Heterologous Production and Anaerobic Purification of His- and StrepII-tagged Recombinant Proteins

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[Abstract] This protocol describes the heterologous expression and purification of proteins related to anoxic hydrogen production of *Chlamydomonas reinhardtii* (Noth *et al*., 2013). For this, the bacterial expression hosts *Escherichia coli* BL21 (DE3) *ΔiscR* (Akhtar MK *et al*., 2008) and *Clostridium acetobutylicum* ATCC 824 are used, which are grown either aerobic or anaerobic with glucose. Two standard chromatographic methods for purification were applied using His- and StrepII-tagged proteins (Figure 1). All procedures have been performed in an anaerobic tent to avoid the access of oxygen.

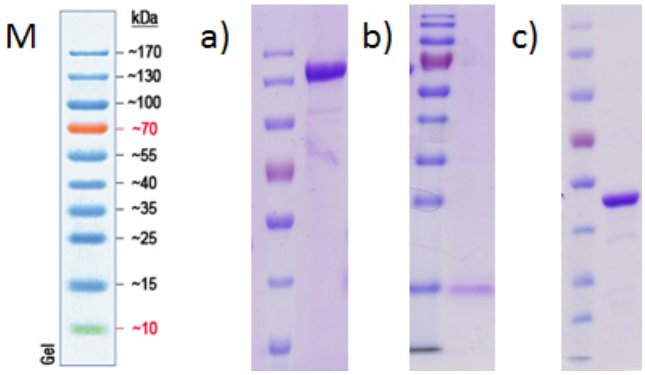

Figure 1. Coomassie stained SDS-PAGE of purified, heterologously expressed proteins from *C. reinhardtii*. M: MW marker PageRuler Prestained Protein Ladder 10-170 kDa; a) purified PFR1 loaded onto a 10% SDS-polyacrylamidgel; b) purified [2Fe2S] ferredoxin (PetF) loaded onto a 15% SDS-polyacrylamidgel; c) purifiedhydrogenase (HydA1) loaded onto a 10% SDS-polyacrylamidgel. Different amounts of protein are loaded onto each gel.

Materials and Reagents

1. Expression vector (pASK-IBA)
2. LB medium (Lennox) (Carl Roth, Germany)
3. Vogel Bonner minimal medium (homemade) (Vogel HJ *et al*., 1956)
4. Thiamin hydrochlorid (Carl Roth, Germany)

5. Resazurin (Riedel-de Haën, Germany)
6. Immidazole (Alfa Aesar, USA)
7. *Escherichia coli* BL21 (DE3) *ΔiscR*
8. *Clostridium acetobutylicum* ATCC 824
9. Ni Sepharose 6 Fast Flow (GE Healthcare)
10. Strep-Tactin Superflow (IBA GmbH, Germany)
11. Ampicillin
12. Anhydrotetracycline
13. Glucose
14. Sodium dithionite (laboratory reagent grade > 85%)
15. Avidin (Affiland)
16. Strep tactin
17. Glycerol
18. d-desthiobiotin (≥ 98%, TLC)
19. Thiamine pyrophosphate
20. PageRuler Prestained Protein Ladder 10-170 kDa (Thermo Fisher Scientific, catalog number: 26616)
21. 0.1 M Tris buffer (pH 8) (see Recipes)
22. Pre-equilibrated gravity flow Ni-NTA (see Recipes)

Equipment

1. Airtight vial
2. Sonicator: Branson Sonifier 250 (Branson, USA)
3. Ultracentrifuge
4. Anaerobic tent (1% H_2, 99% N_2) (Toepffer Lab Systems, Germany)
5. 0.2 µm pore size sterile filter (Sarstedt AG & Co.)
6. NanoDrop (Paqlab, Germany)
7. Batch fermenter (Infors HT, CH)

Procedure

A. Anaerobic expression of pyruvate: ferredoxin oxidoreductase (Noth *et al*., 2013)
   1. Electroporation (Sambrook *et al*., 2006) of 100 µl *E. coli* BL21 (DE3) *ΔiscR* (Akhtar *et al*., 2008) with ~100 ng expression vector (pASK-IBA).
   2. Inoculation of 200 ml LB and aerobic growth of a preculture over night at 37 °C (180 rpm).

