MOLECULAR PHARMACOLOGY, 25:86-91

Specificity of Dye-Ligand Interaction with the Polynucleotide Binding Domain of 1,25-Dihydroxyvitamin D_3-Receptor Complexes of Chicken Intestinal Cytosol

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Received January 19, 1983; Accepted August 11, 1983

SUMMARY

The binding of 1,25-dihydroxyvitamin D_3-receptor complexes from chick intestinal cytosol to DNA-cellulose and isolated intestinal nuclei is inhibited by several dye-ligands in a dose-dependent manner. Concentrations of Cibacron blue F3GA, blue dextran, Procion red HE3B, and Green A dye causing 50% competition for receptor binding to DNA-cellulose ranged from 2.8 to 3.6 μM. A structural analogue of the anthraquinone moiety of Cibacron blue F3GA, bromaminic acid, was 111-fold less potent in inhibiting DNA-cellulose binding. Moreover, the inhibitory effects of these dye-ligands is not due to a simple electrostatic effect, since two other polyanions, heparin and poly-L-glutamate, are much less effective. Whereas dye-ligands can cause the release of receptors bound to DNA-cellulose, they do not alter the dissociation of 1,25-dihydroxyvitamin D_3 from its receptor nor do they affect the apparent equilibrium binding constant of the receptor or the concentration of available sterol-bindng sites. The inhibition of binding by dye-ligands is competitive with respect to DNA-cellulose binding, indicating that the effect of these dyes is at a domain common to polynucleotides.

INTRODUCTION

Receptors isolated from rachitic chicken intestinal cytosol bind $1,25(OH)_2D_3$[1] with high affinity and low capacity (1). While strong evidence has been presented that a hormonal action of $1,25(OH)_2D_3$ is regulation of intestinal calcium transport via gene expression (2), there is no direct evidence that $1,25(OH)_2D_3$ receptors mediate this event. However, the ability of this receptor to discriminate between vitamin D analogues (3), the localization of this receptor in nuclei of target cells (4, 5), and the specificity of this receptor for AT-rich segments of double-stranded DNA (6), suggest a cause-and-effect relationship between the association of $1,25(OH)_2D_3$ with its receptor and the subsequent initiation of a hormone-specific response.

CB has been shown to bind to several nucleotide-binding proteins, including nucleotide-requiring enzymes (7, 8), RNA-polymerase (9), bacterial and HeLa cell DNA-polymerases (10, 11), and the estrogen receptor (12). For several of these proteins, CB binding occurs exclusively at the nucleotide-binding site. Recent evidence suggests that $1,25(OH)_2D_3$-receptor complex binding to dye-ligands occurs through the polynucleotide binding domain (13). This conclusion is based upon the specificity of polyguanylic and polyinosinic acid in desorbing receptors from immobilized dye-ligands (e.g., CB). It is the purpose of this paper to examine carefully the triazinyl dye interaction with chicken intestinal $1,25(OH)_2D_3$-receptor complexes. From the results presented, it is concluded that these dye-ligands bind at the polynucleotide domain of $1,25(OH)_2D_3$ receptors and not at the sterol-binding site.

MATERIALS AND METHODS

Animals. White-Leghorn cockerels were maintained on a vitamin D-deficient diet and housed as described in the accompanying paper (13).

Chemicals. The chemicals used and their sources are as follows: CB (molar absorptivity of 13.6×10^3 M^{-1} cm^{-1} at 610 nm), Pierce Chemical Company (Rockford, Ill.); GA (molar absorptivity of 15.1×10^3 M^{-1} cm^{-1} at 430 nm), generously supplied by Mr. Scott Fulton of the Amicon Company (Danvers, Mass.); hexylene glycol, Eastman Kodak Company (Rochester, N. Y.); cellulose (Cellex 410) and hydroxylapatite, Bio-Rad Laboratories (Richmond, Calif.); Sephadex G-25, Pharmacia (Piscataway, N. J.); bromaminic acid (molar absorptivity of 6.57×10^3 M^{-1} cm^{-1} at 483 nm), Pfaltz and Bauer, Inc. (Stamford, Conn.); and PR (molar absorptivity of 11.3×10^3 M^{-1} cm^{-1} at 522 nm) and calf thymus DNA, Sigma Chemical Company (St. Louis, Mo.). Other reagents and chemicals used are described in the preceding article (13).

