Modulation of 1,25-dihydroxyvitamin D_3 receptor by phospholipids and fatty acids

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Abstract The structural relationship between several lipids and their respective capacities to inhibit the specific binding of [^{3}H]-1,25 $(OH)_2$ vitamin D_3 to chick intestinal cytosol preparations was investigated. The lipids investigated were: *a*) synthetic 3-*sn*-phosphatidylcholines and 3-*sn*-phosphatidic acid, *b*) egg yolk 3-*sn*-phosphatidylcholine and its corresponding phosphatidic acid, and *c*) free unsaturated fatty acids and their esters. The results indicate that at least three structural elements in the phospholipid molecule appear to be important; these are: *1*) the structure of the fatty acid, *2*) the anionic properties of the phospholipid phosphate group, and *3*) the glycerol phosphate portion of the molecule. Our data also demonstrate that the position (1 or 2) and the amount (single vs. double) of unsaturated fatty acids in the phospholipid molecule do not play a major role in the receptor-1,25 $(OH)_2$ vitamin D_3 interaction. Furthermore, under equilibrium conditions, kinetic and Scatchard analysis suggest that phospholipids or free fatty acids may bind at a site different from the 1,25 $(OH)_2$ vitamin D_3 binding site, and therefore inhibit the hormone binding via a noncompetititve conformational change in the receptor molecule. A model for this phospholipid/free fatty acid binding site is proposed.—**Chen, T. C., J. P. Mullen, and N. J. Meglin.** Modulation of 1,25-dihydroxyvitamin D_3 receptor by phospholipids and fatty acids. *J. Lipid Res.* 1984. **25:** 1306–1312.

Supplementary key words protein conformation • chick intestinal cytosol • phosphatidylcholine • phosphatidic acid • fatty acid esters • structure-activity relationship

It is generally accepted that intestinal calcium and phosphate transport are regulated by the hormonally active form of vitamin D_3, 1,25-dihydroxyvitamin D_3 (1,25 $(OH)_2$ D_3) (1). Abundant studies suggest that in the intestine, 1,25 $(OH)_2$ D_3 may function in a manner similar to that proposed for other steroid hormones at a cellular level (2). A key mediator in this model is the specific intracellular receptor (3–5). Upon hormone binding, the properties of the receptor change and the receptor acquires an affinity for the chromatin with which it interacts to modulate the transcription of specific genes for the induction of hormone-dependent proteins (6–10).

In view of the central role the intracellular receptors play in the actions of 1,25 $(OH)_2$ D_3, a detailed understanding of the regulatory mechanism of this protein at the molecular level is of considerable importance. Along this line, we have documented recently that the binding of ^{3}H-labeled 1,25 $(OH)_2$ D_3 to its chick intestinal cytosol preparations can be modulated by phospholipids (11). Because phospholipids are major components of the cellular membrane, their interactions with membrane-bound receptors and enzymes have been studied extensively (12–15). Examples include hormone-sensitive adenylate cyclase and Na,K-ATPase. However, only recently, phospholipids have been indicated to be required for the binding of phorbol ester to an intracellular receptor in EL 4 thymoma cells (16). Unlike the systems of adenylate cyclase and phorbol ester in which phospholipids have stimulatory effects and may serve as structural components of the receptors, the intestinal receptors for 1,25 $(OH)_2$ D_3 do not require phospholipids for their binding activity. On the contrary, phospholipids inhibit the specific ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding and are more likely to play a regulatory rather than a structural role in receptor function.

During the course of our studies on phospholipid modulation of 1,25 $(OH)_2$ D_3 receptors in the chick intestinal cytosol preparation, we have noted a differential inhibitory activity exerted by different kinds of phospholipids (Chen, T. C., J. P. Mullen, H. C. Manuel, N. J. Meglin, and J. B. Puschett, submitted for publication). These differences in activity could be either the result of differing head groups or bases (e.g., choline vs. inositol) and/or differing fatty acid composition (e.g., C-18 vs. C-16 or saturated vs. unsaturated). Therefore, in the present studies, we employed either synthetic 3-*sn*-phosphatidylcholines (PC) with defined fatty acid compositions or fatty acids themselves to investigate the structural requirements in the inhibition of ^{3}H-labeled

Abbreviations: 1,25 $(OH)_2$ D_3, 1,25-dihydroxyvitamin D_3; DTT, dithiothreitol; PC, 3-*sn*-phosphatidylcholine; PA, 3-*sn*-phosphatidic acid; KKD, 50 mM $K_2HPO_4KH_2PO_4$ buffer, pH 7.4, 100 mM KCl, and 1 mM DTT; K_d, equilibrium dissociation constant; Ki, the concentration for 50% maximum inhibition.

