An efficient expression and purification strategy for the production of S100 proteins in *Escherichia coli*

Honglin He,1† Lei Han,1† Wen Guan,1 Jingjing Li,1 Wei Han2* and Yan Yu1*

1Shanghai Municipality Key Laboratory of Veterinary Biotechnology; School of Agriculture and Biology; Shanghai Jiao Tong University; Shanghai, P.R. China; 2Laboratory of Regeneromics; School of Pharmacy; Shanghai Jiao Tong University; Shanghai, P.R. China

†These authors contributed equally to this work.

**Keywords:** S100 protein, S100A4, S100A11, protein expression, protein purification

Submitted: 06/30/12
Revised: 09/03/12
Accepted: 09/11/12

http://dx.doi.org/10.4161/bioe.22172

*Correspondence to: Wei Han and Yan Yu; Email: weihan@sjtu.edu.cn and yanyu@sjtu.edu.cn

S100 proteins belong to a family of small, acidic, EF-hand Ca\(^{2+}\)-binding proteins and have been found to exert both intracellular and extracellular functions in regulation of Ca\(^{2+}\) homeostasis, cytoskeletal dynamics, cell cycle, motility and differentiation. As a result, they have been widely investigated for their association with diseases, such as, neurological diseases, cardiomyopathy, neoplasias and inflammatory diseases.

To facilitate further studies of S100 proteins, we reported a simple and efficient method for the expression and purification of human S100A4 and S100A11 proteins in *Escherichia coli*. Since S100 proteins share many common physical and chemical characteristics, we expect that this approach can be extended to the production of most S100 proteins.

The S100 protein is named after its solubility in 100% saturated ammonium sulfate solution.2 To date, S100 proteins comprise the largest subfamily of EF-hand Ca\(^{2+}\)-binding proteins and more than 20 members in this subfamily have been found. The genes that code for S100 proteins exist only in vertebrates and are typically located in a cluster on human chromosome 1q21, on mouse chromosome 3F1-F2, and on rat chromosome 2q34.2 S100 proteins are low molecular weight (10–12 kDa) acidic proteins and highly conserved between species. They exist as a non-covalent dimer with an antiparallel conformation, with each monomer composed of two different EF-hand motifs linked by a hinge region.3 The binding of calcium to S100 proteins in the two EF-hand motifs induces conformational changes that exposes a hydrophobic surface in each monomer used to interact with their target proteins.4-5 S100 proteins have garnered significant research interest for their association with many diseases, such as, neurological diseases,6,7 cardiomyopathy,8,9 neoplasias,10,11 and inflammatory diseases.12,13 Recent work has further revealed that they have been implicated in intracellular and extracellular regulatory activities.14-16 In particular, S100 proteins are typically localized in the nucleus and cytoplasm, and are essential to signal transduction,17,18 Ca\(^{2+}\) homeostasis,19-21 regulation of enzyme activity,22-24 dynamics of cytoskeleton,25,26 and cell motility.27,28 It is also found that members of S100 family could be secreted into the extracellular space and exert effects in a cytokine-like manner. The receptor for advanced glycation end products (RAGE) has been proved as a common receptor for S100 proteins.29 S100 proteins can regulate a diverse array of processes through RAGE, including cell cycle,30 cell differentiation,31 survival and apoptosis.32 However, the biological functions and pathology of S100 proteins have yet to be well understood. Especially, more and more members of the S100 family have been found to be secreted and have extracellular functions. In addition, there is a lack of understanding on the underlying mechanisms in diverse processes regulated by the S100 proteins. Hence, for in vivo and in vitro studies of S100 proteins, a simple accessible method is needed...
for the production of recombinant S100 proteins. The expression and purification of a few S100 proteins in *E. coli* have been described previously, mostly through the use of fusion proteins and affinity chromatography. However, as fusion proteins contain an additional affinity tag, the structure and function of the isolated protein may be affected. Recently, we reported a simple and efficient method for producing high-purity recombinant human S100A6 from *E. coli* through a two-step chromatography process of ion exchange and gel filtration. The purified protein matched the native human S100A6 exactly without requiring any tag. Here, we describe the improvements made to this method that can be applied to purify other S100 protein members, S100A4 and S100A11.

