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EVIDENCE FOR MULTIPLE MOLECULAR WEIGHT FORMS OF THE CHICK INTESTINAL 1,25-DIHYDROXYVITAMIN D_3 RECEPTOR+

June E. Bishop, Willi Hunziker* and Anthony W. Norman

Department of Biochemistry, University of California, Riverside, CA 92521

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SUMMARY: Studies from many laboratories have reported apparent molecular weights for the chick intestinal 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2D_3$] receptor varying from 47,000 to 67,000 daltons. We report here that in the presence of the protease inhibitor phenylmethylsulfonyl fluoride (PMSF, 0.3 mM) and in the presence or absence of ligand, the apparent molecular weight of the receptor is 99,700 ± 9,400 (SD) daltons (as determined by gel filtration). In the absence of PMSF, however, the unoccupied receptor migrates with an apparent molecular weight of 51,400 ± 5,700 (SD) daltons. This smaller form of the 1,25(OH)$_2D_3$ receptor, upon incubation with [^{3}H]-1,25(OH)$_2D_3$ in the presence of PMSF, then migrates with an apparent molecular weight of 95,900 ± 7,300 (SD) daltons. These results suggest the presence of heretofore unappreciated multiple molecular forms of the chick intestinal 1,25(OH)$_2D_3$ receptor.

INTRODUCTION: The hormonally active form of vitamin D_3, namely 1α,25-dihydroxyvitamin D_3 [1,25(OH)$_2D_3$], is believed to initiate its biological responses in its various target tissues by interaction with a specific receptor; thus this postulated mode of action for 1,25(OH)$_2D_3$ is analogous to that proposed for steroid hormones (1-3). To date, receptors for 1,25(OH)$_2D_3$ have been identified in nineteen tissues of mammals and birds (4) including the intestine, bone, pancreas, and parathyroid gland. The 1,25(OH)$_2D_3$ receptor system most frequently studied, however, has been the chick intestinal mucosal receptor system. Studies in this and other laboratories have reported various apparent molecular weights for the chick intestinal receptor as determined via gel filtration: 47,000 (5), 60,000 (6), 64,000 (7,8), 67,000 (9), and 68,000 (10). These studies were all carried out on cytosol

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*Present address: Department of Veterinary Pharmacology, University of Bern, Länggasstrasse 124, CH 3012, Bern, Switzerland.

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fractions prepared from the intestinal mucosa of vitamin D-deficient chicks in the absence of protease inhibitors; the ligand [^{3}H]1,25(OH)$_2$D$_3$ was, in each instance, bound to the receptor by a subsequent *in vitro* incubation. During the course of our studies which evaluated the effects of protease inhibitors on the 1,25(OH)$_2$D$_3$ receptor (11), we found that phenylmethylsulfonyl fluoride (PMSF) acted as a 1,25(OH)$_2$D$_3$ receptor stabilizer; it is now routinely included in our receptor preparation buffers (12). It was therefore of interest to study the possible effects of this stabilizer on the apparent molecular weight of the 1,25(OH)$_2$D$_3$ receptor.

MATERIALS AND METHODS: Chromatin was prepared from vitamin D-deficient chicks in TED buffer (10 mM Tris, 1.5 mM EDTA, 1.0 mM dithiothreitol, pH 7.4) in the presence (TEDP) or absence of 300 μM PMSF. The receptor was extracted from the chromatin by incubation with 0.3 M KCl at 4°C, 30 min, followed by centrifugation for 30 min at 200,000 x g. The resulting supernatant (unoccupied receptor) was incubated with 8 nM [^{3}H]1,25(OH)$_2$D$_3$ (13) for 2 h at 4°C to generate *in vitro* occupied receptor. *In vivo* occupied receptor was prepared by extraction of chromatin from chicks dosed i.m. with 13 nmoles 1,25(OH)$_2$D$_3$ 2 h prior to sacrifice. Four ml aliquots of these receptor preparations, with Blue Dextran, [^{14}C]bovine serum albumin, and [^{14}C]leucine as molecular weight markers, were applied to a 1.6 x 83 cm Sephacryl S-200 Superfine (Pharmacia) column eluted with KTEDP (TEDP buffer with 0.3 M KCl). One-ml fractions were collected and assayed for receptor. The migration position of *in vitro* occupied receptor was determined by liquid scintillation counting of 0.8 ml aliquots of the fractions. Unoccupied receptor was assayed by incubating 0.4 ml aliquots with 4 nM [^{3}H]1,25(OH)$_2$D$_3$ for 4 h at 4°C, followed by hydroxylapatite assay (14). *In vivo* occupied receptor was assayed by the following variation of our exchange assay (15) to separate receptor-bound from "free" ligand; aliquots of each fraction were incubated overnight with 4 nM [^{3}H]1,25(OH)$_2$D$_3$ in the presence of 0.5 M NaSCN. TEDP was added to the incubations to give a final concentration of 0.2 M NaSCN, incubation was continued for 2 h, and the hydroxylapatite assay was performed. The Sephacryl S-200 column was calibrated using Blue Dextran (void volume marker), aldolase (158,000 daltons), bovine serum albumin (67,000 daltons), ovalbumin (44,000 daltons), and [^{14}C]leucine (salt volume marker). Apparent molecular weights were calculated using a K_D *vs.* log molecular weight plot (16).

