We reported previously that the rate of previtamin D$_3$ (preD$_3$) $\rightarrow$ vitamin D$_3$ isomerization was enhanced by about 10 times in the skin compared with that in organic solvents. To elucidate the mechanism by which the rate of this reaction is enhanced in the skin, we developed a liposomal model that mimicked the enhanced isomerization of preD$_3$ to vitamin D$_3$ that was described in human skin. Using this model we studied the effect of changing the polarity of preD$_3$ as well as changing the chain length and the degree of saturation of liposomal phospholipids on the kinetics of preD$_3$ $\rightarrow$ vitamin D$_3$ isomerization. We found that a decrease in the hydrophilic interaction of the preD$_3$ with liposomal phospholipids by an esterification of the 3$\beta$-hydroxy of preD$_3$ (previtamin D$_3$-3$\beta$-acetate) reduced the rate of the isomerization by 67%. The addition of a hydroxyl on C-25 of the hydrophobic side chain (25-hydroxyprevitamin D$_3$), which decreased the hydrophobic interaction of preD$_3$ with the phospholipids, reduced the rate by 87%. In contrast, in an isotropic n-hexane solution, there was little difference among the rates of the conversion of preD$_3$ its 3$\beta$-acetate, and 25-hydroxy derivatives to their corresponding vitamin D$_3$ compounds. We also determined rate constants ($k$) of preD$_3$ $\rightarrow$ vitamin D$_3$ isomerization in liposomes containing phosphatidylcholines with different carbon chain lengths. The rates of the reaction were found to be enhanced as the number of carbons ($C_n$) in the hydrocarbon chain of the phospholipids increased from 10 to 18. In conclusion, these results support our hypothesis that amphipathic interactions between preD$_3$ and membrane phospholipids stabilize preD$_3$ in its “cholesterol-like” cZe-conformer, the only conformer of preD$_3$ that can convert to vitamin D$_3$. The stronger these interactions were, the more preD$_3$ was likely in its cZe conformation at any moment and the faster was the rate of its conversion to vitamin D$_3$.

During evolution, many poikilothermic and homeothermic terrestrial vertebrates including humans acquired the ability to photosynthesize vitamin D$_3$ in their skin (1, 2) and to use vitamin D$_3$ to enhance the efficiency of dietary calcium absorption to maintain a healthy and mineralized skeleton (3, 4). Cutaneous synthesis of vitamin D$_3$ consists of both photo- and thermal reactions (5, 6) (Fig. 1). When exposed to sunlight, ultraviolet-B (UV-B) (290–315 nm) radiation photolyzes previtamin D$_3$ (7-dehydrocholesterol (7-DHC)$^*$) (Fig. 1A), a $\Delta^{5,7}$-sterol synthesized in the skin, into a 9,10-seco B sterol, cZe-previtamin D$_3$ (cZe-preD$_3$) (Fig. 1B). This novel structure enables the seco-B to change its configuration between the preD form and the vitamin D form via a 1,7-sigmatropic hydrogen shift (Fig. 1, B and C) (7–11), one of the pericyclic processes defined by Woodward and Hoffmann (8). This thermally dependent reaction is the final step to produce vitamin D$_3$ in the skin and represents one of the best known examples of a concerted reaction that occurs in vivo. Although it has long been noted that, like other concerted reactions, the interconversion between preD$_3$ $\rightarrow$ vitamin D$_3$ is not influenced by a solvent effect when carried out in isotropic solutions (12), our recent data have revealed that both kinetics and thermodynamics of this reaction may change significantly in many anisotropic microenvironments (13, 14). Therefore, this simple yet physiologically important reaction provides us an ideal model to study the mechanism by which the kinetics of a concerted reaction is modified by an anisotropic medium. The seco-B ring of preD$_3$ consists of a conjugated triene system, which confers preD$_3$ with high conformational mobility (Fig. 1). Although the middle double bond in the triene system of preD$_3$ is in the cis (Z) configuration, two conformations arise from rotation around the single bonds C$_9$-C$_{10}$ and C$_7$-C$_8$ within the triene system i.e. cZe or s-cis,s-cis conformations (Fig. 1B) and sZe or s-trans,s-cis conformations (Fig. 1D) (9–11). It is known that to chemically isomerize to vitamin D$_3$, preD$_3$ is required to be in the cZe conformation (Fig. 1B) (9–11). From a structure-reactivity point of view, it is important to know whether there is any effect of the conformational restraints imposed by anisotropic media on the chemical transformation of preD$_3$ into vitamin D$_3$. We have previously shown that the rate of the formation of vitamin D$_3$ from preD$_3$ was enhanced by about 10-fold in the skin of terrestrial vertebrates compared with the rate of the same isomerization in an isotropic solution (1, 2, 13). Based on the finding that the major fraction of cellular 7-DHC and preD$_3$ are present in the cell membrane (13), we proposed a mechanism for the membrane-enhanced isomerization of preD$_3$ to vitamin D$_3$. We hypothesized that within the anisotropic membrane bilayers, amphipathic interactions between preD$_3$ and phospholipids stabilized the “cholesterol-like” cZe...
conformation of preD₃ (Fig. 2) and shifted the conformational equilibrium of preD₃ toward cZc-preD₃, the only conformation of preD₃ that isomerizes to vitamin D₃. Therefore, the rate of preD₃ → vitamin D₃ reaction is enhanced. To test this hypothesis, the effect of changing the polarity of 7-DHC as well as changing the chain length and the degree of saturation of the liposomal phospholipids on the kinetics of preD₃ → vitamin D₃ reaction were investigated. We have demonstrated for the first time that there is a positive correlation between the strength of the amphipathic interactions of preD₃ with liposomal phospholipids and the rate of preD₃ → vitamin D₃ isomerization.