1,25 $(OH)_2$ D_3 binding to chick intestinal cytosol preparations.

MATERIALS AND METHODS

Animals

One-day-old white Leghorn cockerels (Nolls Hatcher Farm, Kleinfeltersville, PA) were fed a vitamin D-deficient soy protein diet (Teklad #170245) and received water ad lib for 4–5 weeks. All animals were maintained in a vivarium at 25–26°C on an alternating 12-hr incandescent light and dark cycle.

Chemicals

L-α-phosphatidylcholine (PC) prepared from egg yolk (P-5388); synthetic L-α-PC-dilinoleoyl (P-7649) (dilinoleoyl PC); synthetic L-α-PC-β-linoleoyl-γ-palmitoyl (P-9648) (1-palmitoyl,2-linoleoyl PC); synthetic L-α-PC-β-stearoyl-γ-oleoyl (P-9774) (1-oleoyl,2-stearoyl PC); synthetic L-α-PC-β-oleoyl-γ-palmitoyl (P-3017) (1-palmitoyl,2-oleoyl PC); synthetic L-α-PC-β-palmitoyl-γ-oleoyl (P-4142) (1-oleoyl,2-palmitoyl PC); synthetic L-α-PC-dimyristoyl (P-0888) (dimyristoyl PC); synthetic L-α-PC-distearoyl (P-6517) (distearoyl PC); synthetic L-α-PC-diarachidoyl (P-9898) (diarachidoyl PC); synthetic L-α-PC-dipalmitoyl (P-0763) (dipalmitoyl PC); L-α-phosphatidic acid (P-9511) (PA) prepared by phospholipase D hydrolysis of egg yolk PC (P-5388); synthetic L-α-PA-distearoyl (P-4899) (distearoyl PA); oleic acid (O-0750); oleic acid methyl ester (O-1000); linoleic acid (L-1376); linoleic acid ethyl ester (L-1751); linolenic acid (L-2376); and linolenic acid ethyl ester were obtained from Sigma Chemical Company, St. Louis, MO. Nonradioactive 1,25 $(OH)_2$ D_3 was a gift from the Hoffman-LaRoche Co. of Nutley, NJ. 1,25 $(OH)_2$ [23,24 (n)-^{3}H]D_3 (85 Ci/mmol) was purchased from Amersham Corporation, Arlington Heights, IL. All other chemicals were reagent grade and were obtained from commercial sources.

Cytosol preparations

The mucosa of chicken duodenal loops was scraped free of serosa and washed two times with an ice-cold buffer containing 50 mM K_2HPO_4–KH_2PO_4, pH 7.4, 100 mM KCl, and 1 mM DTT (KKD buffer) (17). Washed mucosa was homogenized in two volumes of the same buffer using a Polytron, type PT-20 (Brinkman Instruments, Westbury, NY). Cytosol was obtained by centrifuging the homogenate at 166,000 *g* for 60 min at 4°C in a Beckman L5-50 ultracentrifuge using a Ti-50 rotor. The cytosol was stored in aliquots in a liquid N_2 freezer until used. Cytosol protein concentration was determined by the method of Bradford (18) using crystalline bovine serum albumin as a standard.

Equilibrium binding studies and incubation procedure

The addition of phospholipids or fatty acids into the assay tubes was carried out as follows: an aliquot of phospholipid in chloroform–methanol 9:1 (v/v) was pipetted into assay tubes and dried under a stream of dry N_2 until the last trace of solvent was removed. The phospholipid was then redissolved in 50 μl of methanol with or without 6.35 pmol of nonradioactive 1,25 $(OH)_2$ D_3 with constant vortexing. In studies including free and esterified fatty acids, the lipids were dissolved in methanol and added directly into the assay tubes containing 6.35 pmol of nonradioactive 1,25 $(OH)_2$ D_3 in methanol or methanol only. The total binding activity was determined by adding 0.03–0.035 pmol of ^{3}H-labeled 1,25 $(OH)_2$ D_3 in 10 μl of methanol and 1 ml of cytosol containing 0.21 mg of protein in KKD buffer to tubes without 6.35 pmol of nonradioactive 1,25 $(OH)_2$ D_3 in the presence and absence of lipids. The nonspecific 1,25 $(OH)_2$ D_3 binding was measured by a parallel incubation of the cytosol with 6.35 pmol of nonradioactive 1,25 $(OH)_2$ D_3 in the presence and absence of lipids. The assay tubes were incubated with shaking in a water bath for 1 hr at 23°C and then placed on ice. Subsequently, the unbound 1,25 $(OH)_2$ D_3 was removed from the cytosol by the dextran-coated charcoal technique (19). The specific receptor binding was calculated as the difference between total and nonspecific binding and was expressed as per cent of control in the absence of phospholipids or inhibitors.

