A simple method for non-denaturing purification of biotin-tagged proteins through competitive elution with free biotin

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ABSTRACT

The use of avidin or streptavidin in the purification of biotinylated proteins has been highly restricted due to the harsh and denaturing elution conditions. Here, we use biotinylated bovine serum albumin as a working model to demonstrate a simple and rapid method for biotin-tagged protein purification under non-denaturing conditions. The biotinylated bovine serum albumin is specifically bound to the anti-biotin antibody agarose beads and competitively eluted with free biotin under near-neutral conditions. The optimized elution conditions include using 4 mg/ml biotin (pH 8.5) as the elution buffer and allowing the buffer to incubate with agarose beads for 30 min prior to elution. The elution recovery rate is over 85% without apparent protein denaturation. The method is applicable for both immunoprecipitation and column chromatography.

METHOD SUMMARY

We describe a method for the purification of biotin-tagged proteins under non-denaturing conditions with no change in protein natural structure or function. Anti-biotin antibody agarose is employed to specifically bind the biotinylated proteins, followed by competitive elution with free biotin at near-neutral conditions. This approach is applicable for both immunoprecipitation and column chromatography.

KEYWORDS

affinity chromatography • anti-biotin antibody agarose • biotinylated protein • competitive elution • immunoprecipitation • purification

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Enzymatic biotinylation by the *Escherichia coli* biotinylase BirA is highly specific in covalently attaching biotin to a 15-amino-acid peptide (GLNDIFEAQKIEWHE), also known as AviTag, which can be added genetically at the N-terminus or C-terminus or in exposed loops of a target protein [1]. The biotinylated proteins can then be isolated and purified with avidin or streptavidin [2–6]. Avidin/streptavidin–biotin binding is rapid and specific and is considered to be the strongest noncovalent interaction known in nature ($K_D = 10^{-14}$ – 10^{-15} M). However, elution of the bound proteins from avidin or streptavidin requires harsh and denaturing conditions, which inevitably destroy the natural protein structure and are often incompatible with downstream processing [7,8]. To avoid this denaturing process, on-bead digestion of bound proteins has been introduced [9,10]; however, it may cause undesirable contamination of avidin/streptavidin peptides and the digesting enzymes [11]. Development of a new method for eluting bound biotin-tagged proteins from the affinity matrix without protein denaturation is therefore necessary.

Anti-biotin antibodies have been introduced recently for biotinylated protein purification [12,13]. Compared with avidin or streptavidin, the binding of anti-biotin antibodies to biotin molecules is a milder affinity interaction, allowing the bound molecules to be released much more easily. Also, it has been reported that the anti-biotin antibodies are able to detect a much broader range of biotinylated sites in cells compared with streptavidin [12]. The current elution strategy uses acid solution to disrupt the antibody–antigen binding. In brief, beads conjugated with anti-biotin antibodies are used to specifically bind the molecules of interest. After washing the

beads with phosphate-buffered saline (PBS), a mild acid solution (pH ≤ 2.5) is employed to elute the bound biotinylated molecules. Although this method has been successful in the purification of biotinylated peptides, it is not applicable for a large number of proteins that are not stable at such a low pH. Using biotinylated bovine serum albumin (biotin-BSA) as our working model and the strategy of anti-biotin antibody binding and free biotin competitive elution at near-neutral conditions, our aim was to develop a non-invasive method for biotinylated protein purification from anti-biotin antibody beads.

GENERAL PROTOCOL FOR IMMUNOPRECIPITATION

Bead equilibration

Wash and equilibrate 50 μ l of anti-biotin antibody agarose beads (cat. no. ICP0615; ImmuneChem Pharmaceuticals, BC, Canada) in a 0.5-ml microcentrifuge tube three times with PBS buffer containing 0.05% Tween 20 (PBST) by resuspending in 500 μ l of PBST and centrifuging at 200 $\times$ g for 30 s. After each centrifuge, carefully remove the supernatant by pipette without agitating the beads.