**EXPERIMENTAL PROCEDURES**

**Materials**

7-DHC and vitamin D₃ were purchased from Sigma. 7-DHC-3β-acetate was purchased from Steroids Inc. (Wilton, NH). 25-Hydroxy-7-dehydrocholesterol (25(OH)7-DHC) was a kind gift from Dr. Rick Gray (Amoco Bioproducts, Naperville, IL). All the above chemicals were purified by high performance liquid chromatography (HPLC) and stored at −20 °C before use. Saturated lipids including didecanoylphosphatidylcholine (DDPC, C₁₀:0) (99%), dlauraylophosphatidylcholine (D LPC, C₁₂:0) (99%), dipalmitoylphosphatidylcholine (DPPC, C₁₆:0) (99%), distearoylphosphatidylcholine (DSPC, C₁₈:0) (99%), di- racchidoylphosphatidylcholine (DAPC, C₂₀:0) (99%), and unsaturated lipids dipalmitoleoylphosphatidylcholine (C₁₆:1, cis-9) (99%) were obtained from Sigma.

HPLC was performed with a P1000 pump equipped with a UV2000 UV-visible absorption detector (Thermo Separation Products, San Jose, CA). An Econosphere silica column (4.6 mm, 5 µm; Alltech Associate, Inc., Deerfield, IL) was used to separate the various vitamin D metabolites except their 3β-acetate derivatives, which were separated by a Cyclobond I 2000 column (100 × 4.6 mm, 5 µm; Advanced Separation Technologies Inc., Whippany, NJ).

UV spectra of vitamin D₃, preD₃ and their photoisomers as well as their 25-hydroxylated derivatives were recorded either by a UV spectrometer (UV-2000, Hitachi Instruments, Inc., Stoughton, MA) or by an online HPLC UV detector (UV2000).

**Methods**

**Preparation of Liposomes and Human Skin Samples—**Liposomes were prepared by a modified procedure reported by Wiseman et al. (15, 16), whereby 7-DHC and vitamin D₃ were incorporated into liposomes. The different lipids were dissolved in pure chloroform to give a final concentration of 20 mg/ml. An aliquot of the stock solution was taken and mixed with an equal volume of one of the following standard solutions of 7-DHC, 25(OH)7-DHC, and 7-DHC-3β-acetate. The organic solvent was evaporated by a stream of nitrogen. When necessary, the vials containing lipids were left under high vacuum overnight to remove trace solvent. The thin lipid film was resuspended in a 10 mM phosphate buffer (pH = 7.4) followed by vortexing and sonication. The resulting liposomes (lipid concentration, 6.75 mM; molar ratio of lipid to incorporated sterol, 100:2) were sealed in ampules that had previously been flushed with argon. The preparation of liposomes (hydration and vortexing) was performed above the transition temperature (Tₚₒ) of the corresponding lipid mixture (DMPC, 23 °C; DPPC, 41.5 °C; and DSPC, 58 °C) (17).

