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Affinity partitioning of proteins tagged with choline-binding modules in aqueous two-phase systems

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ABSTRACT

We present a novel procedure for affinity partitioning of recombinant proteins fused to the choline-binding module C-LytA in aqueous two-phase systems containing poly(ethylene glycol) (PEG). Proteins tagged with the C-LytA module and exposed to the two-phase systems are quantitatively localized in the PEG-rich phase, whereas subsequent addition of the natural ligand choline specifically shifts their localization to the PEG-poor phase by displacement of the polymer from the binding sites. The described procedure is simple, scalable and reproducible, and has been successfully applied to the purification of four diverse proteins, resulting in high yields and purity.

1. Introduction

Protein separation and purification represent biotechnological events of unquestionable importance, accounting in some cases for 50–90% of total production costs at the industrial level [1]. Affinity adsorption on a solid chromatography support constitutes the usual procedure for recombinant, tagged polypeptide separation, either aimed towards purification or to bioreactor setup [2–4]. However, the need for resin preparation and recycling, and other negative aspects and problems like column fouling or changes in the stability and enzymatic parameters of the adsorbed proteins, have fostered the search for alternative procedures. In this sense, aqueous two-phase systems (ATPSs) have been successfully used in the purification of many proteins of interest [5,6], and may be implemented in industrial downstream processes [7]. ATPSs are formed when two polymer solutions, or a polymer and a salt, are mixed at a concentration higher than a critical value, so that they separate into two phases at equilibrium. Most commonly used ATPSs involve the mixing of polyethylene glycol (PEG) or related polymers like thermoseparating ethylene-oxide-propylene-oxide copolymers [8,9] with dextran or phosphate salts. These systems present interesting advantages both for laboratory and industry processes. They are cost-effective, easy to scale up [10] and suitable for continuous operation [11]. Many variables can be manipulated to improve the partition, and compatibility with detergents allows the purification of membrane proteins [12]. Moreover, polypeptides partitioned in ATPSs are exposed to mild physical–chemical conditions as both phases consist mainly of water (70–90%) and the interfacial tension between them is very low [13], favouring mass transfer in enzymatic reactions. However, the use of ATPSs for protein purification on a routine basis, either at industrial or laboratory level has been hampered by the generally poor predictability of the partition coefficient of any given protein in a particular ATPS, as this parameter results from a complex interplay of macromolecular properties such as molecular weight, amino acid composition, hydrophobicity and electrostatic forces [13–16]. Several approaches take advantage of the affinity for a certain ligand in order to direct the localization of the protein of interest to a particular phase [17]. On many occasions, the use of translational fusions as different polypeptide tags, such as tryptophan and tyrosine-rich, hydrophobic sequences [18], poly-histidine tails [19] or combinations of both [20] is necessary. However, these affinity-enhanced partitioning systems are not free of disadvantages, such as a decreased protein expression and solubility, or the need for derivatization of PEG [17].

The C-LytA module belongs to the choline-binding domain family (Pfam ID code PF01473: http://www.sanger.ac.uk/cgi-bin/Pfam/getacc?PF01473). C-LytA constitutes the C-terminal part of the LytA

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amidase from *Streptococcus pneumoniae* and is responsible for the attachment of this enzyme to the choline residues on the surface cell wall [21]. The C-LytA polypeptide is a 135-amino acid repeat protein, built up from six conserved β-hairpins that configure four choline-binding sites [22]. Each choline-binding site is constituted by two aromatic residues from one hairpin and another from the next, with the contribution of an additional hydrophobic side chain. The ligand is bound by hydrophobic and cation–π interactions, that significantly increase the stability of the protein [23,24]. The affinity of C-LytA for choline and choline structural analogs [25,26] allows its use as an affinity tag for single-step purification of hybrid proteins expressed in *Escherichia coli*, by specific adsorption to simple amine-containing chromatographic resins like DEAE-cellulose, followed by specific elution with choline [27–32]. We therefore decided to check whether the C-LytA tag might also be employed in affinity partitioning in PEG-containing ATPSs. Here we show that C-LytA may bind PEG molecules in the choline-binding sites, which can be used to accumulate both C-LytA and C-LytA-tagged proteins in the PEG phase, whereas addition of choline reverses this interaction and directs the protein to the PEG-poor phase. This allowed the purification of four diverse proteins and suggests that choline-binding polypeptide tags may be used in easily modulated systems for the predictable partitioning and easy purification of recombinant proteins in ATPSs.

2. Experimental

2.1. Materials

PEG8000, dextran DxT500 and choline chloride were purchased from Sigma (St. Louis, MO, USA).