Protein binding

Add 150 μ l of ImmuneChem biotin-BSA (cat no. ICP0614) solution (1 mg/ml in PBST), and resuspend the beads. Incubate the slurry at room temperature for 15 min with gentle shaking. Centrifuge at 200 $\times$ g for 30 s. Remove the supernatant by pipette without agitating the beads.

Bead wash

Wash the beads four times with PBST and subsequently two times with normal saline solution by resuspending in 500 μ l of liquid and centrifuging at 200 $\times$ g for

Figure 1. Effect of pH on biotin-BSA elution efficiency. (A) Protein concentrations of elution fractions and (B) biotin elution recovery were analyzed when the beads were eluted with 1 mg/ml of biotin solution at various pH values after 30 min of biotin incubation. With the increase of pH value, the elution recovery increased until pH reached 8.5, after which the recovery rate plateaued.

Figure 2. Effect of biotin concentration on biotin-BSA elution efficiency. (A) Protein concentrations of elution fractions and (B) biotin elution recovery were analyzed when the beads were eluted with various concentrations of biotin solutions at pH 8.5 after 30 min of biotin incubation. No significant increase in elution efficiency was observed when the concentration was higher than 4 mg/ml.

30 s. Carefully remove the supernatant by pipette without agitating the beads after each centrifuge. After the last wash, completely remove the excess liquid by inserting the pipette tip to the tube bottom and sucking out the liquid thoroughly. Avoid sucking beads into the pipette tip.

Elution with free biotin

Add 150 µl of biotin (Sigma-Aldrich, ON, Canada) solution (concentration 1–8 mg/ml, pH 6–9) and resuspend the beads. Incubate the slurry at room temperature for 0–60 min with gentle shaking to prevent bead sediment (a process referred to as ‘biotin incubation’ hereafter). Centrifuge at 200×g for 30 s. Insert the pipette tip to the tube bottom and thoroughly suck out the supernatant. Avoid sucking beads into the pipette tip. Repeat the elution three more times without the step of biotin incubation.

Elution with mild acid

After the competitive elution with free biotin, add 150 µl of acetic acid solution (2% v/v, pH 2.5), and resuspend the beads. Centrifuge at 200×g for 30 s. Insert the pipette tip to the tube bottom and thoroughly suck out the supernatant. Avoid sucking beads into the pipette tip. Repeat the elution three more times.

We first studied the effect of pH on biotin elution efficiency. Due to the difficulty of dissolving the biotin at low pH, a pH range of 6–9 was tested. One mg/ml of biotin was prepared in 25-mM phosphate buffer containing 0.3-M NaCl at pH 6.0, 6.5, 7.0 and 7.5 and in 25-mM Tris-HCl buffer containing 0.3-M NaCl at pH 8.0, 8.5 and 9.0. The tests were performed according to the general protocol with 30 min of biotin incubation. The protein concentrations of the various elution fractions were determined using the BCA Protein Assay Kit (Thermo Fisher Scientific, MA, USA) according to the manufacturer’s enhanced test tube protocol. The biotin elution recovery was determined by calculating the percentage ratio of the protein amount eluted with biotin to the total protein quantity eluted with the biotin and acid solution.

Interestingly, a positive correlation between elution efficiency and pH values was observed. With the increase of pH values, the elution recovery increased accordingly until the pH reached 8.5, but there was no

significant difference between pH 8.5 and pH 9.0, suggesting that pH 8.5 is an appropriate value for biotin elution (Figure 1).

We then optimized the biotin concentration for higher elution efficiency. At pH 8.5 and with 30 min of biotin incubation, 1 mg/ml, 2 mg/ml, 4 mg/ml and 8 mg/ml of biotin solution, repetitively, were employed to elute the bound biotin-BSA. As expected, the higher the biotin concentration, the more efficiently the target protein was eluted. However, when the concentration increased to over 4 mg/ml, very little increase in elution efficiency was obtained (Figure 2). Therefore, 4 mg/ml was determined to be the optimum concentration.

