IN VITRO 25-HYDROXYLATION OF 1α,24-DIHYDROXYVITAMIN D_3 IN VARIOUS RAT TISSUES

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1. Introduction

The 25-hydroxylation of vitamin D_3 in the liver is the first step for vitamin D_3 activation [1–3]. 25-OH-D_3 was shown formed from vitamin D_3 in intact rats but not to a significant level in the hepatectomized rats [1,2]. This suggests that the transformation of vitamin D_3 to 25-OH-D_3 takes place mainly, if not only, in the liver. The transformation of vitamin D_3 to 25-OH-D_3 was demonstrated using both perfused rat liver and rat liver homogenate systems [3]. However, vitamin D_3-25-hydroxylase activity was reported present in the kidney and intestine as well as in the liver of chick [4], indicating the distribution of the enzyme in various tissues.

We have shown that 1α,24-$(OH)_2D_3$ is metabolized rapidly to 1α,24,25-$(OH)_3D_3$ in rats [5,6] and the 25-hydroxylation occurs mainly in the liver [5]. However, the possibility of 25-hydroxylation in the tissues other than the liver has been suggested also, since a small amount of 1α,24,25-$(OH)_3D_3$ was formed from 1α,24-$(OH)_2D_3$ in the hepatectomized rats.

This paper describes the 25-hydroxylation of 1α,24-$(OH)_2D_3$ and 1α-OH-D_3 using homogenates of rat liver, kidney, intestine, bone, adrenal and muscle.

2. Materials and methods

2.1. *Compounds*

Tritium-labeled compounds, 1α-OH-[24(*S*)-^{3}H]D_3 (3.8 Ci/mmol), 1α,24(*R*)-$(OH)_2$[24-^{3}H]D_3 (3.0 Ci/mmol) and 1α,24(*S*)-$(OH)_2$[24-^{3}H]D_3 (3.0 Ci/mmol), were synthesized as in [5,7]. Non-radioactive 1α,25-$(OH)_2D_3$, 1α,24(*R*)25-$(OH)_3D_3$ and 1α,24(*S*)25-$(OH)_3D_3$ were synthesized as in [8] and [9], respectively.

Abbreviations: 1α-OH-D_3, 1α-hydroxyvitamin D_3; 1α,24(*R*)-$(OH)_2D_3$, 1α,24(*R*)-dihydroxyvitamin D_3; 1α,24(*S*)-$(OH)_2D_3$, 1α,24(*S*)-dihydroxyvitamin D_3; 1α,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 1α,24(*R*)25-$(OH)_3D_3$, 1α,24(*R*)25-trihydroxyvitamin D_3; 1α,24(*S*)25-$(OH)_3D_3$, 1α,24(*S*)25-trihydroxyvitamin D_3; DHT_3, dihydrotachysterol$_3$

2.2. *Incubation of liver homogenates*

Weanling male Wistar rats were fed a vitamin D-deficient low calcium diet for 6 weeks [5]. 10% liver homogenates in 0.25 M sucrose were prepared with the aid of a Potter-Elvehjem homogenizer fitted with a teflon pestle. A portion (5 ml) of the homogenates was then mixed with 10 ml phosphate buffer (pH 7.4) containing Mg- and NADPH-generating systems as in [3]. The final concentrations were: 25 mM phosphate buffer (pH 7.4), 1.25 mM $MgCl_2$, 25 mM KCl, 5 mM ATP, 0.1 mM NADP, 40 mM nicotinamide, 5 mM sodium succinate, 5.6 mM glucose 6-phosphate and 0.9 units glucose 6-phosphate dehydrogenase. The reaction was initiated by adding 1.0 μg 1α,24(*R*)-$(OH)_2$[^{3}H]D_3, 1α,24(*S*)-$(OH)_2$[^{3}H]D_3 or 1α-OH-[^{3}H]D_3 in 200 μl ethanol. The incubation was carried out with gentle shaking at 37°C for 2 h and 30 ml methanol:chloroform (1:1) was added to stop the reaction.

