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ELP-z and ELP-zz capturing scaffolds for the purification of immunoglobulins by affinity precipitation

Bhawna Madan[a], Garima Chaudhary[b], Steven M. Cramer[c], Wilfred Chen[a,*]

[a] Department of Chemical and Biomolecular Engineering, University of Delaware, Newark, DE 19716, United States
[b] Department of Chemical and Environmental Engineering, University California Riverside, Riverside, CA 92521, United States
[c] Isermann Department of Chemical and Biological Engineering, Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, United States

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ABSTRACT

The increasing demand of monoclonal antibodies for therapeutic applications along with the high manufacturing cost have made it necessary to evaluate better process options and technologies for their purification. Affinity precipitation is an attractive alternative to traditional chromatographic methods by affording effective purification using a simple environmental trigger. The feature of elastin-like-protein (ELP) fused with antibody binding domains has already been explored for the purification of antibodies. However, ELP when fused with the bulkier domains such as Protein A, resulted in lower protein production. In this study, ELP was fused to smaller synthetic IgG binding domains such as the z or zz domain, resulting in up to 10-fold higher level of production. Both ELP-z and ELP-zz bind tightly to human immunoglobulin (HIgG) with a dissociation constant of 768 ± 142 nM and 68 ± 23 nM, respectively. Owing to the higher binding affinity, the use of ELP-zz resulted in more than 99% recovery of HIgG in four repeated binding and elution cycles with no observable decrease in the purification performance. The same binding and elution cycle was successfully implemented for the purification of monoclonal antibodies from hybridoma culture supernatant with close to 100% recovery.

1. Introduction

The ability of antibodies to recognize specific antigenic targets and trigger responses from the immune system has made them attractive therapeutic agents. The demand for monoclonal antibodies with exigent purity requirements and better purification yield has encouraged the search for novel purification strategies. Affinity chromatographic techniques based on immobilized antibody-binding proteins (either protein A, G, or L) are highly advantageous over less selective purification processes as these techniques involve single-step purification with a much higher yield (Taipa et al., 1998; Anastase-Ravion et al., 2001; Roque et al., 2004, 2007; Linhult et al., 2004; Hober et al., 2007). However, these affinity resins are 30% more expensive than ion-exchange resins, and the binding proteins are susceptible to degradation under harsh conditions (Anastase-Ravion et al., 2001). In addition, manufacturing scale chromatographic processes can have volumetric throughput limitations along with other complexities such as the need to efficiently pack these large scale columns, limited lifetimes, and the need for complicated accompanying equipment.

Affinity precipitation is a viable alternative to affinity chromatography (Anastase-Ravion et al., 2001; Taipa et al., 2001; Hilbrig and Freitag, 2003), in which immunoglobulin (IgG) purification is based on a simple environmental trigger and the specificity of an IgG binding protein. Elastin-like polypeptides (ELPs), which are thermal responsive biopolymers (Urry, 1997; Li et al., 2001a, 2001b) composed of the repeating pentapeptide VPGVG, have been utilized for protein (Meyer and Chilkoti, 1999; Stiborova et al., 2003; Kim et al., 2005a; Trabbic-Carlson et al., 2004; Lim et al., 2007; Hassouneh et al., 2010) and plasmid DNA (Lao et al., 2007) purification. These biopolymers are water soluble below their transition temperature but undergo a reversible phase transition to form aggregates upon an increase in the salt concentration and temperature above the transition temperature (Kostal et al., 2001). Recently, we generated ELP fusions to protein A (SpA), enabling rapid purification of any IgG of interest using the reversible phase transition for simple recovery (Kim et al., 2005a, 2005b). However, ELP fused with the bulkier SpA resulted in lower expression compared with ELP fused with other smaller proteins (Kostal et al., 2001). The z-domain, a shorter synthetic domain derived from the B-domain of SpA, has been shown to have similar IgG-capturing efficiency as B-domain of SpA (Nilsson et al., 1987). In addition, the divalent form of the z-domain (zz domain), has been reported to have up to 10-fold improved binding affinity to the Fc region of IgG (Jendeberg et al., 1995, 1996; Chen et al., 2006; Brockelband et al.,

* Corresponding author. Tel.: +1 302 831 6327; fax: +1 302 831 8201.
E-mail address: wilfred@udel.edu (W. Chen).

