Appl Biochem Biotechnol
DOI 10.1007/s12010-017-2502-8

Efficient Antibody Assembly in *E. coli* Periplasm by Disulfide Bond Folding Factor Co-expression and Culture Optimization

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Received: 29 March 2017 / Accepted: 1 May 2017

Abstract Molecular chaperones and protein folding factors of bacterial periplasmic space play important roles in assisting disulfide bond formation and proper protein folding. In this study, effects of disulfide bond protein (Dsb) families were investigated on assembly of 3F3 Fab, an antibody inhibitor targeting matrix metalloproteinase-14 (MMP-14). By optimizing DsbA/C co-expression, promoter for 3F3 Fab, host strains, and culture media and conditions, a high yield of 30-mg purified 3F3 Fab per liter culture was achieved. Produced 3F3 Fab exhibited binding affinity of 34 nM and inhibition potency of 970 nM. This established method of DsbA/C co-expression can be applied to produce other important disulfide bond-dependent recombinant proteins in *E. coli* periplasm.

Keywords Fab · IgG · Periplasm · Over-expression · Protein folding factor · DsbA · DsbC

Abbreviations

CDR	complementarity determining region
DsbA/C	disulfide bond A and C
ELISA	enzyme-linked immunosorbent assay
Fab	fragment of antigen binding
FRET	fluorescence resonance energy transfer assay

Electronic supplementary material The online version of this article (doi:10.1007/s12010-017-2502-8) contains supplementary material, which is available to authorized users.

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Published online: 10 May 2017

IgG	immunoglobulin G
IPTG	isopropyl β-D-1-thiogalactopyranoside
MMP	matrix metalloprotease
PCR	polymerase chain reaction
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
VH	variable heavy
VL	variable light

Introduction

With fast growth, low cost, ease of genetic manipulation, and many molecular tools and protocols at hand, *Escherichia coli* is one of the most widely used microorganism species for producing recombinant proteins [1]. Particularly, as the space between its inner and outer membranes, *E. coli* periplasm provides beneficial properties for protein expression [2], such as multiple molecular chaperones (e.g., Skp, FkpA, SurA) [3–5], quality control mechanisms via the secretion machinery [6, 7], and the oxidizing environment and folding modulators facile for disulfide bond formation. With extensive understandings of recombinant protein expression in *E. coli* periplasmic space, many challenging eukaryotic proteins with multiple disulfide bonds have been successfully produced [8–12].

Our lab recently isolated a panel of monoclonal antibody Fabs (e.g., 2B5, 3A2, and 3E9) which inhibited matrix metalloproteinase-14 (MMP-14) with high potency and selectivity [13]. However, one potentially valuable Fab clone 3F3 exhibiting an inhibition potency at hundreds nM range, failed to be produced at milligram level needed for full characterizations. Further analysis of 3F3 Fab production profiles demonstrated that both its light chain (VL-CL-FLAG tag) and heavy chain (VH-CH1-His tag) were expressed in *E. coli* periplasm and were able to be separately purified via their associated tags (Fig. 1). But 3F3 Fab was not well assembled in vivo (<10 μg per liter of culture media) in contrast to other Fab clones (typical yields of 0.5–2 mg/L). Encouraged by numerous pioneering works of multiple intramolecular and intermolecular disulfide bond formation by co-expressing folding factors involved in *E. coli* secretion expression systems [8–12, 14], we hypothesized that similar approaches can efficiently facilitate 3F3 Fab assembly. In this study, we report the effects of Dsb (disulfide bond) protein family on 3F3 Fab production. In addition, the promoter, host strain, and culture media and conditions were also systematically optimized to yield milligrams of purified 3F3 Fab.