This work was supported in part by National Institutes of Health Program Project Grant AM-27234 and the Graduate School of the University of Wisconsin-Madison. A preliminary report of a portion of this work was presented at the Endocrine Society Meeting, San Francisco, Calif., 1982.

[1] The abbreviations used are: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; CB, Cibacron blue F3GA; PR, Procion red HE3B; GA, Green A dye; TMDK; 50 mM Tris-HCl, 0.5 mM $MgCl_2$, 0.5 mM dithiothreitol, and KCl of various molarities.

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All chemicals, except CB, were used without further purification. CB was purified by preparative thin-layer chromatography on activated silica gel (2-mm thick; heated at 100° for 1.0 hr just prior to chromatography), obtained from EM Laboratories (Elmsford, N. Y.). After the plates were developed in tetrahydrofuran-water (48:7, v/v) (14), the major blue band migrating with an R_F of 0.53 was removed by scraping the plate; the dye was eluted from the silica gel with methanol. The solvent was removed by rotary evaporation, and the remaining dye was oven-dried at 100° for 4.0 hr and stored under vacuum with silica gel. Chromatography (silica gel plates, 0.25 mm) of the purified dye gave one major band (R_F 0.52) with one trace contaminant (R_F 0.49).

Preparation of cytoplasmic binding protein. Cytosol was prepared using TMDK-0.3 buffer (pH 7.5) and stored as described in the accompanying paper (13). The protein concentration was determined by the method of Bradford (15), using crystalline bovine serum albumin as a standard.

Isolation of chicken intestinal nuclei. Nuclei were isolated by the procedure described by Wray and co-workers (16), utilizing hexylene glycol buffer (0.5 M hexylene glycol, 0.1 mM $CaCl_2$, 5 mM Tris, and 0.5 mM dithiothreitol, pH 7.5). The final nuclear pellets were diluted in the appropriate TMDK buffer for studies using intact nuclei. The DNA content of aliquots was quantitated by the method of Richards (17).

Receptor labeling. Cytosol was incubated with 2.0 nM $1,25(OH)_2[26,27\text{-}^3H]D_3$ (148–160 Ci/mmole) at 0–4° in TMDK-0.3 buffer as described in the accompanying paper (13). Equilibrium binding studies were conducted as described by Mellon and DeLuca (1) except that the incubations were carried out in the presence or absence of CB at 0–4° for 16 hr, and sterol binding was quantitated using the hydroxylapatite batch assay technique (18). The data were analyzed by the method of Scatchard (19) to determine the equilibrium dissociation constant (K_d) from the slope of the plot with an x-intercept value equaling the molarity of binding in solution. Regression analyses utilizing least squares were performed to obtain the best fit. Dissociation rates were measured by the procedure outlined previously (1), utilizing cytosol that had been labeled at 0–4° with 2.0 nM $1,25(OH)_2[^3H]D_3$. Again, the hydroxylapatite batch-assay technique was employed for quantitating sterol-bound receptors. A plot of logarithm bound radioactive $1,25(OH)_2D_3$ versus time of incubation gives a straight line for the pseudo first-order dissociation reaction where the slope is equal to k_d in units of time^{-1}. To ensure that the dissociation process was not resulting from degradation of the hormone-receptor complex, parallel incubations were conducted without nonradioactive $1,25(OH)_2D_3$. Regression analysis was performed to obtain the best fit.

Receptor binding to DNA-cellulose. The preparation of DNA-cellulose was performed as described previously (6). Quantitation of $1,25(OH)_2[^3H]D_3$-receptor complex binding to DNA-cellulose (50 μg of calf thymus DNA per pellet, unless otherwise stated) in TMDK-0.03 buffer was conducted by the method of Radparvar and Mellon (6).

