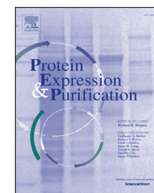
Protein Expression and Purification 121 (2016) 81–87

Contents lists available at ScienceDirect

Protein Expression and Purification

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



Recombinant production and purification of short hydrophobic Elastin-like polypeptides with low transition temperatures

Laure Bataille [a, b, c, d], Wilfrid Dieryck [e, f], Agnès Hocquellet [e, f], Charlotte Cabanne [e, f], Katell Bathany [g, h, i], Sébastien Lecommandoux [a, b, c], Bertrand Garbay [e, f, *], Elisabeth Garanger [a, b, c, d]

[a] University of Bordeaux, LCPO, UMR 5629, F-33600, Pessac, France
[b] CNRS, LCPO, UMR 5629, F-33600, Pessac, France
[c] Bordeaux INP, LCPO, UMR 5629, F-33600, Pessac, France
[d] Institut Européen de Chimie et Biologie, F-33600, Pessac, France
[e] University of Bordeaux, BPRVS, EA4135, F-33000, Bordeaux, France
[f] Bordeaux INP, BPRVS, EA4135, F-33000, Bordeaux, France
[g] University of Bordeaux, CBMN, UMR 5248, F-33600, Pessac, France
[h] CNRS, CBMN, UMR5248, F-33600, Pessac, France
[i] Bordeaux INP, CBMN, UMR5248, F-33600, Pessac, France

A R T I C L E I N F O

Article history:
Received 16 December 2015
Received in revised form
14 January 2016
Accepted 19 January 2016
Available online 21 January 2016

Keywords:
Elastin-like polypeptides
Mass spectrometry
Precision polymers
Recombinant expression
Transition temperature

A B S T R A C T

Elastin-like polypeptides (ELPs) are biodegradable polymers with interesting physico-chemical properties for biomedical and biotechnological applications. We report herein the recombinant expression of three hydrophobic ELPs $(VPGIG)_n$ with variable lengths (n = 20, 40, 60) and sub-ambient transition temperatures. These ELPs were purified from the cytoplasmic soluble fraction of *Escherichia coli* by inverse transition cycling, and their exact molecular weight was confirmed by various mass spectrometry techniques. Transition temperatures of ELP20, ELP40, and ELP60 were measured at 18.6 °C, 12.4 °C and 11.7 °C, respectively.

© 2016 Elsevier Inc. All rights reserved.

1. Introduction

Elastin-like polypeptides (ELPs) are peptide polymers derived from the hydrophobic region of tropoelastin [1]. They consist of multiple repeats of Val-Pro-Gly-*Xaa*-Gly pentapeptides, where the guest residue (*Xaa*) can be any amino acid except proline. ELPs are characterized by an inverse transition temperature (T_t) [2,3]. Below T_t, the free polymer chains of ELP remain disordered, fully hydrated and thus soluble in aqueous solution. Above T_t, the ELP chains fold hydrophobically into more ordered structures and together assemble to form insoluble aggregates. This phenomenon is fully reversible. T_t is influenced by the nature of the guest residue *Xaa* (*e.g.* polar, charged, hydrophobic) and chain length [4]. It also depends on the polypeptide concentration as well as the nature and concentration of salts. The majority of ELPs studied so far have been produced by using recombinant techniques [5–9]. Indeed, bioproduction allows the precise control of the polypeptide sequence and molecular weight at a level that cannot be achieved by traditional polymerization syntheses [10]. There is a wide range of applications for ELPs, from biomedical (*e.g.* cell culture, tissue engineering and drug delivery) to biotechnological applications (*e.g.* protein purification, nanobiotechnology, hydrogels, *etc.*) [5,7,9,11–13]. Biomedical uses are appealing, so the majority of thermo-responsive ELPs designed so far have a T_t in the 30–45 °C range. However, ELPs with lower T_ts in the 10–20 °C range would be useful for specific purposes. For example, ELPs can be used for

Abbreviations used: ELP, elastin-like polypeptides; *Tt*, transition temperature; *E. coli*, *Escherichia coli*; LB, lysogeny broth; IPTG, isopropyl β-D-1-thiogalactopyranoside; ITC, inverse transition cycling; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; PBS, phosphate-buffered saline.

