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Characterization of the Chromatin Acceptor Sites for the Avian Oviduct Progesterone Receptor Using Monoclonal Antibodies†

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ABSTRACT: Monoclonal antibodies (MAb) against the chromatin acceptor sites for the avian oviduct progesterone receptor were prepared with highly purified hen oviduct acceptor proteins reconstituted to hen DNA. Addition of the MAbs to a cell-free assay blocked progesterone receptor from chick oviduct (PRov) binding to native-like acceptor sites on nucleoacidic protein (NAP) representing a partially deproteinized chromatin, which has been shown to be enriched in these binding sites. However, the antibodies do not block PRov binding to pure DNA, nor do they affect the receptor itself. Estrogen receptor binding to NAP was not inhibited, supporting a receptor specificity of the PRov acceptor sites as reported previously from direct competition studies. These data support earlier studies showing that (1) the reconstituted PRov acceptor sites resemble the native sites, (2) the acceptor sites are receptor specific, and (3) the PRov binding sites of NAP are different from those of pure DNA. While some animal-species specificity in the PRov binding inhibition was observed, no tissue specificity was seen. Direct binding of the antibodies to native acceptor sites was demonstrated in an enzyme-linked immunosorbent assay (ELISA) system. The antibodies showed little recognition of free acceptor protein or DNA alone, indicating specificity for the protein–DNA complex. A partial evolutionary conservation of the nuclear acceptor sites for PRov was shown by the fact that about 50% of the inhibition seen with hen NAP was obtained with NAPs from several other species, and this partial cross-reactivity of the MAbs with the same NAPs from other animal species was also seen in the ELISA.

It is widely accepted that steroids bind to specific receptor proteins which in turn bind nuclear "acceptor" sites to regulate gene expression (Jensen & DeSombre, 1972). Specific nuclear binding sites for the avian oviduct progesterone receptor (PRov),[1] which appear to be composed of a group of acceptor proteins bound to certain DNA species, have been identified in avian oviduct chromatin [reviewed in Spelsberg et al. (1979, 1983), Thrall et al. (1978), and Spelsberg, (1982)]. These sites bind PRov in a saturable, high-affinity manner and display receptor dependency (Pikler et al., 1976), receptor specificity (Kon & Spelsberg, 1982), and an in vivo like pattern

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[1] Abbreviations: NAP, nucleoacidic protein (a 4 M Gdn·HCl extract of chromatin); Gdn·HCl, guanidine hydrochloride; [^{3}H]PRov, [^{3}H]-progesterone receptor from chick oviduct; [^{3}H]ERov, [^{3}H]estrogen receptor from chick oviduct; MAb, monoclonal antibody; ELISA, enzyme-linked immunosorbent assay; PBS, 0.15 M NaCl and 0.01 M NaH_2PO_4, pH 7.2; EDTA, ethylenediaminetetraacetic acid; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; Ig, immunoglobulin.

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of binding (Boyd & Spelsberg, 1979; Spelsberg & Halberg, 1980; Boyd-Leinen et al., 1982, 1984). Similar protein–DNA complexes serving as acceptors have been found for estrogen and progesterone receptors in cow, rabbit, and hamster uteri (Ruh et al., 1981; Ross & Ruh, 1984; Singh et al., 1984; Cobb & Leavitt, 1985) as well as in sheep brain (Perry & Lopez, 1978), androgen receptors in the rat prostate (Klyzsejko-Stefanowicz et al., 1976), and estrogen receptors (ERov) in chick oviduct (Ruh & Spelsberg, 1982). These chromatin acceptor sites seem to be different from those described in other systems, which consist of the DNA sequences flanking the 5′ end of steroid-regulated structural genes (Mulvihill et al., 1982; Payvar et al., 1983; von der Ahe et al., 1985).

