Identification of Phosphorylated Sites in the Mouse Glucocorticoid Receptor*

(Received for publication, October 15, 1990)

Jack E. Bodwell‡, Eduardo Orti, James M. Coull§, Darryl J. C. Pappin*, Lynda I. Smith†, and Fiona Swift

From the Department of Physiology, Dartmouth Medical School, Hanover, New Hampshire 03756 and §Milligen/Biosearch, Burlington, Massachusetts 01803

Glucocorticoid receptors in vivo are phosphorylated in the absence of hormone and become hyperphosphorylated in the presence of glucocorticoid agonist but not antagonists (Orti, E., Mendel, D. B., Smith, L. I., and Munck, A. (1989) J. Biol. Chem. 264, 9728–9731). As a preliminary step to elucidating the functional significance of receptor phosphorylation, we have identified seven phosphorylated sites on the mouse receptor. Tryptic phosphopeptides from [32P]-labeled receptors were purified from glucocorticoid-treated mouse thymoma cells (WEHI-7) and from stably transfected Chinese hamster ovary cells (WCL2) that express large numbers of mouse receptors. Phosphopeptide maps of receptors from these two cell types were almost indistinguishable. Solid phase sequencing revealed phosphorylation at serines 122, 150, 212, 220, 234, and 315 and threonine 159. Serines 122, 150, 212, 220, and 234 and the sequences surrounding them are conserved in the homologous regions of the rat and human receptors, but threonine 159 and serine 315 have no homologues in the human receptor. The seven phosphorylated sites are in the amino-terminal domain of the receptor. All but serine 315 are within transactivation domains identified in the human and/or rat receptors. Serines 212, 220, and 234 are in a highly acidic region that in the mouse receptor is necessary for full transcription initiation activity and reduces nonspecific DNA binding. Serines 212, 220, and 234 and threonine 159 are in consensus sequences for proline-directed kinase and/or p34<sup>++</sup> kinase. Serine 122 is a consensus sequence for casein kinase II whereas serines 150 and 315 do not appear to be in any known kinase consensus sequence. The location of many of these sites suggests a role of phosphorylation in transactivation.

Many glucocorticoid target cells respond to changes in hormone levels by altering the expression of specific genes. Transduction between hormone levels and gene expression is mediated by the GR, a ~100-kDa steroid-binding protein that in the absence of hormone is present as a soluble oligomeric complex (Sherman and Stevens, 1984; Pratt, 1987; Carson-Jurica et al., 1990).

At physiological temperatures, binding of glucocorticoid agonists to the GR results in dissociation of the complex (Raaka and Samuels, 1983; Vedeckis, 1983; Holbrook et al., 1983; Pratt, 1987; Mendel and Orti, 1988). The hormone-receptor complex then interacts with specific glucocorticoid response elements in target genes and alters rates of transcription (Beato, 1989; Burnstein and Cidlowski, 1989; Yamamoto, 1985; Carson-Jurica et al., 1990).

Mutagenesis studies have determined a number of functions for different portions of the GR (for a general review see Carson-Jurica et al., 1990). DNA binding has been localized to a central cysteine-rich area. This area contains two zinc fingers that are thought to be necessary for binding to DNA. The carboxyl-terminal portion contains the hormone binding domain, nuclear localization signals, and a transactivation region. Transactivation regions have also been identified in the amino-terminal domain of the human (Hollenberg and Evans, 1988), rat (Godowski et al., 1988), and mouse (Danielson et al., 1987) receptors.

Glucocorticoid and other steroid receptors are known to be phosphorylated (Housley and Pratt, 1983; Carson-Jurica et al., 1990). The unliganded receptor from WEHI-7 mouse thymoma cells contains 2–3 mol of phosphate/mol of protein (Mendel et al., 1987). The addition of glucocorticoid agonists, but not of the antagonist RU 486, increases phosphorylation by 50–70% (Orti et al., 1989a). These changes imply a role for phosphorylation in the function of the GR. As a step toward understanding how phosphorylation may affect function of the GR we have determined the location of seven in vivo phosphorylated sites on the mouse GR.

