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Catalyzed Thermal Isomerization between Previtamin D_3 and Vitamin D_3 via β-Cyclodextrin Complexation*

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To examine the effect of microenviroments on previtamin D_3 ⇌ vitamin D_3 isomerization, we have conducted kinetic studies of the reaction in an aqueous solution of β-cyclodextrin. Our results showed that at 5 °C, the forward (k_1) and reverse (k_2) rate constants for previtamin D_3 ⇌ vitamin D_3 isomerization were increased by more than 40 and 600 times, respectively, compared with those in *n*-hexane (k_1, 8.65×10^{-6} *versus* 1.76×10^{-7} s^{-1}; k_2, 8.48×10^{-6} *versus* 1.40×10^{-8} s^{-1}), the fastest rate of this isomerization ever reported at this temperature. Thermodynamic studies revealed that the equilibrium constant of the reaction was significantly reduced by more than 12-fold when compared to that in *n*-hexane at 5 °C, and the percentage of vitamin D_3 at equilibrium was increased as the temperature was increased in β-cyclodextrin. When complexed with β-cyclodextrin, the previtamin D_3 ⇌ vitamin D_3 isomerization became endothermic (ΔH° = 13.05 kJ mol^{-1}) in contrast to being exothermic in other media. We propose that thermodynamically unfavorable *cZc* conformers of previtamin D_3 are stabilized by β-cyclodextrin, and thus the rate of the isomerization is increased. This conformation-controlled process may play an important role in the modulation of previtamin D_3 ⇌ vitamin D_3 endocrine system *in vivo* such as in the sea urchin.

The photobiogenesis of vitamin D_3 in the skin consists of two sequential pericyclic reactions (Fig. 1) (Havinga, 1973; Holick *et al.*, 1980; Moriarty *et al.*, 1980; MacLaughlin *et al.*, 1982). The first step involves the ultraviolet-B- (UV-B,[1] 290–315 nm) induced electrocyclic ring opening of 7-dehydrocholesterol (7-DHC) between C_9 and C_{10} to form a *seco*-sterol, previtamin D_3 (preD_3) (Woodward and Hoffmann, 1965; Havinga, 1973; Esvelt *et al.*, 1978; Jacobs and Havinga, 1979; Holick *et al.*, 1979, 1980). PreD_3 is an obligatory precursor for the biogenesis of vitamin D_3. Once formed, preD_3 begins to thermally isomerize to vitamin D_3 via an antarafacial [1,7]-sigmatropic hydrogen shift from C_{19} to C_9 (Dauben and Funhoff, 1988a, 1988b; Yamamoto and Borch, 1988; Curtin and Okamura, 1991). The thermal rearrangement of preD_3 ⇌ vitamin D_3 is an intramolecular concerted process. Due to the reversibility of this isomerization, vitamin D_3 and its precursor preD_3 always coexist and constantly interconvert. This contrasts markedly with all other steroids.

The relevance of preD_3 ⇌ vitamin D_3 endocrine system to biological activity was recently implicated in studies (Norman *et al.*, 1993; Dormanen *et al.*, 1994) suggesting the existence of different forms of the 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$) receptor: the classic nuclear receptor for 1,25-$(OH)_2D_3$ associated with genomic activity as well as the uncharacterized membrane receptors for both 1,25-$(OH)_2D_3$ and 1,25-dihydroxyprevitamin D_3 (1, 25-$(OH)_2$preD_3) associated with nongenomic activity. Hobbs *et al.* (1987) reported the first example that preD_3 ⇌ vitamin D_3 isomerization could be altered *in vivo* in the sea urchin *Psammechinus miliaris*. There were three remarkable features for the reaction in the sea urchin. First and most important, the equilibrium of the reaction is dramatically altered and shifted toward preD_3 at equilibrium (45% in the sea urchin *versus* 8% in *n*-hexane at 10 °C). The second striking feature for the reaction in the sea urchin was that the rate of conversion of vitamin D_3 → preD_3 was greatly increased. For example, at 10 °C less than 5% of vitamin D_3 converted to previtamin D_3 in *n*-hexane after 1 month (Tian *et al.*, 1993). In contrast in the sea urchin at the same temperature, it took only about 1–2 days to convert as much as 30–45% of vitamin D_3 into preD_3 (Hobbs *et al.*, 1987). Last and most unusual was the percentage of vitamin D_3 at equilibrium was increased as temperature was increased (72 and 78% at 17.5 and 20 °C, respectively), which is in contrast to all other known reaction systems reported to date which showed a decrease in the amount of vitamin D_3 with increasing temperature (Cassis and Weiss, 1982; Yamamoto and Borch, 1985; Tian *et al.*, 1993, 1994).

