Pyruvate:ferredoxin Oxidoreductase (PFR1) Activity Assays Using Methyl Viologen as Artificial Electron Acceptor

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[Abstract] Here we describe the activity measurements of heterologous expressed pyruvate:ferredoxin oxidoreductase (Noth *et al.*, 2013) from *Chlamydomonas reinhardtii*. This enzyme catalyzes the reversible reaction (I) from pyruvate to acetyl CoA and CO_2 generating low potential electrons which are *in vivo* transferred to ferredoxin.

$$\text{Pyruvate} + \text{CoA} + 2\ FDX_{ox} \leftrightarrow CO_2 + \text{acetyl-CoA} + 2\ FDX_{red} \quad \text{(I)}$$

In this assay we use methyl viologen as artificial electron acceptor which turns into dark violet (ε_{604} = 13.6/Mm/cm) (Mayhew, 1978) in its reduced state (Figure 1).

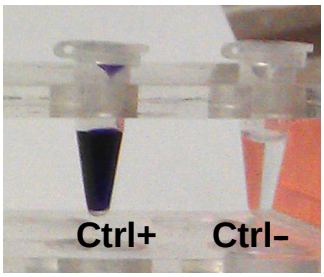

Figure 1. Activity assay using pyruvate, coenzyme A and methyl viologen (Ctrl+). In the absence of pyruvate, no methyl viologen reduction occurs (Ctr-).

Materials and Reagents

Note: All Reagents are dissolved freshly in an anaerobic tent.

1. Purified pyruvate: ferredoxin oxidoreductase (PFR1)
2. Sodium pyruvate (≥ 99%, Stock 500 mM) (Sigma-Aldrich)
3. Sodium coenzyme A (≥ 85%, Stock 20 mM) (Sigma-Aldrich)
4. Thiamine pyrophosphate (≥ 95%, Stock 250 mM) (Sigma-Aldrich)
5. Methyl viologen (98%, Stock 1 M) (Sigma-Aldrich)
6. Dithioerythriol (≥ 99%, Stock 100 mM) (Carl Roth)

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7. 0.1 M Tris-HCl buffer (pH 8.0) (see Recipes)

Equipment

1. Anaerobic tent (1% H_2, 99% N_2) (Toepffer Lab Systems)
2. 96 well plate reader (Beckman Coulter, catalog number: Paradigm1113)
3. PC running Multimode analysis software (Beckman Coulter)
4. NanoDrop (Paqlab)

Procedure

1. Protein concentration of heterologous expressed and purified PFR1 from 2 L of cell culture is measured at A_{280nm} using NanoDrop (ref bio-protocol: Heterologous Production and Anaerobic Purification of His- and StrepII-tagged Recombinant Proteins).
2. All reduction assays are performed under anaerobic atmosphere (1% H_2, 99% N_2) at room temperature.
3. The reaction mixture contains 10 mM sodium pyruvate, 2 mM sodium coenzyme A, 5 mM thiamine pyrophosphate, 10 mM methyl viologen and 16 mM dithioerythritol in 0.1 mM Tris-HCl (pH 8).
4. To start catalysis a final concentration of 1.4 µM PFR1 is added to the reaction mixture and absorbance (A_{604}, 96 well plate reader) can be monitored time resolved every 30 seconds until saturation is reached.
5. To determine enzyme activity the molar extinction coefficient ε_{604} = 13.6 mM^{-1} cm^{-1} (Mayhew, 1978) can be used applying Lambert Beer Law (Eq.I). One unit was defined as the conversion of 1 mol of pyruvate or CoA and the reduction of 2 mol of methyl viologen, respectively, per minute.

$$\mathbf{E_\lambda = \varepsilon_\lambda \cdot c \cdot d} \qquad \text{(Eq.I)}$$

E_λ: extinction
ε_λ: extinction coefficient
c: concentration
d: layer thickness

Recipes

1. 0.1 M Tris-HCl buffer (pH 8.0) (1,000 ml)
   Mix 12.114 g of Tris base with 800 ml dH_2O

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Adjust pH to 8 with HCl
Add ddH_2O to 1,000 ml
Autoclave for 20 minutes at 121 °C
Store at 4 °C

Acknowledgments

Kinetics for enzyme dependent methyl viologen reduction is adapted from Zeikus *et al.* (1977). Research on the pyruvate:ferredoxin oxidoreductase from *C. reinhardtii* was scientifically supported by Anja Hemschemeier and Thomas Happe.

References

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