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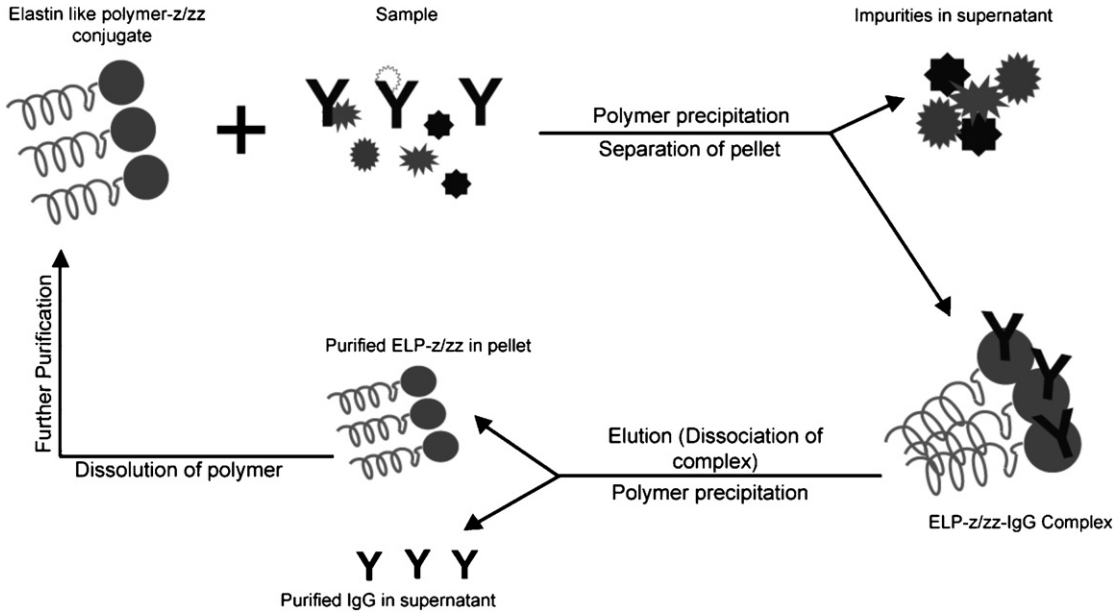

Fig. 1. A schematic of IgG purification using ELP-z/ELP-zz.

2006; Lewis and Rehm, 2009) than the z-domain. To bypass the lower expression levels previously observed with ELP fused with SpA, the current work examines the utility of both the z and zz domains fused to ELP containing 78 repeats (ELP78) for antibody purification using the procedure outlined in Fig. 1.

2. Materials and methods

2.1. Materials

The hybridoma cell culture supernatant (C1B7) was purchased from Developmental Studies Hybridoma Bank (Iowa City, IA). The Human IgG was purchased from Sigma-Aldrich (St. Louis, MO). Goat anti-mouse IgG-alkaline phosphatase (AP) conjugate and AP reagent were purchased from Bio-Rad (Hercules, CA).

2.2. Bacterial strains and plasmids

Escherichia coli strain NEB5α (fhuA2Δ(argF-lacZ)U169 phoA glnV44 Φ80Δ (lacZ)M15 gyrA96 recA1 relA1 endA1 thi-1 hsdR17) was obtained from NEB and was used as a host cell of cloning and subcloning. *E. coli* strain BLR (F^- *ompT hsdS*B(rB^- mB^-) *gal dcm* (DE3) Δ(*srl-recA*)*306*::Tn*10* (Tet^R) was used as a host for protein overexpression. Plasmid pET-ELP78H6 (Kostal et al., 2001) containing 78 VPGVG repeats were used as to construct the expression vectors for ELP-z and ELP-zz fusions.

2.3. Construction of ELP-z fusions

The z-domain (208 bp) was constructed by overlapping oligonucleotides Z-FL1, Z-FL2, Z-FL3, Z-FL4, Z-FL5, Z-RL1, Z-RL2, Z-RL3, Z-RL4 and Z-RL5 (Table 1). The oligonucleotides were annealed by heating the mixture of ten oligonucleotides (2 μM) to 95 °C, followed by gradually cooling to room temperature. The annealed oligonucleotides were then phosphorylated using 20 U of T4 polynucleotide kinase in total volume of 20 μL. Plasmid pET-ELP78H6 was digested with *Xma* I and *Bam* HI and enzymatically dephosphorylated using CIP. The digested vector (~0.1 pmol) was ligated to ~1 pmole of phosphorylated annealed oligonucleotides containing the same *Xma* I and *Bam* HI compatible ends in a 10 μl reaction with 100 Weiss Units of T4 DNA ligase and incubated at 16 °C overnight. The ligation mixture was transformed into chemically competent *E. coli* NEB5α competent cells. The transformants were spread on LB-Ampicillin plates and incubated at 37 °C. Colonies were initially screened by colony PCR and later confirmed by sequencing at Institute for Integrative Genomic Biology Instrumentation Facility, UCR and designated as pELP-z. To construct pELP-zz, the z domain was amplified using primers *Xma*I-zz FP and *Bam*HI-zz RP (Table 1) and was cloned between the *Xma*I and *Bam*HI site of pET-ELP78H6 to generate pELPz1. The second z domain was amplified using primers *Bam*H1-zz FP TD and *Bam*H1-zz RP TD (Table 1) and was cloned into the *Bam*H1site in ELPz1. The construct pELPzz was confirmed by sequencing and genotyping center, Delaware Biotechnology Institute, University of Delaware.

