yadav

Department of Botany, University of Delhi, North Campus, Delhi-110007, India

ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form 5 December 2009
Accepted 2 January 2010
Available online 11 January 2010

Keywords:
Artificial oil bodies
Biotechnological applications
Oleosins
Oil bodies

ABSTRACT

Oil bodies obtained from oilseeds have been exploited for a variety of applications in biotechnology in the recent past. These applications are based on their non-coalescing nature, ease of extraction and presence of unique membrane proteins—oleosins. In suspension, oil bodies exist as separate entities and, hence, they can serve as emulsifying agent for a wide variety of products, ranging from vaccines, food, cosmetics and personal care products. Oil bodies have found significant uses in the production and purification of recombinant proteins with specific applications. The desired protein can be targeted to oil bodies in oilseeds by affinity tag or by fusing it directly to the N or C terminal of oleosins. Upon targeting, the hydrophobic domain of oleosin embeds into the TAG matrix of oil body, whereas the protein fused with N and/or C termini is exposed on the oil body surface, where it acquires correct confirmation spontaneously. Oil bodies with the attached foreign protein can be separated easily from other cellular components. They can be used directly or the protein can be cleaved from the fusion. The desired protein can be a pharmaceutically important polypeptide (e.g. hirudin, insulin and epidermal growth factor), a neutraceutical polypeptide (somatotropin), a commercially important enzyme (e.g. xylanase), a protein important for improvement of crops (e.g. chitinase) or a multimeric protein. These applications can further be widened as oil bodies can also be made artificially and oleosin gene can be expressed in bacterial systems. Thus, a protein fused to oleosin can be expressed in *Escherichia coli* and after cell lysis it can be incorporated into artificial oil bodies, thereby facilitating the extraction and purification of the desired protein. Artificial oil bodies can also be used for encapsulation of probiotics. The manipulation of oleosin gene for the expression of polyoleosins has further expanded the arena of the applications of oil bodies in biotechnology.

Contents

* Corresponding author. Tel./fax: +91 11 27666744.
E-mail address: bhatlasc@gmail.com (S.C. Bhatla).

0734-9750/$ – see front matter
doi:10.1016/j.biotechadv.2010.01.001

1. Introduction

Oil bodies are spherical structures having a matrix comprised of triacylglycerols (TAGs) which is bounded by a phospholipid monolayer embedded with unique proteins, principally oleosins, caleosins and steroleosins (Murphy, 2005). The TAGs stored in oil bodies are mobilized during seed germination to meet the energy requirements of the growing seedling (Sadeghipour and Bhatla, 2002). Oleosins, the principal membrane proteins, endow the oil bodies with peculiar structural properties. Oleosins are alkaline and hydrophobic proteins having three domains: amphipathic N and C termini, and central hydrophobic domain. The central hydrophobic domain is highly conserved and penetrates through the phospholipid monolayer into the oil body matrix. N and C termini of oleosin polypeptide are located on the oil body surface exposed to the cytoplasm such that their negatively charged residues provide steric hindrance and electrostatic repulsion, thus maintaining oil bodies as separate entities (Huang, 1992; Tzen et al., 1992; Purkrtova et al., 2008). Oleosins are known to exist in 2–4 isoforms of 15 to 26 kDa (Tzen et al., 1990; Sadeghipour and Bhatla 2002). They exhibit a very systematic and differential expression pattern during seed development (Kaushik et al., 2010) and mobilization accompanying seed germination (Sadeghipour and Bhatla, 2002; Vandana and Bhatla, 2006; Bhatla et al., 2009). It is the structure and topological orientation of oleosins which make oil bodies and oleosins useful in biotechnological applications, such as emulsifying agents, carriers of recombinant proteins and immobilization matrices. Apart from naturally occurring oil bodies, artificial oil bodies (AOBs) have also been successfully constituted and used for purification, refolding and immobilization of recombinant proteins, as well as for encapsulation of probiotics. The present review highlights the significant applications of oil bodies which have a potential for further exploitation in the near future.