**EXPERIMENTAL PROCEDURES**

*Materials—Sources for the materials used in this study have been listed previously (Mendel et al., 1987; Smith et al., 1988; Smith et al., 1989; Orti et al., 1989a). Methylated trypsin was obtained from Fromega Biotech, Madison, WI. The WCL2 cell line was a generous gift from Dr. Margaret A. Hirst (Hirst et al., 1990).

**Buffers**—The following buffers were used: AB, 1.0 mM ammonium bicarbonate, pH 7.8; FT, 25 mM TES, pH 8.2, at 3 °C, 2 mM EDTA, 2 mM EGTA, 50 mM sodium fluoride, 20 mM sodium molybdate, and 10% glycerol (v/v); FTT, FT buffer plus 0.2% Triton X-100; FTN, FT buffer plus 0.4 mM sodium chloride; FTS buffer, FT buffer plus 10% SDS; FTNT, FTN buffer plus 0.2% Triton X-100; HBS buffer, 10 mM HEPES, pH 7.35, and 150 mM sodium chloride; KRBG, Krebs-Ringer bicarbonate buffer supplemented with 5.5 mM glucose, 25 mM HEPES, pH 7.35, and 1 mg of bovine serum albumin/ml; PST, 50 mM sodium pyrophosphate, pH 9.5, 2% SDS, and 1% Triton X-100; TST, 50 mM Tris, pH 9.0, 2% SDS, and 1% Triton X-100; TSTG, 40 mM Tris, pH 7.8, 4% SDS, 10% glycerol (v/v), and 20 mM...
diethiothreitol; UAB, 8.0 M urea, 100 mM ammonium bicarbonate, pH 7.8.

Cell Culture—Culture conditions for WEHI-7 cells have been described previously (Smith et al., 1989). WCL2 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal calf serum (HyClone Laboratories, Sterile Systems Inc., Logan UT), 39.5 μg of proline/ml, and 3 × 10⁻⁴ M methotrexate. Cells were maintained at 37 °C with 5% CO₂.

Whole Cell Labeling and Preparation of Cytosol—This procedure has been described in detail (Orti et al., 1989a). WEHI-7 cells were collected by centrifugation and incubated twice with phosphate-free Dulbecco's modified Eagle's medium containing 10% dialyzed fetal bovine serum (100 ml/2 x 10⁶ cells, 30 min, 37 °C). Cells were distributed to 162-ml flasks (Costar, Cambridge, MA) in 200 ml of medium at a concentration of 1.3 × 10⁶ cells/ml. [³²P]Orthophosphoric acid was added to four flasks (6.25 μCi/flask), after which the cells were incubated for approximately 4 h at 37 °C. In order to obtain enough counts, a second set of four flasks was prepared similarly the following day, and the two preparations were combined after the immunopurification step. Tramcinolone acetonide (10 μM stock in ethanol) was added to the cultures at a final concentration of 2.5 × 10⁻⁴ M for the last 30 min of the incubation with [³²P]. Cells were collected by centrifugation (2.5 min at 700 g), washed with 10 ml of cold phosphate-free KRBBG, and the cytosol prepared by the freeze/thaw procedure (4 volumes of FT buffer/volume of packed cells) as previously (Orti et al., 1989a).

WCL2 cells (80–100% confluent) in 162-ml flasks were incubated twice with phosphate-free Dulbecco's modified Eagle's medium containing 2% dialyzed fetal bovine serum (75 ml/flask, 30 min, 37 °C). Phosphate-free Dulbecco's modified Eagle's medium (50 ml) containing 10% dialyzed fetal bovine serum was added to each flask. [³²P]Orthophosphoric acid was added to four flasks (6.25 μCi/flask) and incubated for approximately 4 h. There was no difference in the shape of the phosphopeptide tryptic map if incubations went for 14 h as compared with 10 h. Protein A-Sepharose was collected by twirling slowly for 4–14 h. Protein A-Sepharose was washed once with 15 ml of HBS buffer (-10 °C) and the supernatant decanted. This procedure was repeated until all of the receptor had been precipitated. The pellet was dissolved in 15 ml of HBSS buffer (-23 °C) after which they were treated with 2 ml of 0.1% trypsin/flask (-23 °C). After 5 min the cells were dislodged by directing the flow of 10 ml of medium at a concentration of 1.3 × 10⁶ cells/ml. The cells were used for the WEHI-7 and 2-10 ml of packed cells with the WEHI-7. The WCL2 preparation used for the results in Fig. 1 was prepared similarly the following day, and the two preparations were combined.