For studying the effect of phospholipids and fatty acids on the equilibrium dissociation constant (K_d) of the hormone-receptor complex and the total number of hormone binding sites, Scatchard analyses (20) were performed on binding data obtained by incubating various concentrations of 1,25 $(OH)_2$ D_3 (ranging from 0.03 to 0.15 pmol) with cytosol (0.21 mg of protein) in the presence and absence of lipids for 1 hr at 23°C. The phospholipid concentration was measured according to the method of Chen, Toribara, and Warner (21).

RESULTS

Among the synthetic phosphatidylcholines (PC) available, seven analogues with different fatty acid compositions were chosen (**Table 1**). At a concentration of 30 μM, PC with one or two C-18 unsaturated fatty acids were shown to be more active than those with two saturated fatty acids of C-14, C-16, C-18, or C-20 chain length. These data clearly suggest that the fatty acid moiety of the PC molecule plays a very important part in the modulation of 1,25 $(OH)_2$ D_3-receptor interaction.

To further investigate the role of the fatty acyl

TABLE 1. Inhibition of specific binding of ^{3}H-labeled 1,25(OH)$_2$D$_3$ to its cytosolic receptor by egg yolk and synthetic phosphatidylcholines

Phosphatidylcholine (30 μM)[a]	Specific [^{3}H]-1,25(OH)$_2$D$_3$ Bound (% of Control in Absence of PC)[b]
Control	100
PC (from egg yolk)	30 ± 6
Dilinoleoyl PC	35 ± 1
1-Palmitoyl,2-linoleoyl PC	40 ± 2
1-Oleoyl,2-stearoyl PC	48 ± 3
Dimyristoyl PC	61 ± 3
Distearoyl PC	77 ± 1
Diarachidoyl PC	78 ± 3
Dipalmitoyl PC	80 ± 3

[a] The molecular weight of egg yolk PC was assumed to be 770 (22).
[b] Each value represents mean ± SEM of eight determinations from four separate experiments.

group, we examined the effects of various fatty acid structures and their positional distribution on the specific binding of ^{3}H-labeled 1,25 (OH)$_2$ D$_3$ to its chick intestinal cytosolic receptor (**Fig. 1**). The difference in inhibitory potency as expressed by the concentration for 50% maximum inhibition (Ki) (13 μM vs. 16 μM) by the two positional isomers, 1-oleoyl,2-palmitoyl PC and 1-palmitoyl,2-oleoyl PC, is relatively small. Likewise, the inhibitory activity of 1-palmitoyl,2-oleoyl PC (Ki = 16 μM) is only slightly higher than that of 1-palmitoyl,2-linoleoyl PC (Ki = 22 μM). Interestingly, a twofold difference in Ki was noted between the two PC with the same unsaturated fatty acid at the 1-position but with different saturated fatty acids at the 2-position; 1-oleoyl,2-palmitoyl PC had a Ki of 13 μM whereas 1-oleoyl,2-stearoyl PC had a Ki of 30 μM. Next, we studied the effects of the amount of unsaturated fatty acids in the PC molecule

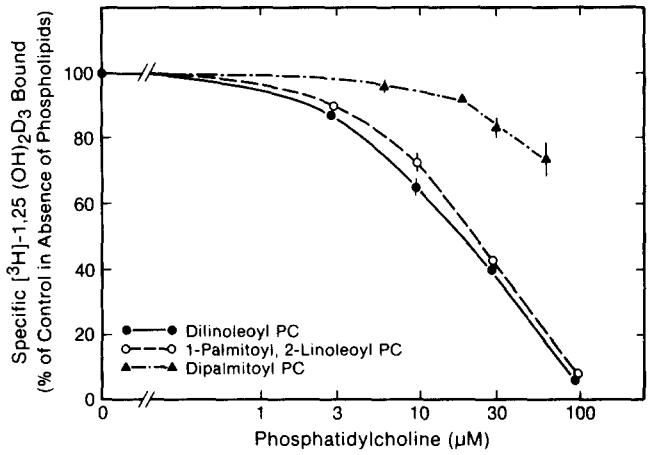

Fig. 2. Modulation of the specific [^{3}H]-1,25 (OH)$_2$D$_3$ binding to chick intestinal cytosolic receptor by phosphatidylcholines (PC) composed of two saturated, two unsaturated, or mixed fatty acid acyl residues. Results are expressed as the means ± SEM of six determinations from three separate experiments.

