A Heat Shock Protein Complex Isolated from Rabbit Reticulocyte Lysate Can Reconstitute a Functional Glucocorticoid Receptor–Hsp90 Complex†

Lawrence C. Scherrer,‡ Kevin A. Hutchison,‡ Edwin R. Sanchez,§ Stephen K. Randall,‖ and William B. Pratt*,‡

Department of Pharmacology, The University of Michigan Medical School, Ann Arbor, Michigan 48109, Department of Pharmacology, Medical College of Ohio, Toledo, Ohio 43699, and Department of Biology, Purdue University School of Science at Indianapolis, 1125 East 38th Street, Indianapolis, Indiana 46205

Received March 18, 1992; Revised Manuscript Received May 6, 1992

ABSTRACT: When unliganded glucocorticoid receptor that has been stripped free of associated proteins is incubated with rabbit reticulocyte lysate, the receptor becomes associated with the 70- and 90-kDa heat shock proteins (hsp70 and hsp90), and the untransformed state of the receptor is functionally reconstituted [Scherrer, L. C., Dalman, F. C., Massa, E., Meshinchi, S., & Pratt, W. B. (1990) *J. Biol. Chem. 265*, 21397–21400]. Recently, an hsp70-containing protein complex (200–250 kDa) purified from rabbit reticulocyte lysate was shown to maintain a fusion protein bearing the mitochondrial matrix-targeting signal in a state that is competent for mitochondrial import [Sheffield, W. P., Shore, G. C., & Randall, S. K. (1990) *J. Biol. Chem. 265*, 11069–11076]. In this work, we show that this partially purified mitochondrial import-competent fraction contains both hsp90 and hsp70. When the purified fraction is immunoadsorbed with a monoclonal antibody specific for hsp90, a significant portion of the hsp70 is coimmunoadsorbed, suggesting that hsp90 and hsp70 are present together as a complex. The partially purified fraction maintains a hybrid precursor protein containing the mitochondrial matrix-targeting signal of rat preornithine carbamyl transferase in an import-competent state. Incubation of immunopurified glucocoticoid receptor with this fraction of reticulocyte lysate results in ATP-dependent association of the receptor with both hsp70 and hsp90, and the resulting complexes are functional as assessed by return of the receptor to the high-affinity steroid binding conformation. The glucocorticoid receptor heterocomplex reconstituting activity of the lysate fraction is low relative to its mitochondrial import activity. Importantly, however, this is the first demonstration of the functional and structural reconstitution of the untransformed state of any steroid receptor utilizing a partially purified system.

The glucocorticoid receptor (GR)[1] is recovered from hormone-free cells as a heteromeric complex containing the steroid binding protein and hsp90 [for reviews, see Pratt (1987, 1990)]. When the GR is bound to hsp90, it is maintained in a high-affinity steroid binding conformation, and it does not bind DNA; the hsp90-free GR does not bind steroid, but it binds DNA with high affinity (Sanchez et al., 1987; Bresnick et al., 1989; Dalman et al., 1989; Scherrer et al., 1990). The progesterone receptor is recovered from hormone-free cells in a heteromeric complex that contains hsp70 as well as hsp90 (Estes et al., 1987; Kost et al., 1989; Smith et al., 1990a). Although cytosolic GR complexes usually contain very little or no hsp70, mouse GR overexpressed in CHO cells are, like progesterone receptors, located in the nuclei of hormone-free cells and are recovered in cytosolic complexes containing both hsp70 and hsp90 (Sanchez, et. al., 1990b).

The binding of steroid receptors to hsp90 does not represent a simple freely reversible equilibrium such that purified receptor and hsp90 spontaneously associate. The heterocomplex was first formed under cell-free conditions by translating the mouse or rat GR in rabbit reticulocyte lysate and demonstrating that the newly translated product was bound to rabbit hsp90 (Dalman et al., 1989; Denis & Gustafsson, 1989). Because the GR–hsp90 complex formed in rabbit reticulocyte lysate is quite stable under conditions that normally cause dissociation of the complex in cytosol preparations, Smith et al. (1990b) asked if the reticulocyte lysate could reassociate hsp90 with the receptor. They showed that incubation of chick oviduct progesterone receptor with rabbit reticulocyte lysate resulted in association of the chicken receptor with rabbit hsp70 and hsp90.