The shell tissue of sea urchin has been found to have the greatest ability to alter the rate and equilibrium of preD_3 ⇌ vitamin D_3 isomerization (Hobbs *et al.*, 1987). However, the active agent within the shell has not been identified. It is known that more than 90% of mollusk shell consists of inorganic salts, mainly calcium carbonate, and the remainder are proteins and polysaccharides (Rieke *et al.*, 1992). Both protein component and pure mineral salts fail to catalyze the isomerization, and the effect of saccharides has not been examined (Hobbs *et al.*, 1987).

Cyclodextrins, the naturally occurring, truncated cone-shaped oligosaccharides, have received increasing attention in recent years for their ability to complex a variety of guest molecules including steroids into their hydrophobic cavities in aqueous solution (Saenger, 1984; Liu *et al.*, 1990; Albers and Muller, 1992). These microheteroenvironments have been shown to modify both energetics and dynamics of many chemical reactions (Ueno and Osa, 1991; Pitchumani and Ramamurthy, 1994). Of great importance is their ability to catalyze reactions of a wide variety of guest molecules (Breslow, 1984; Tabushi, 1984; Chen and Pardue, 1993). It is known that β-cy-

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[1] The abbreviations used are: UV-B, ultraviolet-B; 7-DHC, 7-dehydrocholesterol; preD_3, previtamin D_3; 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 1,25-$(OH)_2$preD_3, 1,25-dihydroxyprevitamin D_3; HPLC, high performance liquid chromatography.

