

Dechlorination of Trichloroacetic Acid by Mn(III)/oxalate System and the Enzymatic System of Manganese Peroxidase/Mn(II)/oxalate*¹

Akio NISHIYAMA*², Takefumi HATTORI*² and Mikio SHIMADA*²

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Introduction

The accumulation of recalcitrant halocarbonated compounds in soil has caused serious environmental pollutions. However, a variety of environmental pollutants, such as chlorophenol, benzopyrene, and dioxins, have been reported to be degraded by white-rot fungi including *Phanerochaete chrysosporium*¹.

The active oxygen species generated in lignin biodegradation system have been considered to be promising for dehalogenation of halogenated compounds. For example, Aust *et al.*² reported dehalogenation of CCl₄ by CO₂⁻ derived from oxalate in lignin peroxidase (LiP)/veratryl alcohol/oxalate system. We report here the enzyme-mimetic dechlorination of trichloroacetic acid (TCA) by Mn(III)/oxalate system and manganese peroxidase (MnP)/Mn(II)/oxalate system for the first time.

Materials and Methods

The reaction mixture contained 66.7 mM oxalate (pH 5.0), 10 mM Mn(III) acetate, and 33.3 mM TCA was incubated at 30°C. Similarly, 33.3 mM TCA was incubated with 66.7 mM oxalate (pH 5.0), 10 mM MnSO₄, 6.3 mU of MnP, and 0.3 mM H₂O₂ at 30°C. After oxalate was removed with Ca(NO₃)₂ by centrifugation, the amount of Cl⁻ derived from TCA was determined spectrophotometrically³.

Results and Discussion

TCA was found to be nonenzymatically dechlorinated by Mn(III)/oxalate system. The yield reached the maximum (3.2%) by 80 minutes (Fig. 1). Both oxalate and Mn(III) were required for the dechlorination of TCA.

The optimum concentration of Mn(III) in the reaction mixture was 6 mM and the yield decreased as Mn(III) concentration increased. The yield of Cl⁻ derived from TCA under anaerobic condition was 5 times higher than that of under the aerobic condition. A proposed mechanism for the dechlorination is shown in Fig. 2. As

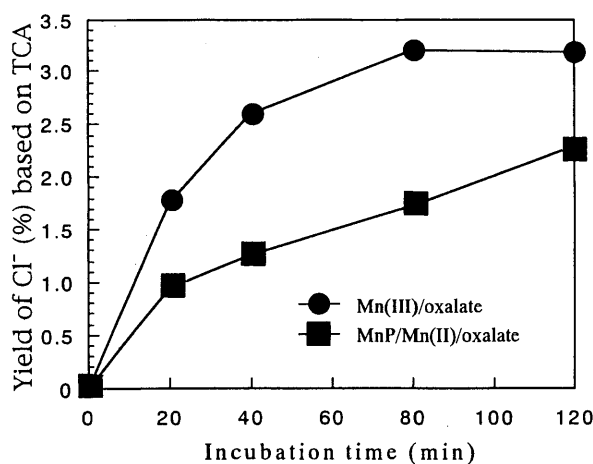


Fig. 1. Enzymatic (MnP/Mn(II)/oxalate) and bio-mimetic (Mn(III)/oxalate) dechlorination of trichloroacetic acid (TCA).

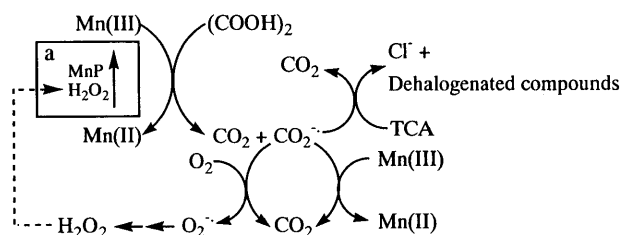


Fig. 2. A proposed mechanism for the dechlorination of TCA by MnP/Mn(II)oxalate system. note: a, Mn(II) is oxidized to Mn(III) in the enzymatic reaction.

shown by Aust *et al.*⁴, the reducing active species, such as CO₂⁻ derived from oxalate, were probably responsible for dechlorination of TCA. Because the yield of dechlorination was greater when the reaction mixture showed higher reducing activity of nitrobluetetrazolium (Data not shown), although the author did not confirm the production of CO₂⁻ by ESR. On the other hand, another reducing agent such as O₂⁻ might also be produced during the reaction. However, in separate investigation, TCA was not dechlorinated by O₂⁻ derived from K₂O (Data not shown), which also supports that CO₂⁻ was an active species for dechlorination.

Similarly, TCA was enzymatically dechlorinated by

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*² Laboratory of Biochemical Control, Wood Research Institute, Kyoto University, Uji, Kyoto 611-0011, Japan.

MnP/Mn(II)/oxalate system (Fig. 1). However, Mn(III) was expected to be regenerated by MnP in the presence of H_2O_2 . Interestingly, in contrast to the Mn(III) oxalate system, dechlorination reaction occurred in 86% yield based on TCA dechlorinated of the complete reaction system, even though H_2O_2 was not added exogenously, suggesting that H_2O_2 was produced from O_2 by the reduction with $\text{CO}_2^{\cdot-}$ (Fig. 2). Thus, more Cl^- would be released in enzyme system than in its biomimetic system during longer periods of the reaction. A possible mechanism for the enzymatic dechlorination of TCA is essentially the same as the non-enzymatic one for the

Mn(III)/oxalate system.

Further research is needed to detect active reaction species such as $\text{CO}_2^{\cdot-}$ by ESR.

References

- 1) D. BARR and D. AUST: *Environ. Sci. Technol.*, **28**, 78A–87A (1994).
- 2) A. KHINDARIA and S. AUST: *Environ. Sci. Technol.*, **29**, 719–725 (1995).
- 3) W. FISCHER and H. KEIN: *J. Anal. Chem.*, **128**, 443 (1948).
- 4) A. KHINDARIA and S. AUST: *Arch. Biochem. Biophys.*, **314**, 301–306 (1994).