*E. coli* is the most commonly used expression system for production of numerous proteins, due to the advantages of low cost, high efficiency and high yield. A typical drawback of *E. coli* is the lack of post-translational capability, in particular the glycosylation of proteins. However, this does not pose a problem for S100 proteins as there have been no reports of glycosylated S100 proteins to date. Thus, *E. coli* BL21 (DE3; Novagen, USA) was chosen as the recombinant host strain for our method. BL21 was genetically engineered to contain the gene of T7 RNA polymerase which is regulated by the lac promoter and the lac operator.

The expression of T7 RNA polymerase gene can be induced by isopropyl-β-D-thiogalactopyranoside (IPTG). The plasmid pET30a(+) (Novagen) was used as the expression vector. Since pET30a(+) has both T7 promoter and lac operator, the target gene can be transcribed under the combined action of IPTG and T7 RNA polymerase. To match the native S100 gene without changing or adding nucleotides, the *Nde*I site was chosen as one of the insert sites for the S100 gene in pET30a(+) and *Xho*I site as the other insert site. Human S100A4 and S100A11 genes were amplified by polymerase chain reaction (PCR) using sense 5′-CGA TCA TAT GGC GTG CCC TCT GGA G-3′ and antisense 5′-CGA CCT GCC TGT GCT GCT T-3′ primers for hS100A4 and sense 5′-ACA GCA TAT.
well before most non-specific proteins when the conductivity reached 5 ms/cm, revealed that S100 proteins were eluted.

The fractions containing S100 protein from the anion exchange chromatography were further purified by gel filtration, Superdex 75 prep grade (GE Healthcare). Here, we used phosphate buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM NaHPO4, 1.4 mM KH2PO4, pH 7.4). Different elution fractions were collected based on UV absorption at 280 nm. Since S100 proteins have smaller molecular weight than most of non-specific proteins, they were eluted after the non-specific proteins.

Following the two chromatography steps, rhS100A4 and rhS100A11 were purified to above 95% (Fig. 2). The amount of recombinant S100 proteins obtained from 1 L of culture was more than 20 mg, which was sufficient for both the in vivo and in vitro studies of their functions.

Since the S100 family proteins have many common physical and chemical properties, we expect that our method of anion exchange chromatography and gel filtration can be extended for the purification of most S100 proteins, based on their acidic pl value and small molecular weight, respectively.

Table 1. Molecular weight and theoretical pl of human S100 protein

| Protein | MW(kDa) | Theoretical pl | Number of AAs | Number of Cys |
|---------|---------|----------------|--------------|---------------|
| S100A1  | 10.55   | 4.39           | 98           | 1             |
| S100A2  | 10.99   | 4.68           | 97           | 4             |
| S100A3  | 11.71   | 4.71           | 101          | 10            |
| S100A4  | 11.73   | 5.85           | 101          | 4             |
| S100A5  | 10.74   | 5.01           | 92           | 2             |
| S100A6  | 10.38   | 5.32           | 90           | 0             |
| S100A7  | 11.47   | 6.27           | 101          | 2             |
| S100A8  | 10.83   | 6.50           | 93           | 1             |
| S100A9  | 13.24   | 5.71           | 114          | 1             |
| S100A10 | 11.20   | 6.82           | 97           | 2             |
| S100A11 | 11.74   | 6.56           | 105          | 2             |
| S100A12 | 10.58   | 5.81           | 92           | 0             |
| S100A13 | 11.47   | 5.90           | 98           | 0             |
| S100A14 | 11.66   | 5.16           | 104          | 3             |
| S100A15 | 11.30   | 6.89           | 101          | 2             |
| S100A16 | 11.80   | 6.28           | 103          | 1             |
| S100B   | 10.71   | 4.52           | 92           | 2             |
| S100G   | 9.62    | 4.69           | 79           | 0             |
| S100P   | 10.40   | 4.75           | 95           | 1             |
| S100Z   | 11.62   | 7.73           | 99           | 2             |