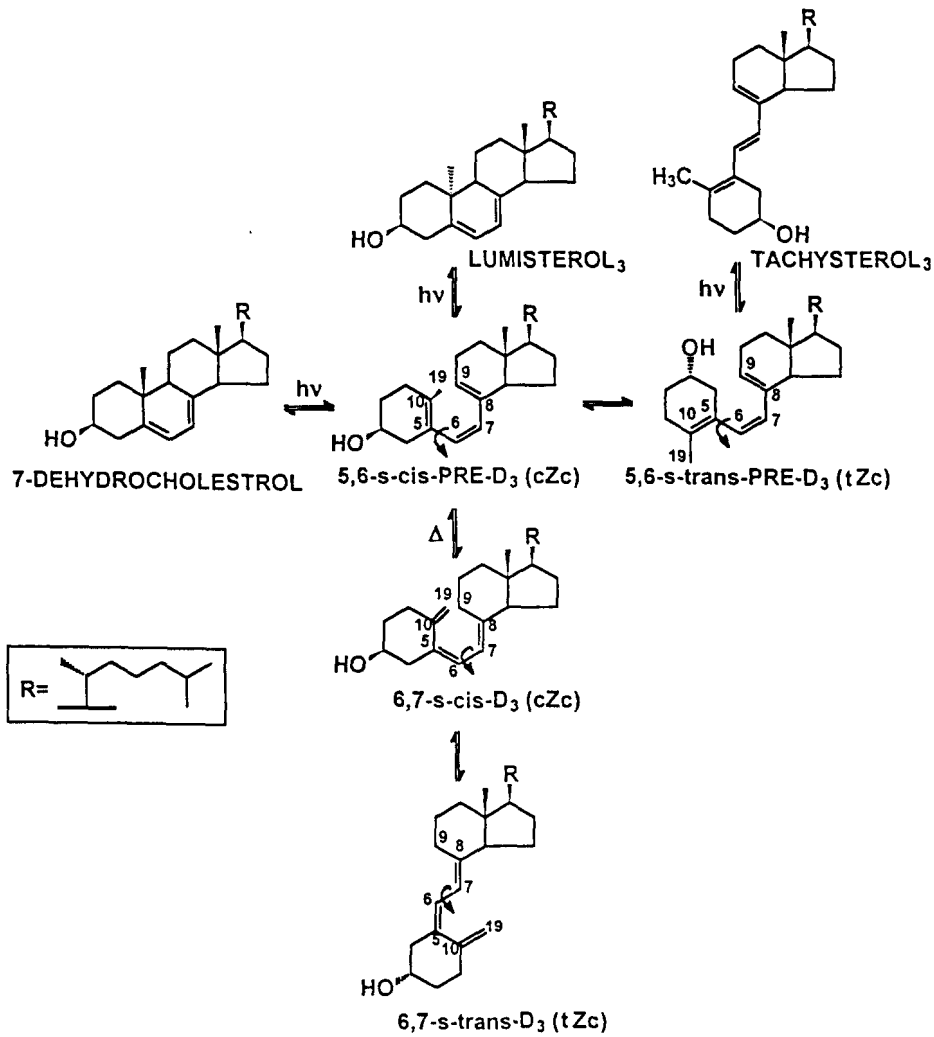

FIG. 1. **Schema for conformation-controlled photolysis of 7-dehydrocholesterol and thermal isomerization between previtamin D_3 and vitamin D_3.** 7-Dehydrocholesterol is first converted by ultraviolet-B irradiation into a *seco*-steroid, previtamin D_3. Unlike its precursor, previtamin D_3 is conformationally mobile, which undergoes rotation around the 5,6 carbon-carbon single bond to create two distinct conformers, *i.e.* 5,6-s-*cis* (*cZc*) and 5,6-s-*trans* (*tZc*) previtamin D_3. Photochemically, 5,6-s-*cis*-previtamin D_3 is responsible for the formation of lumisterol and 7-dehydrocholesterol, whereas 5,6-s-*trans*-conformer is the precursor of tachysterol. Previtamin D_3 is thermally liable, once formed it begins to isomerize to vitamin D_3 via 5,6-s-*cis*-conformer by a [1,7]-sigmatropic hydrogen shift. Vitamin D_3, like its precursor, is also conformationally flexible and undergoes rotation around the 6,7 carbon-carbon single bond. The 6,7-s-*cis*-conformer of vitamin D_3 is responsible for the thermal isomerization to previtamin D_3.

clodextrin is capable of forming 2:1 (host/guest) inclusion complexes with vitamin D_3 (Szejtli *et al.*, 1980; Szejtli, 1984; Bogoslovsky *et al.*, 1988). Therefore, we evaluated β-cyclodextrin as a possible model to mimic the preD$_3$ ⇌ vitamin D_3 reaction in the sea urchin and investigated the mechanism by which the reaction kinetics was modulated by this constrained medium.

MATERIALS AND METHODS

Chemicals—Crystalline β-cyclodextrin, methyl-β-cyclodextrin (mean degree of substitution, 10.5–14.7), α-cyclodextrin, vitamin D_3 (>99%), and 7-DHC (98%) were purchased from Sigma and were used as received without further purification. *n*-Butanol (>99%) was obtained from Aldrich. High performance liquid chromatography (HPLC) grade *n*-hexane and 2-propanol were obtained from EM Science (Gibbstown, NJ). PreD$_3$ was chemically synthesized by photolysis of 7-DHC solution according to a previous reported method (Tian *et al.*, 1993, 1994). PreD$_3$ in *n*-hexane solution was stored in argon-flushed glass ampoules at −70 °C until use, and the purity was checked by HPLC analysis and its UV absorption spectrum.

Preparation of 7-DHC·Cyclodextrin Complex—The inclusion complex was prepared by a modified method described by Duveneck *et al.* (1989), *i.e.* addition of one volume of 1 mM 7-DHC in ethanol to 50 volumes of aqueous β-cyclodextrin solution (15 mg/ml). The prepared solutions were stirred at room temperature for at least 4 h and then filtered to remove possible precipitates to obtain a clear solution. The formation of the inclusion complex was verified by the appearance of characteristic UV absorption spectrum of 7-DHC (MacLauglin *et al.*, 1982). Due to 7-DHC's very low water solubility, no UV absorption for 7-DHC could be detected when using pure water as the solvent. The same procedure was used to prepare the inclusion complexes of 7-DHC with α-cyclodextrin and methyl-β-cyclodextrin in aqueous solution.

Photolysis of 7-DHC·β-Cyclodextrin Complex—Solutions of 7-DHC inclusion complex were placed in argon-flushed quartz tubes and irradiated on ice by UV-B Medical Sunlamps (National Biological Corp., Cleveland, OH) for 1 min (40 mJ cm^{-2}) (Tian *et al.*, 1993). For kinetic studies, triplicate exposed solutions were incubated at 5, 30, 37, and 50 °C for various durations. Aliquots sampled at each time interval were immediately extracted with a precooled *n*-butanol/*n*-hexane solution (15:85, v/v). The amount of vitamin D_3 and preD$_3$ in each sample was quantified by a previously described HPLC method (Tian *et al.*, 1993, 1994).