3. Inoculation of 4 L Vogel Bonner medium (8 x 500 ml; 2,000 ml Erlenmeyer flasks) supplemented with 100 µg/ml ampicillin, 50 µM thiamin hydrochlorid and 0.2 µM resazurin using 15 ml preculture each.
4. Aerobic growth at 37 °C and 180 rpm until the culture reaches the anaerobic phase at A_{600} of 0.6. At that point, the redox indicator resazurin within the medium turns from blue to pink.
5. Each 2 L of culture are induced by adding 0.2 µg/ml anhydrotetracycline and transferred into sterile 2 L Schott flasks containing 50 ml 20% glucose (5 g/L).
6. Protein expression is carried out over night at 8 °C without stirring.
7. Cells are anaerobically harvested by centrifugation for 20 min at 7,500 *x g*, resuspended in Tris-HCl (pH 8.0), 10% glycerol and stored at -20 °C until purification.

B. Anaerobic purification of pyruvate:ferredoxin oxidoreductase (His-tag)
1. For purification the pellet (2 L of culture) is thawed at room temperature and lysed by sonication while keeping the cells cooled on ice.
   Note: Five times for 30 sec; output, 25; *Branson Sonifier 250.*
2. Sedimentation of cell debris at 200,000 *x g* for 60 min and 4 °C in an ultracentrifuge.
3. The soluble fraction is filtered using a pore size of 0.2 µm to get rid of unwanted material which clogs the column.
4. Then, the sample is loaded on a pre-equilibrated (100 mM Tris-HCl, pH 8.0, 10 mM imidazole, 0.5 mM thiamine pyrophosphate) gravity flow Ni-NTA fast-flow column with a bed volume of 4 ml.
5. Protein purification is achieved via increasing the imidazole concentration from 10 to 20 mM during washing each with 40 ml buffer.
6. The His-tagged PFR1 protein is eluted from the column with 10 ml buffer containing 100 mM imidazole. Nine elution fractions each 1.1 ml are collected.
7. The protein concentration of the brownish main elution fractions 3 and 4 are immediately determined using A_{280}.

C. Aerobic expression of [2Fe2S] ferredoxins (Jacobs *et al*., 2009; Winkler *et al*., 2009) with minor changes
1. *E. coli* BL21 (DE3) *ΔiscR* containing the expression plasmid pASK-IBA7-FDX is grown in Vogel Bonner minimal medium for 4 h after induction at A_{600} of 0.6.
2. Cells are harvested, washed in Tris-HCl (pH 8.0), sedimented again and stored at -20 °C until purification.

D. Anaerobic expression of HydA1 (Girbal *et al*., 2005; von Abendroth *et al*., 2008)

1. Expression plasmid containing *C. acetobutylicum* ATCC 824 strain is grown in CGM-medium and a glucose concentration of 60 g/L anaerobically in a batch fermenter over night at 35-37 °C and 100 rpm.
2. Cells are harvested in an anaerobic tent analog to *E. coli*, resuspended in Tris-HCl (pH 8.0), 10% glycerol containing 10 mM sodium dithionite and stored at -20 °C until purification.

E. Anaerobic expression of bacterial 2[4Fe4S] ferredoxin analog to HydA1 (Girbal *et al*., 2005; von Abendroth *et al*., 2008, Noth *et al*., 2013)
   1. Expression plasmid containing *C. acetobutylicum* ATCC 824 strain is grown in CGM-medium and a glucose concentration of 60 g/L anaerobically in a batch fermenter over night at 35-37 °C and 100 rpm.
   2. Cells are harvested in an anaerobic tent analog to *E. coli*, resuspended in Tris-HCl (pH 8.0), 10% glycerol containing 10 mM sodium dithionite and stored at -20 °C until purification.