We have reported (Scherrer et al., 1990) that incubation of either immunoadsorbed or DNA-bound GR with reticulocyte lysate results in both structural and functional reconstitution of the receptor–hsp90 complex. That is, reassociation of the GR with hsp90 was accompanied by complete repression of DNA binding activity and reactivation of steroid binding activity. As with the progesterone receptor, hsp70 was also present in the complex reconstituted with reticulocyte lysate. Recently, we have shown that incubation of the transforming protein tyrosine kinase pp60^{src} with reticulocyte lysate forms a complex containing pp60^{src}, hsp70, hsp90, and the 50-kDa phosphoprotein component of the native pp60^{src} multiprotein complex (Hutchison et al., 1992).

Although several laboratories have tried to purify the receptor–hsp90 heterocomplex reconstituting activity from reticulocyte lysate, no purification has yet been achieved. The inability to purify the reconstituting activity might reflect the possibility that association of hsp90 with steroid receptors involves the coordinated action of two or more proteins in the

† This investigation was supported by NIH Grants DK31573 to W.B.P. and DK43867 to E.R.S. and by an IUPUI Faculty Development Grant and a Biomedical Research Support Grant to S.K.R.

* To whom correspondence should be addressed.
‡ The University of Michigan Medical School.
§ Medical College of Ohio.
‖ Purdue University School of Science at Indianapolis.

[1] Abbreviations: GR, glucocorticoid receptor; hsp70 and hsp90, 70- and 90-kDa heat shock proteins; triamcinolone acetonide, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16,17-acetonide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; CHO cells, Chinese hamster ovary cells.

0006-2960/92/0431-7325$03.00/0

reticulocyte lysate. For example, in the case of both the steroid receptor and pp60^{src}, reconstitution of the heteroprotein complex is energy-dependent, and it has been speculated that hsp70 may be providing an unfoldase activity that is required for association with the hsp90 component of the complex (Scherrer et al., 1990; Smith et al., 1990b, 1992; Hutchison et al., 1992).

It is known that certain proteins have to be maintained in an unfolded state to pass across the membranes of cellular organelles and that hsp70 plays a critical role in such protein import [for a review, see Pelham (1988) and Rothman (1989)]. For example, hsp70 is required for protein import into mitochondria (Murakami et al., 1988). Sheffield and co-workers (Sheffield et al., 1990; Skerjanc et al., 1990) have prepared a mitochondrial precursor protein in which the mitochondrial matrix-targeting signal of rat preornithine carbamyl transferase is fused to murine cytosolic dihydrofolate reductase (designated pO-DHFR), and they have shown that purified pO-DHFR is imported into purified mitochondria when rabbit reticulocyte lysate is present to maintain the hybrid precursor protein in an import-competent state. Partial purification of reticulocyte lysate proteins showed that the competence activity for pO-DHFR import resides in a large mass protein fraction (200–250 kDa) that contains hsp70 (Sheffield et al., 1990).

Although clearly a speculation, it seemed possible to us that protein import into organelles and the formation of the receptor heteroprotein complex might involve similar hsp70-mediated activities. In this work, we use the mitochondrial protein import-competent fraction partially purified from reticulocyte lysate to carry out reconstitution of the GR–hsp90 complex. We show that the purified import-competent fraction from lysate contains both hsp70 and hsp90, which by coimmunoadsorption criteria seem to be associated with each other in a complex. In the presence of an ATP-generating system, the fraction causes both hsp70 and hsp90 to bind to immunopurified GR. The receptors that are reconstituted with hsp90 are clearly restored to the high-affinity steroid binding conformation, a characteristic of the untransformed state of the glucocorticoid receptor. The extent of heterocomplex reconstitution is about 10% of that obtained with unfractionated lysate, suggesting that other components of the lysate are required for maximum efficacy. Our data show that the additional component or components required for maximum efficacy of reconstitution are in the 50% ammonium sulfate supernatant fraction of rabbit reticulocyte lysate.