$$K_D = \frac{V_e - V_0}{V_s - V_0}$$

where V_0 = void volume, V_e = elution volume, and V_s = salt volume.

RESULTS: When all three types of the chick intestinal 1,25(OH)$_2$D$_3$ receptor (unoccupied, *in vitro* occupied, and *in vivo* occupied) were chromatographed on Sephacryl S-200 Superfine columns, no differences were observed in their apparent molecular weights (Figure 1). All three forms of the receptor migrated with an apparent molecular weight of about 96,900 daltons (Table 1).

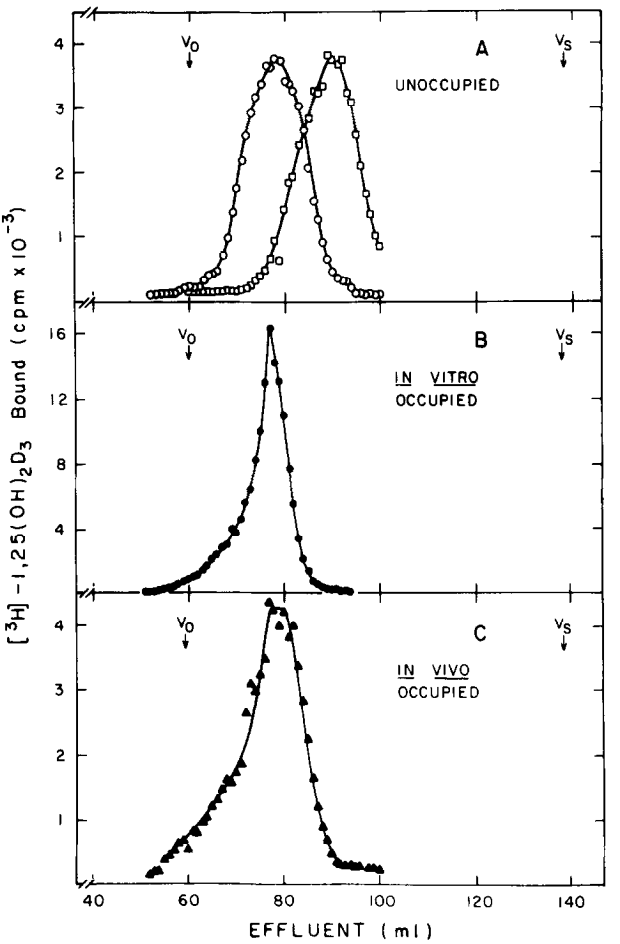

Figure 1. Sephacryl S-200 Superfine gel chromatography of chick intestinal 1,25(OH)$_2$D$_3$ receptor preparation. Panel A: o--o, unoccupied receptor in the presence of PMSF.; □ -- □ , unoccupied receptor in the absence of PMSF. Panel B: ●--●, in vitro occupied receptor in the presence or absence of PMSF. Panel C: ▲--▲, in vivo occupied receptor in the presence or absence of PMSF.

However, when these three forms of the receptor were prepared in the absence of PMSF, only the ligand-occupied forms of the receptor migrated with a large apparent molecular weight. In contrast, the unoccupied form of the 1,25-(OH)$_2$D$_3$ receptor migrated with a lower apparent molecular weight of 51,400 daltons. As shown in Figure 2, when this smaller form of the receptor was pooled, incubated for 4 hr at 4°C with [^{3}H]1,25(OH)$_2$D$_3$ in the presence of PMSF, and rechromatographed on the Sephacryl column, it was found to migrate with an apparent molecular weight of 95,900 daltons (see also Table 2).

TABLE I. Chick Intestinal $1,25(OH)_2D_3$ Receptor: Observed Molecular Weight

$1,25(OH)_2D_3$ Receptor Species	PMSF	n	Molecular Weight ± S.D.
Occupied, *in vivo*	±	2	93,100 ± 5,600
Occupied, *in vitro*	±	8	99,700 ± 9,400
Unoccupied	+	3	91,900 ± 3,300
Unoccupied	-	9	51,400 ± 5,700

Molecular weights of the receptors were approximated by chromatography on a calibrated Sephacryl S-200 column using a K_D *vs* log molecular weight plot (12). PMSF when present was 0.3 mM.