on the specific binding of ^{3}H-labeled 1,25 (OH)$_2$ D$_3$ to its cytosolic receptor (**Fig. 2**). As shown, there was no significant difference between dilinoleoyl PC (Ki = 18 μM) and 1-palmitoyl,2-linoleoyl PC (Ki = 22 μM) on the inhibition of receptor-steroid interaction.

Next, we investigated whether the choline base is critical for inhibitory activity to be manifested by the PC molecule. As shown in **Fig. 3**, phosphatidic acid, prepared by phospholipase D hydrolysis of egg yolk PC, has a Ki of 4.6 μM, which is about fivefold more potent than that of egg yolk PC (Ki = 23 μM). Because the two phospholipids have exactly the same fatty acid composition, the drastic difference in potency in these two compounds suggests that choline not only is nonessential

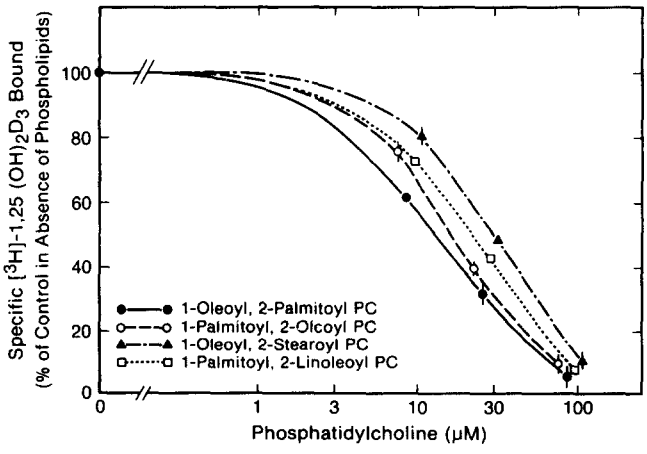

Fig. 1. Effects of the structure of fatty acids and their positional distribution in a phosphatidylcholine molecule on the specific [^{3}H]-1,25 (OH)$_2$D$_3$ binding to chick intestinal cytosolic receptor. Data represent the means ± SEM of six determinations from three separate experiments.

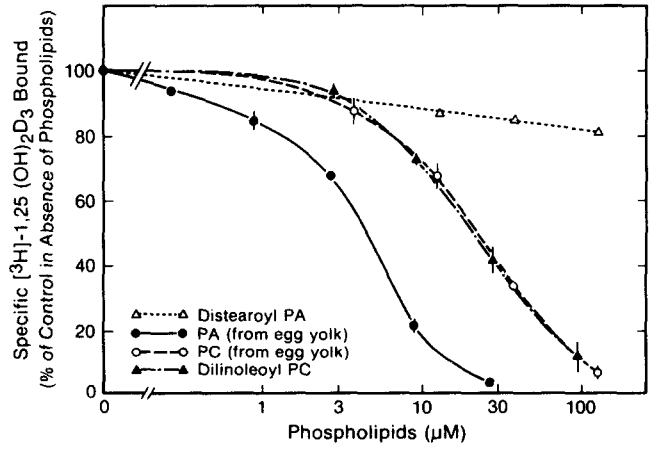

Fig. 3. Significance of the structure of fatty acid acyl group and the choline base on the inhibition of specific [^{3}H]-1,25 (OH)$_2$D$_3$ binding to chick intestinal cytosolic receptor by phosphatidic acids (PA) and phosphatidylcholines (PC). Data represent the means ± SEM of six determinations from three separate experiments.

but also has negative consequences. The negative effects of the choline moiety, however, occurred only when the base was covalently bound to the phospholipid molecule. The addition of choline chloride (100 μM) into the assay mixture neither increased nor decreased the phosphatidic acid activity (**Fig. 4**).