EXPERIMENTAL PROCEDURES

Materials

Rabbit reticulocyte lysate was from Greene-Hectare Inc. (Oregon, WI). Monoclonal anti-β-tubulin antibody was obtained from Sigma Chemicals. EC1 monoclonal antibody (Tai et al., 1986) against hsp56 was kindly provided by Dr. Lee Faber (Medical College of Ohio, Toledo, OH). Rabbit antiserum against both hsp70 and hsp90 (Erhart, et al., 1988) was a generous gift from Dr. Ettore Appella (National Cancer Institute). The BuGR2 (Gametchu & Harrison, 1984) monoclonal antibody against the GR was kindly provided by Dr. Robert W. Harrison III (University of Arkansas for Medical Science) and the 8D3 monoclonal IgM (Perdew, 1988) against hsp90 by Dr. Gary Perdew (Purdue University, West Lafayette, IN).

Methods

Fractionation of Reticulocyte Lysate and Mitochondrial Import-Competent Assay. Fractionation of reticulocyte lysate was performed as described previously (Sheffield et al., 1990). Briefly, a high-speed supernatant of reticulocyte lysate was prepared by centrifugation at 180000*g* for 60 min, and the proteins precipitating between 30% and 50% of $(NH_4)_2SO_4$ saturation were resuspended in 15 mM KP_i (pH 7.5), 10% glycerol, and 1 mM DTT and chromatographed on a column of Sepharose CL-6B. Fractions from the column were combined as described in Sheffield et al. (1990), concentrated 6-fold in a Centricon 30, and stored at −70 °C until use. This material is called fraction 6B.

The chimeric precursor protein pO-DHFR was synthesized in vitro with rabbit reticulocyte lysate and immunopurified as described (Sheffield et al., 1990). Competence assays were conducted as described previously (Sheffield et al., 1990) by diluting urea-denatured, [^{35}S]methionine-labeled pO-DHFR in the presence or absence of 10 μL of lysate or 10 μL of fraction 6B and incubating 2 h at 30 °C. Final incubation conditions were 62.5 mM KCl, 1.25% glycerol, 1.25 mM $MgCl_2$, 125 mM sucrose, 5 mM HEPES (pH 7.5), 1.0 mM ATP, 0.1 mM ADP, 5.0 mM sodium succinate, 5 mM potassium phosphate, and 0.5 mM DTT. Following incubation, 10 μL of lysate was added to all assays followed immediately by 10 μL of mitochondria (5 mg/mL in 5 mM succinate, 1 mM ATP, 0.08 mM ADP, 250 mM sucrose, and 10 mM HEPES, pH 7.5), and the incubation was continued 60 min at 30 °C (import portion of the assay). Import was terminated by microcentrifugation at 4 °C. Proteins in the mitochondrial pellets were resolved by SDS–PAGE (12% gels) and autoradiography.

Receptor Immunoadsorption and Reconstitution of the GR–Hsp90 Complex. L-Cell cytosol was prepared in 10 mM HEPES/1 mM EDTA, pH 7.35, as previously described (Meshinchi et al., 1990), and reconstitution with hsp90 was performed according to Scherrer et al. (1990). Aliquots (350 μL) of cytosol were immunoadsorbed to protein A–Sepharose either with nonimmune mouse IgG or with 4% BuGR monoclonal anti-receptor antibody. Hsp90 was dissociated from the glucocorticoid receptor by incubation of the immunoadsorbed receptor with TEG buffer [10 mM TES, 4 mM EDTA, 10% (w/v) glycerol, and 50 mM NaCl, pH 7.6] plus 500 mM NaCl, for 2 h on ice. The immunoadsorbed pellets were washed 3 times with 1 mL of TEG and then once with 1 mL of 10 mM HEPES, pH 7.4. The pellets were mixed with 100 μL of rabbit reticulocyte lyaste or ammonium sulfate fractions of reticulocyte lyaste or fraction 6B plus 10 μL of an ATP-regenerating system (50 mM ATP, 250 mM creatine phosphate, 20 mM $MgCl_2$, and 100 units/mL creatine phosphokinase) and incubated for 20 min at 30 °C, and the pellets were washed 4 times with 1 mL of TEG buffer containing 20 mM sodium molybdate (TEGM). The washed pellets were divided, and one-seventh of the pellet (equivalent to 50 μL of immunoadsorbed cytosol) was incubated with 50 nM triamcinolone acetonide in TEGM for 3 h on ice, washed with HEPES buffer, and counted for radioactivity in a scintillation counter. The remainder of the pellet was analyzed by SDS–PAGE and Western blotting for receptor, hsp70, and hsp90. Binding of [^{3}H]dexamethasone 21-mesylate was assayed by mixing the TEGM-washed pellets with 100 nM ligand in TEGM for 12 h and then analyzing for labeled receptor by SDS–PAGE and autoradiography.