Kinetic Studies—Due to high excess of β-cyclodextrin (host/guest = 660:1) and the virtual insolubility of free 7-DHC in water, it was expected that 7-DHC, preD$_3$, and vitamin D_3 were completely complexed with β-cyclodextrin forming readily water-soluble inclusion complexes (Szejtli *et al.*, 1980; Szejtli, 1984). Therefore, the following reversible thermal isomerization existed in the exposed solutions:

$$\text{preD}_3\text{-}(\beta\text{-cyclodextrin})_2 \rightleftharpoons \text{vitamin D}_3\text{-}(\beta\text{-cyclodextrin})_2 \qquad \text{(Eq. 1)}$$

In analogy to the thermal interconversion between free preD$_3$ and vitamin D_3 in solutions, rate constants (k_1 and k_2), equilibrium constant (K), and thermodynamic activation parameters were calculated by using standard methods for reversible first-order reactions (Tian *et al.*, 1993, 1994). In brief, the rate constants were obtained from the slopes of the plots of $\ln[(D_e - D_o)/(D_e - D_t)]$ *versus* reaction time t. The equilibrium constants were equal to the ratios of forward rate constants (k_1) over reverse rate constants (k_2). The standard enthalpy change (ΔH°) for the reaction was calculated from the van't Hoff plot, and activation energy (E_a) was obtained from Arrhenius plot. Finally, the activation parameters were calculated from Eyring's equation.

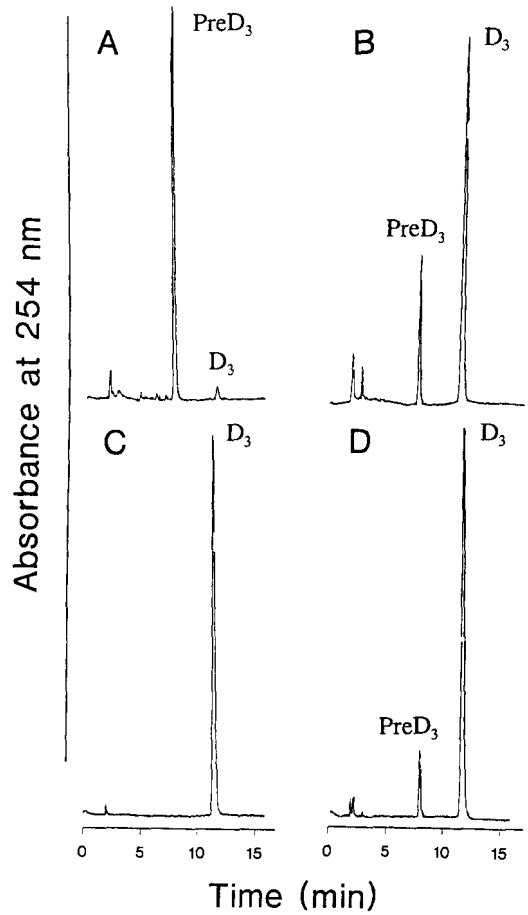

FIG. 2. **HPLC separation and quantification of previtamin D_3 and vitamin D_3.** *A*, the HPLC profile of thermal isomerization of previtamin D_3 into vitamin D_3 in *n*-hexane at 37 °C. 1% of previtamin D_3 is converted into vitamin D_3 at end of 30 min of incubation; *B*, the HPLC profile of previtamin D_3 ⇌ vitamin D_3 isomerization in β-cyclodextrin at the same temperature. 60% of previtamin D_3 is converted into vitamin D_3 during a period of 30 min of incubation; *C*, incubation of vitamin D_3 in *n*-hexane at 37 °C for 1 h. No conversion of vitamin D_3 to preD_3 was detected at the end of the incubation; *D*, whereas incubation of vitamin D_3 in β-cyclodextrin solution at 37 °C for 30 min resulted in 19% of vitamin D_3 being converted into preD_3. Chromatograms were obtained at 254 nm on an Econosphere silica column (250 × 4.6 mm, 5 μm) with mobile phase containing 0.45% 2-propanol in *n*-hexane.

