FK506 Binding to the 56-Kilodalton Immunophilin (Hsp56) in the Glucocorticoid Receptor Heterocomplex Has No Effect on Receptor Folding or Function†

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ABSTRACT: It has recently been reported that the hsp56 component of glucocorticoid receptor heterocomplexes is an immunophilin of the FK506 binding class [Yem, A. W., Tomasselli, A. G., Heinrikson, R. L., Zurcher-Neely, H., Ruff, V. A., Johnson, R. A., & Deibel, M. R. (1992) *J. Biol. Chem. 267*, 2868–2871; Tai, P. K., Albers, M. W., Chang, H., Faber, L. E., & Schreiber, S. L. (1992) *Science 256*, 1315–1318]. The existence of binding proteins for these two potent groups of immunosuppressants in the same molecular complex compels us to ask whether FK506 affects glucocorticoid receptor function. We show here that hsp56 is a component of the native L-cell glucocorticoid receptor heterocomplex and that [^{3}H]FK506 binds to the immunopurified, untransformed receptor complex. However, at concentrations in excess of those required to occupy all of its binding sites on hsp56, FK506 does not affect the steroid binding activity of the receptor nor does it stabilize or dissociate the receptor–hsp90 complex. FK506 does not affect steroid-mediated hsp90 dissociation from the receptor in vitro, and it does not affect steroid-mediated nuclear transfer of the receptor or steroid-mediated transcriptional enhancement from a reporter in intact cells. When immunopurified mouse glucocorticoid receptor is reconstituted into a heat shock protein complex by rabbit reticulocyte lysate, hsp56 is present in the reconstituted complex in addition to hsp90 and hsp70. FK506, however, does not affect reconstitution of the complex or return of the receptor to the steroid binding state, a change of conformation that occurs upon receptor association with hsp90. Although the existence of the glucocorticoid receptor and the FK506 immunophilin in the same heteromolecular complex is indeed provocative, neither enhancement nor inhibition of glucocorticoid receptor function by FK506 is observed at the molecular level.

Steroid receptors are recovered from hormone-free cells in association with hsp90,[1] hsp56, and in some cases hsp70 [see Pratt (1990) for a review]. In intact cells, it is thought that the receptors remain docked to this heat shock protein complex until binding of steroid triggers their dissociation from hsp90 and their progression to high-affinity nuclear binding sites where the primary events in transcriptional activation occur.

The hsp56 component of the heterocomplex was discovered when a monoclonal antibody (KN 382/EC1) prepared against the partially-purified, molybdate-stabilized, rabbit progesterone receptor was found to react with a non-steroid binding ~59-kDa protein in uterine cytosol, but also to cause coimmunoadsorption of a 90-kDa protein and untransformed receptors for progestins, estrogens, androgens, and glucocorticoids (Tai et al., 1986). The EC1 antibody was found to react with a moderately abundant 56-kDa protein with several isomorphs in human IM-9 lymphocyte cytosol (Sanchez et al., 1990). This 56-kDa protein was unique by N-terminal sequencing, and it was immunoadsorbed in association with both the 90-kDa and 70-kDa human heat shock proteins (Sanchez et al., 1990). Because all three proteins were coimmunoadsorbed by the EC1 antibody in great stoichiometric excess of the IM-9 cell glucocorticoid receptor (GR), it was proposed that the proteins existed in a cytosolic complex independent of the presence of receptor (Sanchez et al., 1990). Because two of the proteins in the complex were known heat shock proteins, Sanchez (1990) submitted IM-9 cells to heat shock and demonstrated that the rate of synthesis of the 56-kDa protein was increased. Thus, the EC1 antigen is a novel heat shock protein, and it is now called hsp56. The notion that a cytosolic complex of heat shock proteins exists was confirmed by Perdew and Whitelaw (1991), who immunoadsorbed rat hepatocyte cytosol with a monoclonal antibody against hsp90 and demonstrated coimmunoadsorption of both hsp70 and hsp56. Rexin et al. (1991, 1992) have cross-linked hsp56 to the GR both in cytosol and in intact WEHI cells, suggesting that the two proteins may contact each other, as well as hsp90, in the untransformed receptor heterocomplex.

