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High-Level Production of Spider Silk Protein by Fed-Batch Cultivation of Recombinant *Escherichia coli* and Its Purification

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Abstract

Silk proteins from *Nephila clavipes* are fibrous proteins containing repetitive sequences with both crystalline and amorphous domains. In order to obtain high-level production of silk protein, the synthetic genes had 16 contiguous units of the consensus repeat sequence of the silk protein were expressed in *Escherichia coli* BL21(DE3) under the strong inducible T7 promoter. For production of recombinant silk protein in large amounts, pH-stat fed-batch cultures were carried out. The recombinant silk protein was produced as soluble forms in *E. coli*, and the recombinant silk protein content was as high as 11% of the total protein. When cells were induced at OD_{600} of 60, the amount of silk protein produced was 6.49 g/L. After simple purification steps, 9.2 mg of silk protein that was more than 80% pure was obtained from a 50 mL culture, and the recovery yield was 26.3%.

Introduction

Dragline spider silk has a number of notable mechanical properties such as high tensile strength and elasticity.[1] Strong natural fibers with good tensile and compressive properties would be useful for many applications in medicine, such as sutures, membranes and temporary scaffolds for tissue engineering.[2] This silk protein is composed of two proteins, MaSP 1 and 2, whose partial cDNAs have been cloned and sequenced.[3] Recently, recombinant spider silks have been generated from synthetic spider silk genes using *Escherichia coli* as the heterologous host. While recombinant proteins have been successfully generated from these genetically engineered systems,[4] a significant limitation in these studies has been the difficulties in high-level producing genetically engineered

silk-like proteins.[3] Thus, in this study, we describe the efficient process development during high-cell density cultivation of recombinant *E. coli* for the production of recombinant silk protein. We also describe simple procedure for purification of silk protein.

Materials and methods

Bacterial strain and plasmid. The *E. coli* strain BL21(DE3) (F⁻ *ompT hsdS*$_B$ (r_B⁻ m_B) *gal dcm (DE3)*) (Novagen, Madison, WI) harboring the recombinant plasmid, pSH16A, was used as a host strain for the high cell density cultivation. The plasmid, pSH16A, contains 16 contiguous units of the consensus repeat sequence of the silk protein under the strong inducible T7 promoter.

Fed-Batch cultivation. fed-batch culture was carried out at 37℃ in a 6.6 L jar fermenter (Bioflo 3000; New Brunswick Scientific Co., Edison, N.J.) containing 1.8 liters of R/2 medium.[5] A seed culture (200 mL) was prepared in the same medium. The culture pH was controlled at 6.8 by the addition of 28% (v/v) ammonia water. The dissolved oxygen concentration was kept at 40% of air saturation by automatically increasing the agitation speed to 1,000 rpm and by changing the percentage of pure oxygen. A nutrient feeding solution was added by using the pH-stat (with high limit) feeding strategy. When the pH rose to a value greater than its set point (pH 6.8) by 0.08 due to the depletion of glucose, the appropriate volume of the feeding solution was automatically added to increase the glucose concentration in the culture broth to 5 g/L. Expression of the synthetic silk gene was induced at OD_{600} of 60 by adding IPTG to a final concentration of 2 mM.

Purification of silk protein. Cells were harvested from 50 mL of culture by centrifugation at 6,000 ×g for 10 min at 4℃. The cells were resuspended in 50 mL of resuspension buffer (50 mM K_2HPO_4, 300 mM NaCl, pH 8.0) containing 200 μg/mL lysozyme and Mini Complete protease inhibitor (Boehringer Mannheim, Mannheim, German) and were disrupted by sonication for 30 min at 45% output. The lysate was centrifuged at 14,000 ×g for 30 min at 4℃ to remove cell debris. Finally, silk protein was purified by immobilized metal affinity chromatography. The protein solution was loaded onto a Ni-chealating resin (Qiagen, Valencia, CA) that had been preequilibrated with 50 mM K_2HPO_4,

300 mM NaCl, 5 mM imidazole, pH 8.0, and then protein was eluted with a linear 5 to 250 mM imidazole gradient in the same buffer at a rate of 120 mL/hr. The protein concentration in each fraction was monitored with a UV detector (Bio-Rad).

Results and discussion

Fed-batch cultivation. pH-stat fed-batch cultures of *E. coli* BL21(DE3) harboring pSH16A was carried out as described in materials and methods. Cells were induced with IPTG at OD_{600} of 60. Figure 1 shows the time profiles for cell density (OD_{600}), cell dry weight, and silk protein content expressed as a percentage of the total protein content. When cells were induced at the intermediate cell density (OD_{600}, 60), the fraction of silk protein content again increased for 8 h after induction and then decreased. The cell dry weight and the maximal silk protein content were 59.0 ± 2.9 g/liter and 12% ± 0.4% of the total protein, respectively (Fig. 1).

Recombinant silk protein was purified from 50 mL of culture broth obtained from a fed-batch culture induced at the intermediate cell density as described in materials and methods. The final amount and the recovery yield of purified silk protein were 9.2 mg and 26.3%, respectively. The purity of the silk protein as determined by SDS-PAGE was greater than 80%

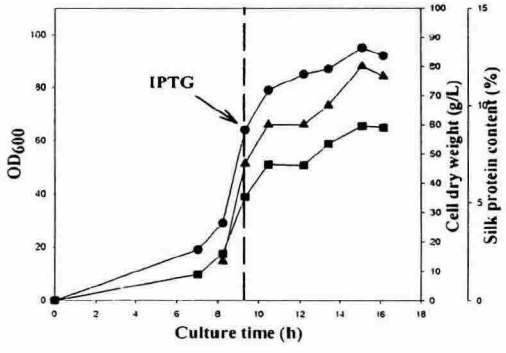

Fig. 1 Time profiles for cell density (OD_{600}) (●), cell dry weight (■), and silk protein content (▲) during fed-batch cultivation with induction at the intermediate cell density.

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Reference

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