2.3. *Incubation of intestinal mucosa homogenates*

Intestines from the vitamin D-deficient rats were rinsed with 0.9% NaCl solution, then the mucosa was scraped off from the serosa with a glass microscope slide. The intestinal mucosa (3 g) was homogenized with 30 ml 0.25 M sucrose in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The incubation was carried out with the same reaction mixture as for the liver homogenate incubations. The

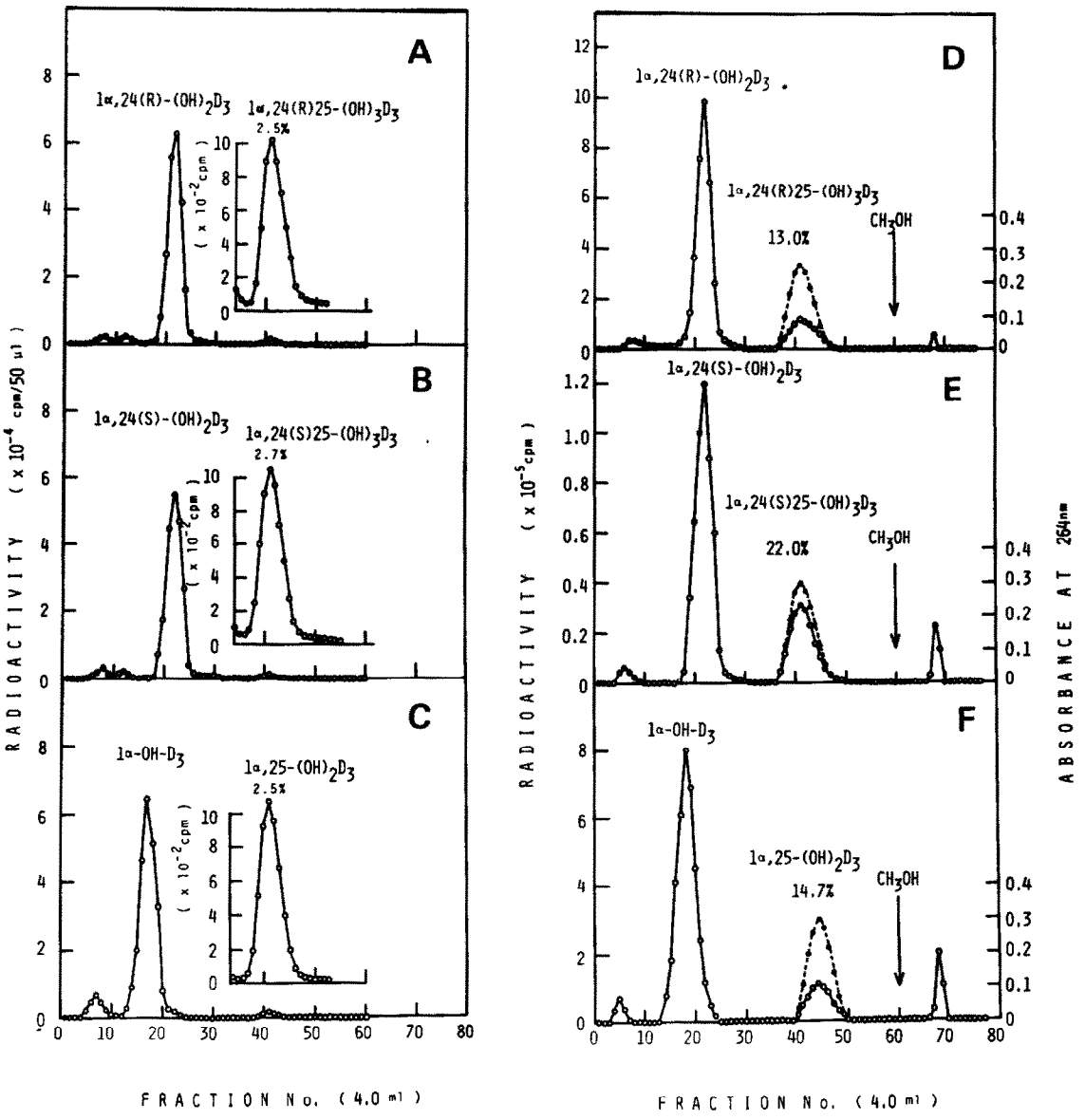

Fig.1. The elution profiles from a Sephadex LH-20 column of the extracts of rat liver homogenates incubated either with 1α,24(*R*)-$(OH)_2[^3H]D_3$ (A), 1α,24(*S*)-$(OH)_2[^3H]D_3$ (B) or 1α-OH-$[^3H]D_3$ (C) for 2 h by the method in [3]. Sephadex LH-20 chromatographic profiles of lipid extracts from rat liver perfusates perfused with 1.0 µg 1α,24(*R*)-$(OH)_2[^3H]D_3$ (D), 1α,24(*S*)-$(OH)_2[^3H]D_3$ (E) or 1α-OH-$[^3H]D_3$ (F) for 2 h by the method in [15] as described [5]. Sephadex LH-20 column (1.5 × 25 cm) developed with chloroform:*n*-hexane:methanol (75:23:2) [16] for 1α,24-$(OH)_2[^3H]D_3$ and chloroform:*n*-hexane (65:35) [17] for 1α-OH-$[^3H]D_3$.

reaction was initiated by adding 50 ng 1α,24(*R*)-$(OH)_2$-[^{3}H]D_3, 1α,24(*S*)-$(OH)_2$[^{3}H]D_3 or 1α-OH-[^{3}H]D_3 in 200 μl ethanol. The incubation was carried out with gentle shaking at 37°C for 2 h.