Recently, hsp56 was shown to be a member of the immunophilin class of proteins (Yem et al., 1992; Tai et al., 1992). The immunophilins are proteins that bind immunosuppressive agents like cyclosporin A, FK506, and rapamycin in a high-affinity and specific manner. All of the members of this family have rotamase (peptidylprolyl *cis–trans*-isomerase) activity in vitro, and it is thought that the immunophilins, in a manner similar to hsp70 and hsp90, play major roles in protein folding and protein trafficking in the cell [see Schreiber (1991) and Walsh et al. (1992)].

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[1] Abbreviations: hsp90, hsp70, and hsp56, 90-, 70-, and 56-kDa heat shock proteins, respectively; GR, glucocorticoid receptor; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TES, 2-[[tris(hydroxymethyl)methyl]amino]ethanesulfonic acid; CAT, chloramphenicol acetyltransferase; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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The predominant cellular immunophilins in the FKBP family were initially identified by SDS–polyacrylamide gel electrophoresis following affinity chromatography of mammalian cell extracts over FK506- and rapamycin–Affigel-10 matrices. Initially, Fretz et al. (1991) passed Jurkat cell extracts over matrices of immobilized FK506 and rapamycin and demonstrated that several proteins could be specifically eluted with competing soluble drug. One of these, a 60-kDa protein, was subsequently shown by Yem et al. (1992) to possess the NH_2-terminal sequence published by Sanchez et al. (1990) for hsp56, as well as sequence homologies to a region near the COOH-terminus of the FK506 binding proteins FKBP-12 and FKBP-13. Importantly, Tai et al. (1992) showed that FK506 binds directly to hsp56 but that the FK506 affinity matrix also selectively retains hsp90, hsp70, and the GR as coadsorbed proteins. Simultaneously, Lebeau et al. (1992) used the EC1 antibody to screen a rabbit liver cDNA library and clone the cDNA for hsp56. They found a segment between amino acids 41 and 134 of the protein that had 55% amino acid homology to FKBP12, with all 10 amino acids in the drug binding site being conserved (Tai et al., 1992).

The existence of the three heat shock proteins, hsp56, hsp70, and hsp90, in a complex implies that they may act together in a spatially organized and temporally coordinated manner. The association of the untransformed GR with a binding protein (hsp56) for another potent immunosuppressant agent (FK506) compels one to ask whether FK506 can affect GR function. In this work, we show that hsp56 is part of the native untransformed GR heterocomplex from L cells and is part of the GR heterocomplex reconstituted by rabbit reticulocyte lysate. FK506 does not, however, affect GR function, steroid-mediated transcriptional activating activity, or reconstitution of GR into the heat shock protein heterocomplex.

EXPERIMENTAL PROCEDURES

Materials. [6,7-^{3}H]Triamcinolone acetonide (42.8 Ci/mmol) and ^{125}I-conjugated goat antimouse and anti-rabbit IgGs were obtained from Du Pont—New England Nuclear. FK506 was purified from fermentation broths of *Streptomyces tsukubaenis* as previously described (Yem et al., 1992) and custom-labeled by tritium exchange (49.1 Ci/mmol) by Amersham (Arlington Heights, IL). Untreated rabbit reticulocyte lysate was from Green-Hectares (Washington, OR). Nonimmune mouse IgG, protein A–Sepharose, and goat antimouse and anti-rabbit IgG–horseradish peroxidase conjugates were from Sigma. Immobilon-P was from Millipore (Bedford, MA). Purified BuGR2 monoclonal antibody against the rat glucocorticoid receptor was from Affinity Bioreagents (Neshanic Station, NJ). The rabbit serum against hsp70 and hsp90 (Erhart et al., 1988) was a generous gift from Dr. Ettore Appella (National Cancer Institute), and the EC1 monoclonal antibody against hsp56 (Nakao et al., 1985) was a gift from Dr. Lee Faber (Medical College of Ohio). The UP30 rabbit antiserum against FK506–Affi-Gel chromatography is described elsewhere (Ruff et al., 1992). Rat 54-5A4 kidney epithelial cells (Cizdzeil et al., 1986) were kindly provided by Dr. Donald DeFranco (University of Pittsburgh).

