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BIOMIMETIC OXIDATION OF VITAMIN D BY IRON-SULFUR MODEL CLUSTER AND DIOXYGEN SYSTEM

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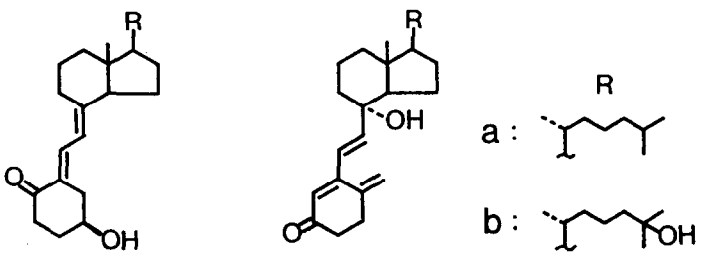
Oxidation of vitamin D_3 (**4**) with a combination of iron-sulfur protein model cluster, $(n\text{-}Bu_4N)_2[Fe_4S_4(SPh)_4]$ (**3**), and molecular oxygen produced (5E)-10-oxo-19-norvitamin D_3 (**1a**) and 8α-hydroxy-9,10-seco-4,6,10(19)-cholestatrien-3-one (**2a**), a new type of vitamin D metabolites, mimicking biological oxidation.

It is now clear that the active vitamin D_3, 1α,25-dihydroxyvitamin D_3, is not only a calcium regulating hormone but also a biological modifier capable of global maturation of target tissues.[2] Recently a new type of vitamin D metabolites, in which the conjugated triene function of vitamin D had been oxidized, was isolated and identified: (5E)-10-oxo-19-norvitamin D derivatives (**1**) from bovine rumen bacteria[3a,b,c], kidney cells in culture[3d,e], and a variety of phagocytic cells[3f,g,h], and 8α,25-dihydroxy-9,10-seco-4,6,10(19)-cholestatrien-3-one (**2b**) from phagocytic cells[4a-d] and liver microsomes.[4e] We report here that these new vitamin D metabolites can be produced chemically by treating vitamin D (**4**) with iron-sulfur protein model cluster $(n\text{-}Bu_4N)_2[Fe_4S_4(SPh)_4]$ (**3**)[5] in the presence of molecular oxygen. The results not only provide evidence for the mechanism of the production of the two new vitamin D metabolites (**1** and **2**) but also afford an insight into the mechanism of the action of iron-sulfur proteins in vivo. This is the first example of a model cluster-dioxygen system mimicking biological oxidation.