Materials and Methods

Construction of 3F3 Fab Expression Plasmids

The fragment encoding VL-CL-VH-CH1 genes of antibody clone 3F3 [13] was amplified by PCR, digested with *Nsi*I and *Sal*I, and cloned into the same sites on a Fab expression vector containing a phoA promoter and STII leader peptide sequences (Fig. 1A, [15]). On pPhoA-Fab3F3, a polyhistidine tag and a FLAG tag were placed at the C-termini of heavy and light chains. To construct 3F3 Fab expression vector with a Lac promoter and pelB leader peptides, its VH and VL fragments were amplified by PCR and cloned into *Nco*I/*Not*I and *Nhe*I/*Hin*dIII

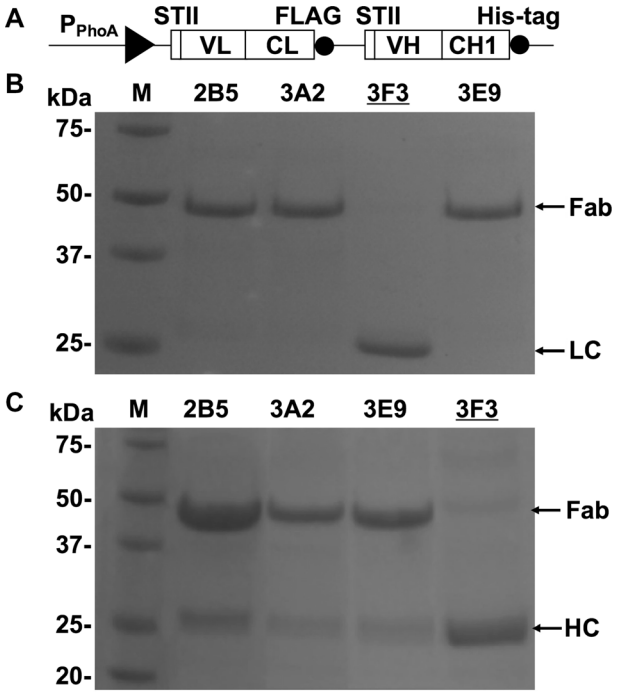

Fig. 1 Expressed 3F3 heavy and light chains failed assembling to Fab. **a** Construct of 3F3 Fab expression cassette with P_{PhoA} promoter and STII leaders. FLAG tag and His-tag are at the C-termini of light and heavy chains. **b** Purification results of four Fabs 2B5, 3A2, 3F3, and 3E9 [13] from their BL21 periplasmic fractions using anti-FLAG resin, resulting in capture of Fabs and light chain (*LC*) fragments (VL-CL). 3F3 LC MW = 27 kDa. **c** Purification results of four Fabs from their BL21 periplasmic fractions using Ni-NTA resin, resulting in capture of Fabs and heavy chain (*HC*) fragments (VH-CH1). 3F3 HC MW = 30 kDa. Further quantification showed assembled 3F3 Fab (MW = 57 kDa) had a yield <10 μg per litter of culture

sites on pMAZ360-IgG [16], respectively, and a 10× His tag with a stop codon was introduced at downstream of CH1 domain to obtain pLac-Fab3F3 (Fig. 4A).

Construction of DsbA/C Plasmids

The plasmids encoding both DsbA and DsbC, either under one promoter (1P) or two separate promoters (2P), were constructed by overlap extension PCR cloning [17]. Briefly, DsbC gene cassette with its promoter region was PCR amplified using pBAD-DsbC [18] as the template, and the 1.6-kb product was gel purified and mixed with pBAD-DsbA [18] for overlap extension PCR. The product was digested with *Dpn*I and transformed to obtain pBAD-DsbAC2P (under two separate promoters) (Fig. 2A). Similarly, DsbC gene cassette without its promoter region was amplified and overlap extended with pBAD-DsbA to construct pBAD-DsbAC1P (under one promoter). All resulting plasmids were confirmed by DNA sequencing.

3F3 Fab Expression

Constructed Fab expression plasmids were transformed into *E. coli* Jude-I or BL21 and cultivated overnight in 2× YT or TB supplemented with 100 μg/mL ampicillin at 30 or 37 °C. For pLac-Fab3F3, cells were induced with 0.1 mM IPTG. To co-express Dsb proteins, pBAD-DsbA/-DsbC/-DsbAC1P/-DsbAC2P were transformed into Jude-I or BL21 harboring pPhoA-Fab3F3 or pLac-Fab3F3. The co-transformed hosts were grown in 2× YT or TB media supplemented with 100 μg/mL ampicillin and 35 μg/mL chloramphenicol at 37 °C. When cell density reached OD_{600} 0.6–0.8, arabinose was added to a final concentration of 0.2% (*w*/*v*) and cells were further cultured for 30 min before 0.1 mM IPTG was added for Fab induction at