Cell Fractionation and GR Immunoadsorption. L929 mouse fibroblasts were grown in monolayer culture, harvested, and ruptured in 1.5 volumes of HE (10 mM HEPES/1 mM EDTA, pH 7.4 at 4 °C) as previously described (Hutchison et al., 1992). Cell homogenates were centrifuged for 1 h at 100000*g*. The supernatant from this centrifugation was removed and is referred to as "cytosol". GR was immunoadsorbed by incubating cytosol (0.3 mL) with 3 μg of BuGR2 antibody or nonimmune IgG overnight, followed by the addition of 0.5 mL of TEG buffer (10 mM TES, 50 mM NaCl, 4 mM EDTA, and 10% glycerol, pH 7.6) and 30 μL of a 20% (v/v) slurry of protein A–Sepharose and rotation for 1 h at 4 °C. In some experiments, receptor or hsp56 was rapidly immunoadsorbed to protein A–Sepharose pellets that had been preincubated with antibody or antiserum. In these cases, cytosol was rotated with the antibody-prebound protein A–Sepharose pellets for 1 h at 4 °C, followed by washing in TEGM buffer (TEG buffer plus 20 mM sodium molybdate).

Reconstitution of the GR–Heat Shock Protein Complex. Associated proteins were stripped from immunoadsorbed GR by rotation in 0.5 M KCl for 2 h at 4 °C. The immune pellets were then washed (3 × 1 mL) with TEG (2 × 1 mL) and with 10 mM HEPES, pH 7.4. The immunoadsorbed, stripped GR was incubated with reticulocyte lysate, and assayed for steroid binding and receptor-associated hsp90 and hsp70 exactly as described by Hutchison et al. (1992).

Glucocorticoid Receptor Nuclear Transfer. Transfer of glucocorticoid receptors to the nuclear fraction of rat kidney epithelial (54-5A4) cells was carried out as described by Qi et al. (1989). Briefly, cells were cultured in McCoy's 5A medium supplemented with 10% fetal calf serum and 5% calf serum at 30 °C under 5% CO_2. When cells reached 70–80% confluence, fresh medium containing charcoal-stripped serum was added, and the cells were incubated an additional 12–18 h. Cells were then treated (one flask per condition) with 1 μM corticosterone, with 100 nM FK506, or with the two together for 2 h at 30 °C. Cells were then harvested by scraping and washed in Earle's balanced salt solution, suspended in 1 mL of HE, and ruptured by Dounce homogenization. The homogenate was centrifuged at 1500*g* for 15 min, and the supernatant (cytoplasmic fraction) was removed. The crude nuclear pellet was washed with 1 mL of buffer (10 mM HEPES, 1 mM EGTA, 3 mM magnesium acetate, and 300 mM sucrose, pH 7.4) and then dissolved in 1 mL of HE containing 2% SDS by heating in boiling water for 10–20 min. The SDS was adsorbed onto Amberlite XAD-16 beads (200-μL dry volume) by rotation of the samples at room temperature for 45 min. Receptors in the cytoplasmic and nuclear fractions were immunoadsorbed to protein A–Sepharose with BuGR2, and the pellets were analyzed by SDS–PAGE followed by Western blotting for receptor.

Immunoblotting. Immunopellets were boiled in SDS sample buffer with 10% β-mercaptoethanol, and proteins were resolved on 7% SDS–PAGE. For samples assayed by two-dimensional gel electrophoresis, protein complexes immunoadsorbed to protein A–Sepharose were prepared for isoelectric focusing by eluting the complexes with lysis buffer (9.5 M urea, 2% NP-40, 5% ampholines, and 5% β-mercaptoethanol). Isoelectric focusing was then performed as previously described (Sanchez et al., 1990), utilizing a mixture of 4% pH 4–8 and 1% pH 3–10 ampholines, and this was followed by SDS–polyacrylamide gel electrophoresis employing 7% acrylamide. Immunoblotting was carried out by transferring proteins from acrylamide slab gels to Immobilon-P membranes. Membranes were then probed with 1.0 μg/mL BuGR2, 1% UP30 (to visualize mouse hsp56), 0.1% EC1 (to visualize rabbit hsp56), or 0.05% anti-hsp70/hsp90 antiserum, followed by ^{125}I-labeled counterantibody to visualize protein bands by autoradiography as described previously (Hutchison et al., 1992).

