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Evolution

Evolutionary importance for the membrane enhancement of the production of vitamin D_3 in the skin of poikilothermic animals

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ABSTRACT **The photoproduction of vitamin D in the skin was essential for the evolutionary development of terrestrial vertebrates. During exposure to sunlight, previtamin D_3 formed in the skin is isomerized to vitamin D_3 (calciol) by a temperature-dependent process. Since early land vertebrates were poikilothermic, the relatively slow conversion of previtamin D_3 to vitamin D_3 at ambient temperature put them at serious risk for developing vitamin D deficiency, thus leading to a poorly mineralized skeleton that could have ultimately halted further evolutionary development of vertebrates on land. We evaluated the rate of isomerization of previtamin D_3 to vitamin D_3 in the skin of iguanas and found the isomerization rate was enhanced by 1100% and 1700% at 25°C and 5°C, respectively. It is likely that the membrane entrapment of previtamin D_3 in its *s-cis,s-cis* conformation is responsible for the markedly enhanced conversion of previtamin D_3 to vitamin D_3. The membrane-enhanced production of vitamin D_3 ensures the critical supply of vitamin D_3 to poikilothermic animals such as iguanas.**

It is well recognized that vitamin D is absolutely essential for the maintenance of calcium and bone metabolism in most terrestrial vertebrates: amphibians, reptiles, birds, and mammals, including humans (1). Vitamin D in nature can only come from the sunlight-mediated photolysis of provitamin D to previtamin D (1). Once formed, previtamin D, a thermodynamically unstable molecule, undergoes a temperature-dependent isomerization to vitamin D (2). As vertebrates evolved in the fertile oceans and began to venture onto the earth's surface, they were confronted with a major problem. Whereas the fertile oceans contained a high amount of calcium, thereby satisfying their calcium requirement, the earliest vertebrates on land ventured into an environment that was deficient in calcium. Vitamin D was absolutely essential to enhance the efficiency of the gastrointestinal track to absorb dietary calcium to maintain a structurally sound, mineralized skeleton. However, the first terrestrial vertebrates were cold-blooded (poikilothermic), and therefore, were faced with a problem in making vitamin D in their skin. The last step of the cutaneous synthesis of vitamin D, the conversion of previtamin D to vitamin D, is a temperature-dependent process. The rate of the reaction is greatly reduced as the temperature is decreased (3, 4). In isotropic solvents, such as hexane or ethanol, it takes 91 and 1200 h for 50% of previtamin D_3 to thermally isomerize to vitamin D_3 (calciol) at 25°C and 5°C, respectively (5). Since previtamin D_3 does not possess any known biologic activity, it must be thermally isomerized to vitamin D_3 before this important hormone can carry out its biologic functions. The slow rate of conversion of previtamin D_3 to vitamin D_3 in cold-blooded vertebrates would have had disastrous consequences because the vitamin D_3 formation rate most likely would have been below the rate of previtamin D_3 degradation by sunlight (3), resulting in vitamin D deficiency and an undermineralized, deformed, and architecturally unsound skeleton. Thus, the evolution of vertebrates on land could have come to an abrupt halt. To solve this paradox, we conducted a study to determine the kinetics of the conversion of previtamin D_3 to vitamin D_3 in the skin of the poikilothermic lizard *Iguana iguana.*

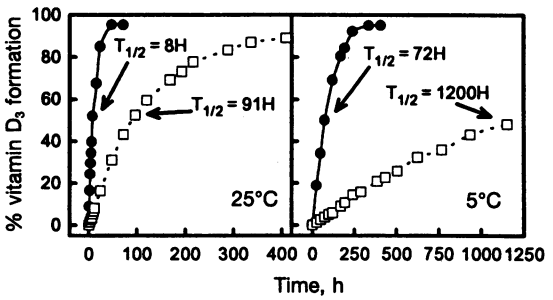

FIG. 1. Thermal isomerization of previtamin D_3 to vitamin D_3 as a function of time in lizard skin (●) and in hexane (□) at 25°C (left) and 5°C (right). Each point represents the mean value from three separate analyses.

METHODS

Skin tissue excised from lizard (*I. iguana*), frog (*Rana temporaria*), and human was exposed to UV-B radiation on ice as described (5, 6) to photosynthesize previtamin D_3 from provitamin D_3 (7-dehydrocholesterol) in the skin. Immediately after exposure, the skin samples were maintained at either 25°C or 5°C for 4 and 16 days, respectively. A similar study was conducted whereby provitamin D_3 in the isotropic solvent hexane (10 μg/ml) was exposed to the same amount of UV-B radiation followed by incubation at either 25°C or 5°C for up to 50 days. At various times, triplicate samples from the hexane solution and from lizard and frog skin were obtained, and the determination of the concentration of previtamin D_3 and vitamin D_3 was made by an HPLC method as described (5, 6).