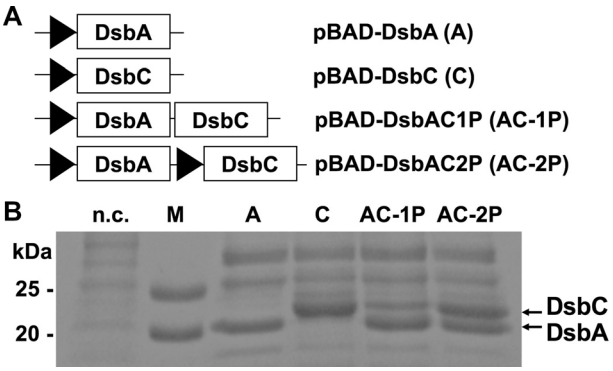

Fig. 2 Folding factor DsbA/C construct designs and expression results. **a** Four constructs were applied in this paper: expression of either DsbA or DsbC under an arabinose promoter P_{BAD}; bicistronic co-expression of DsbA and DsbC under one P_{BAD} promoter (AC-1P); monocistronic co-expression of DsbA and DsbC under two separate P_{BAD} promoters (AC-2P). **b** SDS-PAGE analysis of periplasmic fractions. Cultures were induced with 0.2% arabinose. *DsbA MW* 23 kDa, *DsbC MW* 25 kDa., *n.c.* negative control, *M* protein marker

either 30 or 37 °C overnight. To further improve Fab production in simulated fed-batch and high-glucose conditions [19], BL21 harboring both pLac-Fab3F3 and pBAD-DsbA was cultivated in EnBase media following the manufacturer's instructions (BioSilta).

Periplasmic Fraction Preparation and Western Blotting

A modified cold osmotic shock protocol was applied to release expressed antibody fragments from the periplasmic space [20]. After cells were pelleted by centrifugation at 16,000 ×*g* for 1 min, for every two OD_{600} cells, the pellet was resuspended in 350μL periplasmic buffer (200 mM Tris-HCl, pH 7.5, 20% sucrose, 30 U/μL lysozyme) and incubated at RT for 5 min. Three hundred fifty microliters ice-cold DDW was then added for osmotic shock and incubation on ice for 10 min. The resulting suspensions were centrifuged at 16,000 ×*g* for 2 min and clarified supernatants were subjected to Western blotting. Either anti-His-HRP (Abcam) or anti-Fab-HRP (Sigma-Aldrich) was used as the second antibody. Signals were developed with chemiluminescent substrate (Thermo Scientific) and analyzed with an imager (Bio-Rad).

3F3 Fab Purification

From periplasmic fractions, 3F3 Fab was purified by affinity chromatography using Ni-NTA resin (Qiagen), and dialyzed against 50 mM HEPES (pH 6.8), 150 mM NaCl to remove residual imidazole (a weak inhibitor of MMPs). The homogeneities of purified antibody fragments were verified by SDS-PAGE (non-reducing), and 3F3 Fab concentration was measured with NanoDrop 2000 (Thermo Scientific).

Fab ELISA

Maxisorp 96-well immunoplates (Nunc) were coated with 5 μg/mL streptavidin in TBS buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM $CaCl_2$, 100 μM $ZnCl_2$) overnight at 4 °C and

washed twice with TBST (TBS with 0.1% Tween-20). The plates were blocked with 0.5% gelatin in TBS for 2 h at RT followed by washing twice with TBST. Biotinylated-cdMMP-14 [21] in TBS was incubated at RT for 15 min followed by washing twice with TBST. Purified Fab was serially diluted into cdMMP-14 coated wells and incubated at RT for 1 h. Bound 3F3 Fab was detected using anti-Fab-HRP. TMB (3,3′,5,5′-tetramethylbenzidine) solution was added to develop signals. The reaction was stopped by acidification using 1 M sulfuric acid. The absorbance was measured at 450 nm. The half-maximal effective concentration (EC_{50}) was calculated from a four-parametric logistic curve-fitting analysis.