The importance of fatty acid composition is also demonstrated in Fig. 3. Although the PA, prepared from egg yolk PC, was highly effective in inhibiting ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding to its cytosolic receptor, the synthetic distearoyl PA was far less effective in this regard. This synthetic compound caused only a 20% inhibition at a concentration as high as 120 μM. Furthermore, the data in this figure and in Figs. 1 and 2 show similar dose-response curves by egg yolk PC and synthetic PC with single unsaturated fatty acids. These observations are in agreement with the notion that in most PC and PA prepared from natural sources, one molecule of each pair of fatty acids is saturated and the other unsaturated. Thus, the results obtained from experiments utilizing synthetic and natural phospholipids indicate that their fatty acid compositions play a major role in the modulation of 1,25 $(OH)_2$ D_3-receptor interaction.

Our next step was to examine the possible regulatory role of three C-18 unsaturated fatty acids and their esters in the inhibition of ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding to its cytosolic receptor. As shown in **Table 2,** the specific binding was reduced to about 10%, 24%, and 57% in the presence of oleic acid, linoleic acid, and linolenic acid, respectively, at a concentration of 30 μM. Interestingly, the inhibitory activity of oleic acid methyl ester and linoleic acid ethyl ester was considerably less than that of the corresponding free fatty acid forms. The dose-response curves of the three fatty acids and their esters were further studied (**Figs. 5–7**). The Ki values were 14 μM, 18 μM, and 34 μM in the presence of oleic acid, linoleic acid, and linolenic acid, respectively. On the other hand, the maximal inhibition that could be achieved by the esters was about 25–30%. It appears that the potency of unsaturated fatty acid decreases with an increasing degree of unsaturation. Furthermore, these results strongly suggest that the negative charge derived from the ionization of the carboxyl group at pH 7.4 may also be responsible for the binding of fatty acid to the cytosolic receptor protein through charge-charge interaction in addition to the hydrophobic interaction contributed by the unsaturated hydrocarbon chain of the fatty acid. This conclusion is supported by the data in Fig. 3 showing that PA is about fivefold more potent than PC. Because of its lower pKa, the phosphoric acid group of phosphatidic acid is more negatively charged than that of PC. Furthermore, the positive charge of the choline-ammonium group might interfere with the interaction between the negatively charged phosphoric acid group and cytosolic protein.

TABLE 2. Modulation of specific binding of ^{3}H-labeled 1,25 $(OH)_2D_3$ to its cytosolic receptor by C-18 unsaturated free fatty acids and their esters

Inhibitors (30 μM)	Specific [^{3}H]-1,25 $(OH)_2D_3$ Bound (% of Control in Absence of Inhibitors)[a]
Oleic acid	10 ± 4
Oleic acid methylester	70 ± 2
Linoleic acid	24 ± 4
Linoleic acid ethyl ester	76 ± 1
Linolenic acid	57 ± 9
Linolenic acid ethyl ester	82 ± 2

[a] Data represent means ± SEM of six to eight determinations from three to four separate experiments.

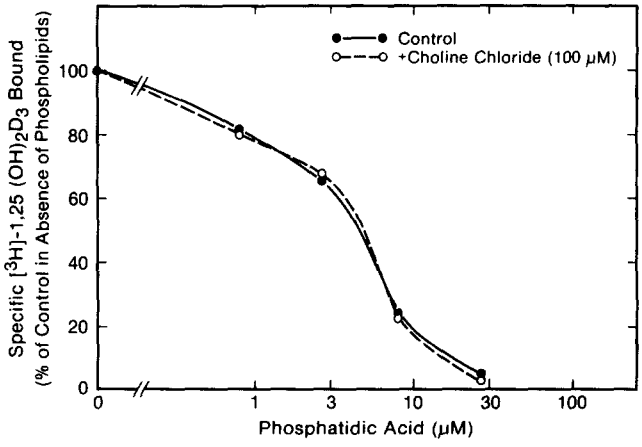

Fig. 4. Lack of choline effect on the inhibition of specific [^{3}H]-1,25 $(OH)_2D_3$ binding to chick intestinal cytosolic receptor by egg yolk phosphatidic acid. Results are the means of four determinations from a single experiment.