DISCUSSION: These results present evidence that the chick intestinal $1,25-(OH)_2D_3$ receptor prepared in the presence of protease inhibitors appears to have a significantly larger molecular weight (96,000) than previously reported values (5-10). This observation is consistent with reports from other steroid hormone receptor systems (17) that proteases can perturb ligand binding to the receptor and modify the receptors' apparent molecular weight. The observation (Table 1) that the unoccupied receptor in the presence of the protease inhibitor PMSF also migrates with an apparent molecular weight of 91,900 is consistent with the observations of Baker *et al.* (18,19) for other steroid hormone receptor systems as well as our own studies (20) on the $1,25(OH)_2D_3$ chick intestinal receptor that some protease inhibitors bind at or near the ligand binding site of the hormone receptor. Thus the "unoccupied" $1,25(OH)_2D_3$ receptor prepared in the presence of PMSF may represent a "pseudo occupied" receptor. In addition, the unoccupied receptor, prepared

TABLE II. Chick Intestinal $1,25(OH)_2D_3$ Receptor: Observed Molecular Weight

$1,25(OH)_2D_3$ Receptor Species	PMSF	n	Molecular Weight ± S.D.
Unoccupied	-	5	56,900 ± 3,300
Followed by			
Occupied, *in vitro*	+	5	95,900 ± 7,300

Molecular weights of the $1,25(OH)_2D_3$-receptor were approximated as described in Table I. PMSF when present was 0.3 mM.

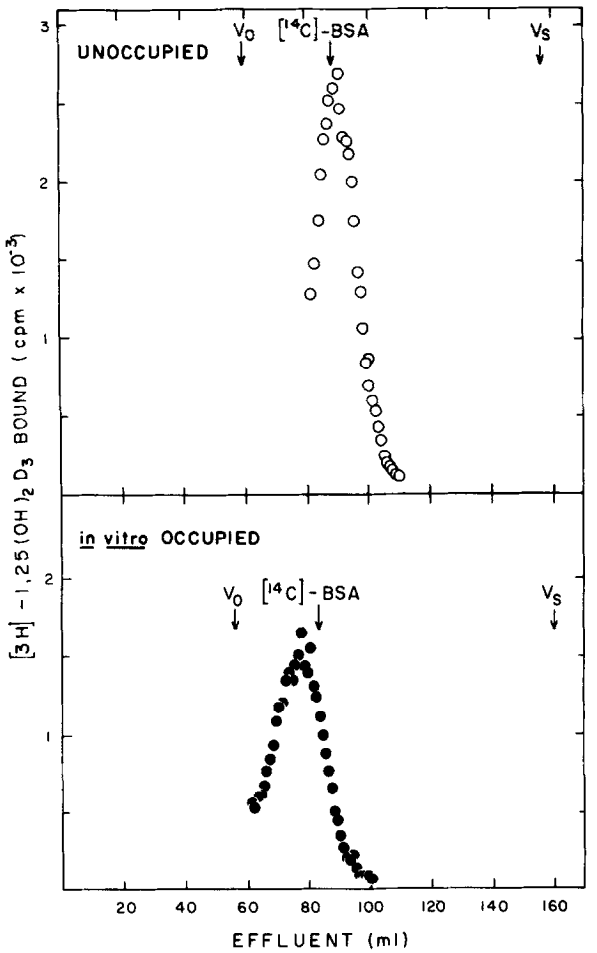

Figure 2. Sephacryl S-200 Superfine gel chromatography of unoccupied then in vitro occupied chick intestinal 1,25(OH)$_2$D$_3$ receptor. Fractions containing the unoccupied 1,25(OH)$_2$D$_3$ receptor from the first passage through the Sephacryl column were pooled, incubated with 4 nM [^{3}H]1,25(OH)$_2$D$_3$ in the presence of PMSF for 4 hr at 4°C and chomatographed a second time on the same Sephacryl column. o--o, Unoccupied receptor. •--•, In vitro occupied receptor.

in the absence of the protease inhibitor PMSF, appears as a smaller (51,400) molecular weight form. While it may be postulated that this smaller form may result from partial proteolysis of the larger form, it is also possible that it may represent dissociated subunits. In our view, proteolytic action is probably not the complete explanation, since the small form can be made to regain its large size upon incubation with [^{3}H]1,25(OH)$_2$D$_3$ in the presence of PMSF. These data, in concert with our finding of differential affinity of occupied and unoccupied receptor forms for DNA/chromatin (20), may help to

elucidate possible transformation/activation steps in the $1,25(OH)_2D_3$ receptor system.

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