MMP Inhibition Assay

The enzymatic activities of 1 nM cdMMP-14 in the presence of 4 nM–3 μM of 3F3 Fab were measured at 37 °C by monitoring the hydrolysis of 1 μM fluorogenic peptide Mca-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH2 (Bachem) at λ_{ex} = 328 nm and λ_{em} = 393 nm in TBS [20, 22]. Fluorescence was recorded continuously for 30 min and the initial reaction rates and inhibition constants were calculated by fitting the data to Eq. (1), where V_i is the initial velocity in the presence of the inhibitor, V_o is the initial velocity in the absence of inhibitor, and $[I]$ is the inhibitor concentration.

$$\frac{V_i}{V_o}\% = \frac{1}{1+\frac{[I]}{IC_{50}}} \times 100 \quad (1)$$

Results

Over-expression of DsbA/C in Periplasm

To construct plasmids encoding both folding factors, DsbC gene cassettes with or without its arabinose-inducible promoter P_{BAD} were cloned into pBAD-DsbA [18] by overlap extension PCR [17]. The resulting plasmids encoded DsbAC either bicistronically (pBAD-DsbAC1P) or monocistronically (pBAD-DsbAC2P) (Fig. 2A). These two plasmids together with pBAD-DsbA/-DsbC were transformed to *E. coli* strain Jude-I, and cells were induced with 0.2% arabinose. Expression profile analysis by SDS-PAGE confirmed that DsbA and DsbC were over-expressed in the periplasmic space with their expected MWs of 23 and 25 kDa (Fig. 2B). In contrast, no DsbA/C associated bands were detected in the control cells without transformation. For AC-2P construct, both Dsb proteins were produced at comparable levels. While for AC-1P, DsbA was expressed twice as much as DsbC, likely because DsbC gene was placed in the downstream of the bicistronic structure. Similar results were observed when BL21 was applied as the expression host (data not shown).

DsbA/C Improved 3F3 Fab Assembly

To test the hypothesis that co-expression of DsbA/C can facilitate 3F3 Fab assembly, and to identify the most efficient Dsb construct for 3F3 Fab production, each plasmid of pBAD-DsbA/-DsbC/-DsbAC1P(one promoter)/-DsbAC2P(two separate promoters) was transformed

to Jude-I cells harboring 3F3 Fab gene which was at downstream of alkaline phosphatase (phoA) promoter and STII signal peptides (Fig. 1A). Arabinose was added to induce DsbA/C, and P_{PhoA} was auto-induced following depletion of phosphate from culture media 2× YT. Periplasmic fractions were prepared and subjected for Western blotting at non-reducing conditions using anti-His-HRP, which recognized the polyhistidine tag at C-terminal of heavy chain (HC). Results indicated that in addition to large amounts of unassembled HC, trace amounts of 3F3 Fab formed (with an apparent MW ~50 kDa) when co-expressed with DsbC, AC-1P, or AC-2P (Fig. 3). However, without Dsb folding factor (n.c. in Fig. 3) or co-expressing DsbA alone showed no assembled 3F3 Fab. Overall, AC-1P promoted the highest Fab expression when P_{PhoA} was used.

Promoter, Host, and Culture Temperature Optimizations

To improve Fab production, 3F3 LC and HC genes were cloned into a periplasmic expression vector carrying a strong inducible P_{Lac} promoter and pelB leader peptides [23]. This pLac-Fab3F3 (Fig. 4A) was co-transformed with each of Dsb plasmids A/C/1P/2P into Jude-I cells. In 2× YT, 0.1 mM IPTG and 0.2% arabinose were used for Fab and DsbA/C inductions. After culture at 30 °C, periplasmic fractions were analyzed by Western blotting (non-reducing) using anti-Fab-HRP, which bound to both assembled 3F3 Fab and unassembled HC/LC fragments. Results indicated that significant amounts of 3F3 Fab were produced (Fig. 4B). In addition, unassembled LC monomer was detected, suggesting that more LC was produced than HC. Associated with this unbalanced expression, LC dimer (with a MW less than that of Fab) was also present. Notably, when co-expressed with DsbA alone, much less LC monomer and no LC dimer were detected, suggesting DsbA was optimal for 3F3 Fab production under P_{Lac} (arrow in Fig. 4B).