Monoclonal antibodies that appear to recognize the native chromatin acceptor sites for the avian oviduct progesterone receptor have recently been reported (Goldberger et al., 1986). Partially purified acceptor protein was reannealed onto hen DNA, and the excess DNA was removed by limited DNase I digestion to give enriched reconstituted acceptor sites. The reconstituted PRov binding sites were previously shown to be similar to native sites (Spelsberg et al., 1984). The MAbs (as ascites fluids) were able to inhibit PRov binding to native acceptor sites without affecting the receptor itself or the nonspecific receptor binding to pure DNA. Direct interaction of the MAbs with native acceptor sites was demonstrated in an ELISA (Goldberger et al., 1986). One of the hybridoma lines, chosen for its ability to inhibit PRov binding to the chromatin acceptor sites on NAP, was subcloned a second time. Three resulting MAbs were used to characterize the PRov nuclear acceptor sites.

Materials and Methods

Receptors. Partially purified chick oviduct [^{3}H]progesterone receptor was prepared as described previously (Boyd & Spelsberg, 1979) for use in cell-free binding assays. Estrogen receptor was isolated from hen oviduct as in Ruh and Spelsberg (1982), except that the length of cytosol incubation with [^{3}H]estradiol was reduced to 2 h.

Chromatin, Nucleoacidic Protein, and DNA. Chromatin and NAP isolations from avian oviduct as well as DNA isolation from hen spleen are described elsewhere (Spelsberg et al., 1984). Native NAP results from the extraction of chromatin with 4.0 M guanidine hydrochloride (Gdn·HCl) and represents enriched acceptor sites from whole chromatin. Chromatin extracted with 3.0 M Gdn·HCl was prepared to test binding of estrogen receptor as described previously (Ruh & Spelsberg, 1982). NAPs for species-specificity studies were made by extraction of the whole chromatin with 3.0 M Gdn·HCl.

Antibodies. The production and initial characterization of monoclonal antibodies against the nuclear acceptor sites for PRov have been described (Goldberger et al., 1986). The ascites fluids from the three hybridoma lines chosen on the basis of their inhibition of PRov binding to NAP were tested for their ability to inhibit PRov binding to whole chromatin. The line giving the best results (1) was subcloned for a second time by limiting dilution. The resulting subclones were tested for production of antibodies that inhibited the specific binding of [^{3}H]PRov to NAP, but did not inhibit nonspecific binding to pure DNA. Three cell lines (D2, A4, H7) were chosen for further characterization and were grown as ascites fluids. For some experiments, the antibodies were purified on protein A–Sepharose. Control ascites fluids contained antibodies against bovine coagulation factor 5. Other controls included purified mouse IgG and a monoclonal IgG against the 90-kilodalton subunit of the chicken oviduct progesterone receptor, which was kindly provided by Dr. David Toft. Titers were determined in an ELISA system utilizing alkaline phosphatase labeled goat anti-mouse IgG (H and L chain specific) and alkaline phosphatase labeled goat anti-mouse IgM (μ chain specific). The IgG concentrations of the ascites fluids were 0.8 (H7), 0.26 (D2), 1 (A4), and 0.5 mg/mL (control). Protein levels in ascites fluids were assayed by the method of Bradford (1976) and were similar for all.