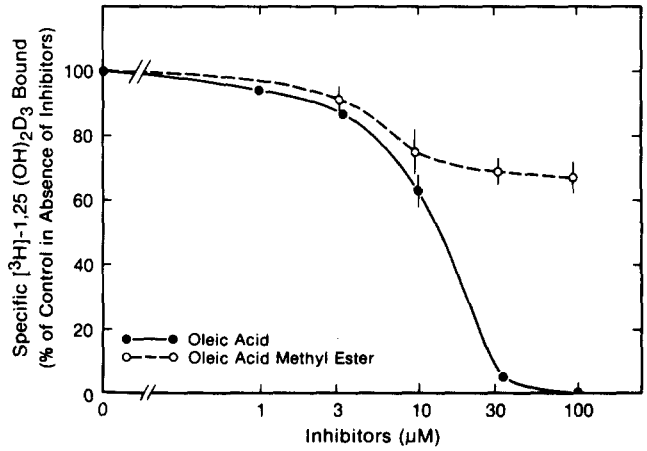

Fig. 5. Oleic acid and oleic acid methyl ester inhibition of the specific [^{3}H]-1,25 $(OH)_2D_3$ binding to chick intestinal cytosol. Each point represents the mean ± SEM of six determinations from three separate experiments.

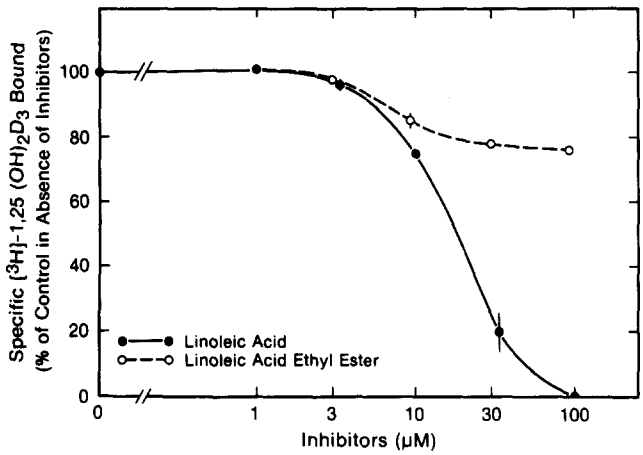

Fig. 6. Linoleic acid and linoleic acid ethyl ester inhibition of the specific [^{3}H]-1,25 $(OH)_2D_3$ binding to chick intestinal cytosol. Each point represents the mean ± SEM of six determinations from three separate experiments.

Finally, Scatchard analysis was performed to examine the nature of phosphatidic acid and oleic acid inhibition of ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding to its receptor (**Fig. 8**). The equilibrium dissociation constant (K_d) was increased slightly in the presence of 17 μM oleic acid (3.9×10^{-11} M) and 9 μM PA (4.4×10^{-11} M) as compared to control (1.8×10^{-11} M). As shown, the maximum number of available binding sites was substantially decreased in the presence of oleic acid (109 fmol/mg protein) and PA (67 fmol/mg protein) vs. control (167 fmol/mg protein). Analysis of the same data utilizing a double reciprocal plot revealed a pattern of noncompetitive inhibition by oleic acid and phosphatidic acid (**Fig. 9**). These results are consistent with our previous findings using phosphatidylinositol as the modulator of ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding to chick intestinal cytosol (11).

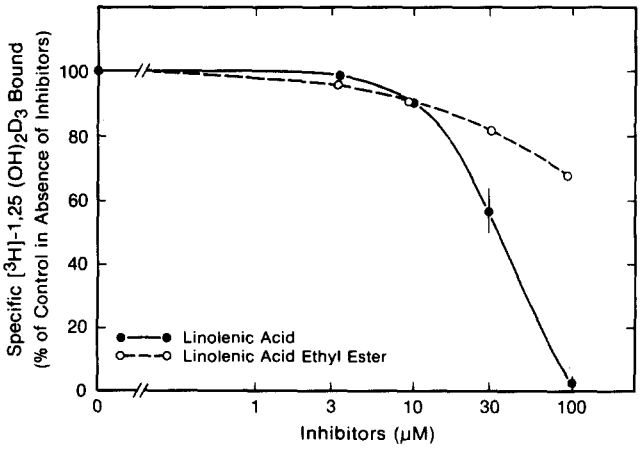

Fig. 7. Linolenic acid and linolenic acid ethyl ester inhibition of the specific [^{3}H]-1,25 $(OH)_2D_3$ binding to chick intestinal cytosol. Each point represents the mean ± SEM of six determinations from three separate experiments.