The same set of Fab3F3-DsbA/C co-expression plasmids was also transformed into BL21, a commonly used B strain with deficiency in lon and ompT proteases. Transformed cells were cultured in 2× YT at 30 °C with 0.2% arabinose and 0.1 mM IPTG. Comparing to same amounts of Jude-I cells, BL21 produced several folds more 3F3 Fab for all cases (Fig. 4B), suggesting that BL21 was superior for Fab expression.

Although low culture temperatures (e.g., 30 °C) were often used for proper protein folding, to further understand the effects of DsbA/C, we repeated the above experiments (Fig. 4B) at culture

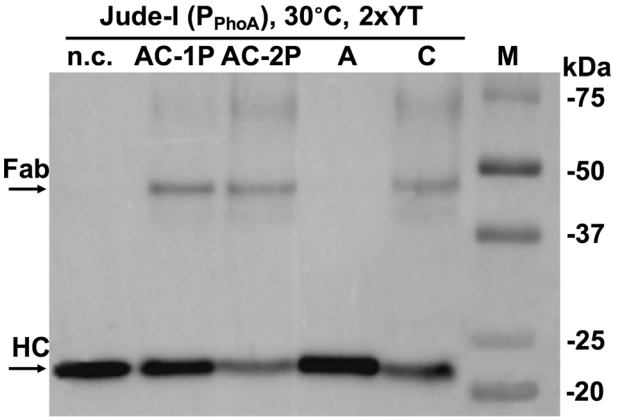

Fig. 3 Effects of disulfide bond enzymes DsbA/C on 3F3 Fab assembly. Western blotting results of overnight cultures at 30 °C in 2× YT. Jude-I was the host. 3F3 Fab was expressed under a P_{PhoA} promoter. No DsbA/C co-expression served as the negative control (*n.c.*). Bands were detected with anti-His-HRP

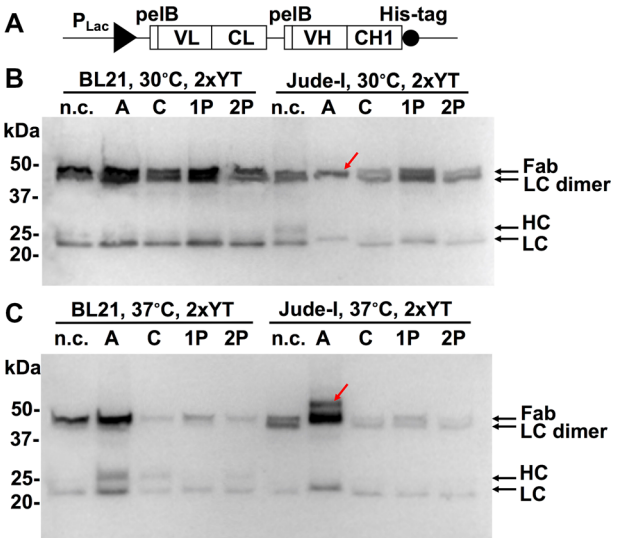

Fig. 4 Promoter, host, and culture temperature optimizations. **a** 3F3 Fab expression construct with P_{Lac} and pelB leaders. 0.2% arabinose was used for DsbA/C expression. 0.1 mM IPTG was used for Fab induction. Western blotting results using host cells Jude-I or BL21 at **b** 30 °C or **c** 37 °C overnight in 2× YT. Bands were detected with anti-Fab-HRP. The arrows in **b** and **c** indicate the assembled 3F3 Fab and 3F3 Fab with leader peptide respectively

temperature of 37 °C. Under this challenge, all constructs except DsbA exhibited inferior 3F3 Fab production as expected (Fig. 4C). Interestingly, when cultured at 37 °C, BL21 co-transformed with pLac-Fab3F3 and pBAD-DsbA produced fully assembled 3F3 Fab as the single major band, with significantly decreased amounts of unassembled LC/HC fragments and only a trace amount of LC dimer. Presumably, the appropriate folding factor (DsbA in this case) facilitated efficiently assembling of produced 3F3 LC and HL, while other constructs failed on Fab assembly resulting in degradation of LC and HC fragments. Culturing the same plasmid combination (pLac-Fab3F3 and pBAD-DsbA) in Jude-I at 37 °C exhibited similar results with an even higher Fab yield. However, there was an additional band of a larger MW than that of Fab (arrow in Fig. 4C), likely associated with Fab molecules carrying unprocessed leader peptides due to the high secretion demand. Collectively, results of Fig. 4 suggested that among all tested conditions, the combination of P_{Lac}, BL21 and DsbA was the most beneficial for 3F3 Fab production.