Cell-Free Receptor Binding Assay. The interaction of PRov with chromatin or NAP and DNA in cell-free binding assays was measured by use of the streptomycin assay (Spelsberg, 1983). Slight modifications (Ruh & Spelsberg, 1982) were made to measure ERov binding. Briefly, 150 μL of [^{3}H]PRov (4.6×10^5 dpm) or [^{3}H]ERov (2.2×10^5 dpm) was added to 50 μg of NAP or chromatin in 0.14 M KCl, 0.001 M EDTA, 0.012 M monothioglycerol, and 0.05 M Tris-HCl, pH 7.4, and incubated for 45 min at 4 °C with intermittent mixing. Inhibition of receptor binding by monoclonal antibodies was tested by incubating the nuclear material with varying amounts of ascites fluid or purified antibody for 15 min prior to the addition of receptor. As the binding assay is sensitive to excess salt, ascites fluids were dialyzed against 10 mM NaCl, 10 mM Tris-HCl, and 1 mM EDTA, pH 7.4, for 1 h prior to use. Triplicates of NAP and DNA were analyzed at each antibody level. The reactions were stopped by the addition of 100 μL of 1% (w/v) streptomycin sulfate in H_2O. In some studies, the nuclear material was collected on filters and processed for analysis of bound ^{3}H-labeled receptors and DNA as previously described (Spelsberg, 1983). A modification of this method involved extraction of the bound [^{3}H]progesterone from the streptomycin precipitates with 95% ethanol for 1 h at room temperature. The DNA was pelleted and quantitated (Burton, 1956) while the supernatant was diluted in a 2:1 (v/v) Phase Combining System (Amersham)/xylene fluor and counted for radioactivity. The receptor binding to NAP was corrected for binding to pure DNA by subtraction of the DNA binding from the binding to NAP. Binding of a nonfunctional receptor (Boyd-Leinen et al., 1984) was used to correct for nonspecific receptor binding to chromatin. Data shown are representative of two to three major experiments.

ELISA. Direct binding of MAbs to NAP, DNA, and partially purified acceptor protein was analyzed in an ELISA system as previously described (Goldberger et al., 1986). The standard assay was washed with 0.05% Tween 20 in PBS after incubation of MAbs and anti-mouse IgG. Studies of binding to NAPs from other species required a high stringency wash of 5 M urea, 0.1 M NaCl, and 0.02 M Tris-HCl, pH 7.5, after incubation of MAbs. Goat anti-mouse IgG–alkaline phosphatase was diluted at 1:500–1:750. Release of *p*-nitrophenol was monitored at 410 nM (A_{410}) on a Dynatech plate reader. The data are representative of repeat analyses with the ELISA.

Results

Inhibition of [^{3}H]PR Binding by MAbs. Figure 1 shows that ascites fluids from all three subclones were able to inhibit specific PRov binding to NAP from hen oviduct. The level of [^{3}H]PR binding to DNA was not affected, while the binding to NAP was reduced to the DNA level by addition of increasing amounts of antibody as reported previously (Goldberger et al., 1986). Control ascites containing antibodies against an unrelated protein did not inhibit specific PRov binding to NAP (Figure 1). The IgG concentration in the ascites fluids was determined in an ELISA, which indicated that about 17 μg of H7, 26 μg of A4, and 8 μg of D2 as IgG gave optimal inhibition of PRov binding. All three ascites fluids as well as the control had similar protein concentrations.

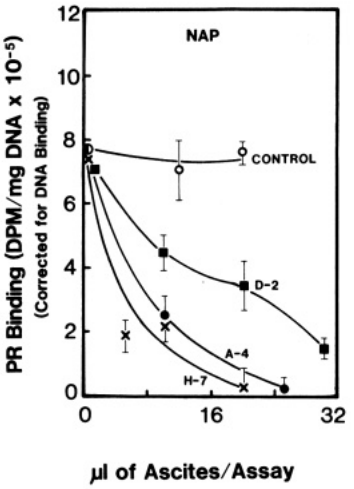

FIGURE 1: Comparative effects of ascites fluids containing MAbs against the PRov acceptor site on PRov binding to NAP. Ascites fluids D2 (■), A4 (●), and H7 (×) containing anti-acceptor site antibodies and a control ascites (○) containing antibody against an unrelated protein were included in the cell-free binding assay with 50 µg of NAP as described. The mean and standard error of triplicate analyses within the same experiment are plotted at each antibody level. Binding of PRov to NAP was corrected for DNA binding.