F. Anaerobic purification of StrepII-tagged proteins (C-E)
   1. All buffers used contain 2 mM sodium dithionite.
   2. For purification the cell pellet is thawed at room temperature and lysed by sonication while keeping the cells cooled on ice.
      Note: Five times for 30 sec; output, 25; *Branson Sonifier 250*
   3. Sedimentation of cell debris at 200,000 *x g* for 60 min and 4 °C in an ultracentrifuge.
   4. Supernatant (40 ml) is incubated for 1 h with 3.5 mg Avidin (Stock 50 mg/ml) at 4 °C.
   5. The soluble fraction is filtered using a pore size of 0.2 µm to get rid of biotinylated, complexed proteins and unwanted material which clogs the column.
   6. Then, the filtered solution is loaded on a Tris-HCl (pH 8.0) equilibrated 2 ml strep tactin gravity flow column.
   7. The unbound proteins are washed from the column using 80 ml Tris-HCl (pH 8.0).
   8. Elution is performed with 10 ml Tris-HCl (pH 8.0), d-desthiobiotin (0.8 mg/ml) in fractions of 1 ml.

Recipes

1. 0.1 M Tris buffer (pH 8) (1,000 ml)
   Mix 12.114 g of Tris base with 800 ml dH_2O
   Add 100 ml Glycerol
   pH to 8 with HCl

Add ddH_2O to 1,000 ml
Autoclave for 20 minutes at 121 °C
Store at 4 °C

2. Pre-equilibrated gravity flow Ni-NTA
   0.1 mM Tris-HCl (pH 8)
   10 mM imidazole
   0.5 mM thiamine pyrophosphate

Acknowledgments

Aerobic expression of [2Fe2S] ferredoxins was adapted from Jacobs *et al.* (2009). Anaerobic expression and purification of the 2[4Fe4S] bacterial type ferredoxin was done according to the previously published isolation of [FeFe]-Hydrogenase HydA1 from *Chlamydomonas reinhardtii* by Girbal *et al.* (2005) and von Abendroth *et al.* (2008), which is also presented here. Research on the pyruvate: ferredoxin oxidoreductase from *C. reinhardtii* was scientifically supported by Anja Hemschemeier and Thomas Happe.

References

1. Akhtar, M. K. and Jones, P. R. (2008). Deletion of iscR stimulates recombinant clostridial Fe-Fe hydrogenase activity and H2-accumulation in *Escherichia coli* BL21(DE3). *Appl Microbiol Biotechnol* 78(5): 853-862.
2. Girbal, L., von Abendroth, G., Winkler, M., Benton, P. M., Meynial-Salles, I., Croux, C., Peters, J. W., Happe, T. and Soucaille, P. (2005). Homologous and heterologous overexpression in *Clostridium acetobutylicum* and characterization of purified clostridial and algal Fe-only hydrogenases with high specific activities. *Appl Environ Microbiol* 71(5): 2777-2781.
3. Jacobs, J., Pudollek, S., Hemschemeier, A. and Happe, T. (2009). A novel, anaerobically induced ferredoxin in *Chlamydomonas reinhardtii*. *FEBS Lett* 583(2): 325-329.
4. Noth, J., Krawietz, D., Hemschemeier, A. and Happe, T. (2013). Pyruvate:ferredoxin oxidoreductase is coupled to light-independent hydrogen production in *Chlamydomonas reinhardtii*. *J Biol Chem* 288(6): 4368-4377.
5. Sambrook, J. and Russell, D. W. (2006). Transformation of *E. coli* by Electroporation. *CSH Protoc* 2006(1).
6. Vogel, H. J. and Bonner, D. M. (1956). Acetylornithinase of *Escherichia coli*: partial purification and some properties. *J Biol Chem* 218(1): 97-106.

7. von Abendroth, G., Stripp, S., Silakov, A., Croux, C., Soucaille, P., Girbal, L. and Happe, T. (2008). Optimized over-expression of [FeFe] hydrogenases with high specific activity in *Clostridium acetobutylicum*. *International Journal of Hydrogen Energy* 33(21): 6076-6081.
8. Winkler, M., Kuhlgert, S., Hippler, M. and Happe, T. (2009). Characterization of the key step for light-driven hydrogen evolution in green algae. *J Biol Chem* 284(52): 36620-36627.