30-mg/L 3F3 Fab Yielded in Rich Culture Media

We next tested the best combination (promoter, host, and Dsb protein) identified with rich media TB and EnBase [19]. As results shown in Fig. 5A, when BL21 (pLac-Fab3F3) was cultured in TB at 30 °C, constructs C/1P/2P reduced 3F3 Fab production levels while DsbA delivered a marginal improvement, comparing to the same cells cultured in 2× YT at 30 °C (Fig. 4B). However, when cultured at the simulated fed-batch and high-glucose conditions using EnBase media, fully assembled 3F3 Fab bands represented >90% the total signal intensities in Western blotting, and unassembled LC/HC or LC dimer was not detected (Fig. 5B). Furthermore, the expression level at 30 °C was twofold higher than that at 37 °C. In addition, there was a band with an apparent MW of ~35 kDa in the 37-°C culture sample (arrow in Fig. 5B), likely due to a low degree of cleavage at the long CDR-H3 region, observed phenomena for certain protease inhibitory antibodies [24, 25]. But the level of truncated Fab was reduced when cultured at 30 °C. Significantly, with OD_{600}/mL reaching 15.7 at the time of harvest, 30 mg purified 3F3 Fab per liter of culture was achieved (inset of Fig. 6), which represented a four orders of magnitude improvement compared to the initial result (<10 μg/L, Fig. 1C).

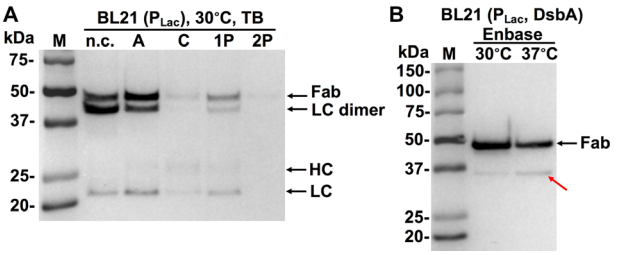

Fig. 5 High yields of 3F3 Fab using **a** rich media TB and **b** fed-batch media EnBase. Co-expression was induced with 0.2% arabinose and 0.1 mM IPTG. Western blotting bands were detected with anti-Fab-HRP. The arrow in **b** indicates truncated Fab

Characterization of Produced 3F3 Fab

From the optimized expression, purified 3F3 Fab was subjected for biochemical characterizations. Its binding affinity was measured by direct ELISA, in which serially diluted 3F3 Fab samples were incubated in 96-well plates sequentially coated with streptavidin and biotinylated cdMMP-14 [21]. Captured 3F3 Fab was then detected with anti-Fab-HRP and signals were developed with chromogenic substrates. As shown in Fig. 6, a sigmoidal dose-response curve was observed allowing the calculation of an affinity EC_{50} value as 34 nM. Next, inhibition function of 3F3 Fab on cdMMP-14 was studied using a fluorogenic peptide substrate. When 3F3 Fab concentrations increased from 4 nM to 3 μM, decreased activity of 1 nM cdMMP-14 displayed a dose-response curve. At the highest concentration tested (3 μM) 3F3 Fab inhibited ~70% activity of cdMMP-14, and the 3F3 Fab concentration gave 50% inhibition (IC_{50}) was determined as 970 nM (Fig. 6).

Discussion

In periplasm of *E. coli*, the formation of protein disulfide bonds is catalyzed by Dsb family folding factors [18, 26]. DsbA serves as an oxidase in vivo and is maintained in its oxidized state by membrane-bound DsbB. DsbC catalyzes disulfide reduction and exhibits isomerization activity. It breaks non-native disulfide bonds and acts as a proofreader for the formation of native disulfide bonds. DsbC is kept reduced by another membrane modulator DsbD. Although DsbA and DsbC catalyze the redox reactions in opposite directions, synergy between them has been reported to facilitate protein folding by reducing mismatched disulfide pairs and forming the proper ones [8, 18, 27].