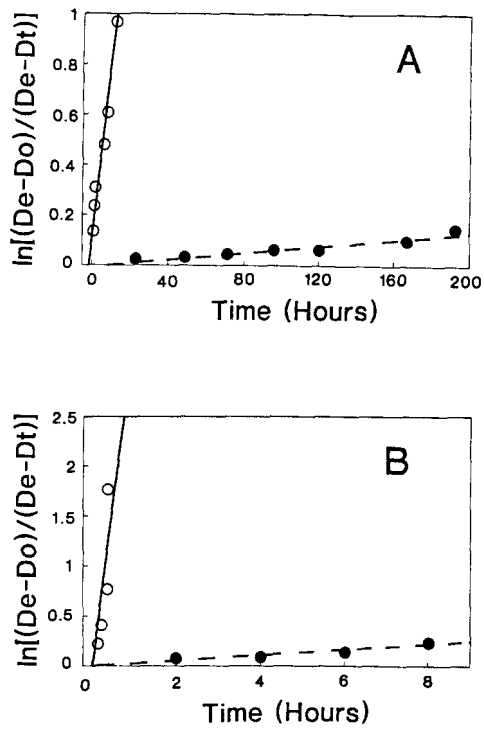

FIG. 3. **Comparison of kinetics of previtamin D_3 ⇌ vitamin D_3 reaction in β-cyclodextrin and in *n*-hexane at 5 °C (*A*) and 37 °C (*B*).** The rate constants of the isomerization (k) in *n*-hexane (●) and in β-cyclodextrin (○) were calculated from the slopes of the straight lines by least-squares analysis. The data presented are means of three determinations.

RESULTS

Kinetic Analysis—Incubation of purified preD_3 in β-cyclodextrin aqueous solution at 37 °C for 30 min resulted in the conversion of 60% of preD_3 into vitamin D_3, in contrast to only 1% of conversion in *n*-hexane (Fig. 2, *A* and *B*). For the reverse reaction, in β-cyclodextrin, 19% of vitamin D_3 was converted into preD_3 at 37 °C within 30 min, whereas in *n*-hexane no conversion of vitamin D_3 into preD_3 was detected at the end of 1 h of incubation (Fig. 2, *C* and *D*).

The integrated rate equation for the thermal interconversion between preD_3 and vitamin D_3 inclusion complexes (Eq. 1) was expressed as

$$\ln[(D_e - D_o)/(D_e - D_t)] = (k_1 + k_2)t = kt \qquad \text{(Eq. 2)}$$

where k was the total rate constant, k_1 and k_2 were the rate constants for the preD_3 → vitamin D_3 reaction and vitamin D_3 → preD_3 reaction, respectively. D_e, D_o, and D_t were vitamin D_3 concentration at time t reached equilibrium, $t = 0$ and $t = t$, respectively. Based on Equation 2, it was expected, and demonstrated (Fig. 3) that the plot of $\ln[(D_e - D_o)/(D_e - D_t)]$ *versus* reaction time t was linear, and the total rate constant was calculated from the slope of the straight line by least-squares analysis. The determined total rate constants in β-cyclodextrin at 5, 30, 37, and 50 °C were 0.0000171 ± 0.0000028 s^{-1} (correlation coefficient r = 0.973), 0.000246 ± 0.000003 s^{-1} (r = 0.985), 0.000477 ± 0.000017 (r = 0.992) and 0.00210 ± 0.00021 s^{-1} (r = 0.993), respectively. The kinetic values (k_1, k_2, and K) for the preD_3 ⇌ vitamin D_3 reaction in *n*-hexane (Tables I and II) were determined at 5 and 37 °C (Fig. 3), and at other temperatures, they were calculated based on previously reported data (Tian *et al.*, 1993). Compared to the preD_3 ⇌ vitamin D_3 reaction in *n*-hexane at 5 °C, complexing with β-cyclodextrin dramatically accelerated the thermal isomerization rates between preD_3 and vitamin D_3 resulting in a 49- and 606-fold catalysis for the forward preD_3 → vitamin D_3 conversion and reverse preD_3 → vitamin D_3 conversion, respectively (Table I), the highest isomerization rate for the reaction reported to date. At equilibrium the percentage of preD_3 in β-cyclodextrin was shifted from less than 8% in *n*-hexane at 5 °C to 49% in β-cyclodextrin.

