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Journal of Molecular Catalysis A: Chemical 97 (1995) L3–L5

JOURNAL OF MOLECULAR CATALYSIS A: CHEMICAL

On the molecular weight of bilirubin oxidase from *Penicillium janthinellum*

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Received 29 August 1994; accepted 25 November 1994

Abstract

When bilirubin oxidase from *Penicillium janthinellum* was treated with bilirubin BOX was dissociated to the compound with the molecular weight between 0.5 kDa and 5 kDa. When BOX was treated with EDTA, a remarkable increase of the reaction rate was observed. The reaction mechanism of these effects is discussed.

Keywords: Bilirubin; Bilirubin oxidase; Biliverdin

Bilirubin oxidase (BOX) catalyzes the oxidation of bilirubin and biliverdin is produced according to 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 hope to 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 molecular weight (MW) of BOX was measured by using gel filtration method. The results are shown in Fig. 1, indicating the MW of BOX was ca. 2000 kDa. Such a tremendously large MW may be caused by the coagulation of BOX.

A typical time dependence of biliverdin formation with BOX is shown in Fig. 2. The biliverdin formation rate accelerated gradually with reaction time and S-shape dependence was observed. The rate acceleration with reaction time may be caused by the increase of the number of active sites with reaction time. After 10 min reaction of the BOX was applied to some types of MW cut-off filters, and both activities of the filtrate and the filter cake were measured. The results are summarized in Table 1. As the MW of BOX is 2000 kDa, BOX does not pass through 30 kDa MW cut-off filter as shown in run No. 1 of Table 1. However, after 10 min contact of BOX with bilirubin, BOX activity was observed both in the filter cake and the filtrate as shown by run No. 2. Similar results were obtained when 10 kDa and 5 kDa

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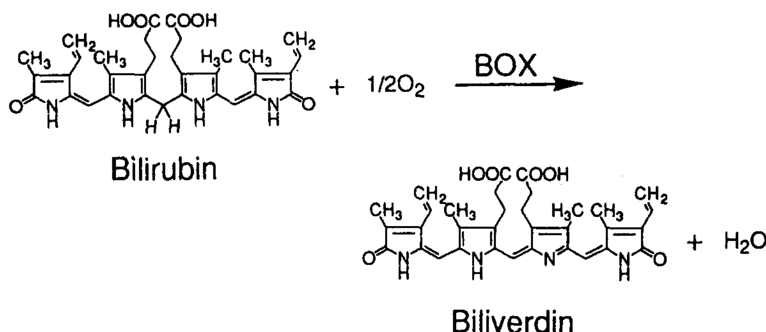
SSDI 1381-1169(94)00061-1



Scheme 1.

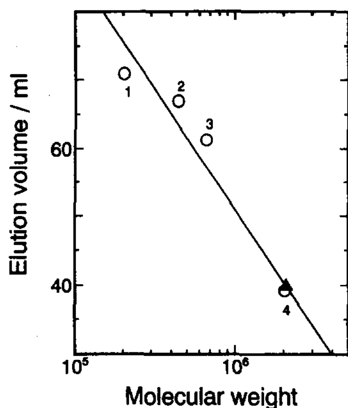


Fig. 1. Measurement of molecular weight of BOX by gel filtration. Column: Superose 6 pg (HR16/50). Standard samples: 1, α-amylase; 2, apoferritin; 3, thyroglobulin; 4, blue dextran. BOX is shown by ▲.

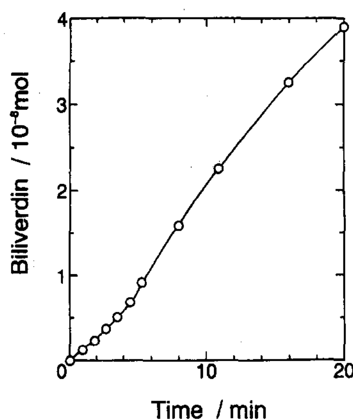


Fig. 2. Typical time dependence of biliverdin foramtion with BOX. The sample solution contains BOX (5.0×10^{-3} U) and bilirubin (1.3×10^{-7} mol). Reaction temp.: 30°C.

MW cut-off filters were used. When 0.5 kDa MW cut-off filter was used, no activity was observed in the filtrate. From the above results, by treatment of BOX with bilirubin BOX was dissociated to the compound with the MW between 0.5 kDa and 5 kDa.

Protein coagulation are often observed by metal ions, and protein dissociation occurs by adding chelating agent. By the treatment of BOX with bilirubin, new active sites may appear by the dissociation of the coagulated BOX with MW 2000 kDa, since the activity increased and the molecular weight of BOX decreased. If the dissociation occurs by the chelation effect of bilirubin, a similar phenomenon will be observed with EDTA. When BOX was treated with EDTA, a remarkable increase of the reaction rate was observed as shown in Fig. 3. When EDTA treated BOX was filtrated with 30 kDa cut-off filter, only 15% of the activity was remained in the filter cake, showing that EDTA also dissociates the coagulated BOX.

From the above results we propose a scheme to dissociate BOX to smaller molecules by the treatment of bilirubin and new active sites appear. EDTA also accelerates the dissociation of BOX.

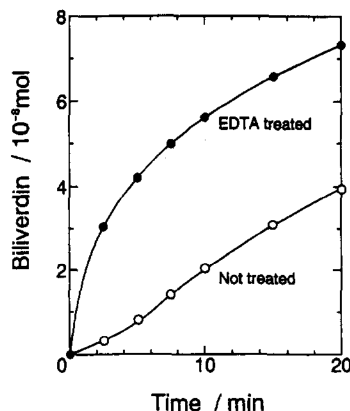


Fig. 3. Effect of EDTA treatment of BOX. BOX (5.0×10^{-3} U) was treated EDTA (2.0×10^{-7} mol) for 10min at 30°C and the solution was filtrated with MW cut-off filter with 30 kDa.

Table 1
BOX activities of filtrate and filter cake

Run No.	MW cut-off filter used	Sample	BOX activity	
			Filter cake	Filtrate
1	30 kDa	BOX	active	inactive
2		BOX + bilirubin	active	active
3	10 kDa	BOX	active	inactive
4		BOX + bilirubin	active	active
5	5 kDa	BOX	active	inactive
6	0.5 kDa	BOX + bilirubin	active	inactive

This work was partially supported by a Grant-in-Aid for Scientific Research on Priority Areas 'Electroorganic Chemistry (No. 06226226)' from the Ministry of Education, Science and Culture, Japan.

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