1α-Hydroxy-25-fluorovitamin D_3: A Potent Analogue of 1α,25-Dihydroxyvitamin D_3†

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ABSTRACT: Chemically synthesized 1α-hydroxy-25-fluorovitamin D_3 was compared to 1,25-dihydroxyvitamin D_3 for potency in the chick intestinal cytosol-binding protein assay, induction of intestinal calcium transport, mobilization of calcium from bone, and epiphyseal plate calcification in the rat. The 25-fluorinated analogue causes 50% displacement of 1,25-dihydroxy[23,24-^{3}H]D_3 at 1.8×10^{-8} M in the competitive protein-binding assay, whereas only 5.6×10^{-11} M of unlabeled 1,25-dihydroxyvitamin D_3 is needed for equal competition. This 315-fold difference between the activities of 1,25-dihydroxyvitamin D_3 and 1α-hydroxy-25-fluorovitamin D_3 indicates that the fluoro analogue is about equipotent with 1α-hydroxyvitamin D_3 in the protein-binding assay. However, 1α-hydroxy-25-fluorovitamin D_3 is 1/50 as active as 1,25-dihydroxyvitamin D_3 in vivo in the stimulation of intestinal calcium transport and bone calcium mobilization in vitamin D deficient rats on a low-calcium diet. Likewise, 1α-hydroxy-25-fluorovitamin D_3 is about 40 times less active than 1,25-dihydroxyvitamin D_3 in inducing endochondrial calcification in rachitic rats. No selective actions of 1α-hydroxy-25-fluorovitamin D_3 were noted. Since the 25 position of the analogue is blocked by a fluorine atom, it appears that 25-hydroxylation of 1α-hydroxylated vitamin D compounds in vivo is not an obligatory requirement for appreciable vitamin D activity.

The hormone 1α,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$][1] induces intestinal calcium and phosphate absorption, bone calcium and phosphate mobilization, calcification, and growth (Omdahl and DeLuca, 1973; Kodicek, 1974; DeLuca and Schnoes, 1976). Naturally, the relationship between the structure of 1,25-$(OH)_2D_3$ and its physiological functions is of considerable interest. Thus, some effort has been devoted to understanding the relative importance of each of the three hydroxy groups in 1,25-$(OH)_2D_3$ for its several functions. For example, several studies have shown that 3-deoxy analogues function in all phases of 1,25-$(OH)_2D_3$-mediated calcium and phosphate metabolism (Lam et al., 1974; Okamura et al., 1974, 1975; Onisko et al., 1977), which demonstrates that the 3-hydroxy group is not absolutely essential in hormone–receptor interactions. Physiological doses of 25-OH-D_3, however, do not produce biological responses unless they undergo 1α-hydroxylation (DeLuca, 1974), whereas large amounts will function without 1α-hydroxylation (Pavlovitch et al., 1973). Therefore, the 1α-hydroxy group is likely a more important structural feature for receptor interaction than the 3-hydroxy.

In contrast to the situation with the 1- and 3-hydroxy, no direct assessment of the 25-hydroxy's contribution to the spectrum of 1,25-$(OH)_2D_3$'s activity in vivo is available. A number of compounds, including dihydrotachysterol$_3$ (Hallick and DeLuca, 1971) and 1α-hydroxyvitamin D_3 (1-OH-D_3) (Holick et al., 1976a,b), undergo rapid 25-hydroxylation in vivo. Although 25-hydroxylation precedes manifestation of activity by these compounds, the hydroxylations occur fast enough so that the analogues act almost as rapidly as 1,25-$(OH)_2D_3$. Thus, it is unclear whether or not the 25-hydroxy is obligatory for in vivo biological response or simply facilitates response. Moreover, administered 1,25-$(OH)_2D_3$ undergoes side-chain cleavage in vivo (Harnden et al., 1976; Kumar et al., 1976; Kumar and DeLuca, 1976). The nature and functional significance of the resulting metabolite are unknown, but the 25-hydroxy group could be central to the cleavage process. This introduces the possibility that a 25-hydroxy group has the triple function of facilitating 1α-hydroxylation, enhancing 1,25-$(OH)_2D_3$–receptor interactions and mediating the further metabolism of 1,25-$(OH)_2D_3$.

One approach to understanding the significance of the 25-hydroxy function is assessing a 1-hydroxyvitamin D_3 analogue, blocked at the 25 position so 25-hydroxylation is precluded. Fluorine in place of hydrogen at the 25 position seems particularly suited for the purpose of preventing 25-hydroxylation, since evidence indicates that carbon–fluorine bonds are not broken in animals (Peters, 1957; Heidelberger et al., 1958). Fluorine also closely approximates hydrogen in atomic dimensions (Pauling, 1960; Cottrell, 1958). This minimizes the possibility of disrupted analogue–protein interactions which might be caused by larger blocking groups. We chose, therefore, to evaluate the biological activity of chemically synthesized 1α-hydroxy-25-fluorovitamin D_3 (1-OH-25-F-D_3).

