Functional and Physical Interactions of Steroid Receptor Coactivator 1 (SRC-1) and the Aryl Hydrocarbon Receptor Complex

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Introduction

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) binds with high affinity to an intracellular cytosolic protein, namely the aryl hydrocarbon receptor (AhR), which has been identified in numerous mammalian species and cells. The AhR-ligand forms a heterodimer with the AhR nuclear translocator (Arnt) protein and this complex is required for initiating AhR-mediated transactivation. Interaction of the nuclear AhR complex with dioxin responsive elements (DREs) (5'-CACGC-A-3') located in 5'-promoter regions of the CYP1A1 and other Ah-responsive genes is also required for induction of gene expression (1).

Research in this laboratory has focused on delineating the interrelationship between the estrogen receptor (ER) and AhR signal transduction pathways; initial studies using transient transfection assays showed that ER expression plasmids restored Ah-responsiveness in ER$^-$ MDA-MB-231 cells (2). This suggested that there was crosstalk between the AhR and ER signaling pathways (3). Recent studies have identified several "coactivator" proteins which modulate the transactivation of the steroid hormone nuclear receptors (4). Results of preliminary studies show that the estrogen receptor associated protein (ERAP) 140 binds to the AhR-Arnt complex. ERAP 140 also upregulates reporter gene activity in MCF-7 cells treated with TCDD and transiently transfected with pRNH11c which contains an Ah-responsive promoter (-1142 to +2434) insert from the human CYP1A1 gene. Since transactivation of protein encoding genes involve numerous transcriptional factors, the effects of steroid receptor coactivator-1 (SRC-1) on AhR-mediated transcriptional activation were investigated in MCF-7 breast cancer cells.

Materials and Methods

Chemicals and Biochemicals: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) was prepared in this laboratory and was determined to be >99% pure by gas chromatography and mass spectroscopy. All other chemicals and biochemicals were of the highest quality available from commercial suppliers.

Cell Maintenance: The human adenocarcinoma MCF-7 cell line was obtained from the American Type Culture Collection (Rockville, MD). MCF-7 cells were grown in MEM (Gibco) medium supplemented with 10% fetal calf serum (FCS, Intergen, Newark, NJ), 10 Fg/L insulin, 2.38 g/L HEPES, 1.0 g/L glucose, 2.2 g/L Na_2HCO_3, 0.11 g/L sodium pyruvate and 2 ml/L antibiotic/antimycotic (Sigma) solution. Cells were grown in a 37°C incubator with a humidified mixture of 5% CO_2 and 95% air.

Expression Vectors: pRNH11c contains the 5'-regulatory region (-1140 to +2434) from the human CYP1A1 gene fused to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene and was kindly provided by Dr. Ronald Hines (Wayne State University). An expression vector encoding for the SRC-1 protein was provided by Dr. Bert O'Malley (Baylor College of Medicine). The plasmid containing the human estrogen receptor (ER) cDNA was provided by Dr. Ming Jer Tsai (Baylor College of Medicine).

Determination of CAT activity: For transient transfection assays, 3μg of chloramphenicol acetyltransferase (CAT)-reporter plasmid or vector only were transfected by calcium phosphate coprecipitation. Where indicated, cells were also transfected with selected concentrations of expression vector for SRC-1 or the ER. Six hr later, cells were shocked with 25% DMSO for 4 min. Cells were treated with 10 nM of TCDD or DMSO vehicle (final concentration 0.2%) eighteen hr after the DMSO shock. Forty-eight hr after treatment the cells were harvested for analysis of CAT activity.

Electrophoretic Mobility Shift Assays Using In Vitro-Translated Proteins: Plasmids containing the AhR, Arnt and SRC-1 cDNAs were used to *in vitro* transcribe and translate the corresponding protein in a rabbit reticulocyte lysate system (Promega). Equal volumes (3μl) of lysate containing the AhR and Arnt complex were transformed with 1 nM TCDD for 2 hours at 25°C. Selected volumes of lysate (1.5 and 3 μl) containing SRC-1 were subsequently added, and incubated for 15 min at 25°C. The labeled DRE probe (60,000 cpm) was then added to the reaction mixtures in the presence of 1 μg of poly(dI-dC) , and the mixtures were incubated for 15 min at 25°C. The reaction was carried out in 20 mM HEPES-5% glycerol, 100 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1mM EDTA in a final volume of 25 μl. Reaction mixtures were loaded onto 5% polyacrylamide gel and run at 110 V in 0.09 M Tris, 0.09 M borate, 2 mM EDTA (pH 8.3). Gels were dried and protein-DNA binding was visualized by autoradiography.