RESULTS

To assess the relative potency of several dye-ligands in competing with DNA for the polynucleotide domain of $1,25(OH)_2D_3$-receptor complexes, DNA-cellulose was incubated in the presence, or absence, of several concentrations of dyes along with $1,25(OH)_2[^3H]D_3$-receptor complexes (Fig. 1). Comparison of binding capabilities was evaluated at the concentration of competitor which resulted in a 50% decrease in specific DNA binding. Concentrations of CB, blue dextran, GA, and PR causing 50% competition ranged from 2.8 to 3.6 μM. At least for the blue chromophore, an attempt was made to estimate whether the anthraquinone moiety of the dye alone would be adequate for receptor binding. Various concetrations of bromaminic acid (1-amino-4-bromo-anthraquinone-2-sulfonate) were much less effective in inhibiting receptor binding to DNA. The 50% competition value was estimated to be 300 μM. Pretreatment of DNA-cellulose with a 5–10 μM dye-ligand mixture, followed by several washes with TMDK-0.03, did not affect subsequent binding of $1,25(OH)_2D_3$-receptor complexes to the matrix, indicating that the previously observed dye-ligand inhibition was not due to a dye interaction with DNA (data not shown).

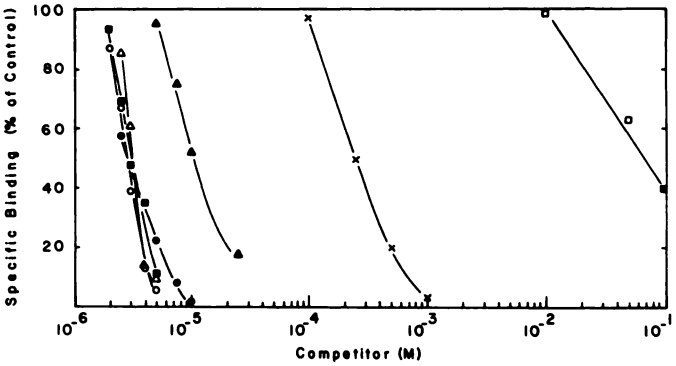

FIG. 1. *Effect of dye-ligands and polyanions on $1,25(OH)_2D_3$-receptor complex binding to DNA-cellulose*

To packed DNA-cellulose pellets or cellulose pellets were added reaction mixtures (0.3–0.5 ml) consisting of receptor-$1,25(OH)_2[^3H]D_3$-complexes (0.05–0.07 nM), KCl (0.03 M), 50 mM Tris, 0.5 mM dithiothreitol, 0.5 mM $MgCl_2$ (pH 7.5), and various concentrations of the following: CB (○), PR (△), dextran blue (●), GA (■), heparin (▲), bromaminic acid (×) and poly-L-glutamic acid (□). Incubations were carried out with constant shaking for 30 min at 4°. At the end of the incubation period, the DNA-cellulose or cellulose slurry was diluted with TMDK-0.03 buffer and washed, and the final pellets were suspended in a scintillation fluid mixture. Specific binding was quantitated by substracting the nonspecific binding (cellulose) from the total binding values (DNA-cellulose). Values represent the mean for three to six determinations.

Since the dye-ligands and bromaminic acid are sulfonated compounds, inhibition of DNA binding might be the result of a nonspecific polyanionic effect. Two polyanions, heparin and poly-L-glutamic acid were tested for their ability to inhibit DNA binding. On the basis of molecular weights for a dissaccharide repeating unit for heparin and for L-glutamic acid, heparin was 5.4-fold and poly-L-glutamic acid was greater than 28,000-fold less potent than CB in competing for 50% of the DNA-bound receptor.

Nuclear and chromatin-associated $1,25(OH)_2[^3H]D_3$ have been detected in intestinal nuclei after administration of radioactive hormone to vitamin D-deficient chickens (4). Therefore, it was of interest to determine whether these dye-ligands could inhibit receptor binding to isolated nuclei. When $1,25(OH)_2[^3H]D_3$-receptor complexes were added to chicken intestinal nuclei in the presence of dye-ligands, there was a dose-dependent inhibition of receptor binding (Table 1).