Gel Electrophoresis and Western Blotting. The immunoadsorbed protein A–Sepharose pellets were extracted with SDS sample buffer, and proteins were resolved on 7% SDS–polyacrylamide gels. Proteins were transferred to Immobilon P membranes, incubated overnight with 1% BuGR antibody

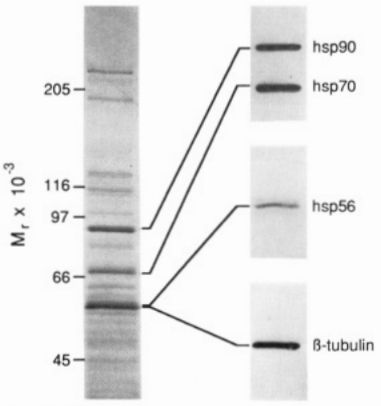

FIGURE 1: Analysis of the protein composition of fraction 6B. Rabbit reticulocyte lysate fraction 6B was analyzed by SDS–PAGE. The left-hand side of the figure shows a Coomassie blue-stained gel of fraction 6B, and the right-hand side of the figure shows strips that were Western-blotted for hsp70, hsp90, hsp56, and tubulin.

to detect the receptor, 0.02% rabbit antiserum to detect hsp90, 0.1% anti-β-tubulin to detect tubulin, or 0.1% EC1 to detect hsp56, and then incubated a second time with the appropriate ^{125}I- or peroxidase-conjugated anti-mouse or anti-rabbit IgG.

RESULTS AND DISCUSSION

In the experiment shown in Figure 1, the mitochondrial import-competent activity was purified by ammonium sulfate fractionation (30–50%) and Sepharose CL-6B chromatography. The fractions from the Sepharose CL-6B column with the highest import-competent activity were pooled, and the proteins in an aliquot of this pooled fraction 6B were resolved by SDS–PAGE. The three major proteins by Coomassie blue staining were identified by Western blotting as hsp90, hsp70, and tubulin. Hsp70 and hsp90 have been found in cytosols in heteromolecular complexes that also contain a 56-kDa protein (Sanchez et al., 1990a; Perdew & Whitelaw, 1991) that is now known to be a previously unrecognized heat shock protein (Sanchez, 1990). A modest amount of this hsp56 is also present in fraction 6B.

When proteins in reticulocyte lysate are fractionally precipitated with $(NH_4)_2SO_4$, a portion (about 40%) of the hsp90 and most (about 70%) of the hsp70 are precipitated in the 30–50% fraction (data not shown). In the experiment of Figure 2A, a 30–50% ammonium sulfate precipitate of lysate (a different preparation from that shown in Figure 1) was chromatographed on Sepharose CL-6B, and the elution of hsp70 and hsp90 was assayed by Western blot. Hsp70 elutes over a broad range of fractions, but hsp90 elutes earlier along with a portion of the hsp70 that elutes as a larger complex than the rest.

A portion of the cytosolic hsp90 and hsp70 have been shown to be bound to each other as a heat shock protein heterocomplex (Sanchez et al., 1990a; Perdew & Whitelaw, 1991). In the experiment of Figure 2B, aliquots of the fraction 6B preparation shown in Figure 1 were immunoadsorbed with 8D3 monoclonal antibody against hsp90. This antibody does not react with hsp70 (Dalman et al., 1989; Perdew, 1988), and the coimmunoadsorption of significant amounts of hsp70 with hsp90 is taken as strong evidence that the two proteins are combined in a complex. It should be noted that the other abundant fraction 6B protein, tubulin, is not coimmunoadsorbed with hsp90 by the 8D3 antibody (data not shown) and we had insufficient material to test for hsp56.