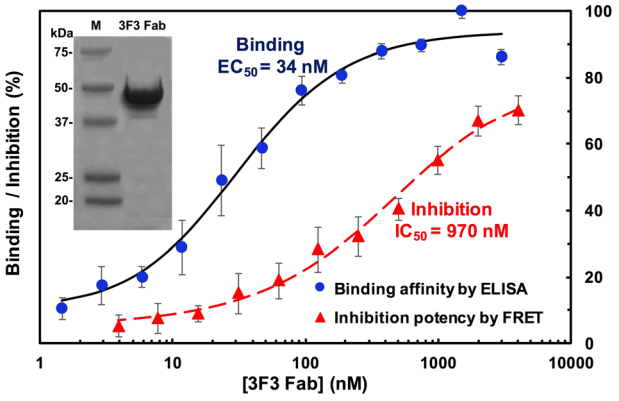

Fig. 6 Binding affinity and inhibition potency of 3F3 Fab. Binding EC_{50} and inhibition IC_{50} were determined using ELISA and FRET assays, respectively. Purified 3F3 Fab sample after concentration (inset) was used for measurements

In this study, the effects of Dsb family on 3F3 Fab production, especially its heavy chain and light chain assembly, were investigated. Particularly, 3F3 Fab was co-expressed with either DsbA/C alone or with both DsbA and DsbC proteins, and the expression levels of Fab as well as unassembled heavy and light chains were analyzed. Results indicated that when phoA promoter was used for Fab expression, DsbC is beneficial for Fab assembly (Fig. 3); while under Lac promoter, DsbA significantly improved Fab production and decreased LC dimer amounts (Fig. 4). Presumably, the fast expression rate of Fab driven by Lac promoter required more DsbA to facilitate the formation of disulfide bonds; while at a low expression rate under phoA promoter, resolving the non-native disulfide bridges by DsbC was apparently more important. All these results demonstrated that the optimal folding factor(s) for disulfide protein formation was sensitive to various factors, therefore, needs to be studied case-by-case.

To promote Fab formation, this study applied a sequential induction protocol, in which DsbA/C (under $P_{BAD,}$ Fig. 2A) was induced first, and cells were cultured for 30 min before Fab induction (under P_{Lac}, Fig. 4A). Presumably, this approach allowed Fab to be synthesized with the presence of DsbA/C. Using this method, we optimized the promoter (Fig. 3), host (Fig. 4), culture temperature (Figs. 4 and 5), and media (Fig. 5) to achieve the yield of 30-mg purified 3F3 Fab per liter of culture, a four orders of magnitude improvement compared to initial expression result (10 μg/L, Fig. 1).

We further demonstrated that co-expressing DsbA and DsbC promoted assembly of aglycosylated IgG in *E. coli* periplasm (Supplementary Fig. 1). Due to toxicity of heterologous proteins to the expression host, it is not uncommon that over-expression often results in unhealthy growth and low cell density. Our results demonstrate that by co-expressing folding factor DsbA/C, the cell culture OD_{600} dramatically increased (Supplementary Fig. 1A). We further showed that construct DsbAC-2P with EnBase media led the production of 2-mg aglycosylated full IgG per liter culture (Supplementary Fig. 1B).

In summary, antibody fragment assembly in *E. coli* periplasm was significantly improved by disulfide bond proteins DsbA and DsbC, and this approach can be applied for production of other important proteins containing disulfide bonds.

Acknowledgements This work was supported by National Science Foundation the Faculty Early Career Development (CAREER) Program 1453645 and National Institutes of Health Grant R01GM115672. C.R. received UCR Chancellor's award for Excellence in Undergraduate Research.

Author Contributions C.R., D.H.N., and X.G. designed the research; C.R., D.H.N., and E.K. performed the research; C.R., D.H.N., and X.G. analyzed the data; and C.R., D.H.N. and, X.G. wrote the paper.

