Specific Binding Protein for Active Metabolites of Vitamin D₃

NORIMASA HOSOYA AND TSUNEYUKI OKU

Nutritional Laboratory, School of Health Sciences, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo
(Post No. 113)

(Received May 14, 1971)

In the recent report from our laboratory, vitamin D₃ binding protein was identified in an intestinal cytoplasm by the analysis of Sephadex G-200 column (1). After the oral administration of ³H-vitamin D₃ to vitamin D deficient rat, three protein peaks showed to be bound with radioactivities in the elution profile of cytoplasmic fraction obtained from intestinal mucosa (Fig. 1). The initial peak of radioactivity was eluted with the peak 1 protein fraction, and the bound radioactivity was identified with ³H-vitamin D₃. However small amounts of radioactivity of peak 2 and 3 were not identified.

The physiological active metabolites of vitamin D₃ were identified as 25-hydroxycholecalciferol (25-HCC) by DeLuca (2), and 1-oxygenated 25-HCC (1,25-dihydroxycholecalciferol; 1,25-DHCC) by Fraser and Kodicek (3). In this report specific proteins binding to 25-HCC and 1,25-DHCC were also identified in mucosal cytoplasm of vitamin D deficient rat intestine.

Vitamin D deficient rats and their intestinal cytoplasmic fraction were prepared according to the previous report (1). 25-HCC-26,27-³H (sp. act. 315 mCi/mM) was purchased from New England Nuclear Co., and 1,25-DHCC-³H was prepared enzymatically from 25-HCC-26,27-³H according to the method of Fraser and Kodicek (3). The binding with active form of vitamin D₃ was assessed in vitro by the supernatant fraction of intestinal mucosa. Two milliliter of cytoplasmic fraction containing about 5 mg/ml protein was incubated for 20 minutes at 2°C with 125 µµ moles of 25-HCC-26,27-³H or the prepared 1,25-DHCC-³H containing about 20

---

1 細谷憲政，奧 恒行
FIG. 2 Separation of the specific proteins binding to 25-HCC and 1,25-DHCC in rat intestinal cytoplasm by gel filtration on Sephadex G-200 in the incubation in vitro with the tritium labeled substrates.

A, 25-HCC binding protein; B, 1,25-DHCC binding protein. The experimental conditions were described in the text.

---, absorbancy at 750 m\(\mu\) per 0.2 ml effluents; •••••, radioactivity (cpm) per 0.5 ml of effluents.

Activity was observed in corresponding with peak 2 fraction of protein (Fig. 2-A). From this result, the second peak of radioactivity (Fig. 1) was considered to be 25-HCC, and peak 2 protein fraction will be specific protein binding to 25-HCC. On the other hand, in the incubation of 1,25-DHCC\(^{3}H\) with the cytoplasmic fraction in vitro, the radioactivity was observed in corresponding with peak 3 protein fraction. Peak 1 and peak 2 protein frations were already confirmed to be bound specifically to vitamin D\(_{3}\) and 25-HCC, respectively. The radioactivity observed in the peak 3 protein fraction will be 1,25-DHCC\(^{3}H\) (Fig. 2-B). From these results, the third peak of radioactivity (Fig. 1) was considered to be 1,25-DHCC, and peak 3 protein fraction will be specific protein binding to 1,25-DHCC.

Further observation was carried out to estimate S value of each protein peaks by sucrose density gradient ultracentrifugation (1). Sedimentation constant of Peak 1, 2 and 3 was 13 S, 8.0~8.6 S and 3.5~4.0 S respectively.

Vitamin D\(_{3}\) and its metabolites were bound to specific proteins in rat intestinal mucosa. Further observation will be reported concerning with their protein characteristics and binding affinities.

REFERENCES