The ability of fraction 6B to maintain the mitochondrial precursor protein pO-DHFR in a import-competent state is

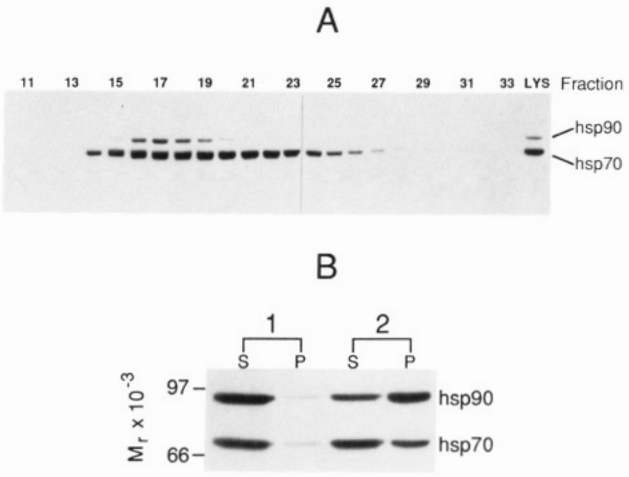

FIGURE 2: Hsp70 and hsp90 exist in a complex in fraction 6B. (A) Western blot of fractions eluted from a column of Sepharose CL-6B. Lysate proteins precipitating between 30 and 50% $(NH_4)_2SO_4$ were chromatographed on Sepharose CL-6B, and an aliquot of each fraction (numbered above lanes) or of the original lysate (LYS) was resolved by SDS–PAGE and Western-blotted with anti-hsp70/hsp90 antiserum. (B) Coimmunoadsorption of hsp70 and hsp90 with the 8D3 monoclonal antibody against hsp90. Fraction 6B was immunoadsorbed to protein A–Sepharose pellets with nonimmune IgM (condition 1) or the 8D3 monoclonal anti-hsp90 antibody (condition 2) exactly as described by Dalman et al. (1989). Proteins in the immunoadsorbed pellet (P) and proteins left in the supernatant after immunoadsorption (S) were resolved by SDS–PAGE and Western-blotted with antiserum against hsp70 and hsp90.

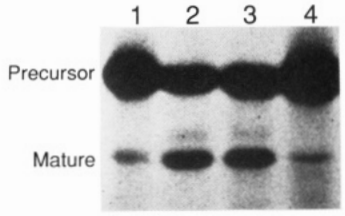

FIGURE 3: Inclusion of either rabbit reticulocyte lysate or fraction 6B maintains the mitochondrial precursor protein pO-DHFR in an import-competent state. [^{35}S]Methionine-labeled pO-DHFR was preincubated with reticulocyte lysate or fraction 6B to maintain import competence, and import into mitochondria was assayed as described under Methods. The figure shows the ^{35}S-labeled pO-DHFR precursor in the mitochondrial pellet and the mature product that was imported into and processed by the mitochondria. Lanes 1 and 4, no additions during preincubation; lane 2, 10 μL of lysate; lane 3, 10 μL of fraction 6B.

shown in Figure 3. In this assay, fraction 6B and reticulocyte lysate have comparable activity in maintaining pO-DHFR in a state such that it can be imported and subsequently processed to the mature form (cf. lanes 2 and 3). It has been shown previously that hsp70 alone (the constitutive form) cannot confer import competence (Sheffield et al., 1990) but the other fraction 6B factor or factors required for activity have not been identified. It is possible that a heat shock protein heterocomplex is required to maintain the pO-DHFR in the appropriate state for import.