Protein Determination—To determine the amount of receptor extracted from the Immobilon membrane, a small sample (approximately 0.10 mm²) was extracted from the membrane by [³⁵S]methionine (see Fig. 3 in Orti et al., 1989b). The procedure was repeated until all of the receptor had been precipitated. The pellet was dissolved in 15 ml of HBSS buffer and the supernatant decanted, and the pellet air dried until the odor of acetone could not be detected. The pellet was dissolved in 50 μl of UAB buffer (Stone et al., 1990) by warming to 37 °C, vortexing, and sonicating for one to 10-s intervals. After adding 15 μl of 1 mM ammonium bicarbonate and 136 μl of water, the tube was vortexed, and methylated with [³⁵S]methionine (see Fig. 3 in Orti et al., 1989a).

Determination of Sites—A 300-μl sample of the combined extract and wash was placed in a 1.5-ml centrifuge tube with 4 volumes of acetone at -80 °C. After 45 min at -20 °C the tube was centrifuged for 10 min at 12,000 × g (−10 °C) and the supernatant decanted. The procedure was repeated until all of the receptor had been precipitated. The pellet was washed once with -80 °C acetone, the supernatant decanted, and the pellet air dried until the odor of acetone could not be detected. The pellet was dissolved in 50 μl of UAB buffer (Stone et al., 1990) by warming to 37 °C, vortexing, and sonicating for one to 10-s intervals. After adding 15 μl of 1 mM ammonium bicarbonate and 136 μl of water, the tube was vortexed, and methylated with [³⁵S]methionine (see Fig. 3 in Orti et al., 1989a).

Purification—To determine the amount of receptor extracted from the Immobilon membrane, a small sample of receptor (approximately 0.10 mm²) was extracted from the membrane by [³⁵S]methionine (see Fig. 3 in Orti et al., 1989). The procedure was repeated until all of the receptor had been precipitated. The pellet was dissolved in 15 ml of HBSS buffer and the supernatant decanted, and the pellet air dried until the odor of acetone could not be detected. The pellet was dissolved in 50 μl of UAB buffer (Stone et al., 1990) by warming to 37 °C, vortexing, and sonicating for one to 10-s intervals. After adding 15 μl of 1 mM ammonium bicarbonate and 136 μl of water, the tube was vortexed, and methylated with [³⁵S]methionine (see Fig. 3 in Orti et al., 1989a).

Current preparations are always sonicated and show no signs of digestion. The sample vortexed, centrifuged (2 min at 12,000 × g), and injected into the HPLC.
Fig. 1. Comparison of tryptic phosphopeptide maps from WEHI-7 and WCL2 cells. Purified GR (10 μg from WEHI-7 and 11 μg from WCL2 cells) labeled with [32P]orthophosphoric acid was digested with trypsin and subjected to reverse phase chromatography. Phosphopeptides from WEHI-7 (3,000 cpm, lower graph) and WCL2 (115,000 cpm, upper graph) cells were injected into the HPLC. After Cerenkov counting, fractions from each phosphopeptide were pooled and sequenced as described under "Experimental Procedures." The number near the apex of each peak identifies each phosphopeptide.

Fig. 2. Sequencing of phosphopeptide peaks from WEHI-7 and WCL2 cells. Peaks isolated from the chromatographs displayed in Fig. 1 were subjected to solid phase sequencing as described under "Experimental Procedures." Initial yields were 1–5 pmol except for phosphopeptide 37 from WEHI-7 cells (all other phosphopeptides were from WCL2 cells) which was less than 1 pmol. The amount of radioactivity applied to the sequenator was 330, 2,780, 1,320, 548, 1,030, 1,078, and 725 cpm for phosphopeptides 37 (WEHI-7), 37 (WCL2), 8, 28, 36, 29 (obtained from a different preparation than that in Fig. 1), and 45, respectively.