This paper relates the binding potency of 1-OH-25-F-D_3 with the chick intestinal cytosol-binding protein and describes the characteristics of 1-OH-25-F-D_3 in mediating calcium metabolism in the rat. The results demonstrate that 1-OH-25-F-D_3 is about 25 to 50 times less active than 1,25-$(OH)_2D_3$ in vivo. Furthermore, 1-OH-25-F-D_3 stimulates all vitamin D responsive systems equally well.

Materials and Methods

General. Ultraviolet spectra were taken in 95% ethanol with

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[1] Abbreviations used: 1,25-$(OH)_2D_3$, 1α,25-dihydroxyvitamin D_3; 1-OH-D_3, 1α-hydroxyvitamin D_3; 1-OH-25-F-D_3, 1α-hydroxy-25-fluorovitamin D_3; LC, high-pressure liquid chromatography; 1,25-$(OH)_2$-[23,24-^{3}H]D_3, 1,25-dihydroxy[23,24-^{3}H]vitamin D_3; 24*R*-OH-D_3, (24*R*)-hydroxyvitamin D_3; 1,24,25-$(OH)_3D_3$, 1,24,25-trihydroxyvitamin D_3; 1,25-$(OH)_2D_2$, 1,25-dihydroxyvitamin D_2; 1,24-$(OH)_2D_3$, 1,24-dihydroxyvitamin D_3; 25-F-1,24-$(OH)_2D_3$, 25-fluoro-1,24-dihydroxyvitamin D_3.

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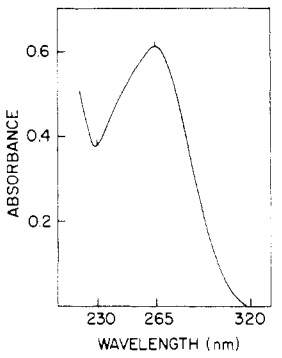

FIGURE 1: Ultraviolet absorbance spectrum of 1-OH-25-F-D_3. A λ_{max} at 265 and λ_{min} at 228 nm is characteristic of and demonstrates that the vitamin D triene chromophore is present.

a Beckman DB-G recording spectrophotometer. High-resolution mass spectrometry was performed at 110 °C above ambient with an A.E.I, MS-9 spectrometer coupled with a DS-50 data system. High-pressure liquid chromatography (LC) was carried out with a 0.7 × 30 cm microparticulate silica gel (10 μm) column connected to a Waters Associates Model ALC/GPC 204 and developed with 5% 2-propanol/hexane. Radioactivity was counted with a Packard Tri-Carb Model 3255 liquid scintillation counter.

Compounds. Crystalline 1,25-$(OH)_2D_3$ was a gift from Hoffmann-LaRoche Co., Nutley, N.J. The 1-OH-25-F-D_3 was synthesized[2] in this laboratory and demonstrated the expected spectral characteristics: UV λ_{max} 265, λ_{min} 228 nm (Figure 1); high-resolution mass spectrum *m/e* (composition, *m/e* calculated) 418.3222 ($C_{27}H_{44}FO_2$, 418.3247), 152.0840 ($C_9H_{12}O_2$, 152.0838), 134.0734 ($C_9H_{10}O$, 134.0732) (Figure 2). LC as described above demonstrated the purity of 1-OH-25-F-D_3 (Figure 3).

Animals. Weanling male rats from the Holtzman Co., Madison, Wis., were housed in individual, overhanging wire cages and fed a vitamin D deficient, low calcium (0.02%) diet or a rachitogenic diet (1.2% calcium, 0.1% phosphate) for 2–3 weeks prior to dosing (Suda et al., 1970).

Intestinal Calcium Transport. Five to seven rats on the low-calcium diet were dosed intrajugularly with either 1,25-$(OH)_2D_3$ or 1-OH-25-F-D_3 in 0.05 mL of 95% ethanol. Controls were dosed with vehicle alone. The animals were decapitated at the times indicated. Duodena were used to assay for intestinal calcium transport by the everted gut sac technique (Martin and DeLuca, 1969). To determine the ^{45}Ca in the mucosal and serosal fluid, aliquots were spotted on filter paper disks, dried, and counted in 10 mL of scintillation counting solution containing 2 g of 2,5-diphenyloxazole and 0.1 g of 1,4-bis[2-(4-methyl-5-phenyloxazolyl)]benzene/L of toluene.

Bone Calcium Mobilization. Blood from the above rats was centrifuged, and 0.1 mL of serum was mixed with 1.9 mL of 0.1% $LaCl_3$. Serum calcium concentration was determined with a Perkin-Elmer Model 403 atomic absorption spectrometer.