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Supplementary Information for

Efficient Antibody Assembly in *E. coli* Periplasm by Disulfide Bond Folding Factor Co-expression and Culture Optimization

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MATERIALS AND METHODS

IgG Cloning and Expression. To construct an IgG periplasmic expression vector pMAZ-Her, VH and VL genes encoding anti-HER2 mAb trastuzumab (Herceptin) were chemically synthesized and cloned into *Nhe*I/*Hind*III and *Nco*I/*Not*I sites on pMAZ360 [16]. pMAZ-Her was co-transformed into Jude-I harboring each folding factor plasmid pBAD-DsbA/-DsbC/AC-1P/AC-2P. Transformed cells were cultivated in TB or EnBase media at 25 °C. After induction with 0.2% arabinose and 0.1 mM IPTG, periplasmic fractions were prepared for Western blotting analysis (non-reducing) using goat anti-human IgG-HRP (Jackson ImmunoResearch).

RESULTS

Production of Aglycosylated IgG. To further demonstrate that DsbA/C can promote proper disulfide formation and protein folding, we next attempted to produce aglycosylated IgG in *E. coli*

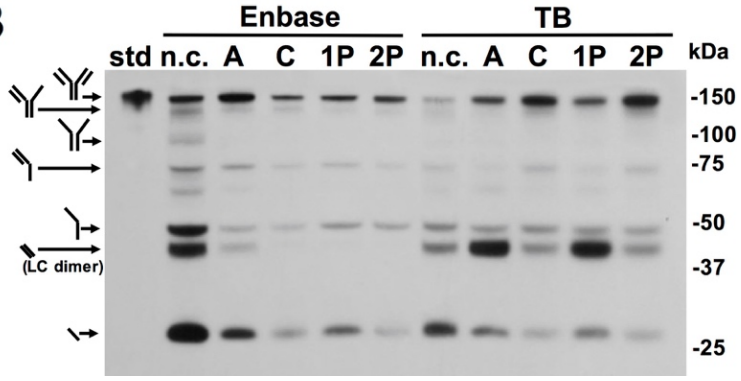
periplasm. The VH and VL fragments of mAb trastuzumab were cloned into a periplasmic IgG expression vector carrying P_{Lac} and pelB leaders, and co-transformed into Jude-I cells with each plasmid pBAD-DsbA/-DsbC/AC-1P/AC-2P. Transformed cells were cultured in TB or EnBase media at 25 ^{o}C with induction. After 24 hr, OD_{600} of TB culture without folding factor was 3.0, while cell densities OD_{600} reached 10.5-11.8 with DsbA/C co-expression (**Supplementary Fig 1A**), suggesting these folding factors significantly improved the cell growth, likely by reducing the toxicity effects of IgG expression on host cells. Similarly, DsbA/C enhanced cell growth in EnBase media from OD_{600} 22.7 (no folding factor) to 25.4-27.6. Western blotting analysis of periplasmic preparations using goat anti-human IgG-HRP indicated that without DsbA/C co-expression, in addition to the band associated with full IgG, as many as six partially assembled antibody fragments were detected, especially for unassembled LC and HC, and LC dimer (**Supplementary Fig 1B**). With DsbA/C co-expression in EnBase media, these fragmented species were much less present, and amounts of fully assembled IgG were significantly increased for the DsbA construct, suggesting improvement of the quality for IgG production. In TB media, all the DsbA/C constructs resulted in more than 5-fold enhancement of the IgG production, and the unassembled fragments were not increased for DsbC and AC-2P constructs. Using construct AC-2P in EnBase, expressed IgG were further purified for quantification. Results indicated that 2 mg purified aglycosylated IgG was obtained per liter of culture.

SUPPLEMENTARY FIGURE 1

A

	n.c.	DsbA	DsbC	AC-1P	AC-2P
TB (induced at OD~1)	3.0	10.5	11.8	10.7	11.3
EB (induced at OD~9)	22.7	27.6	25.5	25.4	25.6

B



Supplementary Figure 1. Assembly of aglycosylated full IgG with DsbA/C co-expression. (A) OD_{600} after 24 hour induction at 25 ^{o}C. **(B)** Western blotting results developed with anti-human IgG-HRP. 2 mg IgG was purified per litter of EnBase media when construct DsbAC-2P was co-expressed.