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Stabilization *In Vitro* of the Untransformed Glucocorticoid Receptor Complex of S49 Lymphocytes by the Immunophilin Ligand FK506

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Untransformed steroid receptors are large heteromeric complexes which have been shown to contain the mammalian heat shock proteins hsp56, hsp70 and hsp90. Based on functional and sequence homology studies, it was recently discovered that hsp56 also belongs to the FKBP class of immunophilin proteins, which are thought to mediate the actions of the immunosuppressive drugs FK506 and rapamycin. This discovery has led to the speculation that FK506 and related drugs could influence the actions of steroid receptors. In this work, we have examined the effects of FK506 on the transformation and hormone-binding properties of glucocorticoid receptors (GR) present in the cytosolic fraction of mouse S49 lymphocyte cells. Based on immunoprecipitation studies, it was found that hsp56 was indeed a component of untransformed GR complexes in S49 cytosols. It was also found that the untransformed but not the transformed GR was retained following affinity chromatography with FK506-affigel resin, reinforcing the possibility that hsp56 within the untransformed GR complex could be a target for the actions of FK506. Using a DNA–cellulose-binding assay, FK506 exhibited a 60% inhibition of dexamethasone (Dex)-induced transformation of the GR to the DNA-binding state, while sodium molybdate, a transition metal oxyanion known to stabilize GR complexes, was 100% effective. This inhibition of GR transformation by FK506 was shown to correlate with an inhibition of Dex-induced GR/hsp90 dissociation, with 10 μM FK506 preventing 48% of the GR/hsp90 complexes from dissociating. Scatchard analysis of GR hormone-binding function was performed, with FK506 treatment of cytosols causing K_d values to decrease (3.36 nM) as compared to vehicle (8.42 nM) and no-addition (9.82 nM) controls. Taken together, our results suggest that FK506 can stabilize the untransformed GR complex of S49 cells and that this stabilization in turn results in an increase in GR ligand-binding affinity. Although we speculate that these actions of FK506 on the GR complex are mediated by the associated hsp56 component, other possible mechanisms are also discussed.

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INTRODUCTION

The glucocorticoid receptor (GR) is a member of the steroid receptor family of proteins which act as ligand-regulated enhancers of specific gene transcription [1–3]. In cells grown in the absence of hormone, the "untransformed" GR is recovered in the cytosolic fraction as a large heteromeric complex containing several members of the mammalian heat shock protein family [see ref. 4 for review]. These non-steroid-binding components of the untransformed GR include hsp90 [5–8], hsp56 [9, 10] and, under certain circumstances, hsp70 [11]. A series of recent reports have shown that the GR and other untransformed steroid receptor complexes also contain members of the immunophilin class of proteins. The initial observation of this kind was made by Yem *et al.* [12] when it was shown that the NH_2-terminus of a $\approx$60 kDa protein capable of binding the immunosuppressive drug FK506 was nearly identical in amino acid sequence to hsp56 (which has also been referred to as p59 [10], FKBP59 [13], FKBP52 [14] and HBI [15]). Since then, the cDNAs of human [14] and rabbit [15] hsp56 have been cloned with each exhibiting sequence homology

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