2.4. Expression and purification of fusion proteins

E. coli strain BLR (DE3) was transformed with pETELP78H6, pELP-z and pELP-zz. Individual colonies were grown in 5 mL Luria broth (LB) medium containing 100 μg/mL ampicillin at 37 °C and 250 rpm overnight. Overnight cultures were subcultured into 100 mL of terrific broth (12 g tryptone, 24 g yeast extract, 2.31 g KH_2PO_4, 12.54 g K_2HPO_4/L) supplemented with 100 μg/mL ampicillin. Expression of ELP-zz was induced with 10 μM of IPTG at OD_{600nm} 3 and was grown for 5 h after induction at 37 °C and 250 rpm. Expression of ELP-z and ELP78H6 was achieved by growing cells for 44–48 h without induction at 37 °C and 250 rpm in an orbital shaker. Proteins were purified by two inverse transition cycles (ITC). Cells were harvested by centrifugation at 4500 g at 4 °C for 15 min and resuspended in phosphate buffer saline (PBS; 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7 with 1X protease inhibitor) to a final OD_{600nm} of 20. Cells were lysed by sonication (5 s pulse on, 5 s pulse off, total 10 min run time) and cell debris were removed by centrifugation for 20 min at 4 °C and 15,000 *g*. The inverse phase transition of the ELP fusions was achieved by the addition of NaCl (1 M). Samples were incubated at 37 °C for 20 min and the aggregates were separated from cell lysate by centrifugation at 15,000 *g*. The aggregates were solubilized in ice-cold PBS and centrifuged at 15,000 *g* at 4 °C for

Table 1
Primer sequences.

Primer name	Sequence 5′ → 3′
Z-FL1	CCGGGTGTAGGTGGCAGCGGCAGCG
Z-FL2	GCAGCGTAGACAACAAATTCAACAAAGAACAACAAAACGCGTTCTATGAG
Z-FL3	ATCTTACATTTACCTAACTTAAACGAAGAACAACGAAACGCCTTC ATCCA
Z-FL4	AAGTTTAAAAGATGACCCAAGCCAAAGCGCTAACCTTTTAGCAGA AGCTA
Z-FL5	AAAAGCTAAATGATGCTCAGGCGCCGAAATAAG
Z-RL1	GATCCTTATTTCGGCGCCTGAGCATCATTTAGC
Z-RL2	TTTTTAGCTTCTGCTAAAAGGTTAGCGCT
Z-RL3	TGGCTTGGGTCATCTTTTAAACTTTGGATGAAGGCGTTTCGTTGTTCTT
Z-RL4	CGTTTAAGTTAGGTAAATGTAAGATCTCATAGAACGCGTTTTGTTGTTCT
Z-RL5	TTGTTGAATTTGTTGTCTACGCTGCCGCTGCCGCTGCCACCTACAC
*Xma*I-ZZ FP	TCCCCCCGGGTGTAGGTGGCAG
*Bam*HI-zz RP	CGCGGATCCTTTCGGCGCCTGAGCATCATTTAG
*Bam*HI-zz FP TD	CGCGGATCCGTAGACAACAAATTCAACAAAG
*Bam*HI-zz RP TD	CGCGGATCCTTATTTCGGCGCCTGAGCATCATTTAG

15 min to remove any insoluble proteins. The inverse transition cycle was repeated again and purified ELP fusion proteins were suspended in cold PBS. The fractions at each step of purification were analyzed on 10% SDS-PAGE and the concentrations of the fusion protein were determined by the spectrophotometric measurement at 215 nm.

2.5. Determination of affinity constants of ELP-z/ELP-zz

ELP-z/ELP-zz (100–800 nM) was mixed with 1 μM of IgG-FITC conjugate in 450 μl of phosphate buffer saline. Thirty micromolar of ELP78H6 (helper ELP) was added as a coaggregant, was mixed and the complexation was allowed to occur for 2 h. To the mixture, NaCl was added to a final concentration of 1 M and incubated at 28 °C for 20 min. The solution was centrifuged at 15,000 *g* for 20 min at 28 °C and the pellet was redissolved in cold PBS buffer. The amount of IgG bound to ELP-z/ELP-zz was measured by the fluorescence intensity of the final solution (λ_{ex} 494 nm, λ_{ex} 520 nm).