RESULTS

At 25°C and 5°C, 50% of the previtamin D_3 converted to vitamin D_3 in 91 and 1200 h, respectively, when the reaction was carried out in hexane (Fig. 1). The analysis of the iguana's skin that was incubated under identical conditions revealed that 50% of the previtamin D_3 had converted to vitamin D_3 within 8 h at 25°C and 72 h at 5°C. Thus, the isomerization of previtamin D_3 to vitamin D_3 was enhanced 11-fold at 25°C and 17-fold at 5°C. An evaluation of the conversion of previtamin D_3 to vitamin D_3 at 25°C in frog and human skin revealed that there was a 12.6- and 10.3-fold enhancement, respectively, in the production of vitamin D_3 at 4 h when compared to conversion in hexane (Table 1).

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Table 1. Percentages of vitamin D_3 formation from previtamin D_3 at 25°C under various conditions

	Time of incubation, h			Rate constant
	1	2	4	$\times 10^6$ s^{-1}
n-Hexane	0.6 ± 0.1	1.4 ± 0.1	3.0 ± 0.5	2.5 ± 0.02
Lizard skin	8.8 ± 1.7*	17 ± 2.2*	30 ± 0.7*	25 ± 0.2*
Frog skin	7.7 ± 1.1*	22 ± 1.1*	38 ± 0.3*	—
Human skin	11 ± 0.3*	18 ± 0.3*	31 ± 0.5*	29*†

A solution of 7-dehydrocholesterol in *n*-hexane (10 μg/ml) and skin from lizard, frog, and human were exposed to UV-B radiation on ice to generate previtamin D_3. The conversion rates of previtamin D_3 to vitamin D_3 were determined at 25°C. The data shown are means ± SD.
*Significantly different ($P < 0.001$) from values determined in *n*-hexane.
†Rate constant at 25°C in human skin was calculated from the reported Arrhenius equation (5).

DISCUSSION

A possible mechanism by which this vitally important thermal isomerization reaction is enhanced in the skin of poikilothermic animals is illustrated in Fig. 2. When provitamin D_3 absorbs solar UV radiation, it causes an isomerization of 5,7-diene and a bond cleavage between C-9 and -10 to form the *s-cis,s-cis*-previtamin D_3. Once formed, the *s-cis,s-cis*-previtamin D_3 conformation is energetically unfavorable because of the steric interactions of the C ring and the C-19 methyl group. As a result, there is a rotation around the C-5 and C-6 single bond to form the energetically stable *s-trans,s-cis*-previtamin D_3 (7, 8). Although the *s-trans,s-cis*-previtamin D_3 cannot isomerize to vitamin D_3, it can revert to *s-cis,s-cis*-previtamin D_3, which then can isomerize to vitamin D_3. It is because of the conversion of the *s-cis,s-cis* to the *s-trans,s-cis* conformer and its slow equilibration back to the energetically less favorable *s-cis,s-cis* conformation that the conversion of previtamin D_3 to vitamin D_3 takes a relatively long time—i.e., 91 and 1200 h for 50% of previtamin D_3 to convert to vitamin D_3 at 25°C and 5°C, respectively.

Evidence exists that provitamin D_3 is located in the membrane fraction of skin cells (5). As illustrated in Fig. 2, it is likely that previtamin D_3 would align itself so that the 3β-hydroxyl group would be near the polar head group of the membrane phospholipids and interact with it through hydrophilic forces, while the nonpolar rings and side chain would be associated with the nonpolar tail by van der Waals interactions. Thus, when provitamin D_3 in the membrane is exposed to UV-B radiation to form the *s-cis,s-cis*-previtamin D_3, this conformation is preserved because a rotation around C-5 and C-6 to form the *s-trans,s-cis* conformer would require energy to disrupt the hydrophilic and van der Waals interactions that were established. The maintenance of the previtamin D_3 in the *s-cis,s-cis* conformation would greatly facilitate its conversion to vitamin D_3; thus, instead of taking 91 and 1200 h for 50% of previtamin D_3 to convert to vitamin D_3 at 25°C and 5°C as in hexane, it took only 8 h and 72 h in the lizard skin at the same temperatures.

During the formation of vitamin D_3 the hydrophilic and hydrophobic interactions of the *s-cis,s-cis*-previtamin D_3 with the phospholipids in the membrane are disrupted, thereby facilitating the specific translocation of vitamin D_3 from the skin cell into the extracellular space.