Since the addition of CB could interfere with receptor-DNA complex formation, the stability of preformed complexes to CB addition was analyzed. After $1,25(OH)_2D_3$-receptor-DNA-cellulose complexes were allowed to form, CB (10 μM) was added and binding was tested for several subsequent times. The addition of CB progressively released $1,25(OH)_2D_3$-receptor complexes (Fig. 2). Approx-

TABLE 1

Effect of dye-ligand on 1,25(OH)$_2$[^{3}H]D$_3$-receptor complex binding to isolated chick intestinal nuclei

For the binding assay, aliquots of suspended nuclei (containing 130–160 μg of DNA) were placed into 7.0-ml glass scintillation vials and washed once with 1.0 ml of TMDK-0.03 buffer. To the nuclear pellets were added the reaction mixtures (0.3–0.5 ml) consisting of receptor-1,25(OH)$_2$[^{3}H]D$_3$-complexes (0.05–0.07 nM), KCl (0.03 M), Tris-HCl (0.05 M), dithiothreitol (0.5 mM), $MgCl_2$ (0.5 mM) (pH 7.5), and various concentrations of dye-ligands. Incubations were carried out with constant shaking for 30 min at 0–4°. At the end of the incubation period, the nuclear suspensions were diluted with TMDK-0.03 buffer and washed as described for the DNA-cellulose assays, and the final pellets were suspended in a scintillation fluid mixture. Specific binding was quantitated by subtracting the nonspecific binding from the total binding values. Values represent the means for triplicate determinations ± standard deviation. Control cytosol (no dye-ligand) had a value of 9–13.2 pg of specific 1,25(OH)$_2$[^{3}H]D$_3$ bound per nuclear pellet.

Dye-ligand	Concentration	Specific 1,25(OH)$_2$[^{3}H]D$_3$ bound/nuclear pellet
	μM	% control
CB	10	29.5 ± 7.1
	25	10.6 ± 7.5
PR	12.5	10.6 ± 4.5
	25	3.7 ± 0.3
GA	5	96.0 ± 16.9
	12.5	13.6 ± 4.8
	25	2.5 ± 1.7

imately 60% of the specific receptor complexes were desorbed at 1 min after CB addition, and desorption progressed to approximately 80% over the next 20 min. In contrast, over the same time period, there was no release of receptors due to addition of TMDK-0.03 buffer.

The observed inhibition of receptor binding to DNA-cellulose by CB could also be mimicked by loss of 1,25(OH)$_2$D$_3$ binding. Thus, if CB causes dissociation of sterol from its receptor, it may be construed as loss of DNA binding. Alternatively, an action by CB at the sterol-binding site may be able to alter the polynucleotide domain in such a way so as to prevent binding to DNA. To test such possibilities, the rate of dissociation (k_d) of 1,25(OH)$_2$D$_3$ at 25° and determination of the apparent equilibrium dissociation constant (K_d) of the chicken intestinal receptor for 1,25(OH)$_2$D$_3$ were tested in the presence or absence of CB. The results in Fig. 3 show that there was essentially no difference in the rates of dissociation, 33.1×10^{-3} min^{-1}and 32.2×10^{-3} min^{-1} for control and CB-treated cytosol, respectively. Furthermore, analysis of 1,25(OH)$_2$D$_3$ binding to chicken intestinal cytosol was conducted in the presence or absence of CB to determine whether the dye could interact with the unoccupied sterol-binding site. Utilizing 5 μM CB, specific saturation of receptor with respect to 1,25(OH)$_2$D$_3$ binding occurred at 0.6–0.8 nM, identical with that for untreated cytosol (Fig. 4). Scatchard analysis of the specific binding data (Fig. 4, *inset*) yielded linear plots. The K_d values derived from the slopes of the lines were 77 pM and 98 pM for untreated and CB-treated cytosol, respectively. In addition, the concentrations of the binding sites estimated from the intercepts of the