* Corresponding author. EA BPRVS 4135, ENSTBB, Bordeaux INP, 146 rue Leo Saignat, 33076 Bordeaux, France.
E-mail address: bertrand.garbay@ipb.fr (B. Garbay).

http://dx.doi.org/10.1016/j.pep.2016.01.010
1046-5928/© 2016 Elsevier Inc. All rights reserved.

temperature-triggered depot formation in tumors [14]. The goal is to deliver the therapeutic compound locally and in a sustained manner. A recent study showed that tumor retention correlates with a decrease in the ELP T_t [15].

ELPs with low T_t can be obtained by different approaches. The first one is to synthesize ELPs of high molecular weight (MW) with repetitions of a moderately hydrophobic repeated sequence such as $(VPGVG)_n$ with n > 230 [16,17]. Such ELPs have a MW ~100 kDa and their T_ts are in the 20–25 °C range. However, it has been demonstrated that heterologous proteins with MWs above 60 kDa are often difficult to express in *Escherichia coli*, resulting in low yields [18]. In addition, the highly repeated DNA sequence in the corresponding genes poses a risk of gene rearrangement, and thus the production of undesirable products [19].

A second strategy relies on the synthesis of ELPs with T_ts > 30 °C, and lowering their T_ts below 20 °C by the addition of large quantities of salts, up to 2 M of NaCl [20,21]. However, such a high salt concentration is more than 10-fold higher than that in human serum (~0.15 M), so this strategy is not suitable for biomedical applications.

A third strategy is to produce short hydrophobic ELPs with low T_t at physiological salt concentration. In the present work, we focused on the design and production of short recombinant ELPs (MW ranging between 9 and 26 kDa) exhibiting T_ts in the 10–20 °C range. We incorporated isoleucine (I) at the guest position of every VPGXG repeat sequence because it has been demonstrated that a chemically synthesized $(VPGIG)_{180}$ polymer of 100 kDa has a T_t value of 10 °C [22]. Three recombinant ELPs were thus designed to contain from 20 to 60 (VPGIG) repeats, namely $(VPGIG)_{20}$ (ELP20), $(VPGIG)_{40}$ (ELP40) and $(VPGIG)_{60}$ (ELP60) with respective MW of 8.9, 17.4 and 25.9 kDa.

The question arose, however, whether the recombinant expression of such hydrophobic ELPs with T_ts < 20 °C would be feasible in *E. coli*. Indeed, previous studies have shown that the expression of hydrophobic ELPs leads to the formation of inclusion bodies or aggregates, which complicates the downstream purification process [23–26]. In addition, the majority of ELPs recombinantly expressed have MWs higher than 20 kDa, while the recombinant expression and purification of lower MW ELPs has been reported to be challenging [27].To circumvent this difficulty, we previously expressed ELP20, ELP40 and ELP60 in fusion with the maltose-binding protein (MBP), which is known to improve the solubility of polypeptides to which it is fused. However, we could not purify to homogeneity ELP40 and ELP60 [28]. We report here the recombinant expression, the purification and the characterization of three hydrophobic ELPs with T_ts < 20 °C in low salt buffer.

2. Materials and methods

2.1. Materials

Enzymes required for recombinant DNA, including restriction endonucleases, T4 DNA polymerase, OneTaq® hot start DNA polymerase, Phusion® Hot Start II DNA polymerase, Antarctic phosphatase and Quick ligation™ kit (T4 DNA ligase), were obtained from New England Biolabs (NEB, Ipswich, MA), except for In-Fusion® HD kit which was obtained from Clontech (Mountain View, CA). The pMal-c5e plasmid and the *E. coli* strain NEB5α-F'Iq were purchased from NEB. The pET-44a(+) plasmid and the *E. coli* strain BLR(DE3) were obtained from Novagen Inc (Madison, WI). The *E. coli* strain Stbl3™ (Invitrogen, Carlsbad, CA) was used for cloning repetitive DNA constructs. Synthesis of the initial VPGIG gene was achieved by Eurofin MWG Operon (Ebersberg, Germany). All custom oligonucleotides were synthesized by Eurogentec (Seraing, Belgium). Sequencing of the different constructs was performed by Millegen (Labege, France). All solvents used for mass spectrometry analysis were HLPC grade and matrix sinapinic acid was purchased from Sigma–Aldrich (Saint-Quentin Fallavier, France).