It is likely that the membrane entrapment of the previtamin D_3 in its *s-cis,s-cis* conformation allowed the efficient conversion of previtamin D_3 to vitamin D_3 for the poikilothermic animals. The enhancement in the conversion of previtamin D_3 to vitamin D_3 was observed in the reptile (*I. iguana*), as well as in the amphibian (*R. temporaria*). This mechanism remains operative today, not only to ensure the efficient production of

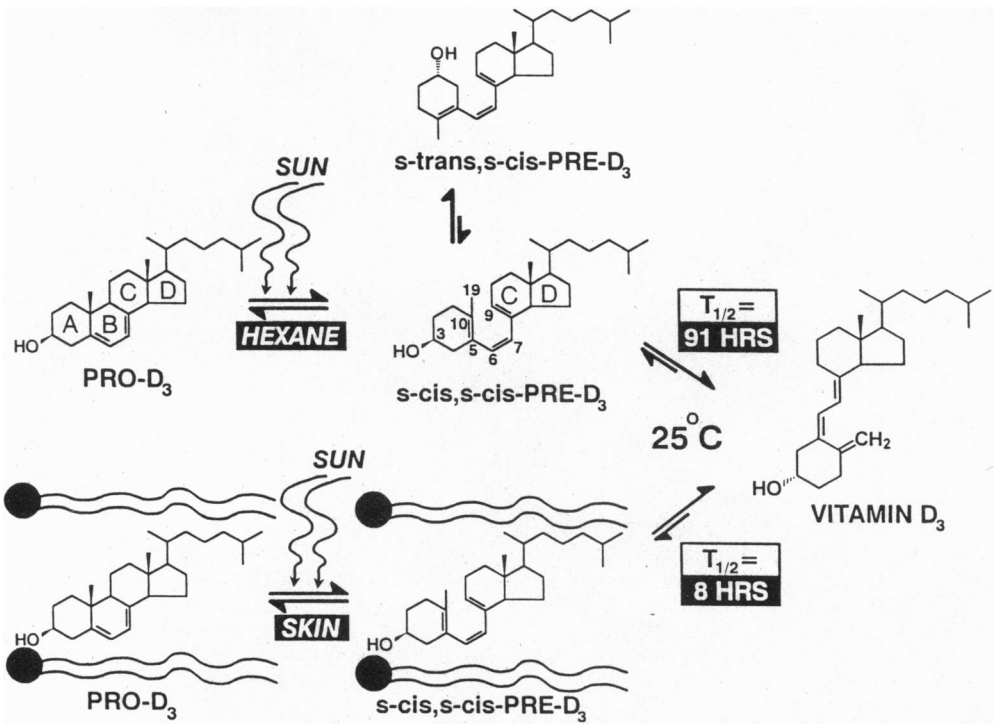

FIG. 2. Photolysis of provitamin D_3 (pro-D_3) into previtamin D_3 (pre-D_3) and its thermal isomerization to vitamin D_3 in hexane and in lizard skin. In hexane pro-D_3 is photolyzed to *s-cis,s-cis*-pre-D_3. Once formed, this energetically unstable conformation undergoes a conformational change to the *s-trans,s-cis*-pre-D_3. Only the *s-cis,s-cis*-pre-D_3 can undergo thermal isomerization to vitamin D_3. The *s-cis,s-cis* conformer of pre-D_3 is stabilized in the phospholipid bilayer by hydrophilic interactions between the 3β-hydroxyl group and the polar head of the lipids, as well as by the van der Waals interactions between the steroid ring and side-chain structure and the hydrophobic tail of the lipids. These interactions significantly decrease the conversion of the *s-cis,s-cis* conformer to the *s-trans,s-cis* conformer, thereby facilitating the thermal isomerization of *s-cis,s-cis*-pre-D_3 to vitamin D_3.

this vital calcium-regulating hormone in reptiles and amphibians, but also in warm-blooded vertebrates. In human skin the enhancement in the conversion of previtamin D_3 to vitamin D_3 at 25°C was similar to that found in lizard and frog skin. Furthermore, the calculated rate constant in human skin was similar to that in iguana skin (Table 1). Although we did not conduct a study in chicken skin at 25°C, we previously reported (5) that at 40°C the determined rate constants in chicken skin and in human skin were similar ($1.30 \times 10^{-4}\ s^{-1}$ vs $1.20 \times 10^{-4}\ s^{-1}$, respectively). Therefore, the enhancement of the conversion of previtamin D_3 to vitamin D_3 appears to have been retained throughout the evolution of terrestrial vertebrates.

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