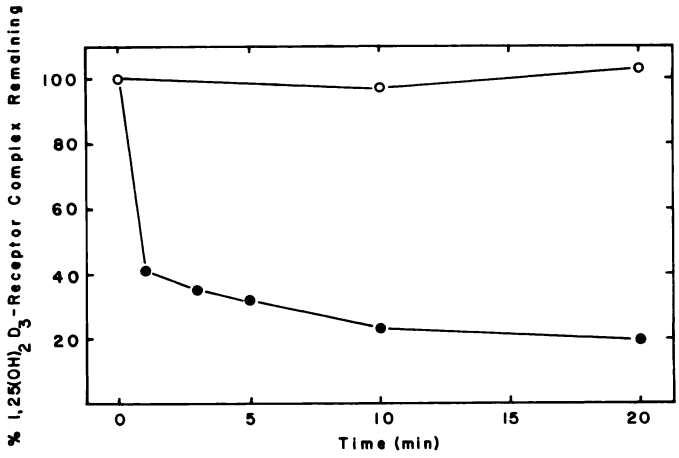

FIG. 2. *Desorption of 1,25(OH)$_2$D$_3$-receptor complexes bound to DNA by addition of CB*

1,25(OH)$_2$D$_3$-receptor complexes were incubated with DNA-cellulose pellets as described under Materials and Methods. Vials were assayed for specifically bound receptor to DNA after addition of TMDK-0.03 buffer (○) or 10 μM CB (●) at various times. Values represent triplicate determinations.

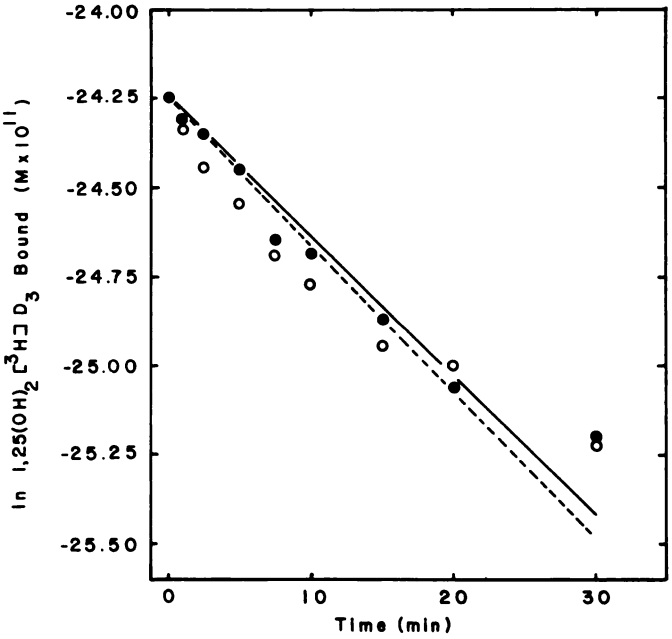

FIG. 3. *Effect of CB on the dissociation of 1,25(OH)$_2$[^{3}H]D$_3$ from chicken intestinal receptor complexes at 25°*

After incubation of chicken intestinal cytosol with 1,25(OH)$_2$[^{3}H]D$_3$ at 4° in the presence (nonspecific) or absence (total) of a 100-fold excess of nonradioactive 1,25(OH)$_2$D$_3$, the incubation temperature was raised to 25°. A 500-fold excess of nonradioactive sterol was added in the presence (○) or absence (●) of 10 μM CB. Samples were withdrawn at varying times and quantitated for specifically bound 1,25(OH)$_2$[^{3}H]D$_3$ (total minus nonspecific values) using the hydroxylapatite batch assay described under Materials and Methods. The dissociation data for 30 min were plotted as the logarithm of bound 1,25(OH)$_2$D$_3$ as a function of time after addition of the 500-fold excess of nonradioactive 1,25(OH)$_2$D$_3$ (1). The dissociation rate constant for the pseudo-first-order reaction was calculated from the slopes of the regression lines to be 33.1×10^{-3} min^{-1} for control (●) and 32.2×10^{-3} min^{-1} for incubation carried out in the presence of CB (○). The curves represent the means of triplicate determinations.