When the pH value and biotin concentration had been optimized, we further studied the biotin incubation time. The result suggested that the incubation step prior to the biotin elution was considerably beneficial for high elution efficiency (Figure 3); however, an incubation time longer than 30 min was not necessary. As such, the optimized elution conditions for immunoprecipitated biotin-tagged proteins included using 4 mg/ml biotin in 25-mM Tris-HCl containing 0.3-M NaCl (pH 8.5) as the elution buffer and incubating the beads with the elution buffer for 30 min before elution. The biotin elution recovery can reach 85–90% using the optimized protocol.

PROTOCOL FOR CHROMATOGRAPHIC COLUMN PURIFICATION

Column packing

Pack a small plastic or glass column with 1 ml of anti-biotin antibody agarose beads. Equilibrate the column with 10 ml PBST.

Sample loading

Prepare 3 ml of biotin-BSA in PBST (~1 mg/ml). Load the sample into the column. Control the loading speed so the sample stays within the beads for at least 5 min.

Bead wash

Wash the beads with 10 ml of PBST followed by washing with 5 ml of normal saline solution.

Biotin elution

Use 4 mg/ml biotin in 25-mM Tris-HCl containing 0.3-M NaCl (pH 8.5) as the elution

Figure 3. Effect of biotin incubation time on biotin-BSA elution efficiency. (A) Protein concentrations of eluting fractions and (B) biotin elution recovery were analyzed when the beads were eluted with 4 mg/ml biotin at pH 8.5. The incubation step prior to elution greatly increased the recovery rate of the target protein.

Figure 4. Elution profile of anti-biotin antibody agarose column chromatography. The test was performed on a column packed with 1 ml of agarose beads. The elution buffer was 4 mg/ml biotin in 25-mM Tris-HCl containing 0.3-M NaCl (pH 8.5) and subsequently a 2% (v/v) acetic acid solution. The eluted samples were collected by 0.5 ml per fraction.

buffer. Add 1 ml of biotin elution buffer into the column. Maintain an eluting speed of 1 ml/min. When the elution buffer has drained out, let the wet beads sit at room temperature for 30 min, then resume eluting the beads with 4 ml of biotin eluting buffer at a flow speed of 1 ml/min.

Acid elution

Elute the beads with 5 ml of acetic acid solution (2% v/v) at a flow speed of 1 ml/min. A typical elution profile obtained by following this protocol is shown in Figure 4. The biotin elution recovery was calculated to be 80.2%.

In conclusion, biotinylation is a powerful tool for biotechnological research in the fields of post-translational modifications, protein–protein interaction and subcellular proteomes. For example, biotin-based proximity labeling techniques, such as BioID and APEX, have recently attracted a great deal of interest due to their ability to capture weak or transient interactions that can be lost in standard affinity purification [14]. This report provides an ideal method for enrichment and purification of biotinylated proteins under neutral elution conditions, thus allowing them to retain their natural structure. Additionally, the ►

Figure 5. Scheme for biotin-tagged protein purification using anti-biotin antibody binding and free biotin elution. The optimized biotin elution buffer was 4 mg/ml biotin in 25-mM Tris-HCl containing 0.3-M NaCl (pH 8.5). PBST: Phosphate-buffered saline buffer containing 0.05% Tween 20; RT: Room temperature.

free biotin in the eluted fractions can be easily removed by conventional desalting approaches, such as dialysis and ultrafiltration, for accurate downstream analysis. The anti-biotin agarose can be regenerated by mild acid wash and is reusable after neutralization with PBS. This method is also applicable for other biotinylated molecules, including biotin-tagged peptides and nucleic acids.