Effects of Temperature on Rate Constants of PreD_3 ⇌ Vitamin D_3 Interconversion—The temperature dependence of the rate constant was defined by Arrhenius' equation

$$k = A\exp(-E_a/RT) \text{ or } \ln k = -E_a/RT + \ln A \qquad \text{(Eq. 3)}$$

where E_a was the activation energy of the reaction, ln$A$ was an integration constant, and A was defined as the frequency factor. Arrhenius plot (ln$k$ against $1/T$) for the preD_3 ⇌ vitamin D_3 interconversion was linear over the experimental temperature range (5–50 °C), with Ea_1 = 82.37 kJ mol^{-1}, E_{a2} = 69.34 kJ mol^{-1}, correlation coefficients r_1 = −0.998 and r_2 = −0.999 (Table III). Compared to the reaction in *n*-hexane, the activation energies were reduced by 2.53 and 31.16 kJ mol^{-1} for the forward and reverse process, respectively.

Effects of Temperature on Equilibrium Constants of PreD_3 ⇌

TABLE I

Temperature dependence on the rate constants of preD$_3$ ⇌ vitamin D$_3$ isomerization in an aqueous solution of β-cyclodextrin (A) and in n-hexane (B)

T	$k_1 \times 10^5$ (s^{-1})		k_{A1}/k_{B1}	$k_2 \times 10^6$ (s^{-1})		k_{A2}/k_{B2}
	A	B		A	B	
°C						
5	0.865	0.0176	49	8.48	0.0140	606
10	1.59	0.0302	53	14.5	0.0269	539
30	14.5	0.320	45	101	0.451	224
37	30.7	0.680	45	170	1.10	155
50	146	2.61	56	637	5.10	125

TABLE II

Temperature dependence on the equilibrium constants of preD$_3$ ⇌ vitamin D$_3$ isomerization in an aqueous solution of β-cyclodextrin and in n-hexane

Temperature	Equilibrium constant	
	β-Cyclodextrin	n-Hexane
°C	K	
5	0.985	12.51
10	1.09	11.10
30	1.57	7.16
37	1.76	6.22
50	2.16	4.87

TABLE III

Arrhenius rate parameters and thermodynamic values for preD$_3$ ⇌ vitamin D$_3$ isomerization in an aqueous solution of β-cyclodextrin (A) and in n-hexane (B)

	$\Delta G°$	$\Delta H°$	E_{a1}	E_{a2}	$\Delta S°$	$\ln A_1$	$\ln A_2$
	kJ mol^{-1}			JK^{-1}mol^{-1}			
A	−0.90	13.05	82.37	69.34	46.80	23.94	18.32
B	−5.14	−15.60	84.9	100.5	−35.25	21.05	25.26

Vitamin D_3 Interconversion—The equilibrium constant for preD$_3$ ⇌ vitamin D$_3$ interconversion depends strongly on temperature. The effect of temperature on the equilibrium constant K was given by the van't Hoff equation

$$\ln K = -\Delta H°/RT + C \quad \text{(Eq. 4)}$$

where $\Delta H°$ was the standard enthalpy change or the heat of the reaction at 25 °C, R was the gas constant, and C was an integration constant. Plotting ln$K$ against the reciprocal of the absolute temperature ($1/T$) gave a straight line ($r = -0.982$) (Fig. 4). The slope of the line ($-\Delta H°/R$) was −1570, and thus the $\Delta H°$ was 13.05 kJ mol^{-1}. Therefore, the determined van't Hoff equation for the isomerization between preD$_3$ and vitamin D$_3$ in β-cyclodextrin was expressed as

$$\ln K = -1570/T + 5.63 \quad \text{(Eq. 5)}$$

Whereas the reported van't Hoff equation (Tian *et al.*, 1993) for the reaction in *n*-hexane was

$$\ln K = 1882/T - 4.24 \quad \text{(Eq. 6)}$$

in which $\Delta H°$ was −15.65 kJ mol^{-1}. These results demonstrate for the first time that whereas in *n*-hexane and other media the preD$_3$ ⇌ vitamin D$_3$ reaction is exothermic, when the reaction was carried out in β-cyclodextrin solution this isomerization became endothermic ($\Delta H° > 0$) (Equations 4 and 5). Fig. 4 compared the effects of temperature on equilibrium constants K in *n*-hexane and in the β-cyclodextrin solution. It was apparent from Fig. 4 that there existed two distinct mechanisms by which the equilibrium were affected by the changes of temperature. The preD$_3$ ⇌ vitamin D$_3$ interconversion in *n*-hexane followed the classic mechanism, *i.e.* as temperature was increased the percentage of vitamin D$_3$ at equilibrium was decreased (Fig. 4 and Equation 6). On the contrary, the isomerization in β-cyclodextrin solution represented a novel mechanism, *i.e.* the percentage of vitamin D$_3$ at equilibrium was increased as temperature was increased (Fig. 4 and Equation 5).