2. Oil bodies as emulsifying agents

Emulsifying agents facilitate the dispersal of one immiscible phase into the other and assist in the stabilization of emulsions. Oleosins have surfactant properties and do not allow oil bodies to coalesce. This non-coalescing nature of oil bodies makes them a suitable agent for emulsification. Since oil body emulsions are prepared from crude seed extracts and contain numerous endogenous seed components, they are likely to be contaminated with non-oil body proteins which may impart undesirable colour, odour, flavour and allerginicity. Thus, care has to be taken while preparing these emulsions so that they are made free from any contaminants by thorough washing of the oil body preparations (Deckers et al., 2004). Such washed and purified oil bodies are more stable, resistant to high temperature and harsh chemical and physical conditions, and exhibit superior water and oil adsorption (Deckers et al., 2003a). Apart from intact oil bodies, enriched oleosin–phospholipid fraction from oil bodies can also be used as an emulsifying agent (Harada et al., 2002). Oil bodies-based emulsions offer an advantage over mineral oil based formulations since oil bodies are derived from renewable and environmentally friendly sources.

Oil bodies-based emulsions can be used in food and feed products, pharmaceutical products, personal care products and industrial products. In food and feed products, oil bodies obtained from plants like sunflower serve as healthier and economical alternative as emulsifying agent since they are rich in polyunsaturated fatty acids and vitamin E. Such products may include mayonnaises, ice creams, vinaigrettes, salad dressings, pudding, juices, icings, fish food, pet food, livestock feed, etc. (Deckers et al., 2001a,b, 2003b; Berry et al., 2005). Use of oil bodies as emulsifying agent in different edible preparations may also provide additional benefits. Presence of oil bodies in juices as clouding agent makes them richer and prevents settling down of solid components. Oil body-based butter has lesser of saturated fatty acids and, thus, it offers a healthier substitute to conventional butter. Emulsion based on oil bodies having recombinant oleosin fused with therapeutic hormonal peptide (growth hormone) has been used to improve fish food (Moloney and Habibi, 2001; Deckers et al., 2001b). Ice creams are costly because of 'milk solids not fat' (MSNF), which is required for the stabilization of fat emulsion and the air phase. Use of oil bodies in ice cream preparations eliminates the need for emulsification step during processing and reduces MSNF usage, thereby offering a low cost alternative to traditional ingredients (Berry et al., 2005).

Oil body-based pharmaceutical formulations include therapeutic, diagnostic and delivery agents. Oil body-based emulsions can be used as adjuvants in vaccines (Deckers et al., 2004), base for dermatological products, component of orally administered medicines, etc. In these products oil bodies may also carry an active ingredient to be delivered to host, if needed. Personal care products wherein oil body-based emulsions may be used, include various cosmetic and cosmeceutical products such as creams, lotions, make up products, hair care products, bath products, such as soaps, washes and cleansers (Deckers et al., 2003a, 2001a). In products like toothpaste, oil bodies may also serve as carriers of components such as flavouring agent, fluoride, silicas, chelating agents, sweetener, etc. Industrial applications of oil body-based emulsions include formulation of lubricants, paints, coatings, inks, polishes, films, paper sizing, latex, etc. (Deckers et al., 2001a). When used in these products, purity of oil bodies is of less significance.

3. Recombinant protein production and purification

In this era of molecular farming, an ideal system for the production of recombinant proteins would be the one which produces the safest biologically active proteins with ease and at minimum cost. Use of plants as expression systems for the production of recombinant proteins offers distinct advantages over bacterial, fungal, insect and mammalian systems. Bacterial and fungal systems are not appropriate for the production of many desired proteins because of the obvious differences in protein processing and codon usage. Furthermore, the expressed heterologous proteins form insoluble aggregates (Pen, 1996). In the baculovirus–Sf9 insect cell system, the infected cells do not survive infection, which must be repeated with new cells and virus preparations if large amount of protein is required (Hudebine, 2009). Both in the mammalian and insect systems, the cost of production is high and yields are low (Giddings et al., 2000; Hudebine, 2009; Ma et al., 2003). Plants are easy to transform and recombinant proteins can be produced cost effectively (Giddings et al., 2000; Twyman et al., 2003). The technology for processing and harvesting plant products already exists (Daniell et al., 2001). Plants also offer very flexible scalability in less time (Twyman et al., 2003). Plants can fold polypeptides and associated subunits as effectively as animal cells (Giddings et al., 2000; Hudebine, 2009). However, they do exhibit