2.6. Comparison of HIgG capturing efficiency of ELP-z and ELP-zz

Purification was carried out using 2.4 μM of HIgG which was incubated with ELP-z/ELP-zz (4.8 μM) at a molar ratio of 1:2 for 2 h at room temperature. To recover the ELP-HIgG complex, NaCl was added to a final concentration of 1 M and was incubated for 20 min at 28 °C. The complex (pellet) was separated from the unbound fraction (supernatant) after centrifugation (15,000 g for 15 min) at 28 °C. The pellet was resolubilized in ice-cold PBS buffer. The effect of helper ELP on the complete recovery of the complex was investigated using 2.4 μM of HIgG at a molar ratio of HIgG: ELP-z/zz of 1:2 by varying helper ELP from 14.4 μM to 28.8 μM. For the comparison of IgG capturing efficiency between ELP-z and ELP-zz, the molar ratios of ELP-z/zz: HIgG were varied from 2:1 to 9:1. For all the experiments, the final concentration of helper ELP was kept at 28.8 μM. Samples from different stages of the purification were analyzed by SDS-PAGE. The quantification of intensity of the protein bands was carried out by Image J software from NIH.

2.7. Elution analysis

The elution of HIgG from the ELP-zz-HIgG complex was studied at mildly acidic buffer pH 3.8 using 0.1 M citrate or 0.5 M Arginine. The ELP-zz-HIgG complex was incubated with these buffers for 2 h at 4 °C. The eluted HIgG was recovered by performing the temperature transition cycle at 37 °C and the samples were analyzed on 10% SDS-PAGE. The effect of elution time was studied by incubating the ELP-zz-IgG complex with 0.5 M arginine buffer pH 3.8 from 30 min to 2 h.

2.8. Reusability of ELP-zz for HIgG purification

For the repeated use of ELP-zz, purification of HIgG was repeated for four consecutive cycles. After each elution step, the ELP-zz was re-solubilized in PBS buffer and used for a subsequent cycle of HIgG purification. The intensity of the protein bands were quantified by Image J software from NIH.

2.9. IgG purification from hybridoma supernatants

For IgG purification from the hybridoma culture supernatant, either 4.8 μM or 9.6 μM of ELP-zz was mixed with 450 μl of hybridoma supernatant (C1B7, 26.1 μg of IgG, 278.4 nM) to a final volume of 0.5 mL. The recovery of the complex and elution was performed as described above. The elution of mouse IgG1 from the complex was carried out with 0.5 M arginine buffer pH 3.8 for 30 min. Samples from different stages of the purification were analyzed by SDS-PAGE followed by silver staining. The intensity of the protein bands were quantified as described above.

3. Results and discussion

3.1. Production of ELP-z and ELP-zz fusion proteins

Although ELP-SpA fusions have been shown to have high binding affinity to antibodies (Kim et al., 2005b), the recombinant protein yield with the bulkier SpA (43 kDa) is low (68 mg/L) (Kim et al., 2005b). In the current work, two shorter antibody-binding domains, the z domain (7 kDa) and the zz domain (14 kDa) derived from the B-domain of SpA, were fused to ELP and explored for the purification of antibodies. The ELP-z and ELP-zz fusion proteins were constructed by fusing these binding domains to the C-terminus of ELP78 (Kostal et al., 2001), joined by a GSGSGS linker to provide flexibility and to minimize interference. The two fusion proteins were expressed in *E. coli* BLR (DE3) and were purified easily by two cycles of inverse phase transition. No loss of protein was observed during purification and the purity was confirmed by SDS-PAGE (Fig. 2). The yield of ELP-z and ELP-zz fusion protein was ~600 mg/L and ~270 mg/L, respectively. This increase in the production of ELP-z and ELP-zz compared with ELP-SpA is consistent with the use of the smaller binding domains and appears to be size dependent.

3.2. Comparison of binding affinity between ELP-z and ELP-zz

The binding affinity of the divalent zz domains for Human IgG (HIgG) has been reported to be 5 to 10-fold higher than the monovalent z domain in the range of 20 nM (Jendeberg et al., 1995). To assess whether the ELP fusion proteins retain similar binding