Calcification. Rats maintained on the rachitogenic diet were dosed intraperitoneally each day for 7 days with either 1,25-$(OH)_2D_3$ or 1-OH-25-F-D_3 in 0.05 mL of 1,2-propanediol. Controls received vehicle alone. Sixteen hours after the last dose, the rats were decapitated and their radii and ulnae were removed. Radii and ulnae were examined for degree of endochondral calcification by the "line-test" method (U.S. Pharmacopoeia, 1955).

Competitive Protein-Binding Assay. The chick intestinal cytosol competitive protein binding assay was done by Mr. Alan Hamstra according to previously described methods (Eisman et al., 1976a,b; Eisman and DeLuca, 1977).

Results

Competitive Binding Assay. Diplacement of bound 1,25-dihydroxy[23,24-^3H]vitamin D_3 [1,25-$(OH)_2$[23,24-^3H]D_3] from the chick intestinal cytosol protein by either 1,25-$(OH)_2D_3$ or 1-OH-25-F-D_3 is presented in Figure 4. Linear-regression analyses were performed on the data in the range of 0.7 to 11.6 × 10^{-11} M for the 1,25-$(OH)_2D_3$ curve and 1.3 to 10.4 × 10^{-8} M for the 1-OH-25-F-D_3 curve. In these areas the log molarity vs. percent displacement is linear. Correlation coefficients were at least 0.98 for each compound. The slopes were equal, indicating that the curves were parallel. Concentration of compound which would cause 50% displacement was calculated from the linear-regression analysis data to be 5.6 × 10^{-11} M for 1,25-$(OH)_2D_3$ and 1.8 × 10^{-8} M for 1-OH-25-F-D_3. The concentration of 1-OH-D_3 (Figure 5) which causes 50% displacement of 1,25-$(OH)_2$[23,24-^3H]D_3 has been reported as 1.9 × 10^{-8} M (Eisman and DeLuca, 1977).

Intestinal Calcium Transport. Stimulation of the intestinal calcium-transport system by single doses of either 1,25-$(OH)_2D_3$ (25 ng) or 1-OH-25-F-D_3 (6.25 μg) was compared at 6, 24, and 48 h (Figure 6). Both compounds produced a significant and nearly equal increase in the serosal/mucosal ratio of ^{45}Ca at 6 h. At 24 h, 1,25-$(OH)_2D_3$ produced a plateau in intestinal calcium transport that was maintained at 48 h. 1-OH-25-F-D_3 duplicated the well-known response with time of rat intestinal calcium transport to 1,25-$(OH)_2D_3$ (Frolik and DeLuca, 1972). But, in contrast to the 6 h observation, the 24- and 48-h points of 1-OH-25-F-D_3 demonstrated a greater stimulation of intestinal calcium transport than did 1,25-$(OH)_2D_3$.

Graded doses of 1-OH-25-F-D_3 were examined for induction of intestinal calcium transport (Table I). A 600-ng dose of 1-OH-25-F-D_3 produced the same response as 25 ng of 1,25-$(OH)_2D_3$ after 24 h. The 250-ng dose of 1-OH-25-F-D_3 resulted in a lower but significant response, whereas increasing the dose to 2500 ng did not augment the response.

Bone Calcium Mobilization. The time course of calcium mobilization from bone produced by 6.25 μg of 1-OH-25-F-D_3 was compared to that produced by 25 ng of 1,25-$(OH)_2D_3$ (Figure 7). At 6 h, the responses did not differ significantly. On the other hand, 1-OH-25-F-D_3 dosage resulted in greater calcium mobilization than did dosage with 1,25-$(OH)_2D_3$ at the peak of activity (14 h). However, the decays in responses remained approximately parallel from 14 through 48 h.

A comparison of lower dose levels (Table I) showed responses produced by 1-OH-25-F-D_3 were greater at 24 than at 6 h. The difference increased with decreasing dose, so that the lower doses did not raise serum calcium at 6 h. After 24 h, however, 600 ng of 1-OH-25-F-D_3 increased the serum calcium concentration as well as 25 ng of 1,25-$(OH)_2D_3$.

Antirachitic Activity. Graded doses of 1-OH-25-F-D_3 were tested for ability to support endochondral calcification in rachitic rats (Figure 8). After seven daily doses, examination of silver nitrate stained radii and ulnae revealed that calcification scores were linear with respect to log daily dose. Linear-regression analysis showed a correlation coefficient of greater than 0.99. In this same assay, bones from rats which received only vehicle scored zero, whereas daily doses of 12.5 ng of 1,25-$(OH)_2D_3$ produced a score of 4.7 ± 0.8 (mean ± SEM

[2] The synthesis of 1-OH-25-F-D_3 will be reported elsewhere.

