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# 1,25-DIHYDROXYVITAMIN $D_3$ RECEPTORS IN RAT KIDNEY CYTOSOL

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Summary: Rat kidney cytosol contains a 3.3 S high affinity binding component for 1,25-dihydroxyvitamin $D_3$ as detected by DNA-cellulose chromatography and subsequent sucrose gradient analysis. The semipurified aporeceptor demonstrates specificity for 1,25-dihydroxyvitamin $D_3$ and an apparent dissociation constant for this sterol-hormone of 3.4 x $10^{-10}$M at 25°C. The physicochemical properties of this binding component are in agreement with those observed for the chick intestinal 1,25-dihydroxyvitamin $D_3$ receptor, suggesting that this component may function as a specific receptor for the hormone in the kidney.

Vitamin D is hydroxylated in the liver and kidney to form 1,25-$(OH)_2D_3$, which promotes the uptake of calcium and phosphate by the intestine. Several aspects of this sterol-mediated induction of mineral absorption have been documented and include 1) a specific cytosolic receptor which conveys the hormone to the nucleus, 2) an effect on nuclear RNA synthesis, and 3) the subsequent appearance of CaBP, thought to be involved in calcium transport (1). The nature of these events suggests that 1,25-$(OH)_2D_3$ may function as a steroid hormone.

In the kidney, a variety of effects have been attributed to the influence of vitamin D, although the metabolite(s) and mechanism(s) involved remain obscure. However, at least three actions of 1,25-$(OH)_2D_3$ have been noted: 1) physiologic doses of the hormone can reverse the increased tubular reabsorption of phosphate characteristic of TPTX-rats (2), 2) 1,25-$(OH)_2D_3$

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Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin $D_3$; 24,25-$(OH)_2D_3$, 24,25-dihydroxyvitamin $D_3$; 25-$(OH)D_3$, 25-hydroxyvitamin $D_3$; CaBP, calcium-binding protein; TPTX, thyroparathyroidectomized; KETT-buffer, 0.01 M Tris-HCl (pH 7.4), 0.001M EDTA, 0.012M thioglycerol, made to the indicated molarity with KCl (KETT-0 = no KCl; KETT-0.1 = KETT-0, 0.1M KCl etc.); STKM-buffer, 0.25M sucrose, 0.05M Tris-HCl (pH 7.4), 0.025M KCl, 0.005M $MgCl_2$, 0.001M EDTA and 0.012M thioglycerol.

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is capable of repairing depressed tubular calcium reabsorption in TPTX-rats (vitamin D and phosphate deficient) (3) and 3) the sterol demonstrates a direct effect on renal vitamin D-(OH)ase activity both in vivo (4) and in cell culture (5). In addition, a vitamin D-dependent CaBP has been detected in kidney (6). Cumulatively, these observations suggest that 1,25-$(OH)_2D_3$ may exert hormonal effects at the renal level. The present report describes the identification and characterization of a specific, high affinity binding component for 1,25-$(OH)_2D_3$ in rat kidney cytosol. This discovery further supports the contention that the kidney is a target tissue for the sterol-hormone.

## MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats (150-200g) were maintained on normal laboratory diets.
Sterols. Non-radioactive 1,25-$(OH)_2D_3$ was obtained from Hoffmann-LaRoche (M. Uskokovic). Tritium-labeled sterols were obtained from The Amersham Corporation at the following specific activities: 25-(OH)[$^3$H]$D_3$ (110 Ci/mmol), 1,25-$(OH)_2$[$^3$H]$D_3$ (110 Ci/mmol), and 24,25-$(OH)_2$[$^3$H]$D_3$ (80 Ci/mmol).
Cytosol Preparation. Tissues were excised, rinsed in cold STKM buffer, and homogenized at 4°C in STKM buffer (2.5 ml/g). Cytosols were prepared, ammonium sulfate precipitated, and resolubilized as in (7).
DNA-Cellulose Chromatography. DNA-cellulose was prepared as described by Alberts and Herrick (8). Chromatography was performed as described in (7).
Sucrose Gradient Analysis. Linear gradients (4.5 ml) of 5-20% sucrose (w/v) in KETT-0.3 were prepared, centrifuged, and fractionated as in (9).
Aporeceptor Preparation. Unliganded receptor material (aporeceptor) was obtained from resolubilized ammonium sulfate precipitates of rat kidney cytosol. The unlabeled material was applied to 12 ml DNA-cellulose columns, washed with 50 ml of KETT-0.15 and then eluted from the column with 15 ml of KETT-0.5. Aporeceptor eluted between 9 and 15 ml and was used immediately for sterol binding studies.
Aporeceptor-binding Studies. Sterols were incubated with aliquots of aporeceptor material in 10% ethanol (v/v) as described in the figure legends. Binding of labeled sterols was determined by the DEAE-filter technique described by Santi, et. al. (10) as modified in (11). Specific binding of 1,25-$(OH)_2$[$^3$H]$D_3$ by aporeceptor was also assayed as described in (11).