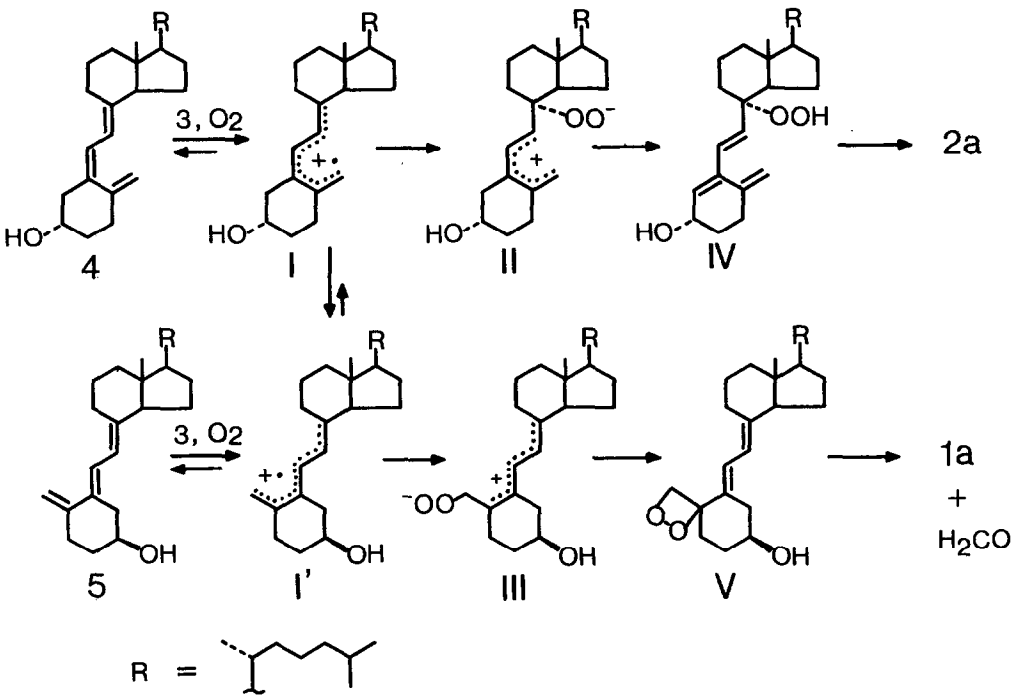


To clarify the mechanism of the biological oxidation of vitamin D triene function, we examined biomimetic oxidation under a variety of conditions. Reaction of vitamin D_3 (**4**) with oxygen in the presence of $FeCl_3$ gave only complex mixture, producing neither **1a** nor **2a**. Potassium superoxide (18-crown-6, DMSO, room temp.) caused no reaction. Under oxygen atmosphere in the presence of $FeSO_4$ (EtOH, room temp.), vitamin D_3 (**4**) produced a small amount of **1a** (0.1-0.3% from more than 1 mg of the substrate and 2-3% from less than 10μg of the substrate), suggesting that a complex of Fe(III) and superoxide anion radical might be the active oxidant.[6] Iron-sulfur model cluster (**3**) caused the oxidation of the triene much more efficiently. Thus, a solution of vitamin D_3 (**4**, 0.13 mmol) and the cluster (**3**, 0.26 mmol) in CH_3CN (14 mL) was stirred with oxygen bubbling at 50°C for 3 h to give **1a**[7] (15%), (5E)-vitamin D_3 (**5**, 8%), and recovered **4** (45%).[8] At room temperature (3 h), the reaction was slower but a small amount (0.6%) of the other metabolite (**2a**)[7] was isolated in addition to **1a** (6%), **5** (4%), and **4** (62%).[8] (5E)-Vitamin D_3 (**5**) was oxidized similarly but more efficiently. After 1 h at room temperature under similar conditions **5** gave **1a** in a yield as high as 41%, together with **2a** (0.7%) and **5** (10%).[8] When **5** was oxidized in the presence of excess dimedone (10 equivalents), the dimedone adduct of formaldehyde was isolated in a yield of 19%, similar to that of **1a** (17%), indicating that C(19) was cleaved off as formaldehyde.

The model cluster (**3**) in the presence of molecular oxygen mimicked the metabolic oxidation of the conjugated triene part of vitamin D.[9] The mechanism of the action of the model cluster (**3**) is not known but it was assumed that some complex formed from the cluster (**3**) and molecular oxygen acts as oxidant.[10] The followings are the mechanism tentatively proposed to explain the products. The active complex formed from **3** and oxygen abstracts an electron from vitamin D to yield radical cation (**I**) which is equilibrating with its 5E form (**I'**). In the equilibrium the latter (**I'**) is expected to be far more predominating than the former (**I**). The cation radicals (**I** and **I'**) react at the 8- or the 19-position with activated dioxygen (species like superoxide) in the complex yielding **II** or **III**.[11,12] The former yields **2a** via intermediate like **IV**, and the latter affords **1a** via 10,19-epidioxide (**V**)[13] whose intermediacy can be deduced from the formation of nearly equal amounts of **1a** and formaldehyde. The major cation radical (**I'**) might yield (5E)-vitamin D (**5**) abstracting an electron from the cluster. The mechanism explains why only the (5Z)-vitamin D (**4**) isomerizes during the oxidation. The facts that the same oxidation products were obtained from (5Z)- and (5E)-vitamin D (**4** and **5**) and that only the 5E isomer (**1a**) of 10-oxo-19-

norvitamin D was produced from both **4** and **5** are consistent with the proposed mechanism.

We showed that the iron-sulfur protein model cluster (**3**) acting as an electron transfer agent effected the reaction of the conjugated triene part of vitamin D (**4** and **5**) and molecular oxygen. Details of the mechanism of the oxidation are currently under investigation.



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7. The structures of all products were confirmed by their ^{1}H NMR, Mass, IR and UV spectra by comparing with those of the synthetic standard samples.[4a,c]
8. In addition to these products derived from the starting vitamin D, diphenyldisulfide (50-70% based on **3**) was isolated in each experiment.
9. It should be noted that, in contrast to the chemical reaction shown here, the production of the 8α-hydroxy-3-oxo-compound (**2b**) was much more predominant than that of the 10-oxo-19-norvitamin D (**1b**) in the metabolism of 25-hydroxyvitamin D_3 in phagocytic cells.[4]
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