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Impact of Fe and Cu Complexes on Manganese Peroxidase (MnP) Activity Determination by UV Spectroscopy

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Abstract

Manganese peroxidase activity can be determine by the formation of Mn(III)-malonate complexes. However, overestimation of the product formed can result from two sources: 1) Oxidation of ferrous ions to ferric ions and the subsequent formation of ferric malonate complex and 2) oxidation of Mn(II) to Mn(III) by the hydroxyl radicals produced by the Fenton reaction. Thus it is important to be aware of impact of these complexes so as to improve the quality of MnP activity results.

1. Introduction

The activity of manganese peroxidase (MnP) can be determined spectrophotometrically by two protocols. These are the direct analysis for Mn(III)-organic acid complexes or the oxidation of phenolic substrate such as 2,6 dimethoxy phenol [1]. The simpler Mn(III)-organic acid method has been used extensively for both the unpurified cell-free spent medium (CFSM) and purified enzymes [2]. It has been shown that using this procedure in the presence of metal ions (Fe, Cu) can affect the test's accuracy through inhibition of MnP activity [2, 3]. But, that might not be the only effect Fe and Cu ions on the MnP assay. The impact of UV radiation absorption should also be considered for organic acid complexes of Fe(II)/Fe(III) and Cu(II). This study will examine the effect of Fe and Cu complexes on the Mn(III)-organic acid method so as to help improve the quality of data obtained for MnP containing samples, especially, the CFSM.

2. Experimental

2.1 Fungal growth and enzyme secretion

Phanerocheate chrysosporium was used for enzyme production in a liquid culture with the same composition as Tien [4]. The initial concentration of trace metals Fe, Cu and Mn in the culture medium were determined by ICP-OES. The fungus was cultured in 25 mL of the medium in a 50 mL sterilized flasks for 3 days. The other culture conditions were pH 3.0 or 4.0, 37°C and no agitation. Finally, the CFSM was harvested by filtration through a 0.2 µm filter and used for MnP assay immediately.

2.2 MnP assay

The MnP activity was detected by the formation of Mn(III)-malonate from the oxidation Mn(II) to Mn(III) and the subsequent complexation with malonate. The product was determined at 270 nm by UV spectroscopy [1]. 1 ml of the assay fluid contained 0.8 mL CFSM, 50 mM malonic acid, 2 mM Mn^{2+} and 0.4 mM H_2O_2. The assay was conducted for 120 secs at pH 4.5 and room temperature.

2.3 Effect of iron and copper

The effect of iron and copper complexes on the assay procedure was determined by replacing the CFSM with varying concentrations of $FeSO_4.7H_2O$ and $CuSO_4.5H_2O$. The assay conditions in Section 2.2 were used, however, variables like H_2O_2 and Mn^{2+} additions were considered.

3. Results and discussion

The absorbance recorded at 270 nm can used to estimate the amount of Mn(III)-malonate formed by enzymes. A preliminary investigation indicated that cultivating the fungus at pH 3.0 was much better for enzyme production than at pH 4.0. This was due to their respective absorbance values of 0.26 (pH 3.0) and 0.092 (pH 4.0). However, these values might have been influenced by the ions in solution. Consequently, the total Mn, Cu and Fe in the two CFSM samples were determined by ICP-OSE as depicted by **Table 1**. The only significant difference between these two CFSM samples was the total Fe concentration, which was approximately 5 time lower in the pH 4.0 sample as a result of precipitation.

Table 1 Concentration soluble metals (μM) before and after 3 days of culture time at medium pH of 3.0 and 4.0

Culture time	pH 3.0			pH 4.0		
(day)	Cu	Fe	Mn	Cu	Fe	Mn
0	26.30	24.13	137.95	24.0	3.64	129.6
3	26.87	24.60	144.52	26.23	5.07	147.18

The ferrous and cupric salts $FeSO_4.7H_2O$ and $CuSO_4.5H_2O$ were used to study the effect metal complexes (**Fig. 1**). Typically, the UV assay for MnP is conducted using a blank that excludes only H_2O_2. Thus when 0.4 mM H_2O_2 was excluded or added to 20 μM Cu^{2+} in the presence of 50 mM malonic acid, the UV absorbance was 0.034. Thus, it can reasonably be assumed that the resulting copper complexes formed were accounted for by the blank solution. On the other hand, for 20 μM Fe^{2+}, the absorbance in the absence and presence of H_2O_2 were 0.028 and 0.157 respectively. This indicates that oxidation of the Fe^{2+} to Fe^{3+} might have occurred and since the ferric malonate complex is more stable [5], the absorbance increased significantly. Another important factor that was considered was the by-products of the Fenton reaction between Fe^{2+} and H_2O_2 as shown by Eqn 1.

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^- + \bullet OH \qquad \{1\}$$

The hydroxyl radical has a higher oxidizing potential than H_2O_2 and thus it is able to successfully oxidize some amount of the 2 mM Mn(II) to Mn(III) when which resulted in a further increase in absorbance to 0.228. This indicates using the blank solution without H_2O_2 could lead to overestimation of MnP activity from two sources. Thus the preliminary absorbance reported for the CFSM did not reflect the actual MnP activity in the CFSM.

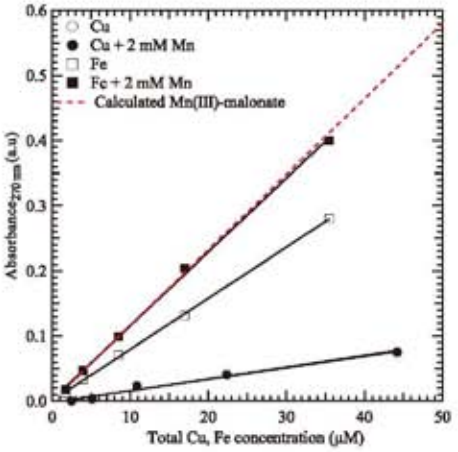

Fig. 1 Effect of total Cu and Fe on absorbance at 270 nm. The calculated Mn(III)- malonate line was drawn using Beer's law and ε=11590 $M^{-1}cm^{-1}$[3].

4. Conclusions

The findings of this report indicates that it is necessary to account for the oxidation of ferrous irons by H_2O_2 so as to correctly determine the MnP activity of CFSM.

References

[1] Wariishi, et al. 1991, *J. Biol. Chem.*, *267*, 23688-23695.
[2] Zeng et al., 2013. *Int. Biodeter. Biodegr.*, 85, 166-172.
[3] Paszczyński, et al, 1988. Meth. Enzymol. 161, 264-270.
[4] Tein M. 1988. *Meth. Enzymol.* 161, 264-270.
[5] Dellien, I., 1977. *Acta. Chem. Scand. A*, 31, 473-479
[6] Xue et al., 2008. *Electrochim. Acta*, 53, 6636-6642