Direct Measurement of Dioxins in Degradation Enzyme Mixture Using Immunoassay

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Introduction

It is important to measure the quantity of Dioxins in enzyme mixture from the viewpoint of biodegradation activity. Presently, quantify of dioxins is measured using high-resolution Gas Chromatograph/ high-resolution Mass Spectrometer (GC/MS) by detecting all isomers assigned with TEF and summing the toxic equivalents. GC/MS requires much cost and time, although it can detect them with high sensitivity. Consequently, measurement of quantity of dioxins has been limited to a small number of cases. It is most reasonable to measure the quantity, using immunoassay method with low cost and short time.

Enzyme-Linked Immunoassay is a bio-assay detection method which is based on the principle of quantifying of dioxin of 2.3.7 tri-chlorinated dioxin with other isomers on an ELISA plate with extraction and purification of a sample. It is useful specially for screening the optimal condition of biodegradation enzyme activity of dioxins in a reaction mixture. In this paper, we report the correlation between Enzyme-Linked Immunoassay with authentic extraction and purification methods and the one without any extraction and purification and measuring the quantity of dioxins contained in an enzyme mixture of a thermophile.

Enzyme Sample

The enzyme samples used for the tests were prepared by the methods to collect thermophile cell lysate by ultra sonication and purify the cell membrane of *Geobacillus midousuji* ATCC No.202050by ultracentrifuge. This dioxin biodegradation enzyme was dried out at low temperature with vacuum centrifugation method and mixed with yeast extract powder: YE (DIFCO).

Method for ELISA with the antibody to 2, 3, 7-trichlorodibenzo-p-dioxin

The antibody to 2, 3, 7-TCDD has cross reactivity to the other dioxin isomers listed in Table 1. Principle of the ELISA method was shown in Figure 1 based on the competitive analysis with biotinilated 2, 3,7 – TCDD, horse radish per oxidase, and its substrate of 3, 3', 5, 5'-tetramethyle benzidine.

ELISA methods, were performed at 4C, 24hours of 1st reaction and 20C , 2 hours of 2nd reaction in citrate buffer and TSB respectively.

Results and Discussion

The results of estimation of ELISA with TSB are shown in Table 2,and Fig.1, 2, 3. Results of this immunoassay in TSB of this antibody show linearity of dioxin concentration and optical density. With addition of DMSO and Ca^{2+} ions and addition/yield of 2, 3, 7-dioxin, comparing in citrate buffer. It was found that as to addition of Dimethylsurfoxid:DMSO (1-5 vol%), Ca^{2+} ion(1mM) show dose response in this ELISA method with TSB. Addition/yield was calculated as follows. Addition = base concentration + additional concentration. Yield = additional concentration – base concentration. Ratio of Yield = Yield / addition. Sixty nano gram of 2,3,7- dioxin was examined as base concentration. We did another 20ng dioxin as low base concentration (data was not shown). These results suggested that the ELISA methods to quantify 2, 3, 7-TCDD in TSBmight show a good reproducibility without any extraction and purification processes.

Fig. 4 indicated the results of linearity of the biodegradation of 2,3,7-TCDD by an enzyme from Geobacillus midousuji. Reaction was performed at 65C, 21hours with 100ng/mL of 2, 3, 7-TCDD by enzyme solution. Enzyme solution contained midousuji enzyme and TSB/YE. The result suggested that the ELISA method might be useful for quantify the degradation rate of dioxin by midosuji.

Acknowledgements

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References

1) Yukio Sugawara et.al. Development of a Highly Sensitive Enzyme-Linked Immunosorbent Assay Based on Polyclonal Antibodies for the Detection of Polychlorinated Dibenzo-*p*-dioxins Analytical Chemistry 1092-1093 70(6) (1998)

2) M. Nakamura et.al. Study Report (K1631) on bioreactor for dioxins degradation system of contaminated in water and soil. Ministry of the Enviroment (2004).

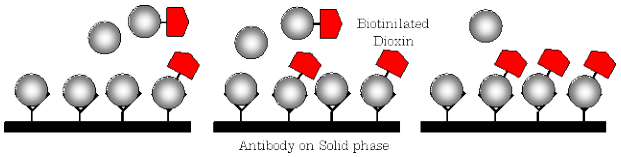
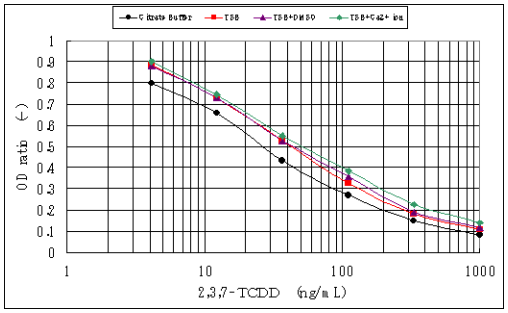


Fig.1 Competitive analysis of ELISA method with chlorinated dioxin and biotinilated dioxin.