Human skin samples were prepared by a previously described method (5, 13). Briefly, neonatal foreskin was cleaned from subcutaneous tissues and cut into small pieces (about 0.3 cm²). Before UV irradiation, skin samples were immersed in a water bath at 60 °C for 30 s (this technique allows the separation of the dermis from epidermis at the stratum basale).

**Photoreaction and Thermal Isomerization—**Heat-treated skin samples, ampules containing liposome preparations, ampules containing a solution of 7-DHC or its analogues in n-hexane (0.135 mM), and ampules containing a solution of 7-DHC-DPPC in n-hexane (7-DHC, 0.135 mM; DPPC, 6.75 mM) were placed on ice and irradiated by UV Medical lamps (this technique allows the separation of the dermis from epidermis at the stratum basale).

**HPLC Analysis—**Immediately after incubation, the heat-treated skin was separated into epidermis and dermis according to a previously described method (5). Epidermis and liposomes were extracted three times with 8% ethyl acetate in n-hexane (5, 13). The organic phase...
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Separation of Vitamin D₃, Previtamin D₃, and Its Photoisomers as Well as Their 25-Hydroxylated Derivatives—PreD₃ was completely separated from its photoisomers and vitamin D₃, respectively. The total rate constant (k) is calculated from the slope of the plot of ln[D₃] versus time t. The equilibrium constant (K) of the reaction is expressed as:

\[ K = \frac{[\text{vitamin D₃}]}{[\text{preD₃}]} \]  

RESULTS

Separation of Vitamin D₃, Previtamin D₃, and Its Photoisomers as Well as Their 25-Hydroxylated Derivatives—PreD₃ was completely separated from its photoisomers and vitamin D₃, as described previously (13). 25-Hydroxyvitamin D₃ (25(OH)preD₃), 25-hydroxyvitamin D₃ (25(OH)D₃), 25-hydroxylumisterol (25(OH)L₃), and 25-hydroxytachysterol (25(OH)T₃) were completely separated from each other by HPLC (Fig. 3). Peak identities of 25(OH)7-DHC and 7-DHC (7-DHC, 0.135 mM; DPPC, 6.75 mM, DPPC liposomes containing 7-DHC (7-DHC, 0.25 mM; DPPC, 6.75 mM, i.e. 2 mol% of 7-DHC) as well as samples of human skin were exposed to UV radiation on ice to generate preD₃. The isomerization of preD₃ into vitamin D₃ was followed by means of HPLC analysis. The results showed that the reaction was of first order whether it was carried out in n-hexane, DPPC/n-hexane, skin, or liposomes (Fig. 4). The rate constants of the isomerization were calculated from the integrated rate equation (Eq. 1) using the least squares method. It was found that in anisotropic microenvironments (human skin and DPPC liposomes), the rates of the isomerization were about 10 times larger than those in isotropic ones (n-hexane and DPPC/n-hexane). At 37 °C, they were 8.62 ± 0.24 × 10⁻⁵ s⁻¹ (regression coefficient, r = 0.999) and 8.72 ± 1.11 × 10⁻⁵ s⁻¹ (r = 0.999) in human skin and DPPC liposomes, respectively, versus 8.08 ± 0.07 × 10⁻⁶ s⁻¹ (r = 0.999) and 8.06 ± 0.13 × 10⁻⁶ s⁻¹ (r = 0.995) in n-hexane and DPPC/n-hexane, respectively. The kinetic plot of the isomerization is shown in Fig. 4. The equilibrium constants (defined by the ratio k/K) were calculated from Eq. 2. They were 11 and 10 in human skin and DPPC liposomes, respectively, versus 6 and 7 in n-hexane and DPPC/n-hexane, respectively.