## RESULTS AND DISCUSSION

It has previously been demonstrated that the 3.3 S chick intestinal receptor for 1,25-$(OH)_2D_3$ can be identified by DNA-cellulose chromatography (12). Hence, we employed this affinity ligand to evaluate rat kidney for 1,25-$(OH)_2D_3$-binding components. We also sought to confirm the presence of this component (14) in rat intestine. Ammonium sulfate preci-

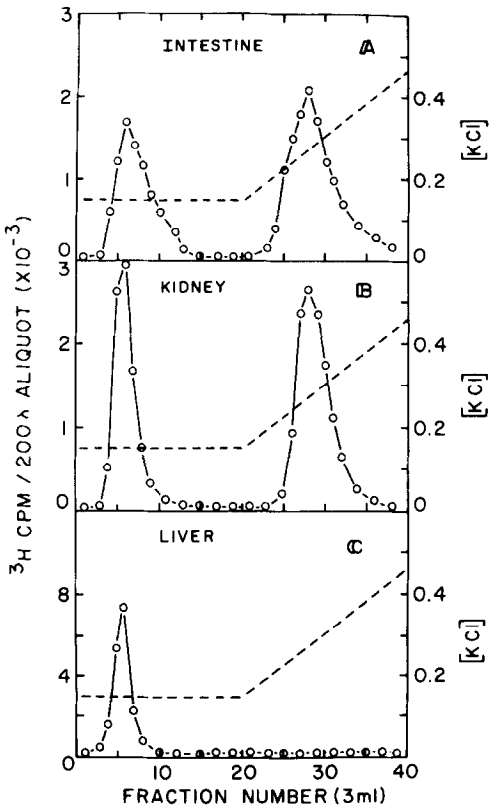


Figure 1. Chromatography of rat tissue 1,25-$(OH)_2D_3$-binding components on DNA-cellulose. Resolubilized ammonium sulfate precipitates of intestinal, kidney, or liver cytosols were incubated with 1.5 nM 1,25-$(OH)_2[^3H]D_3$ for 1 hour at 4°C and then applied to DNA-cellulose columns (12 ml). The columns were washed with 5 volumes of KETT-0.15 and then eluted with a 90 ml linear KCl gradient (0.15-0.6M KCl). Fractions (3 ml) were collected and aliquots counted for tritium (o-o). Salt concentration (---) was monitored by conductivity measurements. Chromatography of (A) intestinal mucosa (2g), (B) kidney cortex (4g), (C) liver (4g).

pitates (40% of saturation) of intestinal, kidney and liver cytosols were incubated with 1,25-$(OH)_2[^3H]D_3$ and then chromatographed on DNA-cellulose. The results (Fig. 1) demonstrate a macromolecular-tritium complex which binds to DNA-cellulose in both intestine (A) and kidney (B) and elutes between 0.2-0.3M KCl. Importantly, this component is not present in a non-target tissue such as liver (C). The chromatographic behavior of the tritium-bound complex in both intestine and kidney is identical to the chick intestinal 1,25-$(OH)_2D_3$-receptor (12). Thus, these results provide strong evidence for the presence of a new receptor in rat kidney.