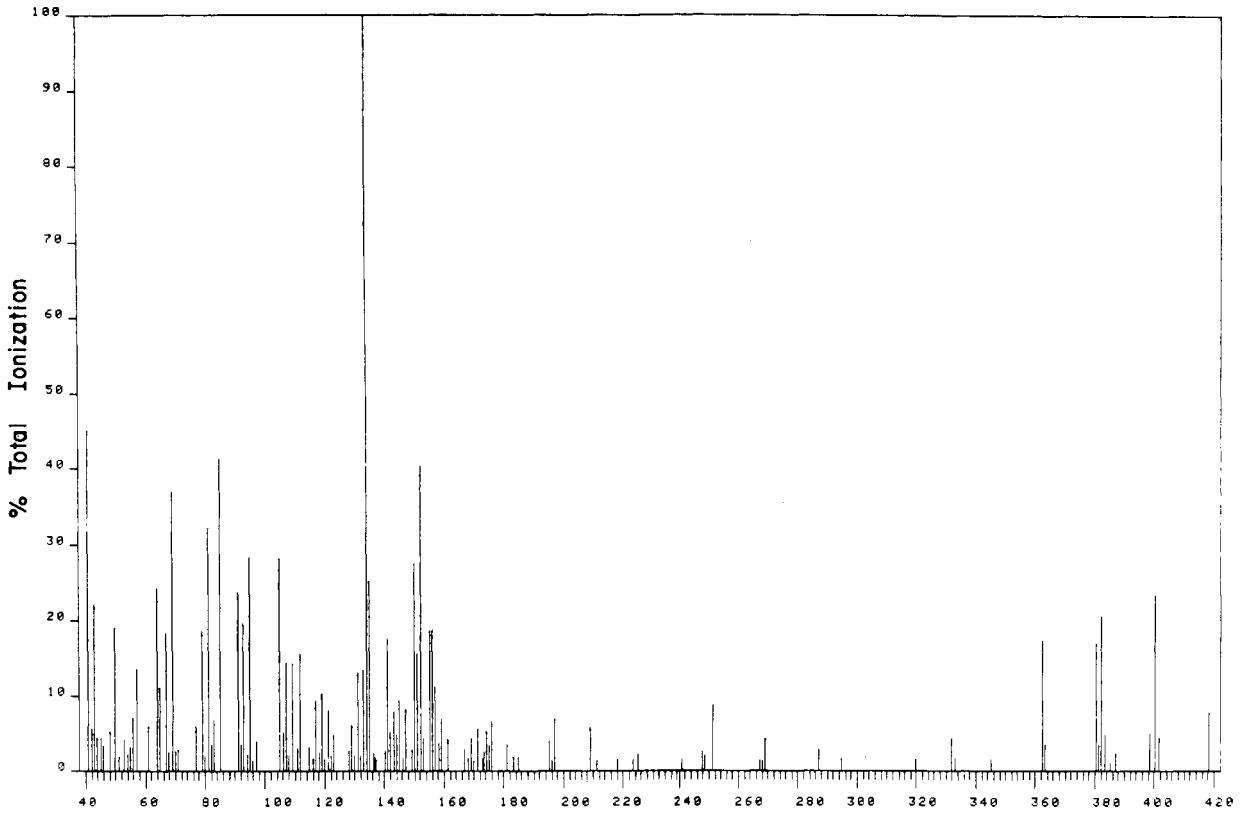

FIGURE 2: High-resolution mass spectrum of 1-OH-25-F-D_3. Note the molecular ion at *m/e* 418 and the fragment at *m/e* 380 which arises by loss of H_2O and HF from the molecular ion. The fragment at *m/e* 152 and base peak at *m/e* 134 are characteristic of 1α-hydroxylated vitamin D like compounds.

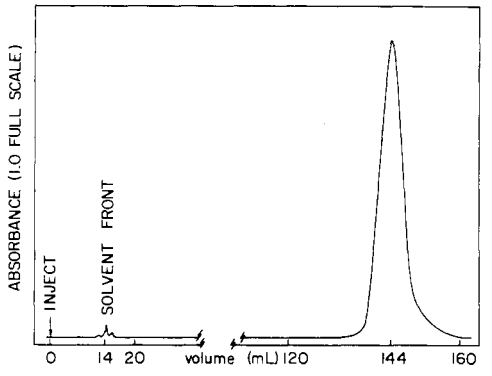

FIGURE 3: High-pressure liquid chromatogram of 1-OH-25-F-D_3 on a microparticulate silica gel column (0.4 × 30 cm) developed with 5% 2-propanol/hexane.