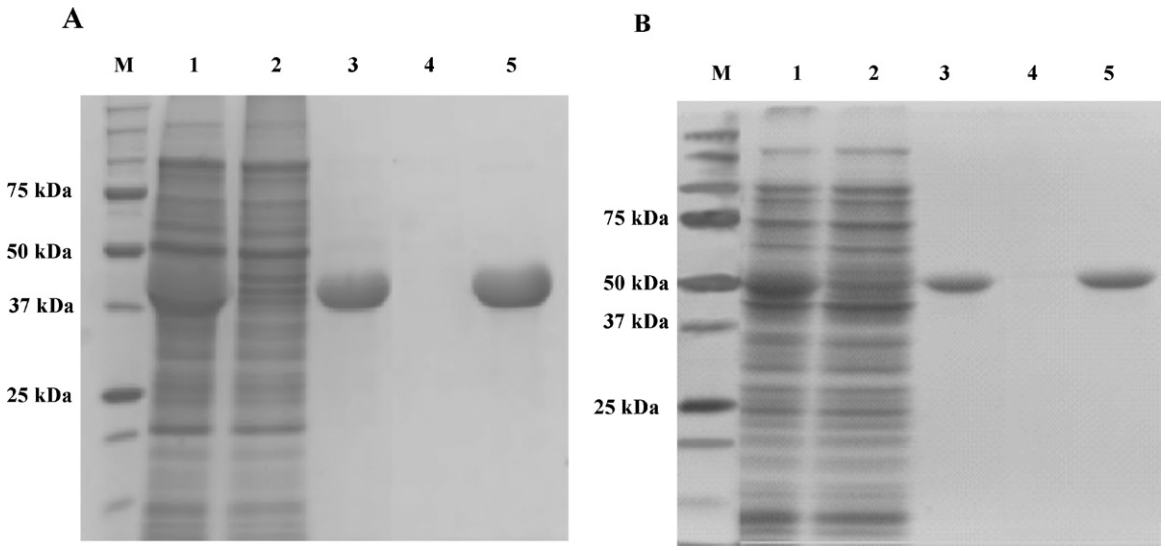

Fig. 2. Purification of ELP-z and ELP-zz by inverse transition cycling. SDS-PAGE of each stage of purification for ELP-z fusion (39.4 kDa, A) and ELP-zz fusion (46.4 kDa, B). Lane M: Marker; Lane 1: soluble lysate; Lane 2: supernatant containing contaminating *E. coli* proteins; Lane 3: resolubilized pellet containing purified fusion proteins; Lane 4: second round supernatant; Lane 5: second round pellet.

affinities, binding experiments were carried out using fluorescein-labeled HIgG. Based on the reported K_D values, a fixed concentration of HIgG of 1 μM was used, while the concentration of the ELP-z or ELP-zz was varied from 100–800 nM. To ensure that all the bound HIgG could be completely recovered by co-precipitation with ELP-z/zz at these very low concentrations, 30 μM of helper ELP (ELP78) was added as a coaggregant to increase the effective ELP concentration as reported previously (Ge and Filipe, 2006; Lao et al., 2007). Using this condition, complete recovery of the added ELP was confirmed by silver strain (data not shown). More importantly, addition of only helper ELP to FITC-HIgG resulted in no detectable fluorescence in the pellet after precipitation, confirming that there was no non-specific entrapment of IgG in the precipitate. The dissociation constant (K_d) of ELP-z or ELP-zz to HIgG was determined using a Scatchard plot as follows:where $[S]_b$ is the concentration of bound IgG, $[S]_f$ is the concentration of free IgG, $[P]$ is the concentration of ELP-z/zz, and K_d is the dissociation constant. The dissociation constant of ELP-z and ELP-zz for HIgG were determined to be 768 ± 142 nM and 68 ± 23 nM, respectively (Fig. 3). These values are similar to those reported by others (Jendeberg et al., 1995, 1996), and they are consistent with the reported 5–10 times high binding affinity for the zz domain. By increasing the HIgG concentration by 10-fold, a saturation binding capacity of 0.5 and 1 HIgG/ELP was observed for ELP-z and ELP-zz, respectively. This two-fold increase in binding capacity for the zz domain is also consistent with other reports indicating the presence of two symmetric binding sites for the z domain on the same Fc region (Jendeberg et al., 1996; Ljungquist et al., 1989).

3.3. IgG purification by ELP-z and ELP-zz

To evaluate the practical utility of ELP-z and ELP-zz for HIgG purification, initial binding experiments were performed using only a 2-fold excess of ELP fusions (2.4 μM) as 100% precipitation of both ELP fusions was detected under this condition. However, only 60% recovery of ELP fusions was observed after precipitation (Fig. 4A). This is due to a substantial change in the transition profile for both ELP fusions when complexed with HIgG (data not shown). Helper ELP (ELP78) was again added to facilitate the recovery, and complete recovery was only observed at a helper ELP concentration of 28.8 μM (Fig. 4B). Consistent with the higher binding affinity for ELP-zz, more than 92% of the added HIgG was captured using ELP-zz, while only 60% was removed using ELP-z (Fig. 4B). The level of removal was in line with the value calculated using the measured binding affinity constants. To further improve the recovery, higher ratios of ELP fusion:HIgG were tested. Complete HIgG recovery was detected for ELP-zz: HIgG at a 4:1 molar ratio, while only 90% recovery was observed for ELP-z under the same condition (data not shown). Because of the substantially higher binding affinity and the need to minimize the amount of added ELP fusions for practical application, only ELP-zz was further investigated.