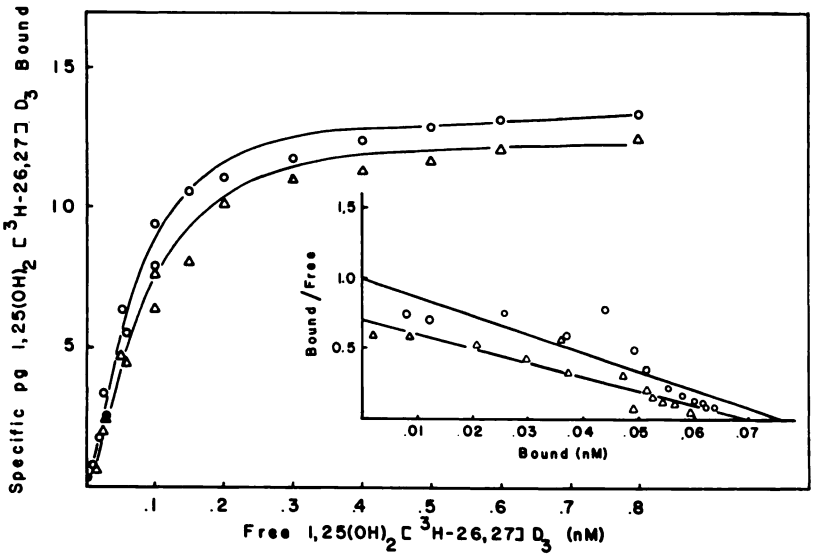

FIG. 4. *Effect of CB on the determination of the apparent equilibrium dissociation constant of chicken intestinal receptor for 1,25(OH)$_2$D$_3$*

Cytosol (0.2 mg) was incubated with increasing concentrations of 1,25(OH)$_2$[26,27-^{3}H]D$_3$ (0.02–0.80 nM) at 0–4° for 16 hr in the presence (nonspecific) or absence (total) of a 200-fold excess of nonradioactive 1,25(OH)$_2$D$_3$ and in the absence (○) or presence (△) of 5 μM CB. Subtraction of nonspecific binding from total binding was used to generate specific binding. Bound radioactivity was quantitated by the hydroxylapatite procedure and analyzed as described under Materials and Methods. Values represent duplicate or quadruplicate determinations combined from two separate experiments. The equilibrium dissociation constants (K_d) for specific 1,25(OH)$_2$D$_3$ binding calculated from the slopes of the regression lines were 77 pM (range of 28–80 pM) and 98 pM (range of 90–103 pM), whereas the concentrations of specific binding sites estimated from the x-intercepts were 0.19 pmole/mg of protein (range of 0.16–0.19 pmole/mg of protein) and 0.17 pmole/mg of protein (range of 0.171–0.175 pmole/mg of protein) for control and CB incubations, respectively.

abscissa were indistinguishable. Control cytosol had a value of 0.19 pmole/mg of protein, whereas cytosol treated with CB was on the order of 0.17 pmole/mg of protein.

The results thus far indicate that several dye-ligands interact with the polynucleotide domain of the 1,25(OH)$_2$D$_3$ receptor and not the sterol-binding site. To characterize further the inhibitory interaction of dye-ligands with respect to DNA binding and to verify that this effect was mediated through a common site on the protein, the inhibitory data were analyzed by double-reciprocal plots. When a constant amount of 1,25(OH)$_2$[^{3}H]D$_3$-receptor complexes was added to increasing concentrations of DNA-cellulose (1.0–5.0 μg of DNA) in the presence or absence of dye-ligand, the reciprocal plots of binding data revealed typical competitive inhibition patterns (Fig. 5). Interestingly, CB bound to dextran (blue dextran) showed analogous competitive inhibition with respect to DNA binding.

The strong inhibitory action of dye-ligands on the DNA-cellulose binding assay can be overcome by filtration of the dye-receptor complex through Sephadex-G25. Data presented in Table 2 demonstrate that labeled cytosol incubated in the presence of 10 μM CB and then subjected to gel filtration restores greater than 90% of the receptor-DNA-cellulose binding. Therefore, the dye-receptor complex is subject to dissociation induced by dilution in the gel matrix and aided by the attraction of CB to Sephadex. Dialysis of the dye-receptor complex into excess TMDK-0.15 buffer also enhances dissociation of the dye-binding equilibrium reaction such that greater than 90% of the DNA binding can be recovered.[2]

[2] W. S. Mellon, unpublished observations.