TABLE I: Dose-Response Relationship of 1-OH-25-F-D_3 in Vitamin D Deficient Rats on a Low-Calcium Diet.[a]

Compound	Dose (ng)	Serum Ca (mg/100 mL) 6 h	Serum Ca (mg/100 mL) 24 h	Serosal ^{45}Ca/Mucosal ^{45}Ca at 24 h
Ethanol		4.6 ± 0.1	4.6 ± 0.1	2.0 ± 0.2
1,25-$(OH)_2D_3$	25	5.7 ± 0.1[b]	5.7 ± 0.2[b]	4.9 ± 0.1[b]
1-OH-25-F-D_3	250	4.5 ± 0.1	5.1 ± 0.2[c]	4.0 ± 0.4[b]
	600	4.6 ± 0.1	5.6 ± 0.2[b]	4.7 ± 0.3[b]
	1250	5.1 ± 0.1[b]	5.8 ± 0.3[b]	4.7 ± 0.4[b]
	2500	5.6 ± 0.2[b]	6.2 ± 0.2[b]	4.5 ± 0.5[b]

[a] Values are expressed as the mean ± SEM of data from five to six rats. [b,c] Differ significantly from controls; $P < 0.001$ and $P < 0.05$, respectively.

of values from six rats). The dose of 1-OH-25-F-D_3 which would produce a score of 4.7 was calculated from the linear-regression analysis data to be 460 ng/day.

Discussion

1-OH-25-F-D_3 is 315 times less effective than 1,25-$(OH)_2D_3$ in the competitive protein-binding assay or about equipotent with 1-OH-D_3. This excludes the possibility, at least with one receptor, that the 25-fluorine enhances protein-receptor interaction. The essentially comparable strength in which 1-OH-D_3 and 1-OH-25-F-D_3 are bound indicates that the fluorine atom in 1-OH-25-F-D_3 is mimicking the hydrogen atom at the 25-position of 1-OH-D_3. These results suggest that incorporation of fluorine into 1-OH-25-F-D_3 should not directly increase its in vivo activity.

Nevertheless, 1-OH-25-F-D_3 is much more active in vivo than would have been predicted by the competitive protein-binding assay results. The 600-ng dose of 1-OH-25-F-D_3 is comparable to the 25-ng dose of 1,25-$(OH)_2D_3$ in stimulating intestinal calcium transport and bone calcium mobilization. Since 12.5 ng (but not less) of 1,25-$(OH)_2D_3$ produces the same response in intestinal calcium transport as 25 ng of 1,25-$(OH)_2D_3$ (Tanaka et al., 1973), 1-OH-25-F-D_3 is about 50 times less active than 1,25-$(OH)_2D_3$ in these systems. Similarly, the antirachitic potency of 1-OH-25-F-D_3 is 40 times less than 1,25-$(OH)_2D_3$. Significantly, the fluoro analogue did not selectively stimulate any one of the vitamin D responsive systems assayed.

It is unlikely that the disparity between 1-OH-25-F-D_3's activity in the chick binding protein assay and in vivo in the rat represents a species difference. Differences between rat and

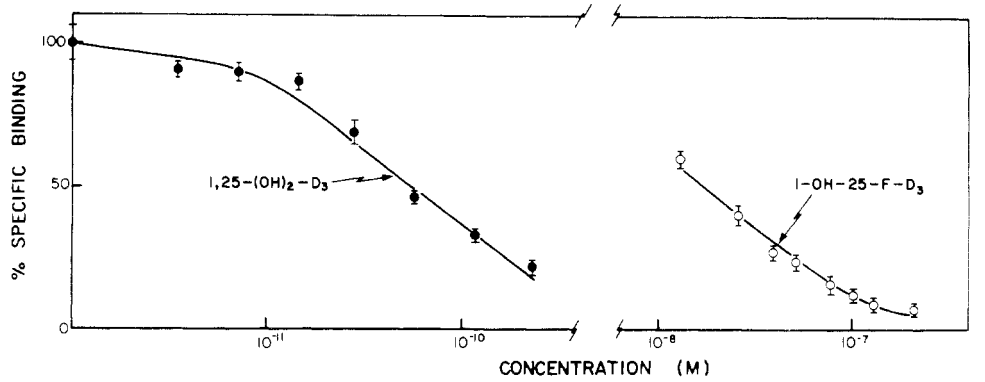

FIGURE 4: Competitive protein binding assay of 1,25-$(OH)_2D_3$ and 1-OH-25-F-D_3. Percent specific binding of 1,25-$(OH)_2$[23,24-^{3}H]D_3 is plotted against log concentration (M) 1,25-$(OH)_2D_3$ (●) or 1-OH-25-F-D_3 (○). Values are expressed at the mean ± SEM of triplet determinations.

