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Short Communication

Time-course and degradation rate of membrane scaffold protein (MSP1D1) during recombinant production

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ABSTRACT

Membrane scaffold proteins (MSPs) are synthetic derivatives of apolipoprotein A-I, a major protein component of human high-density lipoprotein complexes. The most common among these is the variant MSP1D1, which has been in the focus of research on membrane mimetics in the past. As such, the amphipathic MSP1D1 has the ability to self-assemble in the presence of synthetic phospholipids into discoidal nanoparticles, so called nanodiscs. The recombinant production of MSP is exclusively reported using a standard laboratory expression system of the pET family. However, strong variations in both yield and achieved concentration as well as complications related to unspecific degradation are commonly reported. In addition, the time-course of recombinant protein as well as specific protein yields have not yet been quantified conclusively. In this study, the time-course of MSP1D1 concentration was investigated in a standard pET expression system in terms of quantification of production and degradation rates in comparison to a reference protein (eGFP).

1. Introduction

Membrane scaffold protein 1D1 (MSP1D1) is a synthetic derivate of apolipoprotein A-I, which is the major protein element of human high-density lipoproteins [1]. The group of Stephen Sligar (University of Illinois, USA) synthesized the gene expressing the protein and provided the required bacterial translation sequences, optimized codon usage and minimized secondary structures of the corresponding mRNA [2,3]. The amphipathic, synthetic protein has the ability to self-assemble in the presence of synthetic phospholipids into discoidal nanoparticles, so called nanodiscs [2,3]. According to that, nanodiscs are self-organizing model membranes, which are soluble and stable in aqueous solutions and preserve the general state of the phospholipid bilayer architecture [4]. Nanodiscs technology has been developed to overcome the limitations of membrane mimicking systems for studying membrane proteins, applications in biotechnology and medicine [5–7]. Applications for nanodiscs are based on the feature of solubilization of a membrane protein in native conformation or use as a model membrane system (applications are reviewed in more detail in [7]). The microbial production of MSP1D1 is exclusively reported using a standard plasmid-based expression system of the pET family (pET28a) under the control of a T7 promoter, which was established during early research on MSP proteins by the group of Stephen Sligar at the University of Illinois, USA [2,3,5,8]. This expression system is well-established for recombinant protein expression in general because of the high yield of recombinant protein which can represent up to 50% of the total cell protein [9,10]. Even though this expression system is a standard for production of MSP on a laboratory scale, strong variations in yield, achieved concentration as well as complications related to unspecific degradation are commonly reported [3,11,8,12]. Additionally, relevant efficiency parameters such as specific production rates as well as specific yields are rarely stated in literature. Existing reports differ strongly from each other, both in terms of values and units, and therefore fail to provide a conclusive view on the efficiency of this expression system for MSP1D1 production.

Reported concentrations all rely on the pET28a plasmid system in *E. coli* BL21(DE3). Membrane scaffold proteins are first published in 2002 by Bayburt et al., and production of these proteins at a level of about 250 mg per liter of culture were reported [3]. In 2011, a yield of purified membrane scaffold protein between 100 and 200 mg/l of culture was published [11]. A few years later, 2013 Inagaki et al. published a concentration of purified MSP1D1 of ~ 6.5 mg/ml of 6.5–7.0 g wet cell pellet per 1 L culture [8]. In the same year researchers of the University of Connecticut, claimed that 0.5 L cells gave an average of 12–13 mg of membrane scaffold protein [12].

In this study, the time-course of MSP1D1 concentration was

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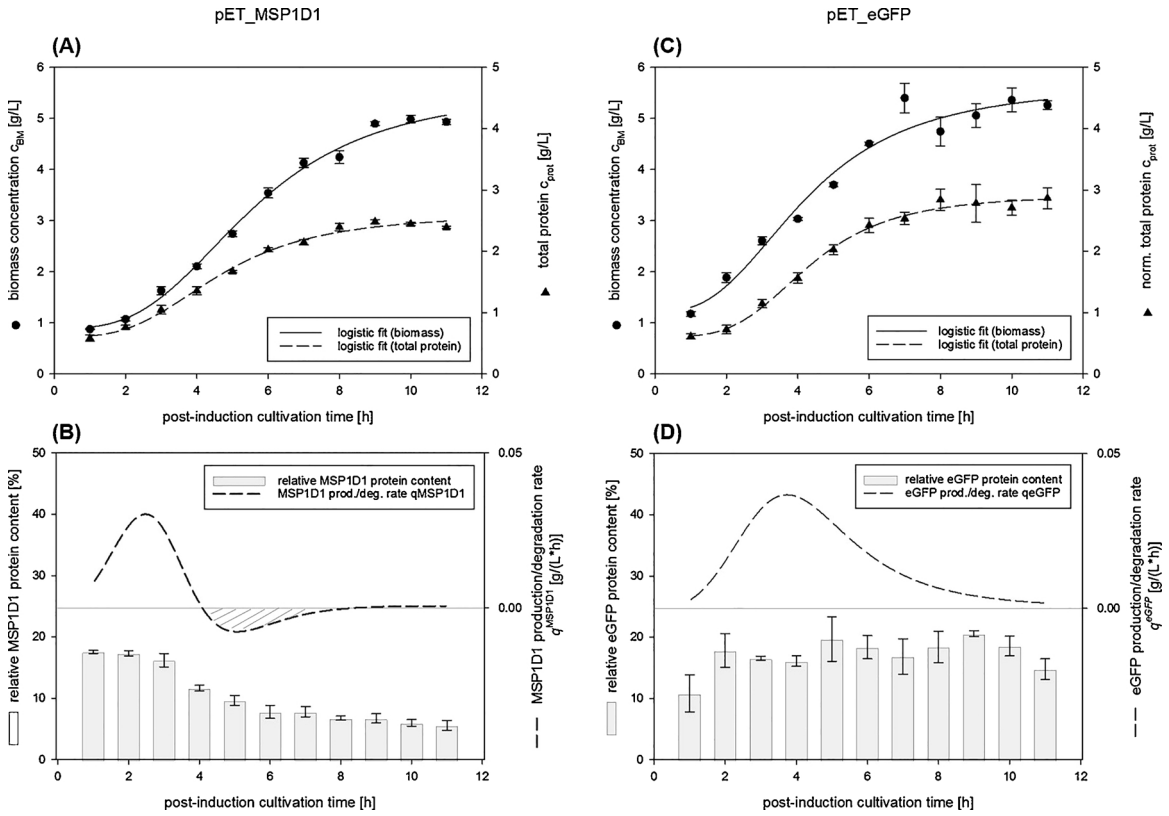