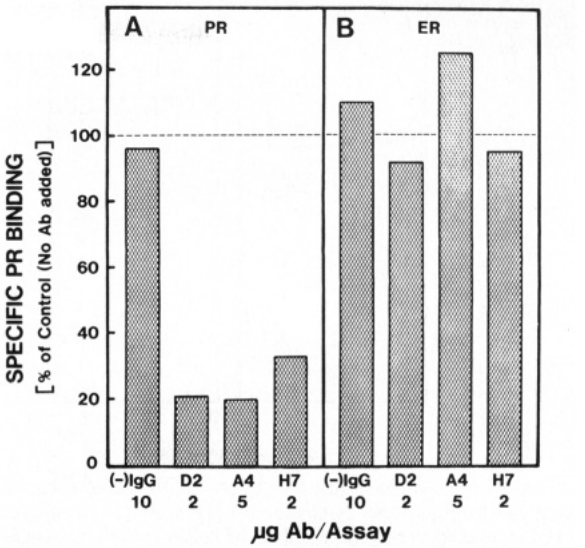

FIGURE 2: Inhibition of ERov nuclear binding by purified MAbs against PRov acceptor sites. NAP was incubated with PRov (A) or ERov (B) in the presence of the indicated amounts of antibody as IgG. MAbs against PRov acceptor were purified from ascites on protein A–Sepharose. The dotted line shows the level of binding without antibody, which was 1×10^6 dpm/mg of DNA for PRov and 30 000 dpm/mg of DNA for ERov. All values were corrected for DNA binding.

Variations in the extent to which the MAbs inhibited PRov binding to NAP sometimes occurred between sets of assays. This may have been due to the known sensitivity of the cell-free binding procedure to several factors (Littlefield & Spelsberg, 1985).

The ability of the three antibodies to inhibit PRov binding to native acceptor sites in whole chromatin was also tested in the cell-free binding assay (data not shown). More antibody (50–75 µL) was required to inhibit PRov binding to chromatin than to NAP, and control (negative) Abs also began to display marked inhibition. This is probably due to the nonspecific adsorption of protein to chromatin (Thrall et al., 1978; Webster et al., 1976), as well as the effects of a large excess of protein on the ionic strength of the reaction mixture. It should be mentioned that the chromatin extensively aggregates when the high levels of ascites or even purified Ab are added. This problem could explain the nonspecific inhibition of the acceptor sites on whole chromatin. Therefore, all of the subsequent studies utilized the more soluble NAP, which appears to contain the same PRov acceptor sites as the native chromatin (Webster et al., 1976; Boyd & Spelsberg, 1979; Spelsberg et al., 1983, 1984; Goldberger et al., 1986).

Specificity of the Inhibition. The receptor specificity of inhibition by the antibodies was also examined. It has been previously shown that the acceptor site for the estrogen receptor is distinct from that of the PRov acceptor (Kon & Spelsberg, 1982). Therefore, the three MAbs were tested for their ability to inhibit binding of ERov to its acceptor site in hen NAP. However, the crude ERov was particularly labile when incubated with ascites fluids. The IgG fractions were therefore purified by chromatography on protein A–Sepharose and again tested for inhibition of both PRov and ERov binding. As shown in Figure 2A, the purified antibodies still inhibited specific PRov binding to NAP. In contrast, none of the MAbs including a control mouse IgG fraction had an effect on ERov binding (Figure 2B).

The antibodies were next screened for their inhibition of PRov binding to NAP from other hen tissues, as well as to NAPs from other species. Figure 3 indicates that two of the MAbs (D2 and H7) were as effective in inhibiting receptor binding to hen spleen NAP as they were in blocking binding