2.4. *Incubation of tissue homogenates other than intestinal mucosa*

A portion (3 g) of the kidney, adrenal or muscle from vitamin D-deficient rats was homogenized with 30 ml 0.25 M sucrose in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. The bones were collected and a 10% homogenate in 0.25 M sucrose was prepared using an Ultra-Turrax. The incubation was as with intestinal mucosa homogenates.

2.5. *Identification of 1α,24,25-(OH)$_3$[^{3}H]D_3*

The polar metabolites of 1α,24(*R*)-$(OH)_2$[^{3}H]D_3 and 1α,24(*S*)-$(OH)_2$[^{3}H]D_3 from a Sephadex LH-20 column were co-chromatographed with synthetic 1α,24(*R*)25-$(OH)_3D_3$ and 1α,24(*S*)25-$(OH)_3D_3$ on a high-pressure liquid chromatography Hitachi model 635, using a Du Pont Zorbax Sil column (2.1 X 250 mm) and 3.5% methanol in dichloromethane as the eluting solvent, as in [10].

3. Results

The homogenates of various tissues from the rat were incubated with 1α,24(*R*)-$(OH)_2D_3$, 1α,24(*S*)-$(OH)_2D_3$ or 1α-OH-D_3 since it had been shown that 1α,24-$(OH)_2D_3$ was metabolized to 1α,24,25-$(OH)_3D_3$ in vivo [5,6]. The chromatographic profiles on Sephadex LH-20 of the extracts of the liver homogenates pre-incubated with the compounds are shown in fig.1. The transformations of 1α,24(*R*)-$(OH)_2D_3$ and 1α,24(*S*)-$(OH)_2D_3$ to the corresponding 25-hydroxylated compounds were demonstrated by Sephadex LH-20 column chromatography (fig.1A,B) and co-chromatography with the synthetic compounds in a high-pressure liquid chromatograph. The transformation rates of 1α,24(*R*)-$(OH)_2D_3$ and 1α,24(*S*)-$(OH)_2D_3$ were 2.5% and 2.7%, respectively, and were almost the same as that of 1α-OH-D_3 to 1α,25-$(OH)_2D_3$. Homogenates that were heated to 100°C for 10 min and incubated with either 1α-OH-D_3 or 1α,24-$(OH)_2D_3$ were incapable of converting 1α-OH-D_3 to 1α,25-$(OH)_2D_3$ or 1α,24-$(OH)_2D_3$ to 1α,24,25-$(OH)_3D_3$.