Table 1. Molecular weight and theoretical pl of human S100 protein

Conclusion

Here, we described a method for producing S100 family proteins. For expression of recombinant S100 proteins, we used BL21 as the host strain and pET30a(+) as the expression vector. Two steps of chromatography, ion exchange and gel filtration, were used for purifying S100 proteins. Using this method, we successfully purified recombinant human S100A4 and S100A11. We expect that this simple yet extremely efficient strategy of producing S100 proteins will provide the framework of support for future studies involving S100 proteins.

Acknowledgments

We would like to thank Junhao Shawn Tan for the critical reading and comments on the manuscript. This work was supported by the Science and Technology Commission of Shanghai Municipality (No. 9540700608).
S100A8, S100A9, and S100A8/A9 induce neutro-
and apoptosis in malignant melanoma. J Biol Chem

calcium-binding protein S100B down-regulates p53
S100A4 (Mts1) in tumor-stroma interplay. J Biol
Comm 2000; 274:865-71; PMID:10924368;

Schäfer BW, Heizmann CW. The S100 family of
extracellular calcium-binding proteins: functional
pathology. Trends Biochem Sci 1996; 21:134-40;
PMID:11878140; http://dx.doi.org/10.1016/S0969-

Gräfin B, Gräfin HH, Bibermann A, Meckel C,
Müller E, Müller N. The calcium-dependent interaction between S100B and the monothiol-AAA ATPase-ATPase and the role of this complex in the cytoprotective processing of ATMD. Mol Cell
2010; 36:2724-36; PMID:20051179; http://
dx.doi.org/10.1016/j.molcel.2010.03.017.

Teyssie S, Sorci M, Robert M, Gris S, Orellana M,
Pérez R, et al. Interaction of S100A1 with the Ca2+-
causal channel (ryanodine receptor of skeletal muscle). Biochemistry 1997; 36:1146-
53); PMID:9298700; http://dx.doi.org/10.1021/

Grut N, Delpicco C, Meldorff GI. Delsolce JC.
Sorci M, Lin S, Qian J, et al. The giant protein AHNAK
4522(00)00238-4.

Shamma F, Förster A, Huether C, Völkl A, Gong T,
Roberts D, et al. The C367R mutation in the human
S100A11 protein: implications for Ca2+-
and Zn2+-binding properties. J Biol Chem 2001;
276:7323-31; PMID:11233244; http://dx.doi.

Borkok H, Koberle T, Vínczé H, Zöldi G, Dénes I,
Debreczeni GM, Kander M, et al. Human S100A12 is a specific target for the calcium-
and interbind-
S100B protein potential implications for Ca2+
homeostasis regulation by S100B. J Biol Chem
2001; 276:7322-23; PMID:11233243; http://

Robitsch R, Huber PR, Schäfer CI G, Bianchi R, Riuzzi F, Tubaro C, Arcuri M, et al. S100B Protein, A Damage-
and Cu(2+)-binding protein family. Biometals
2000; 13:383-97; PMID:10191501; http://dx.doi.