Assay for Chloramphenicol Acetyltransferase. The LM-CAT cell line is an L929 derivative which exhibits gluco-

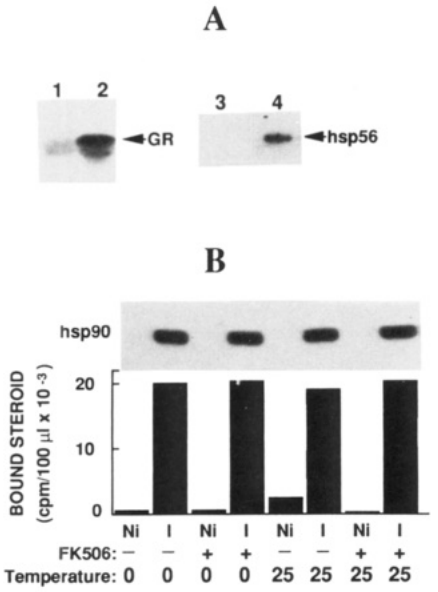

FIGURE 1: Hsp56 is a component of the L-cell GR heterocomplex, but FK506 does not affect steroid binding. (A) Hsp56 is a component of the native, untransformed GR heterocomplex in L-cell cytosol. Aliquots (300 μL) of L-cell cytosol containing 20 mM molybdate were incubated with nonimmune rabbit serum (lane 1), UP30 antiserum against hsp56 (lane 2), nonimmune IgG (lane 3), or BuGR antibody against the GR (lane 4). Samples in lanes 1 and 2 were resolved by SDS–PAGE and Western-blotted for GR. Samples in lanes 3 and 4 were resolved by two-dimensional gel electrophoresis and Western-blotted with UP30 for hsp56. (B) FK506 does not affect steroid binding or hsp90 binding by the GR. Receptors in four aliquots (300 μL) of L-cell cytosol were prebound overnight at 0 °C with 1 μM FK506 where indicated (+), and all samples were then incubated for 1 h at 0 or 25 °C. GR was adsorbed to protein A–Sepharose with BuGR2 antibody (I) or nonimmune IgG (Ni). One-sixth of each washed immunopellet was incubated 2 h at 0 °C with 50 nM [^{3}H]triamicinolone acetonide to assay steroid binding (solid bars) as previously described (Hutchison et al., 1992), and five-sixths of each immunopellet was resolved by SDS–PAGE and Western blotted for hsp90 (autoradiogram at top).

corticoid agonist-mediated expression of a chloramphenicol acetyltransferase (CAT) reporter gene under the control of the mouse mammary tumor virus long terminal repeat (MMTV-LTR) promoter (E. R. Sanchez, S. Zhong, P. Shen, Z. J. Xie, M. Greene, and P. R. Housley, submitted for publication). Measurement of induced CAT enzyme activity in LMCAT cells in response to dexamethasone or FK506 was performed according to the method of Gorman et al. (1982) with minor modifications. Briefly, cell lysates were prepared by sequential freezing and thawing in 0.25 M Tris/5 mM EDTA (pH 7.5) and centrifugation at 8000g. Equal amounts of lysate protein (500 μg) were added to the enzymatic reaction mixture, and the reaction was stopped by extraction with ethyl acetate. The acetylated forms of [^{14}C]chloramphenicol were then separated by thin-layer chromatography and visualized by autoradiography.