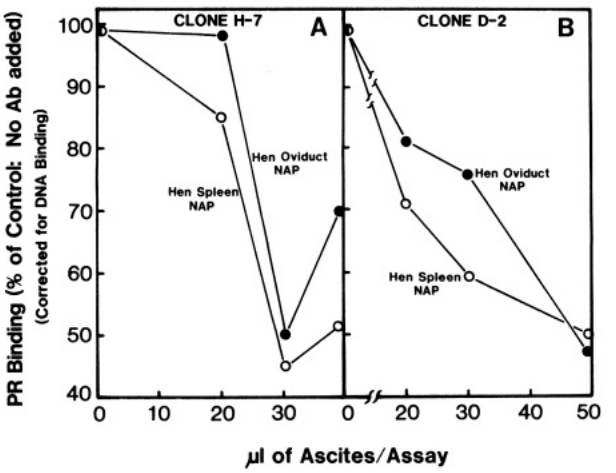

FIGURE 3: Tissue specificity of inhibition by MAbs in ascites. The abilities of MAbs H7 (A) and D2 (B) to block PRov binding to hen oviduct NAP (●) and hen spleen NAP (○) were tested in the cell-free binding assay as described. All values were corrected for receptor binding to pure DNA at the corresponding antibody level. The binding at 100% was $(4–5) \times 10^5$ dpm/mg of DNA for both NAPs.

to hen oviduct NAP. Ascites A4 (data not shown) had a similar effect. The curves were not exactly the same as those in Figure 1 with oviduct NAP. However, the oviduct and spleen curves are very similar within that set of assays. Variations in the extent of inhibition of PRov binding did occur between assays and may have been due to the previously characterized sensitivity of the cell-free binding assay to several factors (Littlefield & Spelsberg, 1985). Different preparations of receptor, NAP, and even Ab yielded some differences in these patterns. Assays performed during the same period of time using the same preparations of the above components of the binding assay showed very similar patterns of binding and amounts of required antibodies.

Studies of the species specificity of inhibition by the antibodies indicated that only the [^{3}H]PRov binding to hen NAP could be completely blocked by all the MAbs (Figure 4). The inhibition of binding to NAPs from tissues of other species

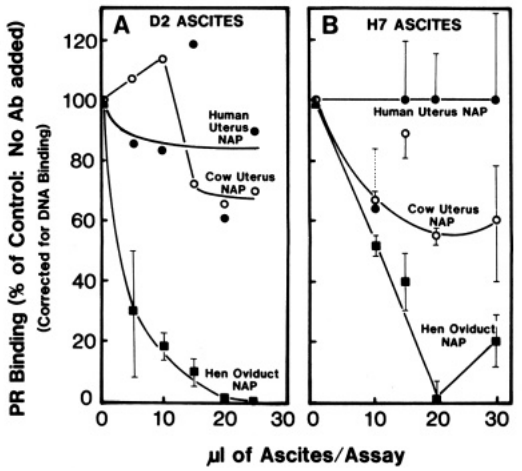

FIGURE 4: Species specificity of antibody inhibition of PRov binding using MAbs in ascites. NAPs from progesterone-responsive tissue of the various species were tested in the cell-free binding assay. Fifty micrograms of each NAP was incubated with increasing amounts of D2 (A) or H7 (B) ascites fluids. The mean and standard error of triplicate analyses at each antibody level are plotted for a representative experiment. All values were corrected for PRov binding to hen DNA at the corresponding antibody levels. Binding at 100% was (2.5–5) $\times 10^5$ dpm/mg of DNA for all three NAPs.

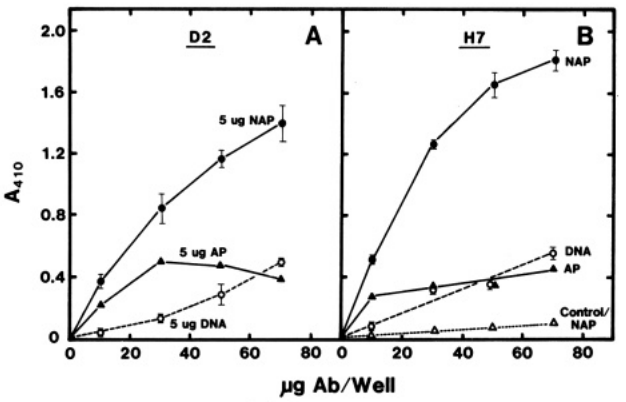

FIGURE 5: Direct binding of purified MAbs to PRov nuclear acceptor sites in an ELISA. The ELISA was performed as described under Materials and Methods. The binding of protein A purified D2 (A) and H7 (B) to NAP (●), DNA (○), and partially purified acceptor protein (▲) was tested. Background binding of a control MAb to NAP was also determined (△). Amounts of antibody per well are given as IgG. Five micrograms of NAP and pure DNA as DNA were plated, while 5 µg of protein was used. Data represent the average of two separate experiments.

varied, but was never more than about 50% of the inhibition seen with hen NAP (Figure 4). Similar results were seen with several other species, including rabbit and frog (data not shown). In the absence of antibody, hen oviduct PRov bound human, rabbit, and cow NAPs at levels close to that seen with hen NAP, while frog oviduct was bound at about 30% and hagfish liver showed little binding (data not shown).

