

Estrogenic Potency of a Bisphenol A Metabolite on Vitellogenin Synthesis in Medaka, *Oryzias latipes*

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(Received September 21, 2004; Accepted October 4, 2004; Published online October 7, 2004)

We investigated the estrogenic activity of a bisphenol A (BPA) metabolite (4-methyl-2,4-bis(*p*-hydroxyphenyl)-pent-1-ene; MBP) in male medaka (*Oryzias latipes*) using vitellogenin (Vg, Vg1 and Vg2) as a biomarker. Male d-rR medaka were exposed to various concentrations of estradiol-17 β (E₂), MBP and BPA for 3 days, and then the serum Vg concentration was measured using specific chemiluminescent immunoassays. The estimated relative estrogenic activities of MBP and BPA compared with E₂ (100%) were 1.3–1.4% and 0.00010–0.00023%, respectively. These findings indicated that MBP has about 10⁴-fold higher estrogenic potency than the parent BPA and about 1/50 that of E₂ for Vg synthesis in medaka. This is the first study to show that MBP can act as a highly potent estrogen agonist in living organisms.

Key words — bisphenol A metabolite, estrogenic activity, vitellogenin, 4-methyl-2,4-bis(*p*-hydroxyphenyl)-pent-1-ene, medaka

INTRODUCTION

Bisphenol A (2,2-bis(4-hydroxyphenyl)propane; BPA) is a raw material that is used in the worldwide production of polycarbonate and epoxy resins. Large production volumes and consumption of BPA have led to its being detected in human biological flu-

ids^{1,2)} and in aquatic environments.^{3–5)} The notion that BPA possesses weak estrogenic activity and disrupts the endocrine system is widely accepted. Yoshihara *et al.* recently found that 4-methyl-2,4-bis(*p*-hydroxyphenyl)-pent-1-ene (MBP), a BPA metabolite in the rat liver S9 fraction, has several hundred- to several thousand-fold higher estrogenic potency than BPA itself.^{6,7)} If BPA is converted to MBP in the environment and if it negatively affects the endocrine system of living organisms, the biological risk of BPA should be reconsidered. However, only one report has described the estrogenic activity of BPA *in vitro*, and the estrogenic impact of MBP has not been investigated *in vivo*. Vitellogenin (Vg), a precursor of yolk protein in oviparous animals, is a good biomarker of estrogenomimetic activity because it is synthesized in the liver and is powerfully regulated by estrogen. Here, we evaluated the estrogenic potency of MBP in medaka *in vivo* using Vg as a biomarker.

MATERIALS AND METHODS

Fish and MBP Exposure — Adult male medaka (d-rR strain⁸⁾) maintained in 60-l glass aquaria under a 14-hr light : 10-hr dark cycle at 25 \pm 1°C were fed daily with a commercial mix of freeze dried, day-old brine shrimp (Artemia Gold; Nagasaki Rikagaku Co., Nagasaki, Japan).

Groups of 3 male fish were transferred to 2-l glass beakers containing dechlorinated tap water. Steroid and chemicals were dissolved in ethanol and added to the water at the following nominal concen-

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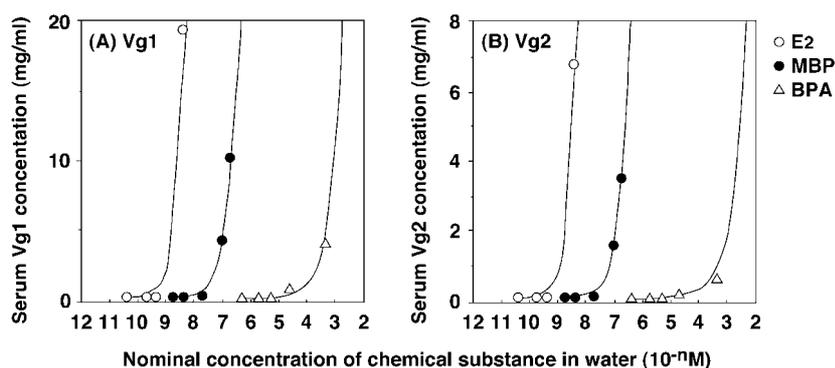


Fig. 1. Serum Vg1 (A) and Vg2 (B) Concentrations in Male Medaka Exposed to E₂ (Open Circles), MBP (Closed Circles) and BPA (Open Rectangles) for 3 days