3.4. Elution of the HIgG from ELP-zz-HIgG complex

It has been reported in literature that the use of lower pH (<3) buffers for the elution of antibodies from Protein A columns can cause conformational rearrangements leading to irreversible unfolding and aggregation of purified antibodies

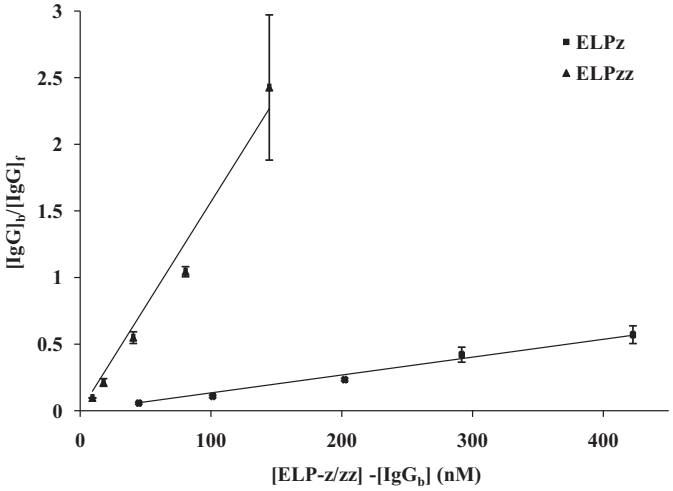

Fig. 3. Scatchard plots for equilibrium binding of ELP-z/ELP-zz conjugate with human IgG. Increasing amounts of ELP-z/ELP-zz (100–800 nM) were mixed with 1 μM of HIgG-FITC in 450 μl of phosphate buffer saline containing 30 μM helper ELP (ELPH6) as a coaggregant. After incubation for 2 h at room temperature, the complex was precipitated by addition of 1 M NaCl and incubation at 37 °C. The amount of IgG complexed with the conjugate was assayed by measuring the fluorescence. Data shown are the mean values (+ standard deviation) obtained from 3 independent experiments.

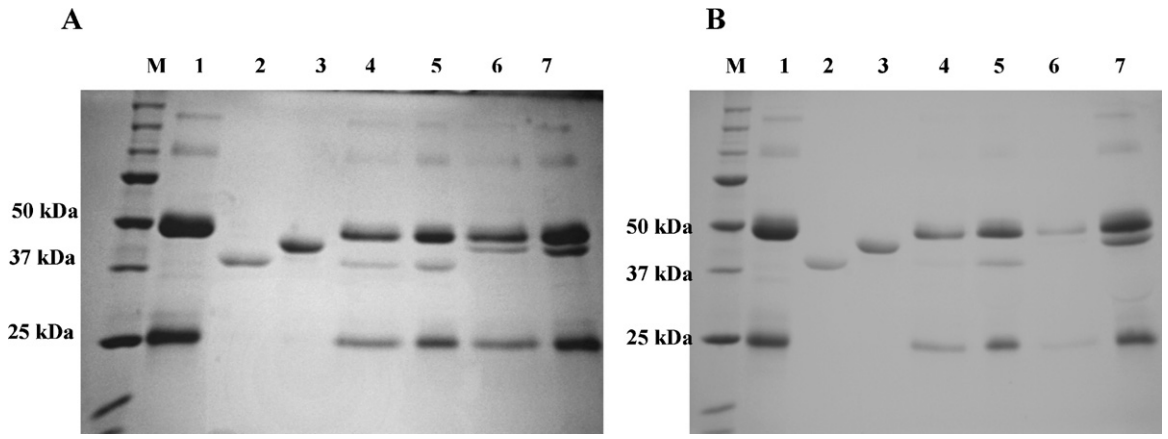

Fig. 4. The HIgG-capturing efficiency of ELP-z/ELP-zz using 2.4 μM of HIgG at a molar ratio of HIgG:ELPz/ELPzz of 1:2 without (A) or with (B) helper ELP. Lane M: Protein marker; Lane 1: HIgG; Lane 2: ELP-z; Lane 3: ELP-zz; Lane 4: Unbound HIgG after precipitation with ELP-z; Lane 5: Recovered ELPz-IgG; Lane 6: Unbound HIgG after precipitation with ELP-zz; Lane 7: Recovered ELPzz-IgG complex.