RESULTS AND DISCUSSION

Because the EC1 antibody does not react with mouse proteins, we have never been able to determine whether or not the well-studied untransformed GR heterocomplex from L cells contains hsp56. In Figure1A, we use the UP30 rabbit antiserum raised against FK506–Affi-Gel affinity-purified Jurkat cell hsp56 to detect the mouse protein. UP30 immunoadsorption of L-cell cytosol causes coimmunoadsorption of the GR (cf. lanes 1 and 2), and immunoadsorption of cytosol with the BuGR monoclonal antibody against the GR causes coimmunoadsorption of hsp56 detected by Western blotting with UP30. Appropriate controls showed that UP30 does not react directly with the GR and BuGR does not react with hsp56 (data not shown). Thus, by coimmunoabsorption criteria, hsp56 is a component of the untransformed mouse GR from L cells.

Table I: Binding of [^{3}H]FK506 by Immunoadsorbed GR Heterocomplex[a]

expt	specifically bound [^{3}H]FK506 (cpm)	specifically bound [^{3}H]triamcinolone acetonide (cpm)	FK506/TA ratio
1	10650	15530	0.69
2	9650	23800	0.41

[a] Replicate aliquots of L-cell cytosol containing unbound GR were immunoadsorbed to protein A–Sepharose with nonimmune IgG or BuGR antireceptor antibody; the immunopellets were washed 3 times with 1 μL of TEG buffer, suspended in HEPES buffer, and incubated 12 h at 0 °C with 200 nM [^{3}H]FK506 or 50 nM [^{3}H]triamcinolone acetonide (TA). Samples were washed twice in HEPES buffer and assayed for radioactivity. GR-specific binding was determined by subtracting the nonimmune value (~30% of the binding in the BuGR pellet for [^{3}H]FK506 and ~2% for [^{3}H]TA) from the binding in the BuGR immunopellet. The specific [^{3}H]FK506 cpm was normalized to account for the slight difference in specific activity of the radiolabeled FK506 and TA stock solutions. The values represent the average of duplicate assays.

Table I shows that [^{3}H]FK506 binds to the immunoadsorbed GR heterocomplex. [^{3}H]Triamcinolone binding was assayed in replicate samples. After normalization of the data to account for the slightly different specific activities of the two radioligands, we calculate the number of FK506 binding sites in the immunoadsorbed, washed GR heterocomplex to be about 55% (41% and 69% for the two experiments, respectively) of the number of binding sites for triamcinolone acetonide. The GR has one steroid binding site, and the hormone binding domain must be bound to hsp90 for it to be in a steroid binding conformation (Bresnick et al., 1989; Scherrer et al., 1990; Hutchison et al., 1992). Analysis of the primary structure of the three domains of hsp56 reveals one rotamase center (Callebaut et al., 1992), and Tai et al. (1992) have shown that hsp56 contains one FK506 binding site with a K_D of 66 nM and saturation of binding sites occurring at ~100 nM free ligand. [^{3}H]FK506 was present at 200 nM in the experiments of Table I. Thus, the data of Table I might be consistent with a stoichiometry of 1 hsp56 per hsp90-bound GR in the immunopurified complex, but with significant loss of the hsp56 relative to hsp90 during heterocomplex isolation. We have noted that hsp56 is clearly a more loosely associated component of the heterocomplex than hsp90, which is tightly bound directly to the hormone binding domain of the receptor (M. J. Czar, unpublished experiments).

Figure 1B shows that addition of 1 μM FK506 to L-cell cytosol does not cause dissociation of hsp90 from the GR and does not affect the steroid binding activity of the native GR heterocomplex. In Figure 1B, FK506 was present at approximately 10-fold the concentration required for complete occupancy of binding sites.