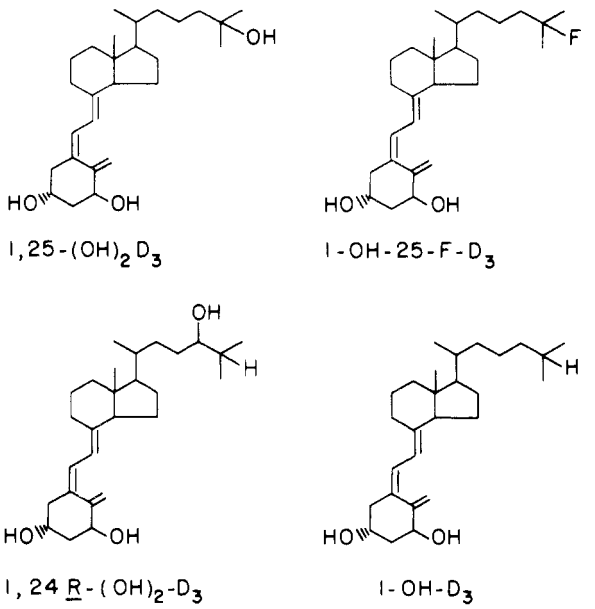

FIGURE 5: Structures of some vitamin D_3 metabolites and analogues.

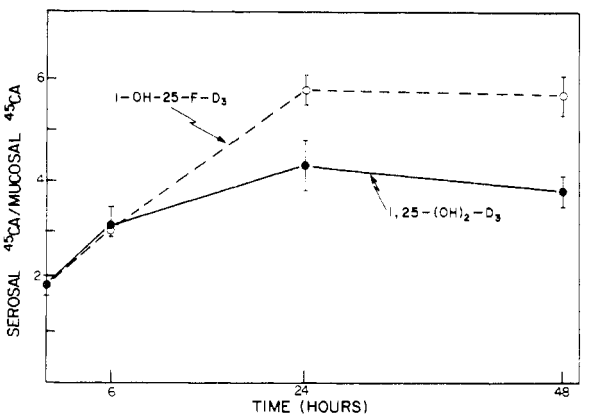

FIGURE 6: Time-course response of rat intestinal calcium transport system. Rats on a low-calcium diet received a single intrajugular dose of either 25 ng of 1,25-$(OH)_2D_3$ (●) or 6.25 μg of 1-OH-25-F-D_3 (○) in 0.05 mL of 95% ethanol. At the indicated times, animals were decapitated and their duodena were used for the determination of intestinal calcium transport. The rate of intestinal calcium transport is represented by the ratio of ^{45}Ca in the serosal medium to ^{45}Ca in the mucosal medium. Each point is the mean ± SEM of determinations from six to seven rats.

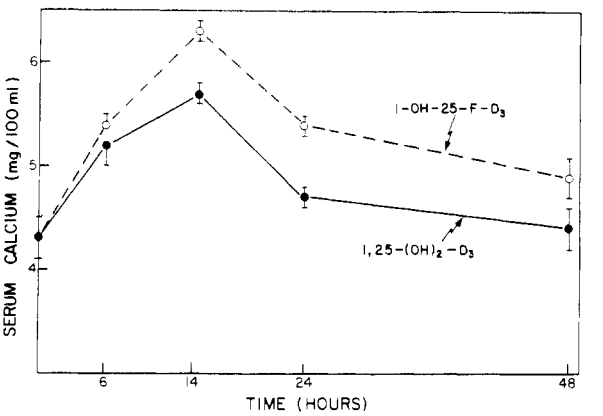

FIGURE 7: Time course response of bone calcium mobilization induced in rats by 25 ng of 1,25-$(OH)_2D_3$ (●) or 6.25 μg of 1-OH-25-F-D_3 (○). Rats on a low-calcium diet received a single intrajugular dose of compound in 0.05 mL of ethanol. At the indicated times, animals were decapitated, blood was collected, and calcium was measured in the serum by atomic absorption spectrometry in the presence of 0.1 % $LaCl_3$. Data are expressed as mg of Ca/100 mL of serum and are the mean ± SEM of five to seven determinations.

chick in the handling of vitamin D compounds have been shown to occur at sites other than the intestinal binding protein (Eisman and DeLuca, 1977; Hughes et al., 1976). Loss of fluorine by 1-OH-25-F-D_3 seems equally unlikely. Much work done with fluoroacetate, 5-fluorouracil, and fluorinated steroids has demonstrated the in vivo stability of carbon–fluorine bonds (Peters, 1957; Heidelberger et al., 1958; Fried and Borman, 1958). Therefore, it appears reasonable to conclude that the 25-fluorine of 1-OH-25-F-D_3 remains intact in vivo. However, although highly unlikely, carbon–fluorine cleavage cannot be absolutely ruled out.