2.2. Construction of expression vector for ELP genes

A synthetic gene corresponding to the MW(VPGIG)20C sequence (sequence A, Fig. 1) provided in pCR2.1 plasmid was extracted from the said plasmid by a double digestion with *Eco*RI and *Hind*III, and was ligated with the Quick ligation™ kit into similarly digested and dephosphorylated pUC19. After transformation into NEB 5α-F'Iq *E. coli* competent cells, pUC19-A was selected by colony PCR with OneTaq® hot start DNA polymerase and verified by DNA sequencing. According to our strategy, the sequence GTTCGCA had to be deleted from pUC19-A to obtain the correct reading frame for the synthesis of ELP20, and to allow subsequent recursive directional ligations (Fig. 1). Deletion was performed by PCR. A linear deleted fragment with 17 bp overlapping extremities was obtained from the plasmid pUC19-A as template by using the following two oligonucleotides: H141 (5′GGCATTGGTTGCTAATCATCGCTGGATCCAA3′) and H142 (5′GATTAGCAACCAATGCCAGGAACGCCGATG3′). Phusion Hot Start II DNA polymerase was used with the GC 5X buffer according to the manufacturer's instructions. The PCR product was then digested by the *Dpn*I enzyme to eliminate the *dam* methylated parental plasmid. The amplified fragment was circularized by using the In-Fusion® HD kit, and was used to transform NEB 5α-F'Iq *E. coli* competent cells. Positive colonies were identified by PCR with OneTaq® hot start DNA polymerase. The sequence of the resultant pUC19-ELP20 plasmid was confirmed by DNA sequencing. The sequence contained in pUC19-ELP20 is termed sequence B.

Sequences coding for ELP40 and ELP60 were obtained by modifying the recursive directional ligation [29]. Sequence A cloned in pUC19-A was double-digested with the enzymes *Btg*Z1 and *Bsm*F1 to generate sequence C (monomer insert), which corresponds to the DNA sequence of MW(VPGIG)20C minus the Met–Trp N-terminal codons and the Cys C-terminal codon. The recipient plasmid pUC19-ELP20 containing sequence B was digested by *Bsm*FI, dephosphorylated by Antarctic phosphatase and ligated to sequence C with the Quick ligation™ kit. Stbl3™-competent cells were transformed with the ligation product. This strain was used because it reduces the recombination of cloned DNA containing repeated sequences, such as ELP's. Positive colonies were identified by colony PCR with OneTaq® hot start DNA polymerase and the resultant pUC19-ELP40 plasmid was checked by DNA sequencing. To obtain the ELP60 sequence, the same strategy was used with pUC19-ELP40 as recipient plasmid and the previously obtained sequence C as monomer insert.

Cloning in the expression vector was as follows: ELP sequences were extracted from pUC19-ELP20, pUC19-ELP40 by a double digestion *Nde*I and *Bam*HI, and ligated independently with the Quick ligation™ kit into similarly digested and dephosphorylated pET-44a(+) plasmid. The different ligation products was then used to transform NEB5α-F'Iq (ELP20 containing plasmids) or Stbl3™ (ELP40 and ELP60 containing plasmids) *E. coli* competent cells. Positive clones were identified by PCR screening. The resultant plasmids pET-ELP20, pET-ELP40, and pET-ELP60 were confirmed by restriction mapping and the DNA sequences of ELP genes were also checked by DNA sequencing. The plasmids were purified and used to transform BLR(DE3)-competent cells for production.

2.3. Bioproduction of recombinant ELPs

Preliminary experiments performed in 250 mL shake flasks with ELP20-producing clones showed that glucose was a better carbon

Download English Version:

https://daneshyari.com/en/article/2020210

Download Persian Version:

https://daneshyari.com/article/2020210

Daneshyari.com