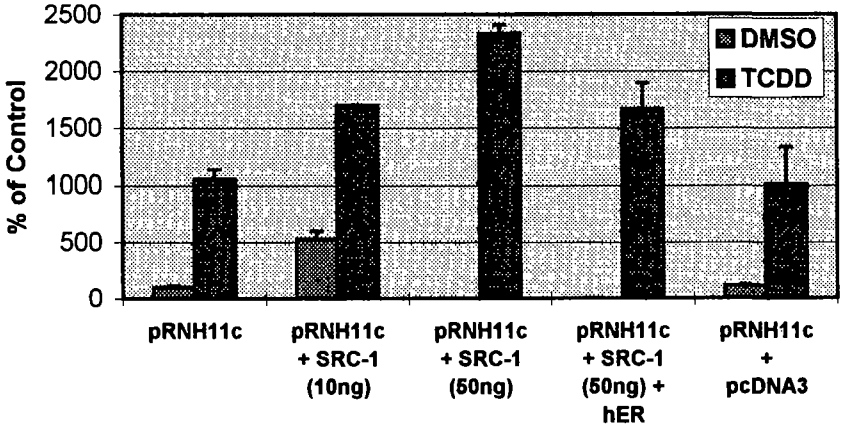
Immunoprecipitation of ERAP 140-AhR/Arnt complex: For immunoprecipitation of SRC-1 monoclonal antibodies against the AhR and Arnt were used. Briefly, the AhR, Arnt, and SRC-1 were *in vitro* transcribed/translated using the rabbit reticulocyte lysate system (Promega). Selected proteins were labeled using [^{35}S]methionine. Equal volumes (10 μl) of lysate containing AhR and Arnt were transformed with 1 nM TCDD for 1 hr at room temperature. Lysate (10 μl) containing SRC-1 was added and the protein complex was incubated for an additional 1 hr. To this mixture, anti-AhR or anti-Arnt antibodies were added and the samples were subsequently incubated for 2 hr on ice. Protein G-agarose beads (Santa Cruz) were added and incubated at 4°C for 5 hr. Immunoprecipitation of protein G-bound proteins was conducted according to the manufacturers recommendations. Proteins were subsequently separated by SDS-PAGE electrophoresis (5% gel) and visualized by autoradiography.

Results and Discussion

MCF-7 cells were transiently transfected with Ah-responsive and a SRC-1 protein expression plasmid. In cells transfected with pRNH11c alone 10 nM TCDD induced CAT activity 10-fold compared to DMSO treated cells. In cells cotransfected with pRNH11c plus SRC-1 there was 2.3-fold increase in the induction of CAT activity by TCDD compared to activity observed in the absence of SRC-1. Since SRC-1 was initially identified as an ER coactivator, the squelching effect of ER on SRC-1-mediated enhancement of AhR transactivaton was tested. Cotransfected of hER with both pRNH11c and SRC-1 resulted in decreased CAT activity 1.5-fold compared to cells transfected with pRNH11c and SRC-1 (Graph 1).

The role of SRC-1 in enhancing Ah-responsiveness was also investigating in gel mobility assays using in vitro transcribed/translated proteins (Figure 2). In the presence of TCDD, there was an increased intensity of a [^{32}P]DRE-AhR retarded band compare the band in samples treated with DMSO. Addition of *in vitro* transcribed/translated SRC-1 significantly increased binding 2-fold of the AhR/Arnt complex to [^{32}P]DRE. This increase binding was ligand and concentration dependent. Although SRC-1 enhanced formation of the AhR/Arnt-DRE retarded band, a super-shifted SRC-1/AhR/Arnt-DRE complex was not observed. Similar results were observed in studies which showed that ER enhanced binding of Sp1 protein to GC-rich elements (ref. Porter et al 1997).

Since ERAP 140 physically interacted with the AhR complex, the enhanced binding and AhR-mediated transactivation by SRC-1 could also be due to protein-protein interactions. Using monoclonal antibodies against the AhR and Arnt proteins, the interaction of [^{35}S]SRC-1 with the AhR/Arnt complex was investigated in coimmunoprecipitation studies (Figure 2). The AhR, Arnt, and SRC-1 proteins were [^{35}S]-labeled and detected at 110 kDA, 97 kDa, and 160 kDa, respectively. A 160 kDa protein was detected after immunoprecipitation of the protein complex using both the anti-AhR and anti-Arnt antibodies.