Fig.2 Standard curve of OD Ratio and 2,3,7-TCDD by ELISA methods. Immunoassay was performed at 4C,24 hours of



1st reaction and 20C,2hours of 2nd reaction with 5vol% of DMSO and Ca^{2+} in TSB or citrate buffer respectively.

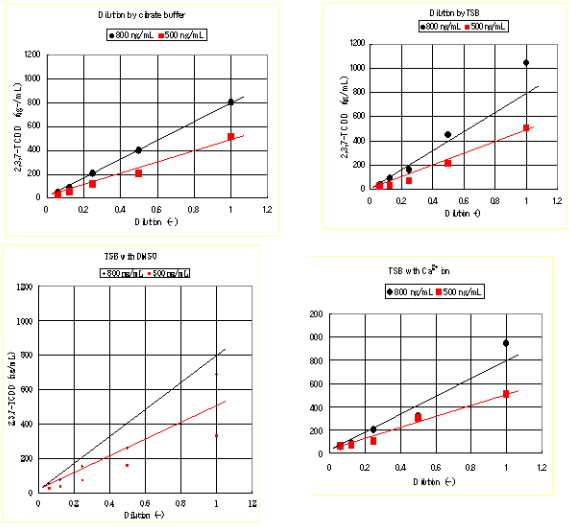


Fig.3 Linearity of measured dioxin concentration and diluted dioxin standard.

Table 1. Cross reactivity of the antibody to 2,3,7-trichloro-Dioxin.

2,3-dichlorodibenzo-*p*-dioxin	4
2,7-dichlorodibenzo-*p*-dioxin	7
2,3,7-trichlorodibenzo-*p*-dioxin	100
2,3,7,8-tetrachlorodibenzo-*p*-dioxin	240
2,3,7,8-tetrachlorodibenzofuran	15
1,2,3,7,8-pentachlorodibenzo-*p*-dioxin	25
1,2,3,4,7,8-hexachlorodibenzo-*p*-dioxin	0.3
1,2,3,4,6,7,8-heptachlorodibenzo-*p*-dioxin	<0.1
1,2,3,4,6,7,8,9-octachlorodibenzo-*p*-dioxin	0.1

Table 2. Addition/Yield of Dioxin with 60ng/mL base concentration in citarate buffer and TSB.

ng/mL	Concentration of addition	Measure 1	Measure 2	Measure 3	Average	Concentration of Yield	Yeild ratio
Citrate	0	29.0	33.9	32.2	31.7	-	-
buffer	19.6	41.0	41.4	37.5	40.0	8.3	42.3
	58.9	68.4	92.4	94.9	85.2	53.5	90.8
dilution	176.7	157.0	270.1	188.0	171.9	140.2	79.3
	530.0	505.0	511.8	515.2	536.4	504.7	95.2
	0	22.4	27.1	27.4	25.6	-	-
dilution	19.6	44.5	44.9	45.9	45.1	19.5	99.5
	58.9	83.3	80.9	79.6	81.3	55.7	94.6
	176.7	225.0	205.5	210.8	213.8	188.1	106.5
	530.0	797.7	596.9	533.8	642.8	617.2	116.5
	0	33.2	28.4	26.3	29.3	-	-
+DMSO	19.6	59.6	43.5	42.0	48.4	19.1	97.4
	58.9	61.5	85.6	86.8	78.0	48.7	82.7
dilution	176.7	216.1	171.5	224.3	204.0	174.7	98.9
	530.0	461.9	370.5	479.6	437.3	408.0	77.0
	0	24.2	31.3	16.2	23.9	-	-
+Ca^2	19.6	60.5	70.7	39.3	56.8	32.9	167.9
	58.9	95.4	89.3	92.7	92.5	68.6	116.5
dilution	176.7	269.8	277.2	238.3	261.8	237.9	134.6
	530.0	526.4	608.2	548.3	561.0	537.1	101.3

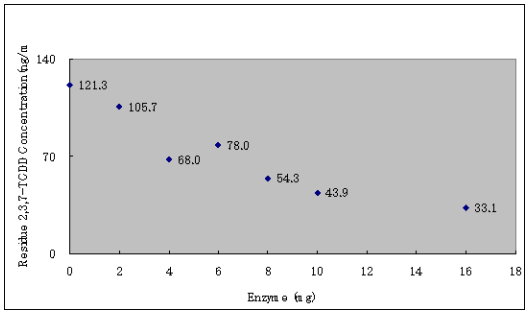


Fig.4

Dose dependent of DIOXIN degradation by bacterial enzyme. Reaction was performed at 65C,21 hours with 100 ng/mL of 2,3,7-TCDD by enzyme solution. Enzyme solution contained midosuji and enzyme TSB.