The difference between the activity of 1-OH-25-F-D_3 in vivo and its binding potency in the competitive binding assay could be explained in a number of ways. The time course of 1-OH-25-F-D_3-produced responses in the rat paralleled those produced by 1,25-$(OH)_2D_3$ except at low doses (1250 ng or less) and early times (6 h). This disparity suggests that 1-OH-25-F-D_3 may undergo metabolism to a more active compound. Since the 25 position is blocked, a logical alternative is 24-hydroxylation. Indeed, 24-hydroxyvitamin D metabolites possess significant activity in calcium metabolism. For example, (24*R*)-hydroxyvitamin D_3 (24*R*-OH-D_3) binds as well as 25-OH-D_3 in the competitive protein-binding assay. Additionally, 1,24,25-trihydroxyvitamin D_3 [1,24,25-$(OH)_2D_3$] is only ten times less active than 1,25-$(OH)_2D_3$ in the same assay—making it the most potent vitamin D_3 metabolite besides 1,25-$(OH)_2D_3$ and 1,25-dihydroxyvitamin D_2 (1,25-$(OH)_2D_2$) (Eisman and DeLuca, 1977). These activity levels are also observed in vivo where 24*R*-OH-D_3 supports calcium metabolism as well as 25-OH-D_3 (Tanaka et al., 1975a,b).

Furthermore, 1,24-dihydroxyvitamin D_3 [1,24-$(OH)_2D_3$]

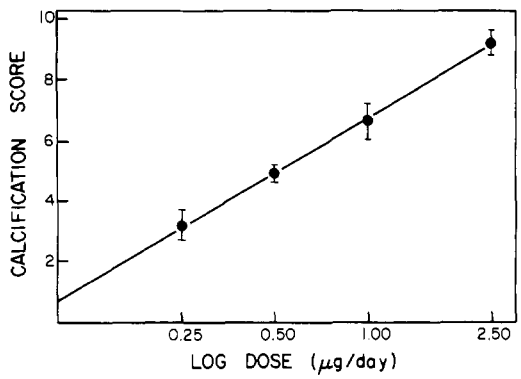

FIGURE 8: Antirachitic activity of 1-OH-25-F-D_3. Doses were administered intraperitoneally on each of 7 days in 0.05 mL of propylene glycol to rachitic rats. Bones were scored as described in the U.S. Pharmacopoeia (1955). Scores are the mean ± SEM of determinations from six to seven rats. In the same assay, animals which received only propylene glycol scored zero. Administration of 12.5 ng of 1,25-$(OH)_2D_3$ for 7 days produced a score of 4.7 ± 0.8.

(Kawashima et al., 1977) and 1,24,25-trihydroxyvitamin D_3 [1,24,25-$(OH)_3D_3$] (Tanaka and Deluca, unpublished results; Holick et al., 1973) are both very active mediators of calcium metabolism in the rat. Thus, the metabolism of 1-OH-25-F-D_3 to 25-fluoro-1,24-dihydroxyvitamin D_3 (25-F-1,24-$(OH)_2D_3$) should result in accumulation of a compound with significant 1,25-$(OH)_2D_3$-like activity.

Alternatively, since fluorine-substituted compounds are inherently more lipophilic than even their hydrocarbon congeners, the pharmacokinetic characteristics of 1-OH-25-F-D_3 might be substantially different from those of 1,25-$(OH)_2D_3$. The in vivo potency of 1-OH-25-F-D_3 could be a pharmacological effect resulting from greater accumulation of 1-OH-25-F-D_3 in the target tissues or resistence to metabolic inactivation. The time-course studies indicate that this explanation is not likely, however.

The 25-hydroxy group may have at least three functions in vitamin D metabolism. It is certainly important for 1-hydroxylation, since 25-OH-D_3, but not vitamin D_3 itself, undergoes 1-hydroxylation in vivo and in vitro (Gray et al., 1972; Tanaka et al., 1977). It also participates in 1,25-$(OH)_2D_3$-receptor interactions. The 300-fold decrease in binding of 1-OH-D_3 with respect to 1,25-$(OH)_2D_3$ in the competitive protein binding assay supports the importance of this 25-hydroxy group in the binding process. Thirdly, the 25-hydroxy group could be required for side-chain metabolism of 1,25-$(OH)_2D_3$.

We synthesized 1-OH-25-F-D_3, which represents the first example of a 1α-hydroxylated, 25-blocked vitamin D_3 derivative, as a tool to study the significance of the 25-hydroxy group in vivo. It is interesting that this analogue, incapable of undergoing 25-hydroxylation, displays substantial 1,25-$(OH)_2D_3$-like activity in the rat. Furthermore, it is probable that this analogue is undergoing metabolism to a more active compound. At least, these results indicate that it may be premature to consider the 25-hydroxy group manditory for manifestation of calcium metabolism activity by vitamin D metabolites and analogues. This study demonstrates that 25-hydroxylation is not necessarily obligatory and raises the possibility that, in certain cases, alternate modes of metabolism, perhaps 24-hydroxylation, could be important substitutes. To probe these possibilities, further work designed to examine the fate of injected 1-OH-25-F-D_3 has been initiated.