Solid Phase Sequencing of Tryptic Phosphopeptides—Each lyophilized phosphopeptide was dissolved in 20 μl of 50% acetonitrile containing 0.1% trifluoroacetic acid by heating the sample tube at 55 °C for 5 min, and then sonicating for 30 s. The peptide solution was applied to a disc of arylamine PVDF membrane (Sequelon-AA, Milligen/Biosearch, Burlington, MA) which was placed on a piece of plastic film resting on a 55 °C heat block. In most experiments, more than 90% of the peptide was transferred to the membrane disc as...
GR Phosphorylation Sites

**Fig. 3.** Relationship of phosphorylated sites in the GR to the tryptic phosphopeptide map. At the top is a diagram of the mouse GR with numbered residues. The shaded region, which is expanded below, contains all seven phosphorylated sites. Positions of individual phosphopeptides are shown by the solid black bars. The letters S, T, and P designate serine, threonine, and phosphate, respectively. At the bottom of the figure is the HPLC phosphopeptide map for WCL2 cells from Fig. 1, with peaks and corresponding phosphopeptides indicated by arrows. Numbers refer to the phosphopeptide peaks as described under "Results."

**Table 1**

| Phosphopeptide no. | Phosphorylated residue no. | Average $^{32}P$ content | Range for $n = 5$ | Relative phosphates $\dagger$ | Sum of peptides $\ddagger$ |
|---------------------|---------------------------|--------------------------|------------------|-------------------------------|--------------------------|
| 5                   | 7                         | 2-11                     | 0.2              | 81                            |
| 6                   | 2                         | 0-3                      | 0.1              |                               |
| 8                   | 150/159                   | 21                       | 17-22            | 0.7                           |
| 11                  | 3                         | 3-5                      | 0.1              |                               |
| 16                  | 2                         | 1-2                      | 0.1              |                               |
| 23                  | 5                         | 4-6                      | 0.2              |                               |
| 28/29               | 315/122                   | 7                        | 5-8              | 0.2                           |
| 34                  | 212                       | 4                        | 2-5              | 0.1                           |
| 35/36               | 212/220                   | 15                       | 14-17            | 0.5                           |
| 37                  | 234                       | 29                       | 26-31            | 1.0                           |
| 38                  | 234                       | 5                        | 5-5              | 0.2                           |

$\dagger$ Number assigned to individual phosphopeptides peaks (see Fig. 1). The results are from five experiments done subsequent to those described in Fig. 1. The amount of peptide 16 in these experiments was much less than in Fig. 1, and peptide 45 was absent in all five of the experiments.

$\ddagger$ Values were calculated from the percent of total radioactivity in the individual peptides after assuming that peptide 37 (serine 234) had 1 mol of phosphate/mol of peptide.

**RESULTS**

We have used two different cell lines to determine the location of phosphates on the mouse receptor. Our basic
procedures and initial results were developed with WEHI-7 cells, with which all of our previous data on phosphorylation were obtained. With these cells, which have about 30,000 receptors/cell, massive preparations of labeled cells are required for sequencing work. WCL2 cells are Chinese hamster ovary cells that have been transfected stably with the mouse GR gene (Hirst et al., 1990). They express about 10^6 receptors/cell. When we established that WCL2 cells gave the same phosphopeptide map as WEHI-7 cells (Fig. 1), the same location for a phosphoserine (Fig. 2, A and B), and exhibit similar hormone dependence and kinetics of phosphorylation, we used them for most of the sequencing work. Our strategy has been to identify first the phosphorylated sites in hormone-treated cells, leaving for later the question of which of these are influenced by hormone treatment.

Fig. 1 (lower graph) shows the elution of WEHI-7 phosphopeptides from reverse phase HPLC. Each peak and corresponding phosphopeptide preparation is named by the approximate percentage of buffer B at the apex of the peak after allowing for a 12-min gradient delay time (the time it takes for changes in the gradient to appear at the detector). The general location in the mouse GR of the phosphopeptides and phosphorylated sites we have identified is shown in Fig. 3.

