

Determination of Quinone Reductase Activity

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[Abstract] We recently demonstrated the presence of a quinone detoxification pathway present in Firmicutes. It is based on two enzyme activities, namely a quinone reductase, YaiB, described here, and a hydroquinone dioxygenase, YaiA, described in a separate protocol. In *Lactococcus lactis* (*L. lactis*), these enzymes are encoded by the *yahCD-yaiAB* operon. The operon is induced by copper to prevent the synergistic toxicity of quinones and copper. The quinone reductase, YaiB, reduces *p*-benzoquinone and a range of quinone derivatives to hydroquinone, using NADPH as a reductant, according to the reaction: *p*-benzoquinone + NADPH + H⁺ → hydroquinone + NADP⁺. We here describe the measurement of quinone reductase activity, based on the spectrophotometric measurement of NADPH-oxidation.

Materials and Reagents

1. Quinone reductase purified from *Escherichia coli* (*E. coli*) by Ni-NTA chromatography as described in Mancini *et al.* (2015).
2. *p*-Benzoquinone (Sigma-Aldrich, catalog number: B10358)
Note: optionally other quinone substrates like 1, 4-penzoquinone, 2-methyl-1, 4-benzoquinone, 2, 5-dimethyl-1, 4-benzoquinone, menadione, naphthoquinone, or 2, 6-dichloro-1, 4-benzoquinone, all available from Sigma-Aldrich.
3. 50 mM NADPH in water; prepare on the day of use (Sigma-Aldrich, catalog number: N5130)
4. 20 mM Tris-Cl buffer (pH 7.5)
5. Flavin mononucleotide (Sigma-Aldrich, catalog number: F1392)
6. *p*-Benzoquinone (see Recipes)
7. NADPH (see Recipes)
8. 20 mM Tris-Cl (see Recipes)
9. 100 μM flavin mononucleotide (see Recipes)

Equipment

1. Thermostated spectrophotometer (Shimadzu, model: UV2600 or similar)

Procedure

1. Mix 970 μl of 20 mM Tris-Cl (pH 7.5), with 10 μl of 100 mM NADPH (final concentration 1 mM) and 10 μl of 100 mM *p*-benzoquinone (final concentration 1 mM) in a cuvette and equilibrate at 30 °C.
2. Zero the spectrophotometer and start the reaction at 30 °C by adding purified quinone reductase and immediately start recording the *decrease* in absorbance at 340 nm due to the oxidation of NADPH to NADP (approximately a 0.1 OD increase at 340 nm in 10 min should be observed, requiring 1 to 10 μg of purified enzyme). In a double-beam spectrophotometer, the measurement can be conducted in the reference beam, which will result in an apparent increase in absorbance. Since YaiB is an enzyme from *L. lactis* which grows best at 30 °C, this temperature was chosen for the assay,
3. A linear absorbance change should be observed within a few seconds. Let the reaction proceed for 5 to 10 min or until a steady reaction rate is observed. Calculate the activity as nmol/min, using an extinction coefficient for NADPH of 6,220 $\text{M}^{-1} \text{cm}^{-1}$.

Enzyme kinetics

- a. Both, NADPH and quinones are substrates of the enzyme reaction. NADPH is the reductant and quinones are the substrates being reduced. To determine the K_m for a substrate (either quinones or NADPH), run enzyme reactions with one substrate at 1, 3, 10, 30, 100, 300 and 1,000 μM , while keeping the other substrate at 1 mM.
- b. Determine the initial reaction rates, v_0 , from the slopes of the earliest linear regions of the recordings and plot v_0 versus the substrate concentrations, S.
- c. Fit the curve to obtain the affinity for the substrate, K_m , and the maximal velocity, v_{max} . Alternatively, plot v_0 versus $1/S$ to obtain a Lineweaver-Burk plot, from which K_m and v_{max} can be determined.

Representative data

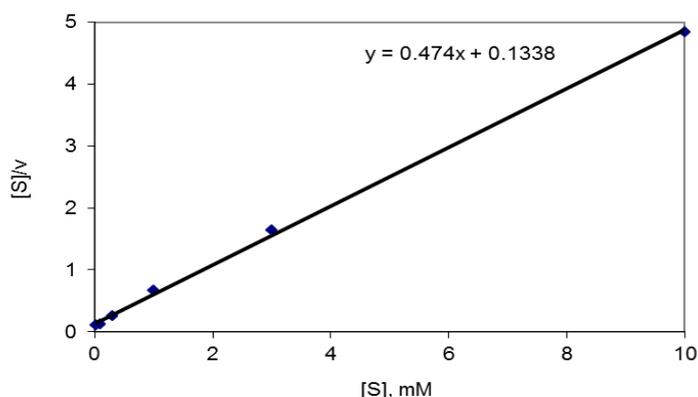


Figure 1. Hanes-Woolf plot of *p*-benzoquinone reduction by YaiB of *L. lactis*. [S] is the mM substrate concentration and v the reaction rate in mmol/min. The slope of the

linear regression line equals $1/V_{\max}$: $V_{\max} = 1/0.474 = 2.11$ mmol/min/mg. K_m is defined by the intercept of the regression line with the ordinate, which equals K_m/V_{\max} : $K_m = 0.1338 \cdot 2.11 = 0.28$ mM.

Notes

1. Quinone reductases are flavoproteins which lose their cofactor quite readily; therefore, all the buffers used for purification or dilution of the enzyme should be supplemented with 10 μ M flavin mononucleotide.
2. NADPH tends to auto-oxidize to NADP in the presence of oxygen; therefore, it must be prepared fresh.

Recipes

1. *p*-Benzoquinone (optionally other quinone substrates like 1,4-penzoquinone, 2-methyl-1,4-benzoquinone, 2,5-dimethyl-1,4-benzoquinone, menadione, naphthoquinone, or 2,6-dichloro-1,4-benzoquinone)
Dissolve substrates at 50 mM in dimethylsulfoxide
Stable at room temperature
2. NADPH
50 mM in water
Prepare fresh on the day of use.
3. 20 mM Tris-Cl (pH 7.5)
Stable at room temperature.
4. 100 μ M flavin mononucleotide
Stored frozen

Acknowledgments

This work was supported by Russian Federation Government Grant 14.Z50.31.0011 to leading scientists. The procedure has previously been described in Mancini *et al.* (2015).

References

1. Mancini, S., Abicht, H. K., Gonskikh, Y. and Solioz, M. (2015). [A copper-induced quinone degradation pathway provides protection against combined copper/quinone stress in *Lactococcus lactis* IL1403](#). *Mol Microbiol* 95(4): 645-659.