In separate experiments, FK506 was shown not to affect the dissociation of hsp90 from steroid-bound receptors when cytosol is incubated at 25 °C (data not shown). As hsp56 may itself be part of a transport form of the receptor, we asked whether FK506 would affect receptor association with the nuclear fraction in rat 54-5A4 kidney epithelial cells. These cells were chosen because Qi et al. (1989) have clearly demonstrated by immunofluorescence that the GR undergoes cytoplasmic to nuclear translocation when the cells are exposed

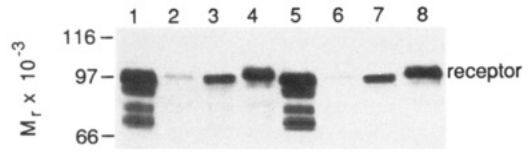

FIGURE 2: FK506 does not affect the steroid-mediated nuclear transfer of glucocorticoid receptors in rat kidney epithelial (54-5A4) cells. Confluent cells were treated for 2 h with 1 μM corticosterone, or 1 μM FK506, or both agents. Receptors were then harvested from the cytosolic and nuclear fractions and analyzed by SDS–PAGE and Western blotting. Lanes 1 and 2, cytoplasmic (lane 1) and nuclear (lane 2) fractions of untreated cells; lanes 3 and 4, cytoplasmic (lane 3) and nuclear (lane 4) fractions of cells treated with corticosterone; lanes 5 and 6, cytoplasmic (lane 5) and nuclear (lane 6) fractions of cells treated with FK506; lanes 7 and 8, cytoplasmic (lane 7) and nuclear (lane 8) fractions of cells treated with both corticosterone and FK506.

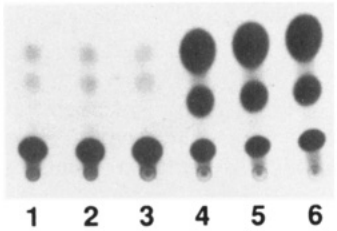

FIGURE 3: FK506 does not affect transcriptional activation of a CAT reporter in L cells. The LMCAT line of L cells was incubated overnight with ethanol vehicle, 10 μM FK506, 1 μM dexamethasone, or both agents, and cell lysates were assayed for CAT activity. Lane 1, no treatment; lane 2, vehicle alone; lane 3, FK506; lane 4, dexamethasone; lane 5, dex plus vehicle; lane 6, dex plus FK506.

to glucocorticoid. As shown in Figure 2, FK506 does not itself provoke nuclear transfer and does not inhibit nuclear transfer mediated by corticosterone. The slower migration of nuclear receptors (Figure 2, lanes 4 and 8) compared to cytoplasmic receptors probably reflects the phosphorylation occurring with GR transformation reported by Hoeck and Groner (1990).

Because none of the functions of the receptor heterocomplex were affected by FK506, we asked if the immunosuppressant would affect steroid-mediated transcriptional enhancement. For this purpose, we used the LMCAT subline of mouse L cells, which has been stably transfected with a plasmid containing a CAT reporter gene under the control of the mouse mammary tumor virus long terminal repeat promoter. In this cell line, steroid-mediated enhancement of CAT expression reflects the action of the endogenous L-cell GR. As shown in Figure 3, FK506 does not itself cause CAT expression (lane 3), and it does not affect dexamethasone-mediated CAT expression (cf. lanes 4 and 6). Thus, it appears that the complete glucocorticoid response pathway is unaffected by FK506.

It is highly likely that hsp56 has rotamase activity (Callebaut et al., 1992) and, like other immunophilins (Schreiber, 1991; Walsh et al., 1992), it is thought to play a role in protein folding events involved in the assembly of protein complexes (Tai et al., 1992). The events that were shown not to be affected by FK506 in Figures 1–3 reflect almost entirely preformed receptor complexes. Tai et al. (1992) have suggested that hsp56 may be involved in the assembly of the inactive GR heterocomplex. Thus, we performed the experiments of Figure 4 to determine if FK506 affected the assembly of the heterocomplex.

