Influence of partition parameters on a recombinant antigen of *Schistosoma mansoni* expressed on *E. coli* using poly(ethylene glycol)–hydroxypropyl starch aqueous two-phase system

A.C. Chaves¹,², E. Silveira², R.P. Bezerra², K.A. Moreira³, N.L.C. Lucena-Silva⁴, F.G.C. Abath⁴, A.L.F. Porto³,⁵, J.M.S. Cabral⁶ and J.L. Lima-Filho³,*

¹Departamento de Patologia, ICB e Ciências Exatas e Naturais, FFPNM, UPE and União das Escolas Superiores de Olinda, UNESF
²Instituto de Ciências Biológicas, UPE
³Laboratório de Imunopatologia Keizo Asami e Departamento de Bioquímica, Universidade Federal De Pernambuco, Av. Prof. Moraes Rego, S/N, Recife, PE, Brazil (UFPE)
⁴Departamento de Imunologia do Centro de Pesquisa Aggeu Magalhães, FIOCRUZ
⁵Departamento de Morfologia e Fisiologia Animal, UFRPE
⁶Centro de Engenharia Biológica do Instituto Superior Técnico, Portugal

*Author for correspondence: Tel.: +55-21-81-32718487, Fax: +55-21-81-32718485, E-mail: zeluiz@hotlink.com.br

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**Summary**

This work describes the partition of a *Schistosoma mansoni* tegumental antigen produced by a recombinant *Escherichia coli* strain using an aqueous two-phase system (ATPS) composed of polyethylene glycol (PEG) and purified hydroxypropyl-starch (Reppal PES 100). The effects of the polymer molecular weight, tie line length and pH on antigen partitioning were investigated. The detection of the antigen in both phases was determined by ELISA. The system composed of PEG 8000 (5.1% w/w) and Reppal PES 100 (13.0% w/w) led to a yield of 92% and a purification factor of 12 concerning the antigen in the PEG-rich phase. It was observed that antigen partition in ATPSs was strongly affected by the pH and tie line length. In addition, it was possible in a single step, to remove the cell debris, which precipitated at the interface of the system.

**Abbreviations:** IPTG – isopropyl-6-D-thiogalactoside; LB – Lurian-Bertani medium; MBPrSm13 – maltose-binding protein-recombinant Sm13 fusion proteins; PEG – polyethylene glycol; Reppal PES 100 – purified hydroxypropyl starch; SDS–PAGE – sodium dodecyl sulphate–polyacrylamide gel electrophoresis

**Introduction**

*Escherichia coli* has been used to produce heterologous proteins for more than 20 years. Fermentation and purification processes have been improved for the production of recombinant proteins at low costs. Much attention has been given to developing methods for the recovery of active intracellular recombinant proteins (Ansejo *et al.* 1994). Extraction in aqueous two-phase systems (ATPSs) is a suitable technology for the first step of a separation procedure and also to partially replace chromatographic steps. This separation procedure, i.e. partition, is influenced by phase system parameters, such as the molecular weight of the polymer, pH, type and concentration of phase-forming components. ATPSs have many advantages over other methodologies usually used for separation (Andrews *et al.* 1996). These systems can be used to extract the target protein directly from the production medium; they have also high capacity and are easily scaled up. The cost of the raw material is not high, mainly due to the possibility of polymer recycling (Shanbhag & Axelsson 1975). Due to the large amount of water employed in both phases (70–80%), these systems are biocompatible, with low tension on interface, which minimizes bioproduct degradation, leading to good resolution and high yields of final product (Kula *et al.* 1982; Hustdet *et al.* 1988). During the past two decades there have been reports on the production of recombinant proteins, including laboratory-scale studies on the production of fusion proteins and their partition in ATPSs (Kohler *et al.* 1989; Strandberg *et al.* 1991). The target protein for the present study is a recombinant form of Sm13, a *Schistosoma mansoni* tegumental membrane antigen. The gene coding for this antigen was cloned, characterized and expressed in *E. coli* (Abath *et al.* 1997; Abath *et al.* 2000). Sm13, a 13 kDa antigen, is one of the principal polypeptides recognized by antibodies from mice protectively vaccinated with...
adult-worm tegumental membranes (Smithers et al. 1990). Moreover, this antigen is also immunogenic in human schistosomiasis (Abath et al. 2000). Schistosomiasis, caused by S. mansoni, is endemic in many tropical areas and responsible for significant morbidity and mortality. In the present paper, MBP-rSm13 (maltose-binding protein-recombinant Sm13 fusion protein) was extracted from bacteria using an ATPS based on polyethylene glycol (PEG)/purified hydroxypropyl-starch (Reppal PES 100). In addition, the effect of the molecular mass of the polymer (PEG), tie line length and pH on the partition of MBP-Sm13 was evaluated.