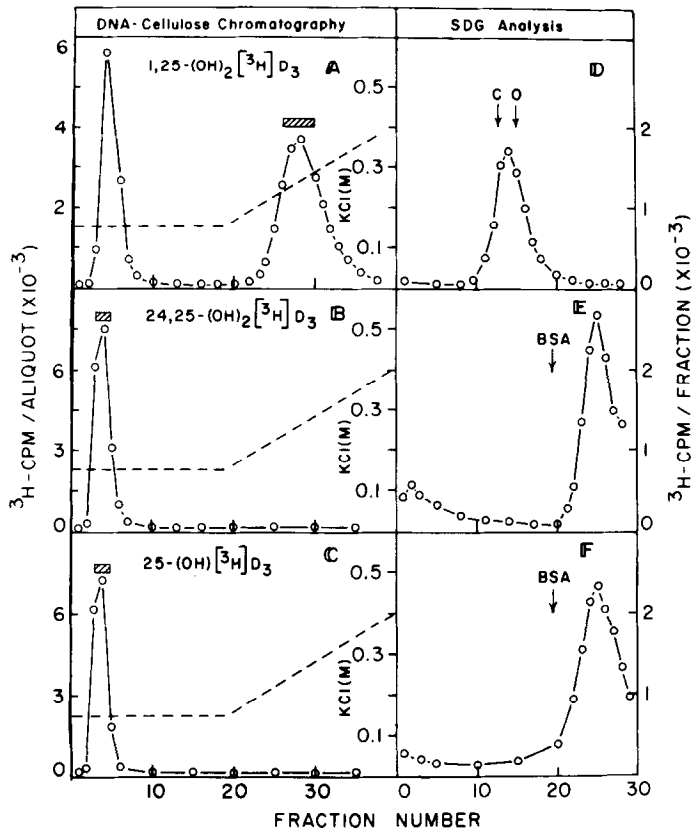


Figure 2. DNA-cellulose chromatography and subsequent sucrose gradient analysis of vitamin D metabolite-binding components in rat kidney. Resolubilized ammonium sulfate precipitates of kidney cytosol were incubated with 1.5 nM (A) $1,25\text{-}(OH)_2[^3H]D_3$, (B) $24,25\text{-}(OH)_2[^3H]D_3$, or (C) $25\text{-}OH[^3H]D_3$ for 1 hour at 4°C and chromatographed on DNA-cellulose as in Fig. 1. Pooled material (//////) was concentrated to 1 ml and 0.5 ml samples were applied to linear 5-20% sucrose gradients (prepared in KETT-0.3): (D) concentrate from A; (E) concentrate from B; (F) concentrate from C. Protein standards are: chymotrypsinogen, C (2.5 S); ovalbumin, O (3.7 S); and bovine serum albumin, BSA (4.4 S). (o-o), tritium profile; (---), KCl gradient (M).

To further define the rat kidney binding component for $1,25\text{-}(OH)_2D_3$, we repeated the experiment described in Fig. 1B and then evaluated the $[^3H]$-eluant by sucrose gradient analysis. The results (Fig. 2A and D) reveal that the semipurified $1,25\text{-}(OH)_2D_3$-binding component sediments at 3.3 S, identically to the chick intestinal receptor (12).

We also investigated the binding of $25\text{-}(OH)D_3$ and $24,25\text{-}(OH)_2D_3$ to cytosolic components of rat kidney since both sterols are metabolized in renal tissue. $25\text{-}(OH)D_3$ is known to associate *in vitro* with a 5.8 S component in cytosol preparations of all nucleated rat tissues (15). However, the binding

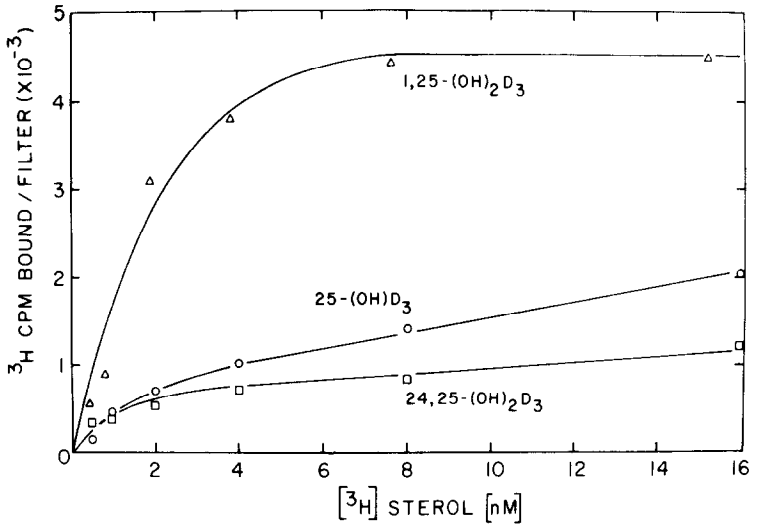


Figure 3. Metabolite specificity of the aporeceptor. Aporeceptor was semipurified by ammonium sulfate precipitation and DNA-cellulose chromatography. Aliquots (0.1 ml) of this material were incubated with increasing concentrations of 1,25-(OH)$_2$[$^3$H]D$_3$ (▲-▲), 24,25-(OH)$_2$[$^3$H]D$_3$ (■-■), or 25-(OH)[$^3$H]D$_3$ (o-o) for 1 hour at 4°C. Total bound sterol was recovered as described in methods and normalized for the specific activity of 1,25-(OH)$_2$[$^3$H]D$_3$(110 Ci/mmol).

of 24,25-(OH)$_2$D$_3$ in kidney cytosol has not been assessed. The results of incubating resolubilized, kidney cytosol precipitates with 25-(OH)[$^3$H]D$_3$ and 24,25-(OH)$_2$[$^3$H]D$_3$ are seen in Fig. 2C and 2B. 25-(OH)D$_3$ binds to a component which does not interact with DNA-cellulose (Fig. 2C) and sediments at about 6 S (Fig. 2F). Similarly, 24,25-(OH)$_2$D$_3$ also associates with a component lacking affinity for DNA-cellulose (Fig. 2B) and sedimenting at 6 S (Fig. 2E). We were unable to demonstrate binding of 24,25-(OH)$_2$D$_3$ to any other component. In contrast to 1,25-(OH)$_2$ D$_3$ neither 25-(OH)D$_3$ nor 24,25-(OH)$_2$ D$_3$ readily associate with the 3.3 S receptor and thus, a receptor-mediated action for the later metabolites in renal tissue is unlikely.