DISCUSSION

It has been suggested by Gorski and Gannon (20) that multiple sites for steroid hormone-receptor interaction exist, one of which could be with DNA, and another for the hormone. Previously, 1,25(OH)$_2$D$_3$ receptors have been shown to bind selectively to synthetic polynucleotides (6). In the present study, evidence is presented which suggests that dye-ligand interactions with chicken

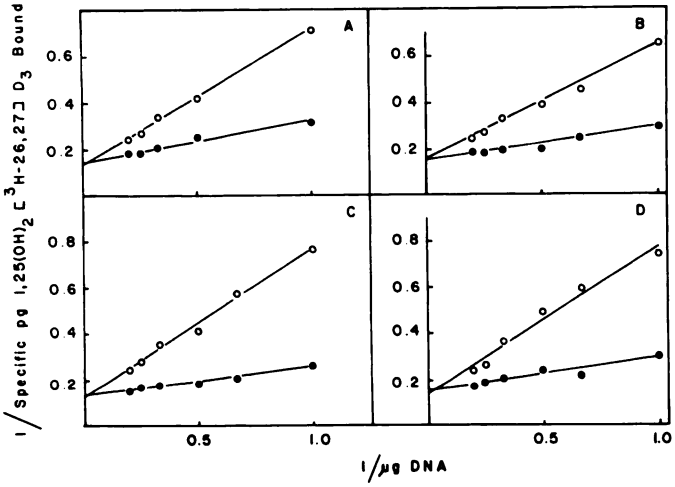

FIG. 5. *Kinetics of inhibition of formation of 1,25(OH)$_2$[^{3}H]D$_3$-receptor-DNA-cellulose complexes by dye-ligands*

To packed DNA-cellulose pellets (containing 1–5 μg of calf thymus DNA per pellet) (total binding) or cellulose pellets (nonspecific binding) were added the reaction mixtures (0.5 ml) as described under Materials and Methods in the presence (○) or absence (●) of blue dextran (1.5 μM) (A), CB (1.5 μM) (B), GA (1.5 μM) (C), or PR (5 μM) (D). Values represent the mean for duplicate determinations.

TABLE 2

Reversal of dye-ligand inhibition of 1,25(OH)$_2$D$_3$-receptor complex binding to DNA-cellulose by gel seiving chromatography

A portion of 1,25(OH)$_2$[^{3}H]D$_3$ cytosol was treated with 10 μM CB and the other was treated with an equal volume of TMDK-0.03 buffer. Portions of CB-treated or control cytosol were applied to Sephadex G-25 columns (1.4 × 5.0 cm) and eluted with TMDK-0.03 buffer. Fractions (0.5 ml) were collected and assayed for radioactivity; those containing labeled receptor were pooled. Aliquots of chromatographed or unchromatographed cytosol (containing 0.1 mg of protein) were assayed for DNA-cellulose binding as described under Materials and Methods.

Treatment[a]	Specific 1,25(OH)$_2$[^{3}H]D$_3$ bound/DNA-cellulose pellet[b]	% of control[c]
	pg	
Cytosol without chromatography	3.66 ± 0.11	100
Cytosol + CB without chromatography	0.26 ± .03	7
Cytosol with chromatography	3.52 ± .08	96
Cytosol + CB with chromatography	3.36 ± 0.15	92

[a] Reversibility also exists for PR and GA.

[b] Values represent the mean ± standard deviation of triplicate determinations.

[c] Calculated using the binding of the cytosol unchromatographed as 100%.

intestinal 1,25(OH)$_2$D$_3$ receptors also may involve the polynucleotide domain of the receptor. In this regard, there are several nucleotide-requiring or DNA-binding proteins that also bind dye-ligands, including estrogen receptor (12), HeLa DNA-polymerase α (10), a bacterial RNA polymerase (9), polynucleotide phosphorylase (8), and DNA polymerases isolated from *Escherichia coli* and bacteriophage T-4 (11).