Materials and methods

Materials

PEG was obtained from Sigma (St. Louis, MO, USA). Reppal PES 100 was obtained from Reppe (Vaxjo, Sweden). All other reagents were analytical grade.

Recombinant strain

An E. coli strain containing the plasmid pMcriA157 was used. The plasmid was selectively maintained by the presence of 100 μg ampicillin/ml. After IPTG induction, a 55 kDa recombinant MPB-Sm13 fusion protein was produced. Details about the construction of the recombinant plasmid can be found elsewhere (Abath et al. 1997).

Production of Sm13 protein

Fermentations were carried out in 250 ml flasks containing 50 ml of LB medium supplemented with ampicillin and inoculated with the recombinant strain. The flasks were incubated in an orbital shaker at 160 rev/min for 8 h. Production of MBP-rSm13 was induced by IPTG at a final concentration of 0.3 mM and 28 °C (Chaves et al. 1999).

Preparation of the aqueous two-phase systems

The systems were prepared from stock solutions of polymers in water. Concentrations of 70, 60 and 50% (w/w) were prepared, for PEG 1000, PEG 3350 and PEG 8000, respectively. A 30% (w/w) stock solution of Reppal PES 100 was prepared by dissolving the powder in cold water. Three tie lines (Table 1) were chosen from the critical point based on the diagram previously published (Almeida et al. 1998). For each tie line the pH values of 6.0, 7.0 and 8.0 were tested.

Protein analysis

The total protein was determined by the Bradford method. The protein extracts were diluted in order to eliminate the interference of the PEG or Reppal PES 100 concentration on the reaction. Blank samples were prepared for each phase. MBP-rSm13 extraction was analysed by ELISA using anti-MPB-Sm13 polyclonal antibodies raised in rabbits (Abath et al. 1997). Quantitation of the protein was performed by ELISA, using known amounts of MBP-rSm13 purified by affinity chromatography using an amylose matrix to construct a standard curve. The degree of the MBP-rSm13 degradation was evaluated on 12.5% SDS–PAGE (Laemmli 1970). The partition coefficient, K, was defined as the ratio between MBP-rSm13 concentration in upper and lower phases and the yield, Y, as the ratio between MBP-rSm13 mass in the top phase and in the total system.

Results and discussion

The partitioning of a protein in ATPS is the result of the combined partition effects of individual factors such as hydrophobicity, electrostatic effects, conformational effects and others. By changing the conditions of the systems, such as the pH, ionic strength, molecular weight and type of polymers, the partition coefficient of a protein will depend on the properties of the protein. In order to understand the partitioning behaviour of MBP-rSm13, a crude extract of E. coli was used. To obtain optimal conditions for extraction of MBP-rSm13 by extraction liquid–liquid partition several parameters were evaluated. In a previous paper we showed that an ATPS composed of PEG and potassium phosphate could lead to an yield of 59% and an antigen purification factor of three in the PEG rich phase (Chaves et al. 2000). In the present paper, yield 92% using a PEG–hydroxypropyl-starch ATPS, a better yield and purification factor could be achieved, extending our previous findings, and improving the purification approach.

Effect of polymer molecular weight

The molecular weight of PEG is an important factor in equilibrium partitioning. Figure 1 shows the partition coefficient of antigen extract in the PEG-Reppal PES 100 system as a function of PEG molecular weight at pH 8.0. This figure shows that log K is dependent on PEG