2.2. Bacterial strains and plasmids

Escherichia coli strains REG-1 and REG-21 were supplied by Biomedal (Seville, Spain). Construction of plasmid vectors is represented in Fig. 1. Plasmid pALEX2-Ca-GFP (coding for the GFP-C-LytA protein) was constructed by insertion of the 718 base pairs (bp) SphI-*Stu*I fragment containing the GFP coding sequence from pJBA111 [33] between the SphI and SmaI sites of commercial vector pALEX2-Ca (Biomedal). For pALEXb-Lip36 construction (C-LytA-Lip36 protein), the 959 bp BamHI-HindIII fragment of p36/LACK gene of *Leishmania infantum* [34] was inserted between the BamHI and HindIII sites of commercial vector pALEXc (Biomedal). For pALEX2c-LacZ construction (LYTAG-β-Galactosidase), the 3116 bp BamHI-HindIII fragment of the *E. coli lacZ* gene encoding β-galactosidase was inserted between the BamHI and HindIII sites of commercial vector pALEX2c (Biomedal). Finally, for pALEXb-ProtA (C-LytA-ProtA) construction, a 433 bp polymerase chain reaction (PCR) product encoding two copies of the *Staphylococcus aureus* protein A IgG affinity domain Z was amplified with primers MAO55 (Forward): 5′-*CGCGGATCCGAAAACCGCGGCTCTTGCGC*-3′and MAO56 (Reverse): 5′-*CGCGGATCCTCAGGTTGACTTCCCCGCGGAGTTCGCGTC*-3′, digested with BamHI and cloned in the BamHI site of commercial vector pALEXb (Biomedal).

2.3. Protein expression

C-LytA protein was purified by affinity chromatography from the overproducing *E. coli* strain RB791 harbouring the pCE17 plasmid [21]. Optimized materials and protocols contained in the C-LYTAG Protein Expression and Purification System (Biomedal)

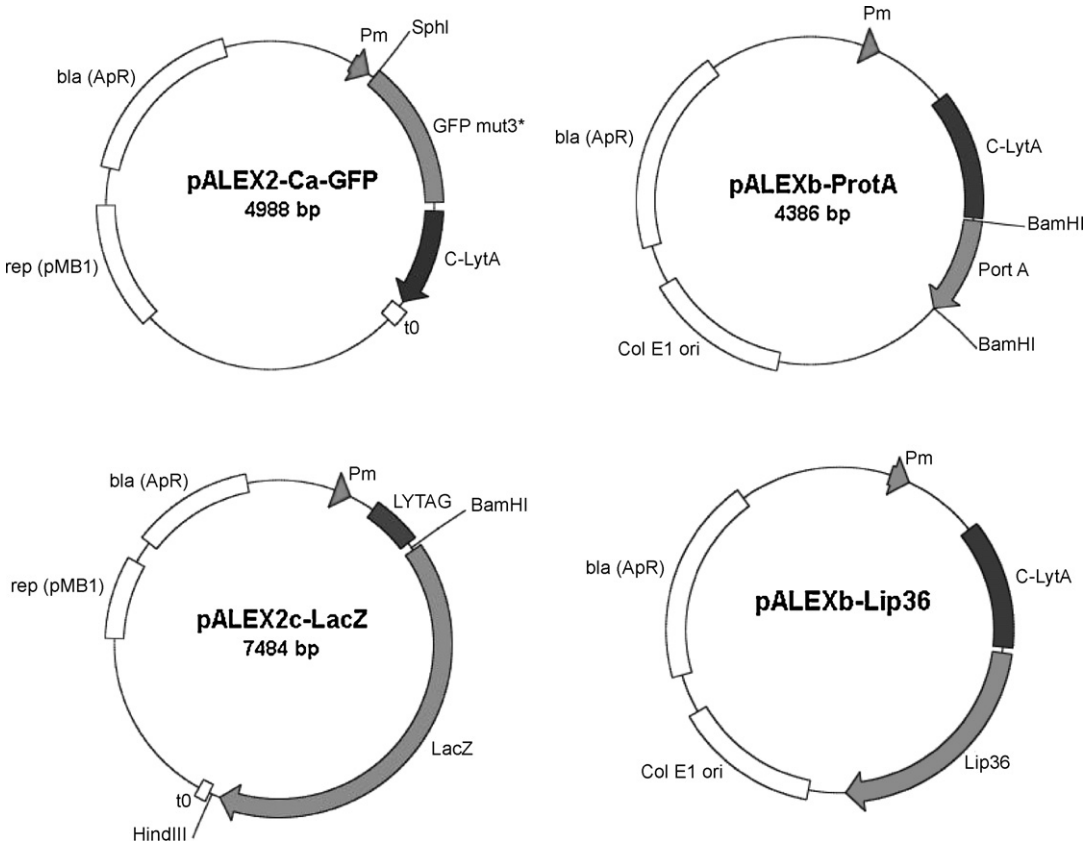

Fig. 1. Plasmid diagrams. All the open reading frames were cloned under Pm promoter control and fused in frame with C-LytA module, or its reduced and improved version LYTAG (pALEX2c-LacZ).