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Letter

Abnormal effect of mercury ions on bilirubin oxidase activity

Yuji Seki [a], Masayuki Takeguchi [a], Sawao Murao [b], Takashi Shin [b], Ichiro Okura [a,*]

[a] *Department of Bioengineering, Tokyo Institute of Technology, Nagatsuta, Yokohama 227, Japan*

[b] *Department of Applied Microbial Technology, Kumamoto, Institute of Technology, Ikeda, Kumamoto 860, Japan*

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Abstract

Purification of bilirubin oxidase (BOX) from *Penicillium janthinellum* was carried out and the effect of metal ions on BOX activity was studied, and in presence of mercury ion, a remarkable increase of the activity was observed.

Keywords: Bilirubin oxidase; *Penicillium janthinellum*; Mercury ion

Bilirubin oxidase (BOX) catalyzes the oxidation of bilirubin and biliverdin is produced as depicted in Scheme 1.

A few types of bilirubin oxidases have been purified and characterized, such as BOX from *Myrothecium verrucaria* [1] and from *Trachyderma tsunodae* [2]. In this communication we describe a new type of BOX from *Penicillium janthinellum*. *P. janthinellum* was cultivated with potato extract and glucose as carbon sources at 28°C. From the membrane fraction of the fungi, BOX was solubilized with Triton X-114 and was applied to DEAE Sepharose fast flow column, and then applied to Sephacryl S-300 HR column after concentration. BOX thus obtained has a specific activity of 3.6 unit/mg-protein. BOX activity was determined by biliverdin production rate.

The effect of metal ions on BOX activity is shown in Table 1. BOX activity was strongly influenced by the addition of metal ions. Among the metal ions used, only mercury ion made the enzyme activity increase remarkably. In the case of cupric and cobalt ions, BOX was completely inhibited, and strongly inhibited by nickel ion. To clarify the reason of the abnormal effect of mercury ion, the following experiments were carried out.

Spectroscopic measurement was carried to find out whether the complex between mercury ion and BOX causes the abnormal effect. By spectroscopic analysis no change in spectrum was observed by the addition of mercury ion to BOX, showing that no complex is formed between BOX and mercury ion. When BOX was contacted with mercury ion in the absence of bilirubin, BOX activity decreased as shown in Fig. 1. When the concentration of mercury ion increased, the activity decreased rapidly and was maintained at more than 50% of the initial activity. The results show that the mercury ion partly inhibits the BOX active site.

Fig. 2 shows the change in spectrum of bilirubin when mercury ion was added. The characteristic absorption band of bilirubin at 450 nm decreased

* Corresponding author.

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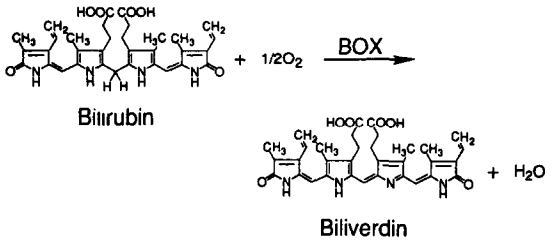


Scheme 1.

Table 1
Effect of metal ions on bilirubin oxidase activity

Metal ion	Relative activity [a]/%
–	100
Hg^{2+}	587
Hg^{2+}	943 [b]
Cu^{2+}	0
Co^{2+}	0
Ni^{2+}	8
Zn^{2+}	n.d.
Fe^{2+}	n.d.
Mg^{2+}	n.d.
Ca^{2+}	n.d.

[a] Bilirubin oxidase: 6.9×10^{-3} U; bilirubin: 8.6×10^{-6} mol dm^{-3}; metal ion: 1.0×10^{-3} mol dm^{-3}; reaction temp.: 37°C. n.d.: not determined.
[b] Hg^{2+}: 3.0×10^{-3} mol dm^{-3}; otherwise conditions same as in [a].

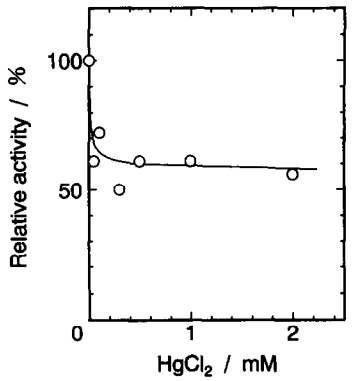


Fig. 1. Effect of mercury ions on bilirubin oxidase activity. Bilirubin oxidase, 6.9×10^{-3} U; bilirubin, 8.6×10^{-6} mol dm^{-3}; reaction temp., 37°C.

and new bands at 280 nm and 635 nm appeared with isosbestic points at 375 nm and 535 nm. The results show that BOX is complexed with mercury ion.

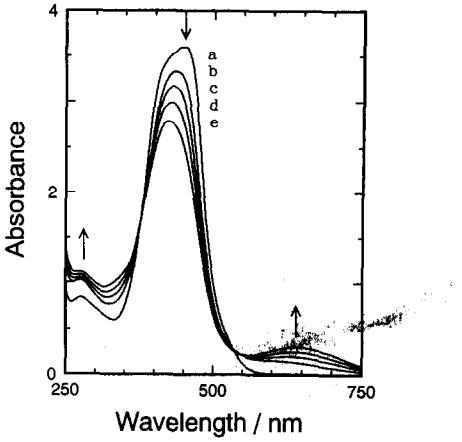
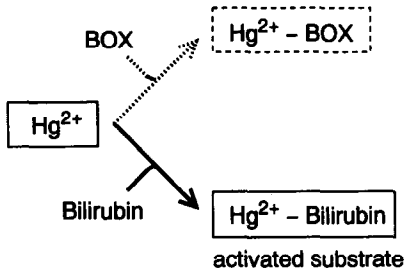


Fig. 2. Change in spectrum of bilirubin by the addition of mercury ions. Bilirubin, 8.6×10^{-6} mol dm^{-3}; Hg^{2+}, 3.0×10^{-3} mol dm^{-3}; reaction temp., 37°C; reaction time, a: 0 min, b: 5 min, c: 10 min, d: 15 min, e: 20 min.



Scheme 2.

From the above results it is concluded that mercury ion binds bilirubin only to form a complex and the complex is a more suitable substrate for BOX than bilirubin as shown in Scheme 2.

The mechanistic study of the reaction is now in progress.

Acknowledgements

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