SYNTHESIS AND Ah RECEPTOR BINDING PROPERTIES OF [^{125}I]-6-METHYL-8-IODO-TRICHLORODIBENZOFURAN: A POTENTIAL TCDD ANTAGONIST

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ABSTRACT

6-Methyl-8-iodo-1,3-dichlorodibenzofuran (I-MCDF) and its radiolabeled analog ^{125}I-MCDF have been synthesized. Incubation of rat liver cytosol with ^{125}I-MCDF followed by velocity sedimentation analysis on sucrose gradients gave a specifically bound peak which sedimented at 9.6 S. This radioactive peak was displaced by coincubation with a 200-fold excess of unlabeled I-MCDF, 6-methyl-1,3,8-trichlorodibenzofuran (MCDF), 2,3,7,8-tetrachlorodibenzofuran (TCDF) and benzo[a]pyrene. Velocity sediment analysis of the nuclear ^{125}I-MCDF-Ah receptor complex which was isolated from rat hepatoma H-4-II E cells gave a specifically-bound peak at 5.6 ±0.8 S. Preliminary studies show that incubation of nuclear extracts of rat hepatoma H-4-II E cells treated with 10^{-6} M I-MCDF and a synthetic oligonucleotide DNA sequence which resembles a consensus dioxin regulatory element (DRE) resulted in the formation of a protein-DNA band which can be detected in a gel retardation assay system.

INTRODUCTION

6-Methyl-1,3,8-trichlorodibenzofuran (MCDF) inhibits the TCDD-mediated induction of aryl hydrocarbon hydroxylase (AHH) activity, cytochrome P-4501A1 and P-4501A2 proteins in immature male rats and rat hepatoma H-4-II E cells in culture (1,2). I-MCDF, an analog of MCDF, exhibits comparable interactive effects with TCDD and inhibits TCDD-induced AHH activity both *in vivo* and *in vitro* (unpublished results). This study reports the synthesis of ^{125}I-MCDF and some of the properties of the ^{125}I-MCDF-Ah receptor complexes.

MATERIALS AND METHODS

Synthesis

I-MCDF was synthesized from 3-methyl-2',4',6'-trichloro-2-biphenylol (M^+286) which was purified by repeated thin-layer chromatography of the products from the diazo coupling of o-cresol and 2,4,6-trichloroaniline as described (1). The substituted biphenyl-2-ol (100 mg) was dissolved in an 85% acetic acid/potassium acetate buffer (pH 3.85) and 0.5 g of potassium iodide and 2.5 ml of 30% hydrogen peroxide was added to the solution with stirring at 20° C. The resulting mixture was stirred for an additional 2 h at 20° C, diluted with 10 ml of water and excess sodium metabisulfite; the 3-methyl-5-iodo-2',4',6'-trichloro-2-biphenylol was purified, cyclized and isolated by thin-layer chromatography and crystallized from methanol/anisole as described for MCDF (1). ^{125}I-MCDF was prepared by radioiodination of 3-methyl-2',4',6'-trichloro-2-biphenylol essentially as described (3) and purified by HPLC. The approximate yield of ^{125}I-MCDF after HPLC purification was 0.5 nmol (ca 16-20% yield) with the specific activity of 2570 Ci/mmol.

Isolation of Rat Hepatic Cytosol, Saturation Binding Studies and Velocity Sedimentation Analysis of Cytosolic Complexes. Rat liver cytosol was prepared as described (1). Cytosolic samples were incubated with 3 nM ^{125}I-MCDF in presence or absence of a 200-fold excess TCDF, MCDF or benzo[a]pyrene at 0° C for 2 h and the uncompeted and competed samples were analyzed by velocity sedimentation analysis on sucrose gradients. The saturation binding studies of ^{125}I-MCDF were determined with freshly prepared rat hepatic cytosol (2 mg protein/ml cytosol) using the hydroxylapatite binding procedure. The cytosols were incubated for 16 h at 0° C in the presence of different concentrations of ^{125}I-MCDF and a 200-fold excess of TCDF.

Nuclear Receptor Complexes

Nuclear ^{125}I-MCDF-Ah receptor complexes were obtained by incubation rat hepatoma H-4-II E cells with ^{125}I-MCDF (0.087 nM) for 1 h at 37° C followed by isolation of nuclear extracts as described (2). For the gel retardation studies, the cells were treated with 10^{-6} M unlabeled I-MCDF and the nuclear receptor complexes were isolated as described (5). The gel retardation analyses were carried out as outlined by Denison and coworkers (5) using a ^{32}P-labeled synthetic consensus DRE sequence of 26 bases (derived from data supplied by Dr. M. Denison, Michigan State University) and nuclear extracts of cells treated with 10^{-6} M I-MCDF.

RESULTS AND DISCUSSION

^{125}I-MCDF was utilized as a radioligand to measure the direct interaction of this putative partial antagonist with the Ah receptor. ^{125}I-MCDF exhibits saturation binding with rat hepatic cytosol; the Bmax and K_D values derived from this data were 2.05 fmol/mg protein and 0.13 nM respectively. In contrast, the values obtained using [^{3}H]-TCDD as a radioligand and the same cytosol were 137 fmol/mg protein and 1.07 nM respectively. It

was apparent from the results that the number of binding sites which were detected using ^{125}I-MCDF were significantly reduced and the explanation for these observations are unknown. Velocity sedimentation analysis of the rat cytosolic Ah receptor ^{125}I-MCDF complex gave a specifically-bound peak at 9.6 S. Competitive binding studies showed that a 200-fold excess of unlabeled I-MCDF, MCDF, benzo[a]pyrene and TCDF all displaced radiolabeled I-MCDF from the 9.6 S complex. The results were similar to those obtained using [^{3}H]-TCDD as a radioligand and confirms the direct interaction of ^{125}I-MCDF with the Ah receptor. The sedimentation properties of the nuclear ^{125}I-MCDF-Ah receptor complex were determined from high salt extracts of nuclei obtained from rat hepatoma H-4-II E cells which were incubated with 0.087 nM ^{125}I-MCDF in the presence or absence of 17.4 nM TCDF. Velocity sedimentation analysis gave a specifically-bound peak which sedimented at 5.6 $\pm$ 0.8 S and this was similar to results obtained using [^{3}H]-TCDD (4). Moreover, the nuclear extracts of cells treated with 10^{-9} M TCDD or 10^{-6} M I-MCDF all retarded the mobility of a ^{32}P-labeled DRE probe using a gel retardation assay system (5). These results suggest that the I-MCDF may be acting as a TCDD antagonist by competing for specific nuclear binding sites.

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