Schindler B, Moi A, Frohne J, Völkl A, Gong T,
Heizmann CW. The multifunctional S100 protein
family. Methods Mol Biol 2002; 172:69-80;
PMID:11889329; http://dx.doi.org/10.1385/1-59259-

Piekarz CD, Zeng F, Chen KL, Lim H, Xie H, et al.
Expression and purification of recombinant human S100A11 in E. coli. Archiv Biochem Biophys 2008; 467:205-12; PMID:18383024; http://

Fujita H, Terasaki S, Shibayama T, Taniguchi M,
Yamaguchi M, et al. Kinetic properties of human
S100A6 in Escherichia coli. Protein Expr Purif 2012; 83:98-103; PMID:22450162; http://

Schneider M, Hanke Z, Shikhli A. A common
mediator of epithelial-mesenchymal transition,
neovascularization and metastasis in mouse and man: from evolution to function and pathology (including an update of the nomenclature). J Transl Med 2009; 7:51; PMID:19387741; http://dx.doi.org/10.1186/1479-

Lundqvist E, Fehr M, Heizmann CW. Gallicic A
S100B and S100A11 differentially modulate cellular survival by interacting with distinct RAGE (receptor for advanced glycation and prod-
and other bacterial extracellular enzymes. Bioeng
Bugs 2011; 2:45-9; PMID:21636987; http://dx.doi.

Hausen MC, Zehnder AM, D’Alessio AN, et al.
Characterisation of the human Ca2+- and Zn2+-
binding properties in chicken skeletal muscles. Eur J Cell Biol 2005; 84:218-27; PMID:16078273; http://dx.doi.org/10.1016/j.ejcb.2004.11.018.

Kumrai M, Cello J, Johnson K, Ares M, et al.
Structural stability and reversible unfolding of human
S100A6 in solution. J Mol Biol 2002; 320:266-
PMID:12145737; http://dx.doi.org/10.1002/jcp.10271.

Yamamoto M, Takeda Y, Inoue H, et al. Identification of a C367R mutant in the human
S100A11 protein: functional significance and RAGE (receptor for advanced glycation end products) binding domain. J Biol Chem 2008; 283:31317-
PMID:18322670; http://dx.doi.org/10.1074/jbc.M709351200.

He H, Yang Y, Su J, Zhang B, Tu T, Guo J, et al.
Expression and purification of recombinant human S100A11 in E. coli. Eur J Biochem 2000; 267:663-70; PMID:10803962; http://dx.doi.org/

Schneider M, Hanke Z, Shikhli A. A common
mediator of epithelial-mesenchymal transition, neovascularization and metastasis in mouse and man: from evolution to function and pathology (including an update of the nomenclature). J Transl Med 2009; 7:51; PMID:19387741; http://dx.doi.org/10.1186/1479-

He H, Li J, Song D, Li M, Yu Y. S100A11: diverse function and pathology corresponding to different target proteins. Cell Birth Rep 2009; 55:117-
PMID:19497045; http://dx.doi.org/10.1016/j.

Tsunakib M, Burbanenkov B, Jermakova N, Savelieva S, Lage J, Heizmann CW. Efficient expression and purifi-
tion for the production of recombinant full-length human S100A6 and other profile proteins. Bioeng Bugs 2011; 2:45-55; PMID:21636987; http://dx.doi.

Merlini NL, Sprat DE, Schrøder KS, Dannevig BI, et al. Selective expression of human S100A11 and S100A1 protein. Protein Expr Purif 2010; 73:58-64; PMID:20441749; http://dx.doi.org/10.1016/j.

Hagiwara A, Lopez MM, Richarson RW, Komanuk G, Ushinov A, Przepiorka, et al. S100A6 in nonmelanocytic cells. In vitro. J Cell Biol 1986; 103:851-6; PMID:3714890; http://dx.doi.org/10.1083/jcb.103.

Hagiwara A, Lopez MM, Richarson RW, Komanuk G, Ushinov A, Przepiorka, et al. S100A6 in nonmelanocytic cells. In vitro. J Cell Biol 1986; 103:851-6; PMID:3714890; http://dx.doi.org/10.1083/jcb.103.

Hagiwara A, Lopez MM, Richarson RW, Komanuk G, Ushinov A, Przepiorka, et al. S100A6 in nonmelanocytic cells. In vitro. J Cell Biol 1986; 103:851-6; PMID:3714890; http://dx.doi.org/10.1083/jcb.103.