Direct Binding of MAbs to Nuclear Sites. The ability of antibodies from the parent hybridoma line to preferentially recognize intact acceptor sites (NAP) over DNA with saturable binding in an ELISA has been demonstrated (Goldberger et al., 1986). Antibodies from two of the subclones, D2 and H7, can also give a high level of saturable binding to NAP in the ELISA, while only low-level, linear binding to DNA was seen (Figure 5). In addition, the recognition of a partially purified acceptor protein fraction by the antibodies was tested. More D2 (Figure 5A) bound to the free protein than to DNA, but the amount was significantly lower than that seen with the intact site. The binding to acceptor protein

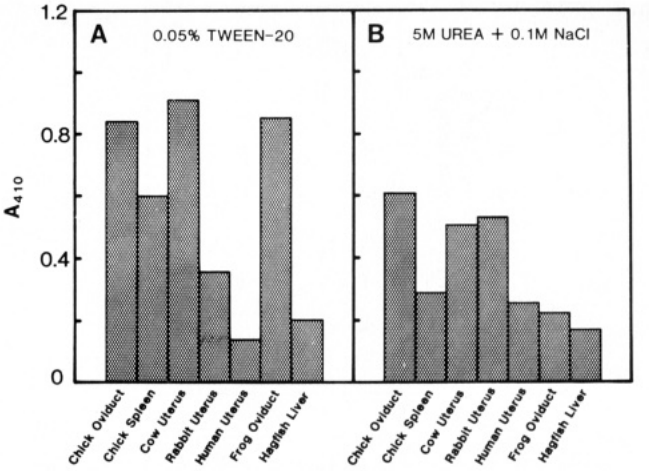

FIGURE 6: Species and tissue specificity of the purified MAb binding to NAPs in the ELISA. Five micrograms of each NAP (as DNA) was plated. Twenty micrograms of purified D2 MAb (as IgG) was added to the wells. (A) Wells were washed with 0.05% Tween 20 after incubation with MAb. (B) Wells were washed with 5 M urea and 0.1 M NaCl after incubation with MAb. The remainder of the assay was processed as described. Data are representative of three experiments.

also saturated at a much lower amount of antibody (30 µg) than the binding to NAP (70 µg), indicating that fewer antigenic sites were present. Binding of H7 to acceptor protein and to DNA was about the same (Figure 5B). Background binding of a control monoclonal antibody to NAP was very low (Figure 5B).

Species and tissue specificities of the direct antibody binding were also assayed. High levels of antibody binding were observed with several NAPs besides chicken oviduct (Figure 6A). The wash stringency was increased to try to eliminate any nonspecific interactions while preserving high-affinity recognition. Higher concentrations of Tween 20 (0.5%) or Tween 80 (0.05 and 0.5%) enhanced all binding as did low concentrations of urea, while salt did not have much effect at all (data not shown). However, the combination of 5 M urea and 0.1 M NaCl, pH 7.2, was effective in reducing antibody interactions with NAPs of several of the species tested (Figure 6B). Chicken oviduct NAP retained the highest MAb binding after the more stringent washing, but rabbit and cow NAPs still gave high values. Similar results were obtained with H7 and A4 (data not shown). However, after the urea/NaCl wash, the interaction of the three MAbs with chicken spleen NAP was significantly less than with chicken oviduct NAP (Figure 6B).