The relative sterol specificity of the kidney 1,25-(OH)$_2$D$_3$-binding component was next determined. Chromatography of unlabeled preparations on DNA-cellulose yields semipurified aporeceptor (see Fig. 2A), which was used to qualitatively assess ligand preference. Aporeceptor was incubated with increasing concentrations of 1,25-(OH)$_2$[$^3$H]D$_3$, 24,25-(OH)$_2$[$^3$H]D$_3$ and 25-(OH)[$^3$H]D$_3$ and total binding determined. The results (Fig. 3) show

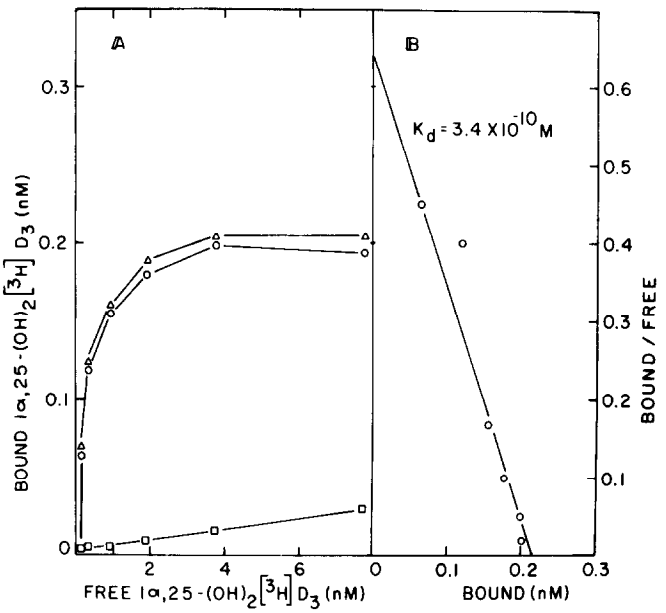


Figure 4. Determination of the dissociation constant for the 1,25-$(OH)_2D_3$-aporeceptor interaction. (A) Aliquots (0.2 ml) of semipurified aporeceptor (as in Fig. 3) were incubated at 25°C for 20 minutes with increasing concentrations of 1,25-$(OH)_2[^3H]D_3$ in the presence (■-■) and absence (▲-▲) of a 100-fold excess of nonradioactive hormone. (B) Scatchard analysis of specific binding (o-o).

that 1,25-$(OH)_2D_3$ is the preferred ligand and saturates the aporeceptor at low concentrations. This specificity and limited capacity imply that the kidney component is a receptor for 1,25-$(OH)_2D_3$.

Finally, since hormone receptors demonstrate high affinity and low capacity for hormonal ligand, we investigated these properties under equilibrium conditions. Semipurified aporeceptor (as above) was incubated with increasing concentrations of 1,25-$(OH)_2[^3H]D_3$ or similarly with the inclusion of a 100-fold excess of nonradioactive hormone and bound hormone determined. The results (Fig. 4A) demonstrate that specific binding of 1,25-$(OH)_2D_3$ occurs at low concentrations ($< 10^{-8}$M) indicating a limited number of hormone binding sites. Scatchard analysis (Fig. 4B) indicates the presence of a single class of binding sites with an apparent dissociation constant (at 25°C) of $3.4 \times 10^{-10}$M.

The evidence described here suggests the presence of a 1,25-$(OH)_2D_3$ receptor in rat kidney cytosol. Its behavior on DNA-cellulose and its sedi-

mentation property indicate a similarity to the 1,25-$(OH)_2D_3$ receptor from chick intestinal cytosol. Preliminary reports have indicated the presence of a 1,25-$(OH)_2D_3$ receptor in rachitic chick kidney (13,16), however, this is the first demonstration of a receptor component in mammalian kidney. Although a functional role for this receptor has not been demonstrated, both renal 24-hydroxylase (17,18) and CaBP (19) induction appear to be manifested through the genome. The role of renal CaBP may be to facilitate the tubular handling of calcium by the kidney. Further work will be required to elucidate the mechanism(s) and site(s) of 1,25-$(OH)_2D_3$ action in the mammalian kidney.

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