We have shown previously that immunoadsorbed, hormone-free GR that has been stripped of associated proteins can be reconstituted into a heterocomplex with hsp90 when it is

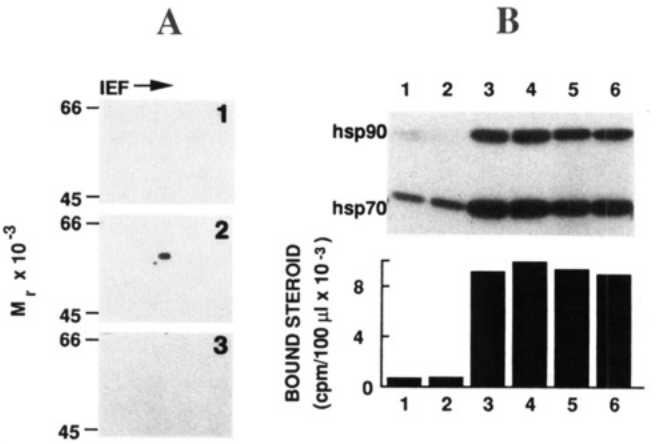

FIGURE 4: GR heterocomplexes reconstituted with rabbit reticulocyte lysate contain hsp56, but FK506 has no effect on heterocomplex reconstitution. (A) Hsp56 is in the reconstituted GR heterocomplexes. L-cell cytosol was immunoadsorbed with BuGR or nonimmune IgG, and the immunopellets were stripped of associated proteins with KCl, incubated with rabbit reticulocyte lysate, and washed. Pellet-associated proteins were resolved by two-dimensional gel electrophoresis, followed by immunoblotting with EC1 for hsp56. Panel 1, immunoadsorbed with BuGR and stripped; panel 2, BuGR-immunoadsorbed, stripped, and incubated with reticulocyte lysate; panel C, nonimmune, stripped and incubated with reticulocyte lysate. (B) FK506 does not affect refolding of the GR into association with the heat shock protein heterocomplex. Immunoadsorbed GR stripped of associated proteins was incubated with reticulocyte lysate that was untreated, treated with vehicle, or treated with 1 μM FK506 or cyclosporin A. After the incubation, pellets were washed and assayed for steroid binding (solid bars) and for receptor-associated hsp90 and hsp70 by Western blotting. Lane 1, stripped immune; lane 2, nonimmune-stripped and then incubated with lysate; lane 3, immune-stripped and incubated with lysate; lane 4, immune-stripped plus lysate pretreated with vehicle; lane 5, immune-stripped plus lysate pretreated with FK506; lane 6, immune-stripped plus lysate pretreated with cyclosporin A.

incubated with rabbit reticulocyte lysate (Scherrer et al., 1990). Heterocomplex reconstitution is an ATP-dependent, enzymatic event that leads to assembly of the GR into a complex with hsp90 and hsp70 (Hutchison et al., 1992). The complex that is formed has the functional properties of the untransformed receptor in that the GR is converted from a DNA binding form back to a non-DNA binding form and from a non-steroid binding form back to a steroid binding form (Scherrer et al., 1990; Hutchison et al., 1992).

It has not been previously determined if hsp56 is a component of the lysate-reconstituted receptor heterocomplexes. In Figure 4A, we show that mouse GR stripped of associated proteins becomes associated with rabbit hsp56 when it is incubated with reticulocyte lysate (cf. panels 2 and 3). Because it is rabbit hsp56 that is in the hybrid (mouse GR–rabbit hsp) heterocomplex, the EC1 monoclonal antibody could be used to detect hsp56 on the Western blot. As shown in Figure 4B, neither FK506 nor another immunosuppressant drug (cyclosporin A) has an effect on reassociation of heat shock proteins with the GR or in restoring the receptor to the steroid binding conformation typical of the untransformed receptor.

It is thought that this conformational change represents an unfolding of the hormone binding domain of the GR with stabilization of the unfolded, steroid binding conformation by the hsp90 component of the heterocomplex (Hutchison et al., 1992). Although hsp56 is present in the heterocomplex, it is not known if it is required for receptor unfolding and association with either hsp70, hsp90, or both. Although immunophilins have rotamase activity in vitro, the role for rotamase activity in cellular events is unknown. By analogy with the well-

described FK506 inhibition of rotamase activity of other more widely studied immunophilins (Schreiber, 1991; Walsh et al., 1992), the data of Figure 4B suggest that any rotamase activity inherent to hsp56 is not required for protein folding events involved in GR heterocomplex assembly.

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