Temperature Dependence on Rate Constants of the Isomerization in DPPC Liposomes—The rate constants of preD₃ → vitamin D₃ reaction in DPPC liposomes was determined at 0, 15, 30, 37, 50, 60, and 70 °C, and they were 2.40 × 10⁻⁶ s⁻¹, 1.25 × 10⁻⁵ s⁻¹, 4.26 × 10⁻⁵ s⁻¹, 8.72 × 10⁻⁵ s⁻¹, 2.12 × 10⁻⁴ s⁻¹, 4.37 × 10⁻⁴ s⁻¹, 1.00 × 10⁻³ s⁻¹, respectively. The activation energy for the isomerization was determined according to the Arrhenius equation,

\[ \ln k = \ln A - \frac{E_a}{RT} \]  

where k is the rate constant for the isomerization, ln A is a constant, E_a is activation energy, R is the molar gas constant, and T is the temperature in degrees Kelvin. The Arrhenius plot (Fig. 5) for the isomerization in DPPC liposomes showed a straight line (r = −0.999) for the entire temperature range examined, which included temperatures well above or below the phase transition temperature (T_m) of DPPC liposomes (T_m = 41.5 °C). This result indicated that the mechanism for the isomerization remained the same whether the DPPC liposomes were in the liquid-crystalline phase or in the gel phase. The
calculated $E_a$ for the isomerization in DPPC liposomes is 66 kJmol$^{-1}$, which is similar to the value determined in human skin (73 kJmol$^{-1}$), and both of them are significantly lower than that determined in n-hexane (87 kJmol$^{-1}$) (13).

Kinetics of the Isomerization of PreD$_3$ and Its Analogues in n-hexane and DPPC Liposomes—Ampules containing 7-DHC, 25(OH)7-DHC, and 7-DHC-3β-acetate in n-hexane and in DPPC liposomes were placed on ice and irradiated with UV radiation to generate preD$_3$ and preD$_3$ analogues. Immediately after irradiation, the exposed ampules were incubated at 37 °C. The formation of vitamin D$_3$ from preD$_3$ was monitored by HPLC. It was found that in an isotropic n-hexane solution, a decrease in the polarity of preD$_3$ at the C$_{3\beta}$ position by esterification (preD$_3$-3β-acetate) or an increase in polarity with the addition of a hydroxyl at C$_{25}$ (25(OH)preD$_3$) had little effect on the rate of the thermal isomerization. The rates for the isomerization of preD$_3$, preD$_3$-3β-acetate, and 25(OH)preD$_3$ in an isotropic solution (n-hexane) were $8.08 \pm 0.07 \times 10^{-6}$ s$^{-1}$, $7.05 \pm 0.42 \times 10^{-6}$ s$^{-1}$, and $8.36 \pm 0.07 \times 10^{-6}$ s$^{-1}$, respectively (Fig. 6A). The rates of the thermal isomerization of these preD$_3$ derivatives were drastically different from each other when incorporated into liposomes (Fig. 6B). In liposomes, the unmodified preD$_3$ had the highest rate of the isomerization, $k = 8.72 \pm 1.11 \times 10^{-6}$ s$^{-1}$. In contrast, the rates for the isomerization of preD$_3$-3β-acetate and 25(OH)preD$_3$ in liposomes were greatly reduced by 67% ($k = 2.86 \pm 0.40 \times 10^{-5}$ s$^{-1}$) and 87% ($1.16 \pm 0.30 \times 10^{-5}$ s$^{-1}$), respectively.

Effects of Lipid Composition of Liposomes on the Rate of the Isomerization—To examine the effects of lipid composition of liposomes on the rate of preD$_3$ = vitamin D$_3$ conversion, 7-DHC was incorporated into liposomes containing phospholipids varying in hydrocarbon chain length i.e. DDPC (C10:0), DLPC (C12:0), DPPC (C16:0), DSPC (C18:0), and DAPC (C20:0). 7-DHC was also incorporated into the liposomes made of cis-un saturated phospholipids, i.e. dipalmitololeoylphosphatidylcholine (C16:1, [cis]-9). Ampules containing each of the above liposomal preparations were placed on ice and irradiated with UV radiation to produce preD$_3$. Exposed samples were incubated at 37 °C for different time intervals. The formation of vitamin D$_3$ from preD$_3$ showed that the rate of the isomerization correlated positively with the length of the hydrocarbon chain of lipids from C10 to C18 and followed the order DDPC (C10:0) ($k = 3.93 \pm 0.05 \times 10^{-5}$ s$^{-1}$) < DLPC (C12:0) ($k = 5.13 \pm 0.16 \times 10^{-5}$ s$^{-1}$) < DPPC (C16:0) ($k = 8.72 \pm 1.11 \times 10^{-5}$ s$^{-1}$) < DSPC (C18:0) ($k = 1.04 \pm 0.02 \times 10^{-4}$ s$^{-1}$). The linear regression equation for the rate constants of the isomerization versus carbon chain length of lipids (Fig. 7) was deduced from the above data and was written as