Peptide 37, the major phosphopeptide from the WEHI-7 cells, was collected and sequenced. Results are shown in Fig. 2A. The sample contained 330 cpm, and less than 1 pmol of GR was sequenced. Despite these constraints 5 of the first 9 residues were identified, enough to identify the phosphopeptide unambiguously as a tryptic peptide starting from serine 224 in the mouse GR (Danielsen et al., 1986). The radioactivity eluted from the sequenator with a peak at cycle 11, which corresponds to serine 234. Peptide 37 probably extends past lysine 254 to lysine 264 since lysines 254 and 262 are followed by proline, which is known to make cleavage sites almost completely resistant to trypsin (Wilkinson, 1986). There are no phosphorylation sites in this additional sequence since we have determined by phosphoamino acid analysis that peptide 37 contains only phosphoserine (data not shown). Thus the major phosphorylated site on the WEHI-7 GR is serine 234.

Peptide 37 is also the major phosphopeptide for the WCL2 cells (Fig. 1, upper graph). In fact, the tryptic map for WCL2 cells is almost indistinguishable from that for WEHI-7 cells. Peptide 45, present only in the WCL2 cells, is an incomplete digestion product that has not appeared in more recent preparations (see below and "Experimental Procedures"). Peptide 37 from the WCL2 cells was the same tryptic peptide, with a phosphorylated serine 234, as that from WEHI-7 cells (Fig. 2B).

Peptides 8, 28, 29, 36 and 45 from WCL2 cells were then isolated and sequenced. The partial sequence of peptide 8 (Fig. 2C) identified it as the tryptic peptide from serine 150 to lysine 169, overlapping peptide 37. It has two phosphorylated sites, radioactivity appearing at serine 150 (cycle 1) and threonine 159 (cycle 10). The sequence for peptide 28 (Fig. 2D) matched the tryptic fragment from leucine 106 to arginine 129 (Fig. 2F). Radioactivity eluted at cycle 17, identifying serine 122 as the phosphorylated site.

Peptides 34 (data not shown), 35 (data not shown), 36, and 45 all had partial sequences corresponding to the tryptic peptide starting with leucine 192 (Fig. 2, E and G), and all had radioactivity that eluted at serine 212. A second site, common to peptides 35, 36, and 45, was identified from peptide 45, which showed radioactivity not only at serine 212 but also at serine 220 (Fig. 2G). 5 residues past the end of the tryptic peptide from leucine 192 to lysine 215. It is clear that in this peptide the bond at lysine 215 was not cleaved. Serine 220 is in the next tryptic peptide from glutamic acid 216 to arginine 223.

The phosphorylated sites at serines 212 and 220 probably account for all of the radioactivity in peptides 34, 35, 36, and 45, but because of incomplete hydrolysis the two sites appear in four peptides. These peptides may have originated as follows. Peptide 34 is the tryptic peptide from leucine 192 to lysine 215 which contains the phosphorylated serine 212. Although barely detectable in the preparation that was sequenced (Fig. 1, upper graph), this peptide is more abundant in recent preparations (see Table I). Peptides 35 and 36 encompass peptide 34 and the next tryptic peptide from glutamic acid 216 to arginine 223. Both have the same primary sequence but differ in the distribution of phosphate between serines 212 and 220. The failure of trypsin to cleave at lysine 215 is probably a result of glutamic acid 216. Adjacent negative charges are known to make cleavage sites very resistant to hydrolysis by trypsin (Wilkinson, 1986). An experiment that used a much higher than usual ratio (1:4) of trypsin to receptor demonstrated that peptides 35 and 36 contain two phosphorylation sites. The larger amount of trypsin produced a substantial decrease in radioactivity in the peak containing peptides 35 and 36, an increase in radioactivity in peptide 34, and a new phosphopeptide, peptide 9, that was not sequenced.

Peptide 45 elutes at a position in the HPLC which is much more hydrophobic than would be expected if it contained only the two tryptic peptides encompassing serines 212 and 220. This preparation was hydrolyzed incompletely (see "Experimental Procedures"), and the bonds at serines 215 and 223 were not cleaved, with the result that peptide 45 contains both peptides 35/36 and 37.

