# A SPECIFIC BINDING PROTEIN FOR 1,25-DIHYDROXYVITAMIN $D_3$ IN RAT INTESTINAL CYTOSOL[+]

by

Barbara E. Kream and Hector F. DeLuca

Department of Biochemistry, College of Agricultural and Life Sciences

University of Wisconsin-Madison, Madison, Wisconsin 53706

Received April 11, 1977

In the presence of 0.3 M potassium chloride and 0.5 mM dithiothreitol, rat intestinal cytosol contains two binding proteins for 1,25-dihydroxyvitamin $D_3$ (1,25-$(OH)_2D_3$)[1] having sedimentation coefficients of 3.2S and 5-6S. The 3.2S protein is specific for 1,25-$(OH)_2D_3$ as determined by competition analysis, whereas the 5-6S protein binds 25-hydroxyvitamin $D_3$ (25-OH-$D_3$) exclusively.

There is now evidence that 1,25-$(OH)_2D_3$, the metabolite of vitamin $D_3$ most active in stimulating intestinal calcium absorption, may likely function at the cellular level in a manner similar to other steroid hormones. To substantiate this idea, high affinity, low capacity, 1,25-$(OH)_2D_3$ binding proteins have been demonstrated in nuclei and cytosol from chick intestinal homogenates (1-4). However, the demonstration of a similar 1,25-$(OH)_2D_3$ binding protein in the intestines of rats and mice has been unsuccessful despite the fact that calcium and phosphate transport are responsive to 1,25-$(OH)_2D_3$ in these animals (5). Only a 5-6S protein having greater affinity for 25-OH-$D_3$ than 1,25-$(OH)_2D_3$ has been detected in rat intestinal cytosol (4,6). The present study shows that rat intestinal cytosol contains a specific 1,25-$(OH)_2D_3$ binding protein which may, as in the chick, partially mediate the action of 1,25-$(OH)_2D_3$ in the intestine.

---

[+]This work was supported by USPHS Grant AM-14881, U.S. Energy Research and Development Administration Contract EY-76-S-02-1668 and the Harry Steenbock Research Fund. B. E. K. was supported by Postdoctoral Fellowship AM-05055 from the USPHS.

[1]Abbreviations: 1,25-dihydroxyvitamin $D_3$, 1,25-$(OH)_2D_3$; 25-hydroxyvitamin $D_3$, 25-OH-$D_3$.





*ISSN 0006-291X*

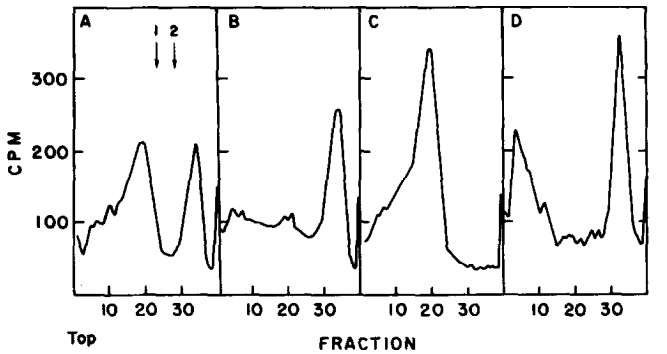


Figure 1: The binding of 1,25-$(OH)_2$-[$^3$H]$D_3$ and 25-OH-[$^3$H]$D_3$ to rat intestinal cytosol and competition for binding. Rat intestinal mucosa was washed and homogenized in buffer. Cytosol (5 mg) was incubated with metabolites and binding was analyzed on 4-20% sucrose gradients. Binding of 0.25 nM 1,25-$(OH)_2$-[$^3$H]$D_3$ (A) and competition for binding by 18 nM 1,25-$(OH)_2D_3$ (B) or 18 nM 25-OH-$D_3$ (C). Binding of 0.38 nM 25-OH-[$^3$H]$D_3$ (D). The arrows indicate the sedimentation of ovalbumin (1) and bovine serum albumin (2).