The same preparation of fraction 6B used for the experiments of Figure 1, 2B, and 3 was incubated with immunopurified GR in the experiment shown in Figure 4. The receptor stripped free of hsp90 is shown in lane 1 of Figure 4A and after incubation with reticulocyte lysate or with fraction 6B in lanes 3 and 5, respectively. In the presence of fraction 6B, the GR becomes associated with hsp90, and formation of the complex requires the presence of the ATP-regenerating system (cf. lanes 5 and 6). It is important to note that the amount of hsp70 in the reconstituted samples containing the GR (lanes 3 and 5) is larger than the amount of hsp70 in

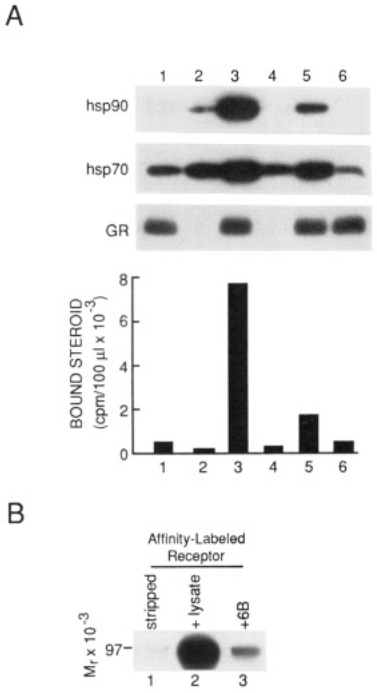

FIGURE 4: Fraction 6B reconstitutes the GR–hsp90 complex and steroid binding activity. GR was immunoadsorbed from L-cell cytosol using the BuGR monoclonal antibody, and the immunopellet was treated with reticulocyte lysate or fraction 6B as described under Methods. (A) Receptor treated with lysate or fraction 6B was analyzed by SDS–PAGE and Western blotting for hsp90, hsp70, and receptor (top of panel A) or for binding of [^{3}H]triamcinolone acetonide (graph, bottom of panel A). Lane 1, BuGR-adsorbed receptor stripped with salt; lanes 2 and 3, nonimmune (lane 2) and BuGR (lane 3)-adsorbed, stripped receptor incubated with reticulocyte lysate; lanes 4 and 5, nonimmune (lane 4) and BuGR (lane 5)-adsorbed, stripped receptor incubated with fraction 6B; lane 6, BuGR-adsorbed, stripped receptor incubated with fraction 6B in the absence of the ATP-regenerating system. (B) Receptors immunoadsorbed with BuGR and stripped with salt were incubated with reticulocyte lysate or fraction 6B and analyzed for binding of [^{3}H]dexamethasone 21-mesylate. The figure shows an autoradiogram of receptors incubated as follows: lane 1, stripped receptor; lanes 2 and 3, stripped receptor incubated with reticulocyte lysate (lane 2) or fraction 6B (lane 3).

control samples that do not contain the GR (lanes 2 and 4). We have noted before that hsp70 is rather sticky and becomes associated with nonimmune IgG–protein A–Sepharose pellets (Scherrer et al., 1990; Hutchison et al., 1992). Nevertheless, we routinely see a larger amount of hsp70 in samples containing the GR, implying that both hsp90 and hsp70 are present in the heterocomplex.

Receptor that has been incubated with reticulocyte lysate binds [^{3}H]triamcinolone acetonide (Figure 4A, bar graph, lane 3) whereas the hsp90-free receptor (lane 1) does not. The GR heterocomplex reconstituted with fraction 6B also has some triamcinolone acetonide binding activity, showing that fraction 6B can return the receptor to the high-affinity steroid binding conformation. Because the amount of steroid binding activity is low, binding activity was confirmed by site-specific affinity labeling with [^{3}H]dexamethasone 21-mesylate (Figure 4B).

By excising and counting the ^{125}I-labeled hsp90 bands in lanes 3 and 5 of Figure 4A, it was determined that fraction 6B formed 10% of the complexes formed by the unfractionated reticulocyte lysate. Similarly, fraction 6B reactivated about 10% of the steroid binding activity in comparison to reactivation by the unfractionated lysate (cf. lanes 3 and 5 in the bar graph). In contrast, fraction 6B had nearly the same