Discussion

We have utilized monoclonal antibodies that appear to be directed against the nuclear acceptor sites for the avian oviduct progesterone receptor to further characterize the acceptor sites themselves. While relationships between acceptor sites in several tissues and species are shown, we have made no attempt to form absolute quantitative comparisons. The antibodies were produced by subcloning one of the hybridoma lines discussed previously (Goldberger et al., 1986). The original immunogen was a reconstituted acceptor site that had been enriched by nuclease digestion. All three MAbs were able to block binding of PRov to the native nuclear acceptor sites in NAP, while the nonsaturable binding to pure DNA was not affected. Control ascites fluid against an irrelevant protein also did not inhibit PRov binding. Considerably more ascites was required to completely block the binding to chromatin than to NAP. Recent additional studies have revealed that control

ascites significantly inhibited PRov binding at these Ab levels. These effects are believed to be due to the marked nonspecific adsorption of protein to chromatin (Webster et al., 1976; Thrall et al., 1978) as well as ionic effects of an excess of protein on cell-free binding assay, which is very sensitive to such factors (Littlefield & Spelsberg, 1985). However, it should be mentioned again that antibodies from the parent hybridoma line did inhibit PRov binding to chromatin at levels where no inhibition was seen by control ascites (Goldberger et al., 1986). Some of the inhibition by these three sublines, therefore, is probably specific.

Comparison of the direct binding of the MAbs to intact nuclear acceptor sites with their binding to the isolated protein and DNA components in the ELISA indicated a marked recognition of the protein–DNA complex, which was significantly greater than the minimal binding by the free protein and the nonsaturable binding to pure DNA. The MAbs also did not recognize the free protein on Western blots (data not shown). These observations support the idea that MAb binding to the free protein is of low affinity and/or specificity compared to the recognition of the intact acceptor site. Such specificity is not surprising, since reconstituted protein–DNA complexes were used to immunize the mice and the resulting hybridomas were screened for the inhibition of PRov binding to native complexes in NAP.

The antibodies did not show tissue specificity in inhibition of PRov binding, as reduction of binding to spleen NAP paralleled the effect on binding to oviduct NAP. This supports previous studies showing that spleen chromatin, which displays little binding of PRov, contained PRov acceptor sites that were "unmasked" by extraction with 4.0 M Gdn·HCl (Webster et al., 1976; Thrall et al., 1978; Spelsberg et al., 1983). These spleen acceptor sites appear to be similar to those of oviduct. Therefore it was not surprising that the antibodies were able to inhibit receptor binding to spleen NAP. However, the direct binding of the MAbs to spleen NAP in the ELISA was more easily reversed by a high-stringency wash than the binding to hen NAP. Whether the sites in spleen chromatin are identical with those in oviduct chromatin remains to be determined.

Studies to determine the species specificity of inhibition of PRov binding by the MAbs showed some cross-reactivity. Progesterone receptor binding to NAP from most of the non-avian species tested was never reduced below 50% of control values when inhibition was seen. Binding to hen NAP was more significantly inhibited at the same MAb levels. The fact that some inhibitory activity of the MAbs was seen with NAPs from other species suggested that the nuclear acceptor sites for PRov may be at least partly conserved over evolution. A similar conclusion has been reached from studies of hen oviduct PRov binding to chromatins from other species (data not shown) and from analysis of PRov binding to reconstituted acceptor containing genomic DNA from other species (Toyoda et al., 1985). Therefore, the nuclear acceptor sites for steroid receptors may be evolutionarily conserved, as are the steroids and their receptors (Buller et al., 1976; Greene et al., 1977).

