Nitrite 이온센싱을 위한 cytochrome cd_1 효소분석연구
Enzyme assay and kinetic studies of cytochrome cd_1 for nitrite ion biosensor

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1. Introduction

Cytochrome cd_1 is a bifunctional enzyme that catalyses the reduction of nitrite to nitric oxide ($NO_2^- + 2H^+ + 1e^- \rightarrow NO + H_2O$) and the reduction of molecular oxygen to water ($O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$). *Paracoccus pantotrophus*[1] cytochrome cd_1 is an homodimer of approximately 120 kDa located in the bacterial periplasm.[2] Each of the two subunits contains a covalent *c*-type and a unique non-covalently bound d_1-type haem group. The spectroscopic changes associated with the redox state of the iron atom in the center of the d_1 and *c* haems in cytochrome cd_1 upon binding of nitrite ions suggests that this enzyme would be ideal for the development of an optical biosensor for the detection of this analyte. To develop an optical biosensor, it is important to immobilize the biomolecule, particularly enzymes, in an environment that protects the molecular recognition element to prevent partial or total loss of physiological activity due to structural alteration of the tertiary structure. In order to obtain the insight on immobilization the enzyme, more detail information about enzyme activity, kinetic and the like is needed because the enzyme activity should be high as much as the level which enzyme can be used as an optical sensing element. Consequently, in this work, enzyme assay and kinetic studies of cytochrome cd_1 from *P. pantotrophus* was conducted to provide the fundamental and possibility for biosensing system for the detection of nitrite.

2. Experimental, Results and Discussion

The activity of cytochrome cd_1 was routinely measured at 600 nm by measuring the re-oxidation of $Na_2S_2O_4$-reduced Methyl Viologen (MV) in the presence of sodium nitrite ($NaNO_2$) under anaerobic conditions at 20 C. Strict anaerobic conditions of all solutions and vessels used in the assay was achieved by purging with oxygen-free nitrogen for 30 min prior to use.

The reaction mixture, in a 3 ml sealed cuvette, initially contained 2.00 ml of 0.1 M TRIS-HCl buffer pH 7.8 with 2 X 10^{-3} M EDTA (standard assay buffer), 0.01 ml of 0.05 M MV solution in 0.1 M TRIS-HCl buffer pH 7.8 and a suitable amount of enzyme (3 X 10^{-8} ~ 3 X 10^{-7} M, final concentration in cuvette). $Na_2S_2O_4$ (0.02 M solution in 0.1 M TRIS-HCl buffer pH 7.8) was added to the assay mixture to give an absorbance of 1.2 1.4 at 600 nm.

The absorbance at 600 nm was followed for 5 min to measure the small background oxidation rate before starting the reaction by injecting 0.010 ml of 0.1 M solution of $NaNO_2$. The reduction

of NO_2^- to nitric oxide (NO) was followed by measuring the rate of change of absorbance at 600 nm with time. The absorption coefficient for reduced MV was taken as 13 700 $M^{-1}cm^{-1}$ at 600 nm. Control assays without nitrite or without enzyme showed little or no bleaching of the reduced MV. The specific activity of the cytochrome *cd1* was determined *cd1* was determined to be 0.6 *mmol* of NO_2^- reduced min^{-1} mg of $protein^{-1}$ at 20 C. Values of specific activity of cytochrome *cd1* nitrite reductases from other bacterial sources have been reported and compared in this study. It should be noted that each reported value was determined using a different system for the reduction of the enzyme and it is possible that the differences in the methods used may account for the considerable variation in the specific activity value obtained.

3. Conclusions

In this work, enzyme assay and kinetic studies of the dissimilatory cytochrome *cd1* nitrite reductase from *P. pantotrophus* was conducted to provide the fundamental and possibility for biosensing system for the detection of nitrite. Kinetic studies of cytochrome cd1 was also conducted to determine the Michaelis-Menton constant (K_m), the maximal reaction rate (V_{max}), and the catalytic constant (k_{cat}) were calculated; 1.70 X 10^{-4} M, 1.62 X 10^{-5} $Mmin^{-1}$ and 2 s^{-1} respectively. Specific activity of cytochrome *cd1* nitrite reductases from *Paracoccus pantotrophus* was calculated in this work. The specific activity valuce was 0.6. The direct comparison was difficult because specific activity of cytochrome *cd1* in the literature values were determined using a different system for the reduction of the enzyme. Although we have to accept that the differences in the methods used , the specific activity value of cytochrome *cd1* in this work is comparatively high for the biosensing purpose. pH dependence of cytochrome *cd1* nitrite reductase activity studies of cytochrome *cd1* from *P. pantotrophus* was also conducted to provide the fundamental and possibility for biosensing system for the detection of nitrite. The results of this study clearly show that the pH range at which the optimum activity of the cytochrome *cd1* occurs is between 6-9. From above results, the purified cytochrome *cd1* nitrite reductase can be used as an useful candidate for an nitrite biosensing system.

References

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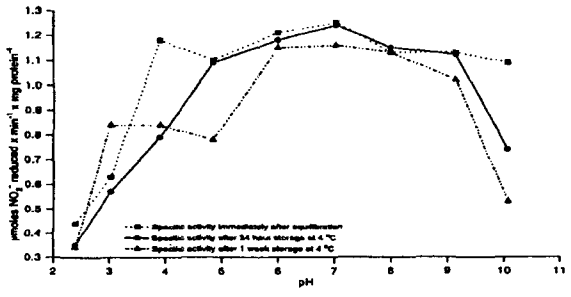

Fig. 1 Measurement of the specific activity of cytochrome *cd1* nitrite reductase as a function of pH over a period of 7 d.