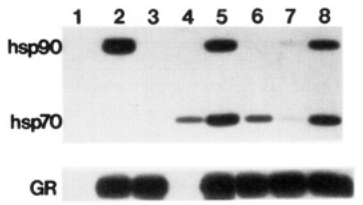

FIGURE 5: Activity present in the 50% ammonium sulfate supernatant of reticulocyte lysate is required for efficient heterocomplex reconstitution. Lysate proteins precipitating between 30 and 50% ammonium sulfate saturation were redissolved in half of the original volume of HEPES buffer, pH 7.35, and the 50% supernatant was dialyzed against HEPES buffer and contracted to half of the original lysate volume. GR was immunoadsorbed from L-cell cytosol using the BuGR antibody, and the immunopellets were incubated with whole reticulocyte lysate, the 30–50% fraction plus an equal volume of buffer, the 50% supernatant plus an equal volume of buffer, or equal volumes of both fractions. Receptor, hsp90, and hsp70 in each sample were assayed by SDS–PAGE and Western blotting. Lanes 1 and 2, receptor immunoadsorbed from L-cell cytosol with nonimmune (lane 1) or BuGR (lane 2) to show native GR-associated hsp90; lane 3, BuGR-adsorbed receptor stripped with salt; lanes 4 and 5, nonimmune (lane 4) and BuGR (lane 5)-adsorbed stripped receptor incubated with reticulocyte lysate; lanes 6–8, stripped receptor incubated with 30–50% ammonium sulfate fraction (lane 6), 50% supernatant (lane 7), or 30–50% fraction and 50% supernatant (lane 8).

effect on import competence as unfractionated lysate in Figure 3. Unlike receptor heterocomplex reconstitution (Figure 4A), import-competent activity does not require ATP (Sheffield et al., 1990). Taken together, these observations show that maintenance of pO-DHFR import competence and reconstitution of the GR–hsp90 complex are different processes, although they may both require the activity of hsp70.

Because most of the GR–hsp90 complex reconstituting activity is lost in the purification steps that produce fraction 6B, it seemed likely that there are other components required for heterocomplex reconstitution in addition to hsp70 and hsp90. We have found that most of the GR–hsp90 reconstituting activity is lost at the original ammonium sulfate reconstitution step, suggesting that additional component(s) may be present in the 50% ammonium sulfate supernatant that is (are) required for efficient reconstitution. The data of Figure 5 show that this is the case. In this experiment, reticulocyte lysate was fractionated with ammonium sulfate, and the reconstituting activities of the 30–50% fraction and the 50% supernatant alone and together were assayed. Lane 2 shows the native GR–hsp90 heterocomplex immunoadsorbed from L-cell cytosol, and lanes 3 and 5 show the receptor stripped of hsp90 (lane 3) and after heterocomplex reconstitution with whole (i.e., unfractionated) reticulocyte lysate (lane 5). In this particular fractionation of the lysate, neither the 30–50% ammonium sulfate fraction (lane 6) nor the 50% supernatant (lane 7) alone yielded significant reconstitution, but the two fractions together (lane 8) yielded nearly as extensive reconstitution of both hsp90 and hsp70 into the heterocomplex as the unfractionated lysate.

We have not yet identified the component or components in the 50% supernatant that permit this efficient heterocomplex reconstitution. It is important to note that we have found that purified hsp90 alone or hsp90 plus purified hsp70 does not yield any reconstitution of the GR–hsp90 complex or reactivation of steroid binding activity. This leads us to speculate that, in addition to the other components that may be required for heterocomplex reconstitution, hsp90 may have to be in a preformed complex with hsp70 for the GR to be properly unfolded and stabilized in the high-affinity steroid binding conformation. Despite the limited extent of recon-

stitution of the GR–hsp90 complex achieved with fraction 6B, the reconstituted complexes are functional in that the receptor is restored to the steroid binding state. It is clear that purifying and reconstituting a multicomponent protein folding system from reticulocyte lysate in a form that has efficient heterocomplex reonstituting activity will be a difficult and long-term process. However, the observations of this paper are the first demonstration of any reconstituting activity by a partially purified system.

ACKNOWLEDGMENT

We thank Robert Harrison III and William Hendry for providing the BuGR antibody, Gary Perdew for the 8D3 antibody, Lee Faber for the EC1 antibody, and Ettore Appella for the rabbit antiserum against hsp70 and hsp90.