Related studies using the ELISA indicated that the NAPs from several species were bound by the antibodies at a level similar to that of hen oviduct. The binding to hen, rabbit, and cow NAPs proved to be more resistant to a high-stringency wash with urea and NaCl than the binding to hagfish and frog oviduct. Although high-affinity recognition appears to be restricted to hen and a few other species, there are sites in the NAPs of several other species that appear to be bound by these MAbs. The direct-binding data also support the idea of some conservation of PRov nuclear acceptor sites through evolution. However, these results also seem to conflict with the PRov inhibition studies, since the MAbs could bind the NAPs under relatively mild conditions but were unable to block PRov binding. The MAbs and PRov may have different affinities for the same acceptor domains in the NAPs from other species, particularly if the antibody binding site is close to but does not exactly correspond to the receptor binding site. A slight change in the antigenic determinant between species could greatly affect antibody binding affinities without affecting receptor binding. Therefore, the antibody could bind NAP, although not tightly enough to prevent PRov binding.

The antibodies do appear to be receptor specific. Inhibition of the binding of PRov, but not ERov, was observed with protein A purified antibodies. These data support previous studies showing that (1) the nuclear acceptor sites for ERov and PRov in avian oviduct are distinct (Kon & Spelsberg, 1982) and that (2) those for glucocorticoid receptor as well as ERov and PRov are distinct (Hager et al., 1980).

These results support earlier work from this laboratory showing that reconstituted nuclear acceptor sites are native-like (Spelsberg et al., 1984). The monoclonal antibodies generated against the reconstituted sites recognize the native acceptor sites on the NAP and can specifically block the binding of receptor to them. In addition, the MAb data support the premise that the PRov nuclear acceptor sites in chromatin and NAP are similar (Spelsberg, 1982; Spelsberg et al., 1983, 1984) but differ from the binding sites on DNA (Thrall & Spelsberg, 1980) as assayed by antibody inhibition of PRov binding. The antibodies should be valuable probes in the further characterization of the intact acceptor sites.

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Control of the Action of Phospholipases A by "Vertical Compression" of the Substrate Monolayer

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ABSTRACT: Monolayers of *rac*-1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol at an air–water interface were "vertically compressed" by substituting an alkylated glass plate for air while maintaining a constant surface pressure of 15 mN m^{-1}. At this surface pressure the overlaying of the lipid film by the alkylated surface resulted in an average increase of 16 $Å^2$/molecule in the mean molecular area of those phospholipid molecules residing at the interface between water and the alkylated glass. Subsequently, the activities of phospholipases A1 and A2 toward the monolayers were measured both in the presence and in the absence of the support. While phospholipase A1 activity was increased 4-fold by the support, the activity of phospholipase A2 was reduced to 15% of the activity measured in the absence of the alkylated surface. These findings indicate that such a "vertical compression" of the monolayer is likely to induce a conformational change in the phospholipid molecules, which in turn would cause the above reciprocal changes in the activities of phospholipases A1 and A2. A molecular model accounting to these findings is presented.

Phospholipases A1 and A2 hydrolyze *sn*-1 and *sn*-2 fatty acyl ester bonds, respectively, of *sn*-3 phospholipids (de Haas et al., 1968). Phospholipase A2 (EC 3.1.1.4, PLA2)[1] is present in pancreatic tissue and juice and in snake and bee venoms [for a review, see Verheij et al. (1981)]. Intracellular PLA2s have been found in almost every type of cell studied (van den Bosch, 1980). PLA2s are the best characterized of the lipolytic enzymes, and their catalytic mechanisms, amino acid sequences, and X-ray crystallographic structures have been worked out (Verheij et al., 1981; Dijkstra et al., 1981a). Far less is known about the properties of phospholipases A1 [PLA1; for a review, see van den Bosch (1982)].

Phospholipases A belong to the group of esterases acting at lipid–water interfaces on water-insoluble substrates. Micellarization of the substrate strongly enhances the rate of catalysis (Verheij et al., 1981), and even 10^4 times higher activities are observed when the critical micellar concentration of the substrate phospholipid is exceeded (Wells, 1978). In spite of the efforts made, no general agreement exists on the molecular basis of this interfacial activation. The most attractive viewpoints are as follows: (a) the substrate theory,

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[1] Abbreviations: PLA, phospholipase A; $diC_{12}PG$, 1,2-didodecanoyl-*sn*-glycero-3-phosphoglycerol; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

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