Most of the phosphopeptides were sequenced at least twice and some three times. In all cases the same sequences were identified, and the radioactivity eluted at the same position. Peptide 45 was sequenced only once, but the results clearly show phosphorylation at serines 212 and 220.

Peptide 16 has virtually disappeared from recent preparations that are hydrolyzed more completely whereas peptide 5 has increased. Peptide 5, however, is not homogeneous. Rechromatography on a C18 reverse phase column separated peptide 5 into at least two peaks. We have not had enough material to sequence either of these two peaks nor peptide 23. Peptide 38 has the same initial sequence as peptide 37 with a phosphorylation site at serine 234 (data not shown), but the exact cleavage product is unknown.

Table I shows the relative abundance of each of the phosphopeptides recovered from the HPLC, with averages and ranges from five experiments. The distribution of phosphate among the sites is reflected in the relative amounts of radioactivity in the individual phosphopeptides. If recoveries of the individual phosphopeptides are similar, the seven phosphorylated sites account for greater than 80% of the phosphate in the receptor.

Serine 234 (peptide 37) is the most heavily phosphorylated site. If it is assumed to be fully phosphorylated and thus to account for 1 mol of phosphate/mol of receptor, then the sum of all the identified phosphopeptides account for 3.4 mol. This rough estimate is reasonably consistent with the range of 4-
We have identified seven phosphorylated sites on the mouse GR: serines 122, 150, 212, 220, 234, and 315 and threonine 159. Although most of the sites were determined from WCL2 cells, the almost identical patterns of phosphorylation found with the mouse receptor, whether in WEHI-7 mouse thymoma cells or overexpressed in Chinese hamster ovary (WCL2) cells, suggest strongly that phosphorylation is not cell type specific.

Although solid phase sequencing of picomol levels of 32P-labeled phosphopeptides has been reported previously (Wettenhall et al., 1991; Aebersold et al., 1989), this is the first study to use covalent attachment to an arylamine PVDF membrane in sequencing these levels of phosphopeptides. We were able to locate phosphoserine and phosphothreonine residues using 1-5 pmol of peptide that contained only a few hundred cpm of radioactivity. These results could be obtained with small quantities of radioactive material because of the efficient way the solid phase sequenator handles the [32P] inorganic phosphate that is released during the Edman degradation. With a gas phase sequenator the phosphate from the amino acid normally remains in the reaction chamber because it is largely insoluble in the nonpolar solvents that are used to extract the amino acid derivatives (Soderling and Walsh, 1982). Covalent attachment of the peptide to arylamine PVDF membranes allows the use of liquid trifluoroacetic acid to remove the phosphate from the reaction vessel efficiently, for subsequent analysis (Coull et al., 1991).

In the mouse GR, phosphoserine accounts for approximately 90% of the total phosphate recovered from the HPLC. Originally we reported only phosphoserine, but our recent experiments using longer exposure times for autoradiograms reveal small amounts of phosphothreonine. Likewise, Dalman et al. (1988) found only phosphoserine in the rat GR, but a more recent study (Hoeck and Groner, 1990) shows a small amount of phosphothreonine. One group (Rao and Fox, 1987) has reported 11% phosphothreonine in the human receptor. However, this phosphothreonine must be at a different site from that equivalent to threonine 159 in the mouse since the amino acid at the homologous position in the human GR is alanine (see below).

Location of the seven phosphorylated sites in the amino-terminal domain between residues 122 and 315 is consistent with previous reports (Dalman et al., 1988; Smith et al., 1989; Hoeck and Groner, 1990) that most of the phosphates are located in that domain. Originally, we detected a phosphorylation site in the carboxyl-terminal domain (Smith et al., 1989), but recent experiments using a more rigorous purification procedure with two different monoclonal antibodies have failed to confirm this site.

Proteolytic cleavage studies (Dalman et al., 1988) of the GR in mouse L cells showed that the 15-kDa tryptic fragment containing the DNA binding domain was phosphorylated. We did not identify any phosphorylated sites in that portion of the GR, but such a site could be contained in one of the phosphopeptides that we were not able to sequence.