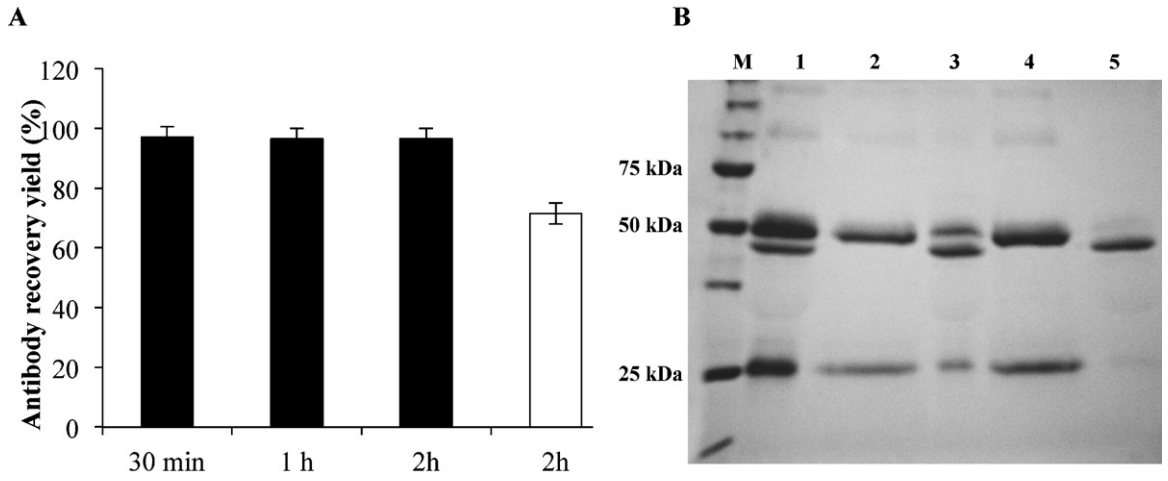

Fig. 5. Elution of HIgG. (A) Elution of HIgG from ELP-zz-HIgG complex at pH 3.8 with 0.5 M Arginine buffer (black bars) or 0.1 M Citrate buffer (white bars). (B) Coomassie stained SDS-PAGE showing HIgG recovered from ELPzz-HIgG complex with 0.1 M Citrate buffer and 0.5 M Arginine buffer. Lane M: Marker; Lane 1: ELPzz-HIgG complex; Lane 2: Recovered HIgG after elution with 0.1 M Citrate buffer; Lane 3: Recovered ELPzz after elution with 0.1 M Citrate buffer; Lane 4: Recovered HIgG after elution with 0.5 M Arginine buffer; Lane 5: Recovered ELPzz after elution with 0.5 M Arginine buffer. Data shown are the mean values (+ standard deviation) obtained from 3 independent experiments.

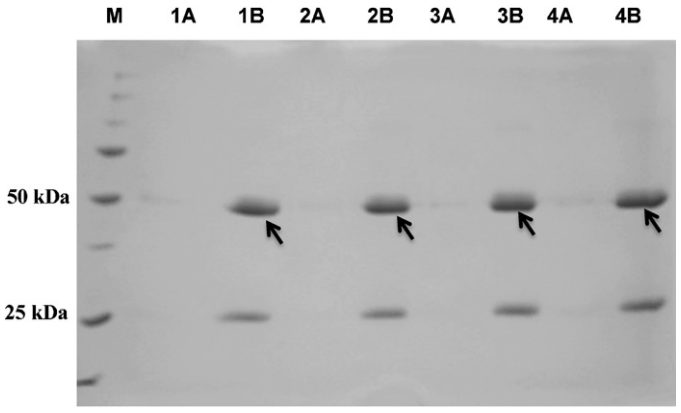

Fig. 6. Purification of HIgG in four (1–4) repeating cycles. The same ELP-zz fusion was reused for purification after IgG elution and fractions were analyzed on coomassie stained SDS-PAGE. A: unbound protein fraction after precipitation; B: recovered IgG after elution, the 50 kDa and 25 kDa bands corresponds to heavy and light chains respectively. Heavy chains of purified IgGs are indicated by an arrow.

(Calmettes et al., 1991; Martsev et al., 1995). To circumvent this problem, elution was performed using either the citrate buffer or the arginine buffer at a milder pH 3.8 for 2 h. Elution with 0.1 M citrate resulted in only 72% HIgG recovery, whereas elution with 0.5 M arginine resulted in over 96% recovery (Fig. 5A and B). Based on the better performance using the arginine buffer, we further investigated whether a shorter incubation is sufficient to achieve the same level of recovery. As shown in Fig. 5A, even 30 min of incubation was sufficient to achieve the same 96% recovery using 0.5 M arginine, (data not shown), indicating the highly efficient nature of this elution buffer. This is consistent with other reports indicating that a similar efficiency for the elution of IgG from Protein A columns can be achieved using the same arginine buffer (Arakawa et al., 2004; Ejima et al., 2005). An added benefit of using arginine as an eluent is the fact that it has been shown to suppress aggregation and is known to dissociate any protein trapped in insoluble aggregates (Shiraki et al., 2002; Arakawa and Tsumoto, 2003).