Table 1. Estrogenic Potency of MBP on Vg Synthesis

Hormones or chemicals	Effective concentration required to induce 10 mg/ml Vg1 in serum	Effective concentration required to induce 5 mg/ml Vg2 in serum
E ₂	2.5×10^{-9} M (100)	3.3×10^{-9} M (100)
MBP	1.8×10^{-7} M (1.4)	2.6×10^{-7} M (1.3)
BPA	1.1×10^{-3} M (0.00023)	3.6×10^{-3} M (0.00010)

Regression formula of test chemical concentration-Vg response curve calculated from data described in Fig. 1. From these results, effective concentrations of test chemicals required to induce 10 mg/ml Vg1 and 5 mg/ml Vg2 in serum were calculated. Values in parenthesis indicate percentage of relative estrogenic activity compared with E₂.

trations: 17 β -estradiol (E₂; Sigma-Aldrich, St. Louis, MO, U.S.A.), 0.01, 0.05, 0.1, 0.5 and 1 ppb; MBP (synthesized and purified MBP as described by Takao *et al.*⁹⁾), 1, 5, 25 and 50 ppb; BPA (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 100, 500, 1000, 5000 and 10000 ppb. Controls contained appropriate volumes of vehicle solvent (0.1 ml/l). The solutions were replaced every day for 3 days and the fish were not fed during exposure to chemicals. The caudal peduncle was severed and blood from the groups was collected into micro-capillary tubes (Microcaps; Drummond Scientific Co., PA, U.S.A.). Serum was separated by centrifugation at $10000 \times g$ for 5 min and stored at -30°C .

Measurement of Serum Vg Concentration

Serum concentrations of Vg1 and Vg2, were measured using specific chemiluminescent immunoassays¹⁰⁾ as follows. Ninety-six well polystyrene luminescence immunoassay plates (LIA plates; Greiner, Frickenhausen, Germany) were coated with either anti-Vg1 or anti Vg2 IgG, and blocked with 1% BSA and 0.1% bovine γ -globulin to prevent non-specific binding. Serum samples or Vg standard were placed into the wells and incubated for 3 hr at 15°C (1st reaction). The plates were washed and acridinium-labeled F(ab')₂ of anti-Vg IgG was

added (2nd reaction) for 1 hr at 15°C . The chemiluminescent reaction (Reagents 1 and 2; CHIRON, Emeryville, CA, U.S.A.) and luminescence detection were controlled using a Luminescencer-JNR (ATTO, Tokyo, Japan). All assays were performed in duplicate.

RESULTS AND DISCUSSION

Figure 1 describes the serum Vg1 and Vg2 concentrations of male medaka exposed to various doses of E₂, MBP and BPA. All chemicals at the tested concentrations dose-dependently increased the serum Vg concentration and regression curves of the 3 exposure groups were almost parallel. The LOEC (Lowest Observed Effect Concentration) values for E₂, MBP and BPA on the synthesis of both Vgs were 3.7×10^{-9} , 9.2×10^{-8} and 2.2×10^{-5} M, respectively. The estimated relative estrogenic activities of MBP and BPA (percentage compared with E₂) calculated from the regression curves were 1.3–1.4 and 0.00010–0.00023%, respectively (Table 1). These findings showed that MBP has extremely high estrogenic activity (about 10^4 -fold that of parent BPA, and about 1/50 of that of E₂) on Vg synthesis in male

medaka. The present findings closely correlated with *in vitro* evaluations of estrogenic MBP activity.⁷⁾

A recent report has indicated that not only sunlight but also fluorescent light can photolyze MBP.⁸⁾ Here, we exposed male medaka to chemicals under white fluorescent light (14 hr/day). Therefore, the estrogenic potency of MBP might be increased by repeating the present study under darkness. Yoshihara *et al.* considered that MBP might become metabolically activated under conditions of poor glucuronidation capacity as in rat and human fetuses, but not under normal circumstances.⁷⁾ A similar situation might be extant during the early development of lower vertebrates. Aquatic animals, fish fry or pre-metamorphous amphibians, can easily absorb BPA from water. The present study revealed an extremely potent estrogenic effect of MBP in teleosts *in vivo*. Therefore, MBP concentrations in these animals should be investigated during early development to enhance understanding of the estrogenic effect of BPA.

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