$$\ln k = -14.02 + 1.68 \ln C_n \quad \text{(Eq. 4)}$$

where $k$ (s$^{-1}$) was the rate constant of the isomerization, and $C_n$ was the number of carbons in the hydrocarbon chains of phosphatidylcholines. The correlation coefficient ($r$) of the determined regression equation was 0.999. Eq. 4 allows the calculation of rate constants of the isomerization in various liposomes and is valid for liposomal lipids containing 10 to 18 carbon atoms in hydrocarbon chain. Increasing the length of liposomes by guest on July 18, 2018http://www.jbc.org/ Downloaded from
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hydrocarbon chain further from C18 to C20 did not enhance but decreased the rate constant ($k_{C18} = 1.04 \pm 0.02 \times 10^{-4}$ s$^{-1}$ versus $k_{C20} = 8.01 \pm 0.08 \times 10^{-5}$ s$^{-1}$). The effect of incorporating cis-unsaturated phospholipids into the liposomes on the rate of the isomerization was also examined. It was found that by introducing a cis double bond at carbon 9 of hydrocarbon tails of DPPC liposomes, the rate of the reaction was significantly reduced by more than 40%, i.e. $k = 4.86 \pm 0.63 \times 10^{-5}$ s$^{-1}$ for liposomes prepared by unsaturated phospholipids (C16:1, cis-9) versus $k = 8.72 \pm 1.11 \times 10^{-5}$ s$^{-1}$ for liposomes constructed by saturated phospholipids of the same carbon chain length (DPPC, C16:0).

We hypothesized that the conformational restraints imposed by amphipathic interactions between preD$_3$ and phospholipids stabilized the “cholesterol like” cZc-preD$_3$ conformer (Fig. 2). Therefore, the conformational equilibrium of cZc-preD$_3$ = tZc-preD$_3$ was shifted to the cZc conformation. According to the hypothesis, the population of preD$_3$ molecules existing in the cZc form at any instant was higher in a lipid bilayer compared with an isotropic solution, and consequently, the rate of the thermal isomerization of preD$_3$ was enhanced (1). If this hypothesis was correct, it was expected that there would be a positive correlation between the strength of the amphipathic interactions and the rate of this thermal isomerization.

DISCUSSION

The first objective of this study was to develop a simple model by which the mechanism of the synthesis of vitamin D$_3$ in the skin could be studied. Based on our previous finding that 7-DHC and its photolyzed product preD$_3$ were present in the skin cell membrane, we incorporated 7-DHC into liposomes to mimic the membrane phospholipid bilayers. We found that the rate for the preD$_3$ = vitamin D$_3$ isomerization in DPPC liposomes and human skin was essentially the same. The rate of the reaction was about 10-fold faster in liposomes (8.72 ± 1.11 × 10$^{-5}$ s$^{-1}$) compared with that in n-hexane (8.08 ± 0.07 × 10$^{-6}$ s$^{-1}$) and similar to human skin (8.62 ± 0.24 × 10$^{-5}$ s$^{-1}$). The equilibrium constant was also significantly higher in liposomes than the values obtained from the isotropic n-hexane solution and similar to human skin. Thus our liposome model closely mimicked the kinetic properties of the preD$_3$ = vitamin D$_3$ isomerization in the skin (1, 2, 13). This is in contrast to the cholesteric liquid-crystalline model used by Cassis and Weiss (18) in which the determined equilibrium constant was smaller instead of larger than that determined in organic solvents.

Next, we examined the effects of isotropic versus anisotropic interactions of DPPC with preD$_3$ on the rate of preD$_3$ = vitamin D$_3$ isomerization. We found that in an isotropic solution of DPPC/n-hexane, the rate of preD$_3$ = vitamin D$_3$ isomerization is essentially the same as in a solution of pure n-hexane (8.06 ± 0.13 × 10$^{-5}$ s$^{-1}$ versus 8.08 ± 0.07 × 10$^{-6}$ s$^{-1}$) (Fig. 4). In contrast, the rate of preD$_3$ = vitamin D$_3$ isomerization in a solution of DPPC liposomes is enhanced by about 10 times compared with that in n-hexane (Fig. 4). Our results indicate that isotropic interactions of phospholipids with preD$_3$ do not affect the rate of preD$_3$ = vitamin D$_3$ conversion, but in an anisotropic microenvironment such as liposomes and skin, the rate of the isomerization is greatly enhanced (Fig. 4).