We have demonstrated a new method for purification of biotin-tag proteins at near-neutral conditions. This method applies anti-biotin antibody conjugated beads to specifically bind biotinylated proteins, followed by elution with free biotin solution at a pH value close to the neutral point (Figure 5). The optimized elution buffer is 4 mg/ml biotin in 25-mM Tris-HCl containing 0.3-M NaCl (pH 8.5). This method is a promising strategy to replace the currently used avidin/streptavidin–biotin purification system in many biotinylation-related research techniques.

AUTHOR CONTRIBUTIONS

H Xiao conceived and designed the protocols. K Lin contributed the data. The experiments were performed by K Lin, Q Yan and A Mitchell. The data were processed by K Lin,

Q Yan, A Mitchell, and C Lu. The paper was written by K Lin, Q Yan, N Funk and H Xiao.

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REFERENCES

1. Fairhead M, Howarth M. Site-specific biotinylation of purified proteins using BirA. *Methods Mol. Biol.* 1266, 171–184 (2015).
2. Cull MG, Schatz PJ. Biotinylation of proteins *in vivo* and *in vitro* using small peptide tags. *Methods Enzymol.* 326, 430–440 (2000).
3. de Boer E, Rodriguez P, Bonte E *et al.* Efficient biotinylation and single-step purification of tagged transcrip-

tion factors in mammalian cells and transgenic mice. *Proc. Natl Acad. Sci. USA* 100(13), 7480–7485 (2003).

4. Kojima N, Matsuo T, Sakai Y. Rapid hepatic cell attachment onto biodegradable polymer surfaces without toxicity using an avidin-biotin binding system. *Biomaterials* 27(28), 4904–4910 (2006).
5. Lowe BM, Sun K, Zeimpekis I, Skylaris CK, Green NG. Field-effect sensors – from pH sensing to biosensing: sensitivity enhancement using streptavidin-biotin as a model system. *Analyst* 142(22), 4173–4200 (2017).
6. de Back DZ, Vlaar R, Beuger B *et al.* A method for red blood cell biotinylation in a closed system. *Transfusion* 58(4), 896–904 (2018).
7. Rybak JN, Scheurer SB, Neri D, Elia G. Purification of biotinylated proteins on streptavidin resin: a protocol for quantitative elution. *Proteomics* 4(8), 2296–2299 (2004).
8. Rösli C, Rybak JN, Neri D, Elia G. Quantitative recovery of biotinylated proteins from streptavidin-based affinity chromatography resins. *Methods Mol. Biol.* 418, 89–100 (2008).
9. Hesketh GG, Youn JY, Samavarchi-Tehrani P, Raught B, Gingras AC. Parallel exploration of interaction space by BiD and affinity purification coupled to mass spectrometry. *Methods Mol. Biol.* 1550, 115–136 (2017).
10. Scheurer SB, Roesli C, Neri D, Elia G. A comparison of different biotinylation reagents, tryptic digestion procedures, and mass spectrometric techniques for 2-D peptide mapping of membrane proteins. *Proteomics* 5(12), 3035–3039 (2005).
11. Cheah JS, Yamada S. A simple elution strategy for biotinylated proteins bound to streptavidin conjugated beads using excess biotin and heat. *Biochem. Biophys. Res. Commun.* 493(4), 1522–1527 (2017).
12. Udeshi ND, Pedram K, Svinkina T *et al.* Antibodies to biotin enable large-scale detection of biotinylation sites on proteins. *Nat. Methods* 14(12), 1167–1170 (2017).
13. Del Olmo T, Lauzier A, Normandin C *et al.* APEX2-mediated RAB proximity labelling identifies a role for RAB21 in clathrin-independent cargo sorting. *EMBO Rep.* 20(2), pii: e47192 (2019).
14. Trinkle-Mulcahy L. Recent advances in proximity-based labelling methods for interactome mapping. *F1000Res.* 8, 135–146 (2019).