REFERENCES

Bresnick, E. H., Dalman, F. C., Sanchez, E. R., & Pratt, W. B. (1989) *J. Biol. Chem. 264*, 4992–4997.

Dalman, F. C., Bresnick, E. H., Patel, P. D., Perdew, G. H., Watson, S. J., & Pratt, W. B. (1989) *J. Biol. Chem. 264*, 19815–19821.

Denis, M., & Gustafsson, J.-A. (1989) *J. Biol. Chem. 264*, 6005–6008.

Erhart, J. C., Duthu, A., Ullrich, S., Appella, E., & May, P. (1988) *Oncogene 3*, 595–603.

Estes, P. A., Suba, E. J., Lawler-Heavner, J., Elashry-Stowers, D., Wei, L. L., Toft, D. O., Sullivan, W. P. Horwitz, K. B., & Edwards, D. P. (1987) *Biochemistry 26*, 6250–6262.

Gametchu, B., & Harrison, R. W. (1984) *Endocrinology 114*, 274–279.

Hutchison, K. A., Brott, B. K., DeLeon, J. H., Perdew, G. H., Jove, R., & Pratt, W. B. (1992) *J. Biol. Chem. 267*, 2902–2908.

Kost, S. L., Smith, D. F., Sullivan, W., Welch, W. J., & Toft, D. O. (1989) *Mol. Cell. Biol. 9*, 3829–3838.

Meshinchi, S., Sanchez, E. R., Martell, K., & Pratt, W. B. (1990) *J. Biol. Chem. 265*, 4863–4870.

Murakami, H., Pain, D., & Blobel, G. (1988) *J. Cell Biol. 107*, 2051–2057.

Pelham, H. (1988) *Nature 332*, 776–777.

Perdew, G. H. (1988) *J. Biol. Chem. 263*, 13802–13805.

Perdew, G. H., & Whitelaw, M. L. (1991) *J. Biol. Chem. 266*, 6708–6713.

Pratt, W. B. (1987) *J. Cell. Biochem. 35*, 51–58.

Pratt, W. B. (1990) *Mol. Cell. Endocrinol. 74*, C69–C76.

Rothman, J. E. (1989) *Cell 59*, 591–601.

Sanchez, E. R. (1990) *J. Biol. Chem. 265*, 22067–22070.

Sanchez, E. R., Meshinchi, S., Tieurungroj, W., Schlesinger, M. J., Toft, D. O., & Pratt, W. B. (1987) *J. Biol. Chem. 262*, 6986–6991.

Sanchez, E. R., Faber, L. E., Henzel, W. J., & Pratt, W. B. (1990a) *Biochemistry 29*, 5145–5152.

Sanchez, E. R., Hirst, M., Scherrer, L. C., Tang, H.-Y., Welsh, M. J., Harmon, J. M., Simons, S. S., Ringold, G. M., & Pratt, W. B. (1990b) *J. Biol. Chem. 265*, 20123–20130.

Scherrer, L. C., Dalman, F. C., Massa, E., Meshinchi, S., & Pratt, W. B. (1990) *J. Biol. Chem. 265*, 21397–21400.

Sheffield, W. P., Shore, G. C., & Randall, S. K. (1990) *J. Biol. Chem. 265*, 11069–11076.

Skerjanc, I. S., Sheffield, W. P., Randall, S. K., Silvius, J. R., & Shore, G. C. (1990) *J. Biol. Chem. 265*, 9444–9451.

Smith, D. F., Faber, L. E., & Toft, D. O. (1990a) *J. Biol. Chem. 265*, 3996–4003.

Smith, D. F., Schowalter, D. B., Kost, S. L., & Toft, D. O. (1990b) *Mol. Endocrinol. 4*, 1704–1711.

Smith, D. F., Stensgard, B. A., Welch, W. J., & Toft, D. O. (1992) *J. Biol. Chem. 267*, 1350–1356.

Tai, P. K., Maeda, Y., Nakao, K., Wakim, N. G., Duhring, J. L., & Faber, L. E. (1986) *Biochemistry 25*, 5269–5275.