## MATERIALS AND METHODS

Male weanling rats were obtained from the Holtzman Company (Madison, Wisconsin) at 21 days of age and maintained for 4-6 weeks on a purified vitamin D-deficient diet supplemented three times a week with vitamins A, E, and K in Wesson oil (7). 25-OH-[23,24-$^3$H]$D_3$ (78 Ci/mmol) was synthesized by the method of Partridge et al. (8) and 1,25-$(OH)_2$-[23,24-$^3$H]$D_3$ (78 Ci/mmol) was synthesized enzymatically from 25-OH-[23,24-$^3$H]$D_3$ by the method of Frolik and DeLuca (9). 25-OH-$D_3$ was the gift of Dr. John Babcock of the Upjohn Company (Kalamazoo, Michigan) and 1,25-$(OH)_2D_3$ was generously supplied by Dr. M. Uskokovic of the Hoffmann-LaRoche Company (Nutley, New Jersey). Each rat was decapitated and 12.5 cm of duodenum distal to the pyloric valve were excised and rinsed with buffer at 4° containing 0.3 M potassium chloride, 10 mM Tris-HCl, 1.5 mM EDTA, and 0.5 mM dithiothreitol, pH 7.4. Mucosa was scraped free of serosa and washed in 10 volumes (w/v) of buffer according to a previously described procedure (4). Washed mucosa was homogenized in 2 or 3 volumes of buffer using a polytron type PT-20 (Brinkman Instruments, New York). Cytosol was the clear supernatant fluid (without the fluffy lipid layer) obtained following centrifugation of the homogenate at 106,500 x g for 1 hour. Cytosol (0.5 ml) was incubated with 0.4 mM radioactive metabolite dissolved in 95% ethanol in presence or absence of competing nonradioactive metabolite. Binding of metabolites to components in rat intestinal cytosol was determined on 4-20% linear sucrose gradients containing 0.3 M potassium chloride, 10 mM Tris-HCl, pH 7.4, and 1.5 mM EDTA as previously described (4).

## RESULTS

The binding of 1,25-$(OH)_2$-[$^3$H]$D_3$ and 25-OH-[$^3$H]$D_3$ to cytosol prepared from rat intestinal mucosa was analyzed by sucrose density gradient centrifugation and compared. Experimental conditions which have been

successful in demonstrating a specific high affinity binding for 1,25-$(OH)_2D_3$ in chick intestinal mucosa were altered for this investigation. Rat intestinal mucosa was washed once prior to homogenization in buffer containing 0.3 M potassium chloride and 0.5 mM dithiothreitol. The cytosol contained two high affinity binding proteins for 1,25-$(OH)_2$-[$^3$H]$D_3$ having sedimentation coefficients of 3.2S (peaks at fraction 19) and 5-6S (peaks at fraction 32) as determined by comparison with ovalbumin and bovine serum albumin. The 1,25-$(OH)_2$-[$^3$H]$D_3$ associated primarily with the 3.2S species (Figure 1A). An excess of nonradioactive 1,25-$(OH)_2D_3$ displaced 3.2S-bound 1,25-$(OH)_2$-[$^3$H]$D_3$ (fraction 19) to the 6S species (fraction 32) (Figure 1B) whereas the same amount of 25-OH-$D_3$ displaced 6S-bound 1,25-$(OH)_2$-[$^3$H]$D_3$ to the 3.2S macromolecule (Figure 1C). 25-OH-[$^3$H]$D_3$ bound only in the 6S region (Figure 1D) and was not displaced onto the 3.2S region by either nonradioactive 1,25-$(OH)_2D_3$ or 25-OH-$D_3$. Therefore, it appeared that the 3.2S and 5-6S macromolecules were specific for 1,25-$(OH)_2D_3$ and 25-OH-$D_3$, respectively. The binding capacity of the 3.2S macromolecule was destroyed by incubation at 37° or by treatment with trypsin indicating that the 3.2S species is at least in part protein.

Previous studies in other labortories (10) and in ours have clearly demonstrated the widespread distribution of the 6S protein in all rat tissues examined. However, in our studies the 3.2S protein for 1,25-$(OH)_2D_3$ was not found in rat tissues not considered responsive to vitamin $D_3$ unlike the distribution of the 6S component. Although further investigation is necessary to fully characterize the 3.2S protein, it possesses the properties of high affinity and low capacity which are important criteria of steroid hormone receptors (11,12).

## References

1. Brumbaugh, P. F. and Haussler, M. R. (1974) J. Biol. Chem. 249, 1258-2162.
2. Brumbaugh, P. F. and Haussler, M. R. (1975) J. Biol. Chem. 250, 1588-1594.
3. Lawson, D. E. M. and Wilson, P. W. (1974) Biochem. J. 144, 573-583.

4. Kream, B. E., Reynolds, R. D., Knutson, J. C., Eisman, J. A., and DeLuca, H. F. (1975) Arch. Biochem. Biophys. 176, 779-787.
5. Omdahl, J. L. and DeLuca, H. F. (1973) Physiol. Rev. 53, 327-372.
6. Haddad, J. G., Hahn, T. J., and Birge, S. F. (1973) Biochim. Biophys. Acta 329, 93-97.
7. DeLuca, H. F., Guroff, G., Steenbock, H., Reiser, S. and Manatt, M. R. (1961) J. Nutr. 75, 175-180.
8. Partridge, J. J., Faber, S. and Uskokovic, M. R. (1974) Helv. Chim. Acta 57, 764-771.
9. Frolik, C. A. and DeLuca, H. F. (1973) J. Clin. Invest. 52, 543-548.
10. Haddad, J. G. and Birge, S. F. (1975) J. Biol. Chem. 250, 299-303.
11. O'Malley, B. W. and Means, A. R. (1974) Science 183, 610-620.
12. Jensen, E. V. and DeSombre, E. R. (1972) Ann. Rev. Biochem. 41, 203-230.