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Primary Structures of N-Terminal Extra Peptide Segments Linked to the Variable and Constant Regions of Immunoglobulin Light Chain Precursors: Implications on the Organization and Controlled Expression of Immunoglobulin Genes†

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ABSTRACT: The mRNA molecules coding for mouse immunoglobulin (Ig) light (L) chains program the cell-free synthesis of precursors in which extra peptide segments precede the N-termini of the mature proteins; i.e., the extra piece is linked to the variable (V) region (the amino terminal half of the L chain). The complete primary structures of the N-terminal extra pieces of three κ-type and three λ-type L-chain precursors were determined. Despite the fact that the extra pieces differ extensively in sequence (up to 73%) they share the following features: a high percentage of hydrophobic residues (69–75%), they are of comparable size (19–22 residues long) and contain fairly long peptide segments (16–19 residues) devoid of any charged residues, a few amino acids are frequently repeated (e.g., five leucines, five serines, and three isoleucines in RPC-20 extra piece), and methionine is the N-terminal residue. These structural features and other experiments indicate that (1) the V region is larger than hitherto realized; (2) the precursor seems to be the immediate translation product in the cell, since the N-terminal Met was identified as the initiator residue; (3) in the cell the precursor is short lived, and the maturation process (cleavage of the extra piece) may regulate secretion of the mature protein; (4) the physiological role of the hydrophobic extra piece may be to favor interaction of the precursor with cell membranes. Two targets for interaction are considered: the endoplasmic membranes and the cell-surface membrane. The N-terminal extra piece can undergo translocation from the V to the C region, as deduced from characterization of the precursor of the κ-type constant (C) region polypeptide fragment (the carboxy terminal half of the L chain) which was found to contain an N-terminal extra piece (17 residues long) with a primary structure identical to that of the extra piece linked to one of the V regions in whole L-chain precursors. These findings can be formally explained by the two genes–one Ig chain hypothesis, if we assume that the DNA coding for the extra piece (Xp DNA) is a constitutive part of the V gene. Alternatively, we raise the speculation that three genes may code for one Ig chain, where the Xp DNA represents a third distinct gene.

It has been recently realized that the mRNA molecules coding for a variety of secretory proteins program the cell-free synthesis of precursors larger than the mature proteins (see Burstein and Schechter, 1977a). Some understanding of the functions of the precursors may be afforded by determination of their structure, i.e., to determine the position (amino- or carboxy-terminal end), size, and sequence of the extra peptide segment. This was first done in 1973 by subjecting to radioactive sequence analysis the precursor of an Ig[1] L chain (Schechter, 1973). The Ig chains comprise a very heterogeneous population of proteins, and they have unique structural features that make the study of their precursors of special interest. In the mature L chain, the V region (the amino-terminal half of the protein) exhibits sequence variability which is responsible for antibody diversity and specificity, and the C region (the carboxy terminal half of the protein) of either κ- or λ-type L chains have a distinct sequence. We have isolated from mouse myeloma tumors L-chain mRNA molecules that program the cell-free synthesis of precursors in which extra pieces (17–22 residues long) precede the N-termini of both the V (precursors of the whole L chain) and C_κ region (precursor of the κ-type C-region fragment). Complete primary structures of the N-terminal extra pieces of the M-41 κ L-chain precursor and M-104E λ_1 L-chain precursor and partial sequences of extra pieces of other L-chain precursors have been reported previously (Burstein and Schechter, 1977a). Here we report the complete primary structures of five additional extra pieces: two of κ L-chain precursors (M-321 and M-63), one of λ_1 L-chain precursor (RPC-20), one of λ_2 L-chain precursor (M-315), and one of the precursor of the C_κ-region polypeptide fragment. The primary structures of these extra pieces and other experiments indicate the function(s) of precursor molecules in general (e.g., interaction with cell membranes, Schechter and Burstein 1976b) and stimulate new ideas concerning the organization and controlled expression of Ig genes (e.g., three genes may control the synthesis of one Ig chain, Burstein et al., 1977a).

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[1] Abbreviations used are: C region, constant region; H chain, heavy chain; Ig, immunoglobulin; L chain, light chain; MOPC-41, MOPC-63, MOPC-104E, MOPC-315 and MOPC-321 are abbreviated to M-41, M-63, M-104E, M-315 and M-321, respectively; V region, variable region; -X-, unknown amino acid residue in a partial sequence; Xp, extra piece; HV, hypervariable.

0006-2960/78/0417-2392$01.00/0