Transactivation domains have been identified within the amino-terminal domain of the human, rat, and mouse receptors. In the human receptor (Hollenberg and Evans, 1988) a transactivation domain designated as tau 1 is contained in amino acid residues 77-262, homologous to residues 86-270 in the mouse receptor. All the phosphorylated sites except serine 315 are in this region. The rat transactivation domain designated enh2 (Godowski et al., 1988) lies between amino acids 237 and 318, corresponds to mouse region 225-306 that includes the major phosphorylated site, serine 234. In the mouse receptor (Danielsen et al., 1987) a highly acidic region between 196 and 295 has been shown to be necessary for maximum initiation activity and also to decrease nonspecific binding to DNA. This region contains three of the phosphorylated sites, serines 212, 220, and 234, increasing its acidity even more. The location of these phosphorylated sites in the transactivation regions suggests a role for phosphorylation in modulating these functions.

The sequences around the mouse GR phosphorylation sites at serines 122, 150, 212, 220, and 234 are conserved in the rat (homologous serines at 134, 161, 224, 232, and 246) and human (homologous serines at 113, 141, 203, 211, and 226) GR. The site for serine 150 is also conserved in the human mineralocorticoid receptor (corresponding to serine 214). The phosphorylation site at threonine 159 is conserved in the rat but not in the human receptor, in which the threonine is replaced by an alanine. Likewise, the homologue to serine 315 is replaced by a proline in the human GR. It therefore seems unlikely that serine 315 and threonine 159 are important for modulating function.

All the phosphorylated sites except serines 150 and 315 are in consensus sequences of known kinases. Serine 122 is the only one of four potential casein kinase II sites (XXX-Ser/Thr-Pro-XXX) that is phosphorylated. There are 11 potential phosphorylation sites that fit the consensus sequence (XXX-Ser/Thr-Pro-XXX) of the proline-directed kinase (Vulliet et al., 1989), and four of them are phosphorylated (serines 212, 220, and 234 and threonine 159). Serines 212 and 220 also fit the consensus sequence for the P34αβ'-kinase (Ser/Thr-Pro-XXX-Arg/Lys) (Moreno and Nurse, 1990); which is important in regulation of the cell cycle (Nurse, 1990).

Recently three phosphorylated sites have been identified in the progesterone receptor from chick oviduct (Denner et al., 1990). There is no extensive homology between the sites on the GR and the progesterone receptor. One of the progesterone receptor sites is located in the hinge region between the DNA and hormone binding domains. However, all three sites on the progesterone receptor have the same motif of a serine followed by proline seen in three of the seven sites found in the mouse GR (serines 212, 220, and 234).

We are currently determining which phosphorylated sites of the GR are influenced by hormone and how phosphorylation at those sites affects GR activity.

Acknowledgments—We wish to thank Dr. Allan Munck for his leadership and guidance during these studies and for reviewing this manuscript. We are most appreciative of the generous gift of WCL2 cells by Dr. Margaret A. Hirst.

REFERENCES

Aebersold, R. H., Nika, H., Pipes, G. D., Wettenhall, R. E. H., Clark, S. M., Hood, L. E., and Kent, S. B. H. (1989) in Methods in Protein Sequence Analysis (Wittmann-Liebold, B., ed.), pp. 79-97, Springer-Verlag, Berlin

Beato, M. (1989) Cell 56, 335-344

Bodwell, J. E., and Meyer, W. L. (1981) Biochemistry 20, 2767-2779

Burnstein, K. L., and Cidlowski, J. A. (1989) Annu. Rev. Physiol. 51, 683-699

Carson-Jurica, M. A., Schrader, W. T., and O'Malley, B. W. (1990) Endocr. Rev. 11, 201-220

Coull, J. M., Pappin, D. J. C., Mark, J., Aebersold, R., and Koester, H. (1991) Anal. Biochem. in press

Dalman, F. C., Sanchez, E. R., Lin, A. L., Y., Perini, F., and Pratt, W. B. (1988) J. Biol. Chem. 263, 12259-12267

Danielsen, M., Northrop, J. P., and Ringold, G. M. (1986) EMBO J. 5, 2513-2522