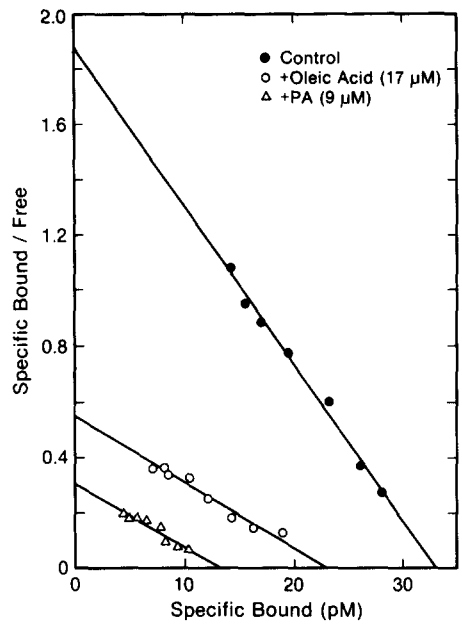

Fig. 8. Scatchard analysis of specific [^{3}H]-1,25 $(OH)_2D_3$ binding to chick intestinal cytosolic receptor in the presence and absence of inhibitor. Each point represents the mean of two determinations from a single experiment. The r value is 0.99 (control), 0.98 (+ oleic acid), and 0.92 (+PA).

DISCUSSION

These experiments demonstrate that there is a structure-activity relationship in the modulation of 1,25 $(OH)_2$ D_3-receptor interaction by phospholipids and

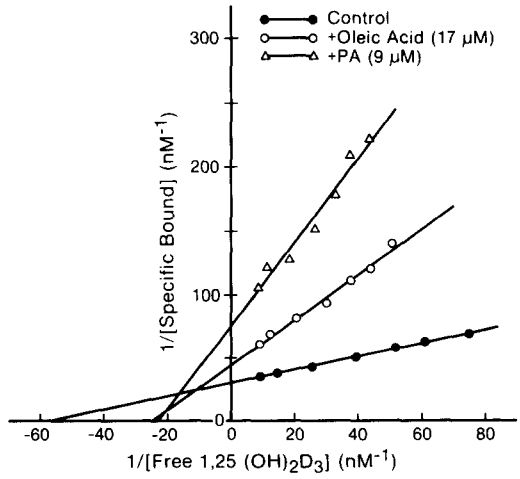

Fig. 9. A plot of 1/specific bound vs. 1/[1,25 $(OH)_2D_3$] in the presence and absence of inhibitor. Each point represents the mean of two determinations from a single experiment. The r value is 0.99 (control), 0.99 (+ oleic acid) and 0.98 (+PA).

fatty acids. At least three structural elements in the phospholipid molecule appear to be critical; these are: *1*) the structure of fatty acids, *2*) the anionic properties of the phospholipid phosphate group, and *3*) the glycerol phosphate portion of the molecule. The importance of the fatty acid structure was clearly demonstrated by utilizing various synthetic PC. A twofold difference in the inhibition of specific ^{3}H-labeled 1,25 $(OH)_2$ D_3 binding was observed between PC containing one unsaturated C-18 fatty acid and that with two C-16, C-18, or C-20 saturated fatty acids at a concentration of 30 μM (Table 1). The difference in Ki between dipalmitoyl PC and 1-palmitoyl, 2-linoleoyl PC (Fig. 2) and between egg yolk PA and distearoyl PA (Fig. 3) could be in the range of 10- to 100-fold. Furthermore, a more than 2-fold difference in Ki between 1-oleoyl,2-palmitoyl PC and 1-oleoyl,2-stearoyl PC may indicate a subtle relationship between two fatty acids within the phospholipid. The intermediate inhibitory activity shown by dimyristoyl PC is difficult to explain at the present time. A requirement for a strong anionic group was clearly demonstrated by a 5-fold difference in inhibitory activity shown by egg yolk PA and its corresponding egg yolk PC (Fig. 3) and by a much larger difference between free acids and their esters.

The contribution by the glycerol phosphate portion of the phospholipid molecule to its overall inhibitory activity was suggested by the finding that the phosphatidic acid is a better inhibitor than oleic acid on a molar basis (Figs. 3, 5, and 8). Our data also demonstrate that *1*) the position of an unsaturated fatty acid (e.g., at the 1- or 2-position) and *2*) the number of unsaturated fatty acids in the phospholipid molecule do not play a critical role in the receptor-1,25 $(OH)_2$ D_3 interaction (Figs. 1 and 2).