Fig. 1. Time-course post induction of biomass concentration, total protein concentration, relative protein content and protein production/degradation rate for MSP1D1 and eGFP as reference. (A) biomass and total protein for MSP1D1, (B) production/degradation rate and relative MSP1D1 protein content, (C) biomass and total protein for eGFP, (D) production/degradation rate and relative eGFP protein content. Fits for production/degradation rates (B) and (D) are calculated from logistic 4-parameter fits of biomass and protein concentration (A) and (C). Shaded area in B indicates negative values of the absolute production rate, indicating MSP1D1 degradation is higher than production rate leading to the deduction that MSP1D1 is actively degraded and removed from the system.

investigated in a standard pET expression system in comparison to a reference protein. For this purpose, relevant efficiency parameters including specific protein content, specific production rates and observed degradation rate are presented.

2. Material and methods

2.1. Materials

Chemicals used were purchased from Carl Roth GmbH & Co. KG (Karlsruhe, Germany), Bio-Rad Laboratories, Inc. (Hercules, USA) and Merck KGaA (Darmstadt, Germany). The membrane scaffold protein 1D1, as lyophilized powder, was purchased from Sigma-Aldrich (Taufkirchen, Germany). The expression plasmid pMSP1D1 was a gift from Stephen Sligar, University of Illinois, Urbana, USA (Addgene plasmid # 20061) [2]. The eGFP standard was purified by IMAC with Ni-NTA columns (HisTrap HP, 5 × 5 ml nickel column; GE Healthcare Life Sciences; Chicago, United States of America) using a chromatography system (ÄKTA start chromatography system; GE Healthcare Life Sciences, Chicago, United States of America).

2.2. Methods

2.2.1. Genetic works, bacterial strain and growth conditions

The eGFP gene (obtained from the pJOE4056.2 plasmid [13,14]) was used to replace the gene for MSP1D1 in the pMSP1D1 plasmid (obtained from [2]) under the control of a T7 promoter. All cultivations and expressions were performed in *E. coli* BL21Gold(DE3). For the overnight culture the required amount of TB medium [5] was supplemented with 50 µg/ml kanamycin, and cultivation was performed in baffled shaking flask. Then 50 µl of a glycerol stock of the strain was added. Incubation was performed at 37 °C and 120 rpm overnight in a shaker incubator (Newbrunswick™/Innova® 44, Eppendorf AG, Hamburg, Germany).

2.2.2. Cultivation and expression of MSP1D1 and eGFP

The overnight culture was diluted with TB medium to an OD_{600nm} of 0.1 for starting the cultivation and the particular antibiotic kanamycin with a concentration of 50 µg/ml was added. The cultivation was performed at 37 °C and 120 rpm in an incubator shaker (Newbrunswick™/Innova® 44; Eppendorf AG, Hamburg, Germany). Induction was started between an OD_{600nm} of 1.2–1.3 with 1 mM of sterile filtered isopropyl-β-D-thiogalactopyranosid (IPTG) and the temperature was decreased to 30 °C. The induction was carried out over 11 h.

2.2.3. Sampling, replicates and sample analysis

All experiments were performed in triplicate as biologically independent experiments and data is shown as average values ± standard deviation. Every hour after the induction a sample was taken and analyzed regarding the OD_{600nm} and biomass. The OD_{600nm} was determined using a spectrophotometer (UV-3100 PC; VWR GmbH, Darmstadt, Germany). The cell pellet was harvested via centrifugation at 4 °C and 10,000 × *g* for 10 min (Microcentrifuge 5430R; Eppendorf AG, Hamburg, Germany). Then a chemical cell disruption was carried out for the intracellular located target proteins (BugBuster Master Mix; Merck KGaA, Darmstadt, Germany). For determination of protein concentration a colorimetric Bradford assay [15] was used (Roti® Quant; Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The determination of protein sizes and quantification of proteins was performed using SDS-PAGE with 12% polyacrylamide gels (TGX Stain-Free™ FastCast™ Acrylamide Solutions; Bio-Rad Laboratories, Inc., Hercules, USA) and coomassie staining (Roti®-Blue quick; Carl Roth GmbH & Co. KG;