3.5. Reusability of ELP-zz polymer

The cost of raw material for downstream processing depends on whether the raw material can be reused. The regeneration and rebinding efficiency of ELP-zz was evaluated with HIgG. The same

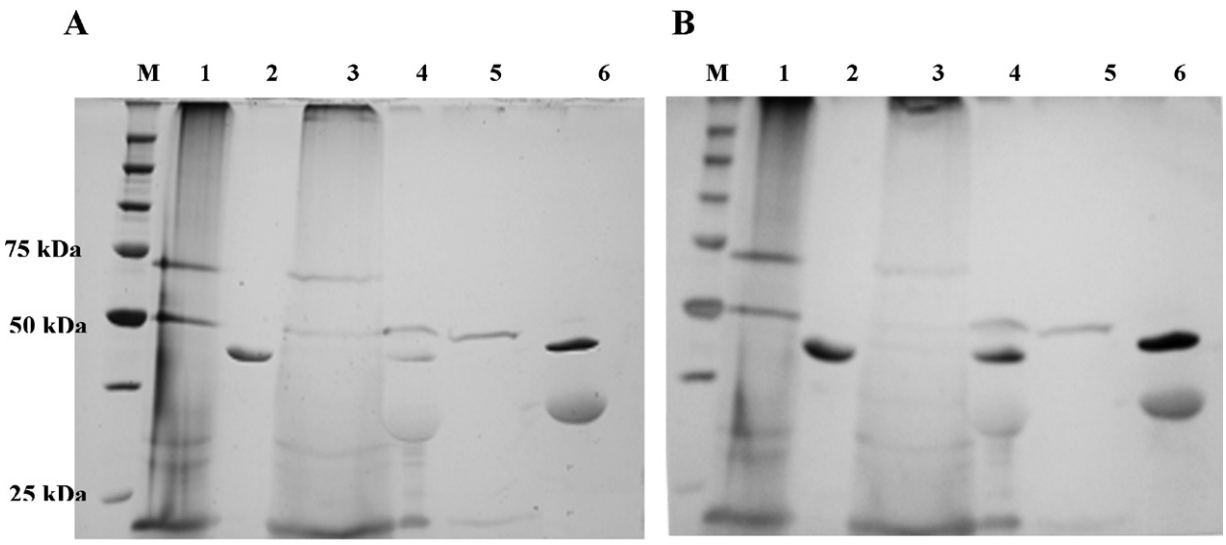

Fig. 7. Silver stained SDS-PAGE showing purification of IgG from the hybridoma culture supernatant at an ELP-zz to IgG ratio of (A) 4:1 and (B) 8:1. Lane M: Protein marker; Lane 1: Supernatant of hybridoma cell culture (C1B7); Lane 2: ELP-zz; Lane 3: Unbound protein fraction after precipitation; Lane 4: Recovered ELP-zz-IgG complex; Lane 5: Recovered IgG after elution; Lane 6: Recovered ELP-zz fusion after elution. A helper ELP concentration of 28.8 μM was used in all experiments.

ELP-zz was reused for four cycles of HIgG purification with no loss in ELP-zz detected in each cycle. More importantly, the same HIgG recovery efficiency was observed in all cycles (Fig. 6). This demonstrated that ELP-zz can be reused several times without losing its IgG binding affinity and inverse transition property.

3.6. Purification of IgG from hydrodoma supernatants

Finally, we evaluate whether ELP-zz is suitable for the purification of IgG directly from hybridoma culture supernatants. A hybridoma cell culture supernatant (C1B7) containing the mouse IgG1 against human acetylcholinesterase was used. After precipitation, the purified fractions were analyzed by silver stain (Fig. 7A and B). The recovery was observed to be around 70% using the same 4:1 ELP-zz:IgG in the presence of helper ELP as described above. This lower recovery of mouse IgG1 from the hybridoma supernatant has also been observed with zz polyester beads and is consistent with the lower binding affinity toward mouse IgG (Lewis and Rehm, 2009). By increasing the ratio to 8:1, the recovery efficiency from the hybridoma supernatant increased to 100%. This is much more efficient than our earlier work with ELP-Protein G fusions, which reported only 64% recovery even at a ratio of 30:1 (Kim et al., 2005a). More importantly, the bound mouse IgG was 100% eluted using the same arginine buffer with no contaminating ELP-zz or helper ELP detected after the separation. Owing to the superior binding affinity and the possibility of complete elution, ELP-zz may prove to be very useful in purifying the growing number of industrial IgG products or chimeras with Fc regions in industry. The recovered ELP-zz/helper ELP can be reused for IgG purification from hybridoma supernatant without any loss of efficiency.

4. Conclusions

While antibody purification has long been dominated by standard affinity chromatography, the limitations of high cost and complex scale-up have prompted the development of alternative non-chromatographic separation methods. In this paper, we generated a new set of thermally responsive ELP-z or zz domain fusions as the capturing scaffolds for the direct purification and recovery of antibodies. Because of the high binding affinity of the zz domain, more than 99% recovery of HIgG was obtained using ELP-zz during four repeated binding and elution cycles with no observable decrease in the performance. Direct purification of monoclonal antibodies from hybridoma culture supernatant was also demonstrated with close to 100% recovery. Because of the conditions involved in precipitation are comparable to those used for IgG purification using ionic exchange chromatography (Zhang et al., 2010), we believe the purified IgGs will retain their functionality as demonstrated. We are currently working with Bristol–Myers Squibb to evaluate the commercial applicability of ELP-zz for large-scale purification of antibodies.

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