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Primary recovery of recombinant human serum albumin from transgenic *Oryza sativa* with a single-step aqueous biphasic system

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ABSTRACT

Recent advances in genetic engineering technology have provided various methods in recombinant proteins production for pharmaceutical purposes. However, the development of the effective purification strategy remains the major challenge for large-scale production of therapeutic proteins because high purity of the proteins is often demanded. In this study, recombinant *Oryza sativa* L. cv Tainung 67 human serum albumin (OsrHSA) was expressed and purified with a single-step polyethylene glycol (PEG)/phosphate aqueous biphasic system (ABS). The optimum condition of PEG/phosphate ABS for the purification of OsrHSA was determined by investigating the effects of PEG molecular weight; pH; tie-line length (TLL); and volume ratio (V_R) of ABS on the OsrHSA purification efficiency. The purification efficiency of OsrHSA was evaluated based on the partition coefficient (K_{HSA}) and recovery yield (R_B) of the OsrHSA in the PEG/phosphate ABS. An R_B of 69.8% of OsrHSA was recovered in the optimum PEG/phosphate ABS consists of TLL of 26.0% (w/w) of pH 7.5. In addition, large-scale ABS were attempted and results showed that the recovery efficiency of 3000 g ABS was consistent with a 10 g ABS. This novel finding has demonstrated that the feasibility of ABS as a potential tool to purify HSA protein in one-step operation.

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1. Introduction

Human serum albumin (HSA) is the most abundant protein component in the human blood plasma which plays a vital role in regulating the plasma oncotic pressure and the fluid distribution between compartments [1]. HSA is a valuable biomarker of various diseases and implantable biomaterials which is also applicable to a variety of biotechnological and biopharmaceutical uses. For instance, HSA can assist in drug delivery, surgical adhesion, and also effective as a cell culture medium supplement [2]. The annual world demand for therapeutic HSA is approximately 500 metric tons which is equivalent to $250 million with a unit price of $3 to $5 per gram and there is a steady rise in prices due to the inadequate supply [3]. In present, all the therapeutic HSA is derived from human plasma of diverse donors via ethanol fractionation. However, this plasma-derived HSA (pdHSA) from random donors would result in transmission of blood-derived pathogens to the recipients [4]. To overcome the shortage of supply and the potential risk of blood pathogens, the development in the production of non-human derived HSA is demanded. In this context, transgenic expression systems as living bioreactors have provided an excellent solution for mass production of HSA in a cost-effective manner.

Transgenic rice is an attractive alternate expression system for the production of recombinant HSA because of the low production cost and safety [5]. In recent decade, *Oryza sativa* recombinant HSA (OsrHSA) has been successfully produced from transgenic rice seeds [6]. The expressed OsrHSA is revealed to be equivalent to the pdHSA in terms of both the structural and functional properties. This discovery has provided an insight to the development of recombinant HSA (rHSA) production. However, the high investment cost associated with the complicated and expensive downstream processes to obtain the rHSA with high purity often limits the application of rHSA in biopharmaceutical field [5].

To achieve high purify of rHSA for biopharmaceutical uses, multiple-steps of the purification strategies are often required, which alternately result in low yield of biopharmaceutical proteins. For instance, despite high purity of rHSA (≥95%) was attained, low yields of rHSA ranging from 20% to 25% were recovered in most studies using multiple-steps chromatographic separation methods [7–9]. Although high purity can be achieved under sequential

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operations of ion exchange, affinity and hydrophobic interaction chromatography, the implementation of these chromatographic separation methods in large-scale production of rHSA are impractical and expensive due to the time-consuming and complicated multiple-operations. Thus, there are increasing demands in finding a cost-effective separation method which could yield high recovery and purity of rHSA in large-scale production. In this context, aqueous biphasic system is introduced to purify and recover the rHSA in a single-step operation.

Aqueous biphasic system (ABS) is a liquid–liquid partitioning technique, comprised of two immiscible phase-forming solutions above the critical concentrations [10]. ABS has emerged as an excellent separation method for recovery of various biological products such as proteins, enzymes, and nucleic acids [11]. The purification of the target bioproduct can be achieved by the selective distribution of the target bioproduct and other impurities between the two phases of the aqueous system. The partition behavior exhibited by the target bioproduct in the system is determined by the physiochemical interactions between the target bioproduct and the phase-forming components. Therefore, types and concentrations of the phase-forming components; pH; volume ratio (V_R) and other additives (*e.g.*, salts or organic compounds) are among the key determinants to the resultant purity of the target bioproduct [10].

ABS featuring short processing time; low materials cost and energy consumption; high resolution; high yield and high capacity could be a promising separation tool for the recovery of rHSA [12]. Thus, the feasibility of inexpensive polyethylene glycol (PEG)/phosphate ABS to recover OsrHSA from cell feedstock was investigated in this study. Different system parameters such as concentration of phase components; pH; concentration of NaCl and the operating temperature were studied to obtain the optimum recovery of OsrHSA with high purity.

2. Materials and methods

2.1. Materials

Polyethylene glycol (PEG) 1000, PEG 2000 and PEG 8000; potassium phosphate monobasic (KH_2PO_4) and potassium phosphate dibasic (K_2HPO_4); human serum albumin (HSA) were purchased from Sigma-Aldrich (St. Loius, USA). Murachige and Skoog (MS) medium was obtained from Duchefa Biochemie (Haarlem, Netherlands). Other chemicals were all of analytical grade.

2.2. Cultivation of transgenic *Oryza sativa* and OsrHSA production

Transgenic *O. sativa* L. cv Tainung 67 (rice cell) was produced and gifted from Plant Physiology and Culture Laboratory, Yuan Ze University, Taiwan. The transgenic *O. sativa* was employed as the expression host for OsrHSA production as described in previous studies [9,13]. A rice suspension cells culture of the transgenic *O. sativa* was established when the transformed calli were subcultured in a culture medium containing Murachige and Skoog (MS) medium, 3% sucrose and 10 µM 2,4-dichlorophenoxyacetic acid. The MS medium consists of major components of 2.99 mM $CaCl_2$; 1.25 mM KH2PO_4; 18.79 mM KNO_3; 1.50 mM $MgSO_4$ and 20.61 mM NH_4NO_3. In addition, MS medium also contains minor components including 0.11 µM $CoCl_2 \bullet 6H_2O$; 0.1 µM $CuSO_4 \bullet 5H_2O$; 100 µM Fe-NaEDTA; 100.27 µM H_3BO_3; 100 uM $MnMoO_4 \bullet 4H_2O$; 1.03 µM $Na_2MoO_4 \bullet 2H_2O$ and 29.91 µM $ZnSO_4 \bullet 7H_2O$. The rice cells suspension was cultivated in the dark at 25 °C and pH 5.8. OsrHSA production was then induced by sucrose-free MS medium in replacement of the MS medium. The resultant OsrHSA culture medium was collected after 2 days for further purification process [9].

2.3. Determination of total protein concentration using Bradford method

The total protein concentrations of rice cells extract and ABS sample extracts were determined using modified Bradford method with bovine serum albumin (BSA) as standard [14].

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