Various concentrations of dye-ligands resulted in a dose-dependent inhibition of 1,25(OH)$_2$D$_3$ receptor binding to DNA-cellulose and isolated nuclei. An examination of the dose-response data reveals that the three dye-ligands, CB, PR, and GA, are approximately equipotent (2.8 to 3.4 μM) (Fig. 1). Therefore, the relatedness of these dye-ligand structures becomes apparent from estimates of their potency in competing for receptor binding to DNA. The potency of inhibition for 1,25(OH)$_2$D$_3$-receptor complex binding to DNA-cellulose by CB is on the same order of magnitude as the inhibition of estrogen receptor binding to DNA- or oligonucleotide-cellulose (12, 21). The reduced potency observed for bromaminic acid indicates that the anthraquinone portion of the CB molecule alone is not entirely sufficient for effective inhibition of receptor-DNA binding. Similar findings have been reported by Beissner and Rudolph (22) for several nucleotide-requiring enzymes.

Interestingly, when the blue chromophore is linked to dextran, it retains its inhibitory potency under conditions of low ionic strength. These results might not have been expected in light of previous findings employing immobilized CB. In the accompanying paper (13), apparent receptor affinity for the blue chromophore, assessed by the ionic strength necessary to cause receptor desorption, was shown to be weaker for blue dextran-agarose than for CB immobilized to agarose via an ether linkage to the triazine ring. Thus, either the unbound conditions allow better access of the dye to the binding sites, where the dextran moiety does not hinder receptor attachment, and/or at low ionic strength the binding of CB and blue dextran is sufficiently tight to be equipotent in competing with DNA for the polynucleotide-binding site.

Since dye-ligands, most especially CB, can interact with non-nucleotide sites on proteins (23), it was necessary to test whether the observed inhibition of DNA binding could have resulted from loss of sterol. Whereas 10 μM CB caused rapid dissociation of the 1,25(OH)$_2$D$_3$-receptor-DNA-cellulose complex (Fig. 2), there was essentially no difference in the rates of dissociation at 25° for the sterol (Fig. 3). Moreover, saturation analysis of 1,25(OH)$_2$D$_3$ binding indicates that CB does not affect the K_d nor the concentration of specific binding sites (Fig. 4). Both the K_d and the concentration of binding sites are essentially identical with those reported previously by Mellon and DeLuca (1). These results indicate that neither the affinity nor the number of unoccupied sterol-binding sites is affected by CB. Therefore, a lack of perturbation at the sterol site suggests that dye-ligands are interacting at a secondary binding domain.

Under equilibrium conditions, the inhibitory action of several dye-ligands was overcome by increasing concentrations of DNA-cellulose (Fig. 5). Analysis of the binding data using double-reciprocal plots indicates that the dye-ligand inhibition was competitive with respect to DNA binding. These results suggest that dye-ligands bind to a site common to that for DNA. As has been suggested by Yon (24), biospecific competition can occur via several possibilities. Competition of the dye-ligands could transpire with a structurally analogous group (e.g., DNA) at an identical site, the limits of which are completely encompassed by both compounds. Evidence against identical binding sites is supported by the observations that receptor complexes formed at 25° (6) or partially digested by trypsin[3] result in loss of DNA binding, whereas binding to immobilized CB is not completely lost but has altered affinity with respect to salt elution. The competition could result from nonspecific electrostatic accidental interaction. Although polyanions can inhibit receptor binding to DNA-cellulose, they are less potent than the dye-ligands (Fig. 1). Most likely the competition of dye-ligand and DNA for 1,25(OH)$_2$D$_3$ receptors occurs by one of several other possibilities. The entire DNA-binding site, or a portion of the DNA site, may be bound by dye-ligands in addition to binding site(s) external to the DNA-binding domain. At this time, neither possibility can be ruled out. Definitive studies will be initiated with purified receptor preparations to ascertain the limits of the dye-ligand and DNA binding domain(s).

ACKNOWLEDGMENTS

The author thanks Rosanne Kosson and David Hinke for their expert technical assistance, and Joni Dick for preparation of the manuscript.

[3] W. S. Mellon, unpublished observation.

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