The kinetic analyses obtained by Scatchard and double reciprocal plots indicate a pattern of noncompetitive inhibition (Fig. 9) and, consequently, a significant decrease in the number of binding sites (Fig. 8) in the presence of phosphatidic acid or oleic acid. These results are in agreement with our previous data employing phosphatidylinositol as an inhibitor (11). Therefore, our previous and present data strongly suggest that a phospholipid-fatty acid binding site, which is most likely different from the 1,25 $(OH)_2$ D_3 binding site, exists in the crude cytosol preparations of chick intestinal mucosa. We propose that this binding site is composed of two parts: *1*) a region lined with nonpolar amino acid side chains that recognize and interact with the hydrocarbon chain of unsaturated fatty acid that is either free or bound to phospholipids, and *2*) a positively charged residue, such as the amino group on the side chain of lysine or arginine, which is responsible for an electrostatic interaction with carboxylate anions of free fatty acids or the phosphate anions of phospholipids. Both Kream, Jose, and DeLuca (23) and Wecksler, Okamura, and Norman (24) have suggested models for a 1,25 $(OH)_2$ D_3 binding site. If their proposals are correct, then it is evident that the steroid binding site has features very different from those of the phospholipid-fatty acid regulatory site that we have presented in this communication. To document the existence and the molecular structure of this putative regulatory site, various physical-chemical studies, utilizing homogeneous receptor preparations, will be required.

The possibility that phospholipid liposomes or fatty acid micelles may trap ^{3}H-labeled 1,25 $(OH)_2$ D_3 and prevent its access to the receptor binding sites, thereby resulting in an apparent inhibition of specific binding, is unlikely for the following reasons. *a*) In the absence of phospholipids or fatty acids, the nonspecific binding was found to be proportional to the concentration of ^{3}H-labeled 1,25 $(OH)_2$ D_3 added to the incubation medium over the range of 0.011 to 0.033 pmol (data not shown). If the inhibition was due to the preferred solubility of ^{3}H-labeled 1,25 $(OH)_2$ D_3 in the lipid aggregate, we should have seen a proportional decrease in nonspecific binding as well as total binding. Our data indicated no such inhibition in nonspecific binding in the presence of 8.8 μM and 26.4 μM phosphatidic acid. On the contrary, the same concentration of phosphatidic acid reduced the total binding and, accordingly, the specific binding to 25% and 5% of the control value, respectively. *b*) There is no direct correlation between the critical micelle concentration (CMC) of phosphatidylcholines with different fatty acid compositions and their inhibitory potency. For example, phosphatidylcholine (egg yolk) and synthetic dipalmitoyl PC have essentially the same CMC (4.6×10^{-10} M) (25), whereas their inhibitory potencies are very different (Table 1, Figs. 3 and 4). *c*) There is tissue or species difference for the cytosol preparation in the inhibition of specific 1,25 $(OH)_2$ D_3 binding by different phospholipids; rat kidney cytosol preparation is more sensitive to phosphatidylcholine (egg yolk) than to phosphatidylinositol or phosphatidic acid inhibition (Chen, T. C., N. J. Meglin, J. P. Mullen, and J. B. Puschett, unpublished data), whereas the reverse is true in the chick intestinal cytosol preparation. *d*) The partially purified receptor preparation was found to be more sensitive to phospholipid inhibition than the unpurified cytosol preparation (Chen, T. C., unpublished data). This result as well as that of tissue or species specificity strongly suggests an interaction between receptor and phospholipid. Furthermore, partial removal of non-receptor phospholipid binding proteins such as phospholipid transfer proteins may allow more phospholipid to interact with the receptor molecules.

Aside from a potential role for the phospholipids and

fatty acids in the mechanism of action of vitamin D in small intestine, our findings have important implications with respect to the clinical measurement of the plasma level of 1,25 $(OH)_2$ D_3 by the chick cytosolic receptor assay technique (26). Because of the high content of phospholipids and fatty acids in the plasma samples, incomplete separation of these materials from the 1,25 $(OH)_2$ D_3 preparations would give false estimations of the 1,25 $(OH)_2$ D_3 levels in the patients' plasma.

We would like to express our sincere appreciation to Dr. Jules B. Puschett for his support and advice in the conduct of this work and in the preparation of this manuscript. We also wish to thank Ms. Jane Wolfe for her excellent secretarial assistance.

Manuscript received 30 March 1984.

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