We used the liposomes as a simple model for the membrane bilayers to study the mechanism by which the rate of preD$_3$ = vitamin D$_3$ conversion was greatly enhanced in the skin. It is known that phospholipids and preD$_3$ are amphipathic molecules (ones with both hydrophilic and hydrophobic parts). Based on structural similarity, it was proposed (1, 19, 20) that, within the ordered lipid bilayer, preD$_3$ molecules oriented themselves in a way similar to cholesterol molecules, i.e. with their hydrophilic C$_{35}$ hydroxyl group close to the polar head groups of lipids, whereas their hydrophobic rings and the side chain aligned along the hydrocarbon chains of membrane lipids (Fig. 2). However, unlike the rigid cholesterol, the sec-o-B ring sterol, preD$_3$, is very conformationally mobile. Rotation about the single C$_{35}$-C$_{36}$ bond of preD$_3$ generates a wide array of conformations extending from cZc-preD$_3$ to tZc-preD$_3$ (Fig. 1). It was well established that the thermal isomerization between preD$_3$ = vitamin D$_3$ was a conformation-controlled process and required a cyclic transition state, possible only for cZc conformers but not possible for tZc and other conformers (Fig. 1) (10, 11).
only on the polarity of preD₃ but also on the hydrophobicity of phospholipids. To evaluate the effect of changing structures of phospholipids by altering either the carbon chain length or chain saturation on the rate of preD₃ ⇌ vitamin D₃ isomerization was examined.

We first carried out kinetic studies of preD₃ ⇌ vitamin D₃ isomerization in liposomes prepared by phospholipids with different hydrocarbon chain lengths. We observed a chain length-dependent rate enhancement of the isomerization. Fig. 7 showed that the rate of the thermal isomerization in the different liposomes followed the order DSPC (C18:0) > DPPC (C16:0) > DLPC (C12:0) > DDPC (C10:0). Least squares analysis of the data revealed a positive linear relationship (r = 0.999) between ln k (k, rate constant) and ln Cₙ (Cₙ, number of carbon atoms in the hydrocarbon chains of phospholipids) (Fig. 7). Because the strength of the amphipathic interactions is directly related to the chain length of phospholipids, our observation further supports the hypothesis that there is a positive correlation between the strength of the anisotropic interactions between the amphiphiles and the rate of the isomerization.

We found that DSPC (C18:0) liposomes had the maximum effect on the rate enhancement of preD₃ ⇌ vitamin D₃ isomerization (Fig. 7). It indicated that the optimal amphipathic interactions between preD₃ and phospholipids were achieved in DSPC (18:0) liposomes. Based on this information, we estimated that the “effective hydrophobic length of preD₃” corresponded to the length of a 18-carbon chain, which was similar to the reported value of cholesterol in phospholipid bilayers (the length of a 17-carbon chain or about 17.5 angstroms) (24–27).

To gain further insight into the mechanism of membrane-enhanced preD₃ ⇌ vitamin D₃ isomerization, we carried out kinetic studies of the reaction in liposomes prepared with unsaturated phospholipids. We found that the rate of the isomerization was reduced by more than 40% when the reaction was carried out in C16:1 (cis-9) liposomes compared with the reaction in saturated C16:0 liposomes. It is known that the cis double bonds cause rigid kinks of 30° in the hydrocarbon chains. These kinks cause disorder in the packing of the hydrophobic chains and increase the distance between hydrocarbon chains of phospholipids and the incorporated preD₃, which are expected to reduce the strength of van der Waals interaction between these amphiphiles (28). This reduced van der Waals interaction would be less effective in stabilizing the cholesterol-like cZc-preD₃ conformer. Accordingly, the rate of the isomerization was more than 40% slower in cis-unsat-urated liposomes than that in saturated ones of comparable carbon chain length (Fig. 7).