The perfusion of rat liver with the perfusate con-

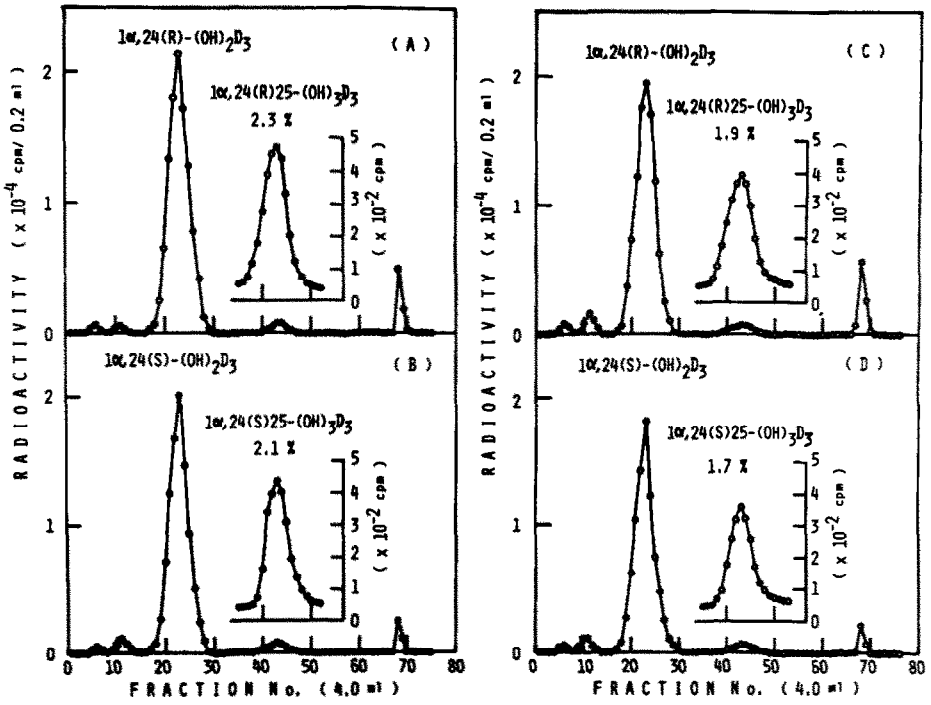

Fig.2. Sephadex LH-20 chromatographic profiles of lipid extracts from rat kidney homogenates or rat intestinal mucosa homogenates incubated with 50 ng 1α,24(*R*)-$(OH)_2$[^{3}H]D_3 (A,C) or 1α,24(*S*)-$(OH)_2$[^{3}H]D_3 (B,D) for 2 h respectively. Sephadex LH-20 column chromatography was as in fig.1.

taining 1α,24(*R*)-$(OH)_2D_3$, 1α,24(*S*)-$(OH)_2D_3$ or 1α-OH-D_3 was carried out and the extracts of the perfusate were analyzed by chromatography on Sephadex LH-20. As shown in fig.1D–F, 13% of 1α,24(*R*)-$(OH)_2D_3$, 22% of 1α,24(*S*)-$(OH)_2D_3$ and 14.7% of 1α-OH-D_3 were metabolized to the 25-hydroxylated compounds.

Fig.2 shows the results of the incubation of rat kidney homogenates with 1α,24(*R*)-$(OH)_2D_3$ or 1α,24(*S*)-$(OH)_2D_3$. Both compounds were metabolized to 1α,24(*R*)25-$(OH)_3D_3$ (2.3%) and 1α,24(*S*)25-$(OH)_3D_3$ (2.1%) (fig.2A,B); the fractions of both metabolites gave a single peak by Sephadex LH-20 rechromatography and high-pressure liquid chromatography.

The incubation of 1α,24(*R*)-$(OH)_2D_3$ or 1α,24(*S*)-$(OH)_2D_3$ with the intestinal mucosa homogenates also gave the corresponding 25-hydroxylated metabolites; the transformation rates were 1.9% and 1.7%, respectively (fig.2C,D). On the other hand, incubation of 1α,24(*R*)-$(OH)_2D_3$ or 1α,24(*S*)-$(OH)_2D_3$ with homogenates of bone, adrenal or muscle did not result in the formation of any metabolites (not shown).

As in the case of 1α,24-$(OH)_2D_3$, 1α,25-$(OH)_2D_3$ was produced by the incubation of 1α-OH-D_3 with homogenates of the liver, kidney and intestine but none of the metabolite was formed by the incubation of bone, adrenal or muscle homogenates.

4. Discussions

The 25-hydroxylation of vitamin D_3 takes place in the liver [1–3]. 25-Hydroxylation of vitamin D_3 and 1α-OH-D_3 was studied using homogenates of liver and intestine of the rat and hydroxylation reported to occur in the liver but not in the intestine [11]. However, we have explored the presence of 25-hydroxylase in various tissues of rats since, when 1α-OH-D_3 or 1α,24-$(OH)_2D_3$ was administered to hepatectomized rats, the corresponding 25-hydroxy derivative was detected, suggesting the presence of 25-hydroxylase in other tissues as well as in the liver (S. I. et al in preparation). The result indicated that 25-hydroxylase exists in liver, kidney and intestine of rats but not in bone, adrenal and muscle. The corresponding 25-hydroxy derivative of 1α-OH-D_3 or 1α,24-$(OH)_2D_3$ gradually increased during the incubation period of 2 h and attained a maximal level. The 25-hydroxylation of 1α-OH-D_3 or 1α,24-$(OH)_2D_3$ did not occur when the steroid was incubated with boiled homogenates of various tissues. The above implies that 25-hydroxylation is not oxidation by air but an enzymic reaction.

It is well known that there are two types of 25-hydroxylation in rat liver [12–14]. 25-Hydroxylation of vitamin D_3 was compared with that of dihydrotachysterol$_3$ (DHT_3) [12] and it was concluded that the latter was different from the former which was regulated by metabolic control. It was reported [13,14] that 25-hydroxylation of 1α-OH-D_3 was the same type with DHT_3 and we have found [5] that of 1α,24-$(OH)_2D_3$ in liver to be DHT_3 kind. Our next problem is whether the 25-hydroxylation of 1α,24-$(OH)_2D_3$ in kidney and intestine is under metabolic control.

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