Graph 1: Transient transfection of pRNH11c was measured.

These studies were initiated to determine if SRC-1 modulated AhR-mediated transactivation in MCF-7 breast cancer cells. SRC-1 enhanced reporter gene activity of an Ah-responsive construct and also increased AhR/Arnt binding to DRE; in addition, SRC-1 physically interacted with AhR and Arnt proteins by coimmunoprecipitation using antibodies directed against AhR and Arnt. Thus, these data show that SRC-1 may also be a coactivator for the AhR complex in MCF-7 human breast cancer cells.

Acknowledgment

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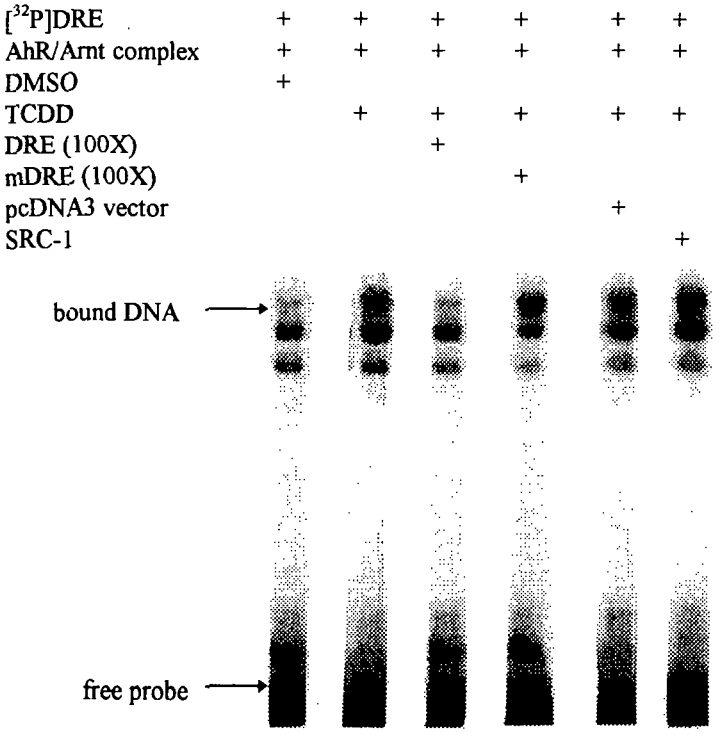

Figure 1: Electrophoretic mobility shift analysis of *in vitro* transcribed/translated protein. SRC-1, AhR, and Arnt were expressed using the rabbit reticulocyte system. Binding of AhR/Arnt to [^{32}P]DRE was determined in the presence of increased concentration of SRC-1.

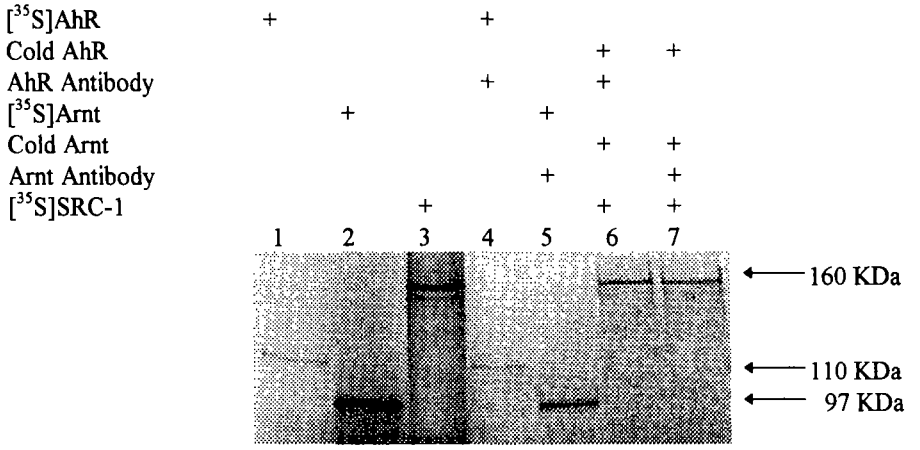

Figure 2: Immunoprecipitation of SRC-1. AhR, Arnt, and SRC-1 were [^{35}S]-labeled using the rabbit reticulocyte lysate system.

References

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