We concluded that in an ordered lipid bilayer, conformational restraints imposed by amphipathic interactions stabilized the cholesterol-like cZc conformer of preD₃. The stronger the amphipathic interactions were, the more preD₃ was in cZc conformation, and the faster was the rate of its conversion to vitamin D₃.

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REFERENCES

1. Holick, M. F., Tian, X. Q., and Allen, M. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3124–3126
2. Tian, X. Q., Chen, T. C., Lu, Z., Shao, Q., and Holick, M. F. (1994) Endocrinology 135, 655–661
3. Holick, M. F. (1989) in Vertebrate Endocrinology: Fundamentals and Implications (Pang, P. R. T., and Schreibman, M. P., eds) Vol. 3, pp. 7–34, Academic Press, Inc., Orlando, FL
4. Holick, M. F. (1994) Am. J. Clin. Nutr. 60, 619–630
5. Holick, M. F., MacLaughlin, J. A., Clark, M. B., Holick, S. A., Potts, J. T., Jr., Anderson, R. R., Blank, I. H., Parrish, J. A., and Elias, P. (1980) Science 210, 203–205
6. MacLaughlin, J. A., Anderson, R. R., and Holick, M. F. (1982) Science 216, 1091–1094
7. Havinga, E. (1973) Experientia (Basel) 29, 1181–1192
8. Woodward R. B., and Hoffmann, R. (1965) J. Am. Chem. Soc. 87, 2511–2513
9. Okamura, W. H., Midland, M. M., Hammond, M. W., Rahman, N. A., Dormanen, M. C., Nemere, I., and Norman, A. W. (1995) J. Steroid Biochem. Mol. Biol. 53, 603–613
10. Dauben, W. G., and Funhoff, D. J. H. (1987) J. Org. Chem. 52, 5070–5075
11. Dauben, W. G., and Funhoff, D. J. H. (1988) J. Org. Chem. 53, 5376–5379
12. Schlattmann, J. L. M. A., Pot, J., and Havinga, E. (1964) Rec. Trav. Chim. Pays-Bas 80, 1173–1185
13. Tian, X. Q., Chen, T. C., Matsuoka, L. Y., Wartsman, J., and Holick, M. F. (1993) J. Biol. Chem. 268, 14888–14892
14. Tian, X. Q., and Holick, M. F. (1995) J. Biol. Chem. 270, 8706–8711
15. Wiseman, H., Cannon, M., Armstrong, H. R. V., and Halliwell, B. (1990) FEBS Lett. 274, 203–205
16. Wiseman, H. (1993) FEBS Lett. 336, 285–288
17. Woodle, M. C., and Papahadjopoulos, D. (1989) Methods Enzymol. 171, 203–217
18. Cassis, E. G., and Weiss, R. G. (1982) Photochem. Photobiol. 35, 439–444
19. Moriarty, R., Schwartz, R. N., Lee, C., and Curtis, V. (1980) J. Am. Chem. Soc. 102, 4257–4259
20. Tian, X. Q., Holick, M. F., and Allen, M. (1995) in Biologic Effects of Light 1995 (Holick, M. F., and Jung, E. G. eds) pp. 39–48, Walter de Gruyter Co., Berlin
21. Villalain, J. (1996) Eur. J. Biochem. 241, 586–593
22. Theunissen, J. J. H., Jackson, R. L., Kempen, H. J. M., and Demel, R. A. (1986) Biochim. Biophys. Acta 860, 66–74
23. Verma, S., Philippet, J. R., and Wallach, D. F. H. (1983) Biochemistry 22, 4587–4591
24. Wu, W.-G., and Chi, L.-M. (1991) J. Am. Chem. Soc. 113, 4683–4685
25. McMullen, T. P. W., Lewis, R. N. A. H., and McElhaney, R. N. (1993) Biochemistry 32, 516–522
26. McMullen, T. P. W., Lewis, R. N. A. H., and McElhaney, R. N. (1994) Biophys. J. 66, 741–752
27. Bittman, R. (1997) Subcell. Biochem. 28, 145–171
28. Lantce-Hermens, A. M. W., and Kruijff, B. (1977) Biochim. Biophys. Acta 470, 141–151
A Liposomal Model That Mimics the Cutaneous Production of Vitamin D₃: Studies of the Mechanism of the Membrane-Enhanced Thermal Isomerization of Previtamin D₃ to Vitamin D₃

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