Phanerochaete chrysosporium glyoxal oxidase reactivation by coper ion loadings

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INTRODUCTION

Glyoxal oxidase (GLOX) is an extracellular H_2O_2-generating metalloenzyme produced by various wood-degrading fungi, one of them being *Phanerochaete chrysosporium*, a white-rot fungus with the ability to completely degrade lignin. Because of the presence of one copper ion in its active site, GLOX is classified as radical copper oxidase, forming the AA5 family in the CAZymes system. In order to determine copper in metalloproteins, a method known as Zincon assay was used. The method is based on the use of metal chelator Zincon, a colorimetric indicator used for spectrophotometric determination of zinc, copper and mercury.

RESULTS

a) Copper loading – Zincon assay

The amount of GLOX being loaded with copper was determinated by spectrophotometric method using the zincon assay. For the calibration curve (Fig 1), $CuCl_2$ solution was measured with the concentrations given in Table 1.

Table 1. Standards concentrations

Standards	
Stock concentration [µM]	**Final concentration [µM]**
0	0
10	1
20	2
50	5
100	10
200	20
300	30
400	40

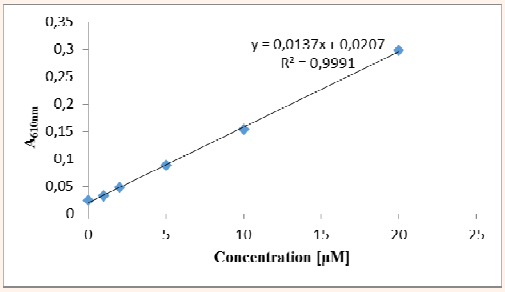

Fig 1. Calibration curve generated with $CuCl_2$

To be active, GLOX needs and can contain only one copper in its active site. Relaying on this data that previous studies confirmed, obtained values (protein and copper concentrations) were put in a ratio to calculate the percentage of active enzyme produced in *P. pastoris* and *T. reesei*. Ratios shows that 65.9% of rGLOX produced in *P. pastoris* is active and 51.7% of rGLOX from *T. ressei* (Table 2).

Table 2. Percentage of active enzyme produced in *P. pastoris* and *T. reesei*.

Sample	Absorbance		Protein concentration	Cu^{2+} concentration	Ratio
	A_{280nm}	A_{610nm}	[µM]	[µM]	[%]
P.pastoris	0.239	0.082	6.765	4.552	65.9
T.reesei	0.356	0.092	10.081	5.349	51.7

b) GLOX reconstitution with Cu^{2+}

The calculated ratios showed that a bit more than half of the enzyme preparation was active, which leaves 34 – 48% of inactive enzyme. For that reason, an attempt for rGLOX reconstitution was made. Reconstitution was carried out in a different conditions with activity measurements after 23 h from the beginning of the incubation (Fig 2).

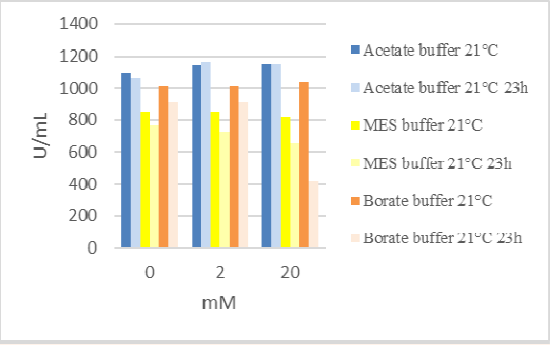

Fig 2. Enzyme activity after loading of 2mM and 20mM of CuSO4 solution at 21°C and 23 h of incubation.

CONCLUSIONS

The reconstitution attempt was not successful. Enzyme activity remained the same or slightly decreased under investigated conditions.