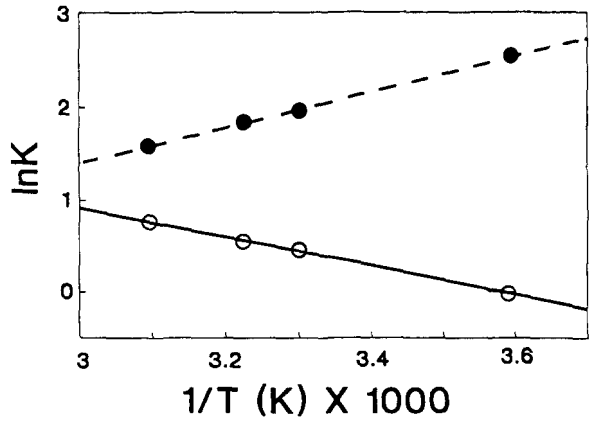

FIG. 4. **Comparison of temperature dependence of equilibrium constants for previtamin D$_3$ ⇌ vitamin D$_3$ isomerization in β-cyclodextrin (○) and in *n*-hexane (●).** Effects of temperature on equilibrium constants for the reaction in these two media are opposite, which was determined by the sign of the standard enthalpy changes, *i.e.* $\Delta H° > 0$ or $\Delta H° < 0$. For details see "Results" and "Discussion."

Thermodynamic Analysis—The determined values of standard thermodynamic parameters $\Delta G°$, $\Delta H°$, and $\Delta S°$ for preD$_3$ ⇌ vitamin D$_3$ interconversion in β-cyclodextrin solution are given in Table III together with the reported values for the reaction carried out in *n*-hexane (Tian *et al.*, 1993).

Eyring's Transition-State Theory and the Activation Parameters—According to transition-state theory, the rate of a reaction at any given temperature depends solely on the concentration of the high energy activated complex. Eyring's equation relates the rate constant to quasithermodynamic parameters by the following expressions

$$k = (k_B T\kappa/h) \exp(-\Delta G^‡/RT) =$$

$$(k_B T\kappa/h) \exp(\Delta S^‡/R - \Delta H^‡/RT) \quad \text{(Eq. 7)}$$

where k_B was Boltzmann's constant, h was Planck's constant, and κ was the transmission coefficient and its value was assumed to be unity during the calculations. The enthalpy and entropy of activation $\Delta H^‡$ and $\Delta S^‡$ are measures of the heat and entropy changes when reactants are converted to activated complexes, and the determined values for the reaction in β-cyclodextrin solution are listed in Table IV and compared with the reported values for the reaction in *n*-hexane (Tian *et al.*, 1993). The free energies of activation, $\Delta G_1^‡$ (preD$_3$ → vitamin D$_3$) and $\Delta G_2^‡$ (vitamin D$_3$ → preD$_3$) were smaller for the reaction carried out in β-cyclodextrin than those in *n*-hexane (Table IV), which means less energy was needed to bring the reactants to their transition states, and therefore the rate constants were increased for the reaction in β-cyclodextrin solution compared to those in *n*-hexane (Equation 7 and Table I).

Effects of Cavity Size and Hydroxyl Groups of Cyclodextrin on Reaction Rate and Equilibrium of PreD$_3$ ⇌ Vitamin D$_3$ Isomerization—Compared to the rate constant in β-cyclodextrin at 37 °C, the determined k values in α-cyclodextrin ((2.18 ± 0.0035)×10^{-5} s^{-1}) and in methyl-β-cyclodextrin ((7.66 ± 0.038) × 10^{-5} s^{-1}) were decreased by more than 20- and 6-fold, respectively. Whereas the percentage of vitamin D$_3$ at equilibrium at 37 °C were increased from 64.2 ± 1.8 in β-cyclodextrin to 96.7 ± 0.2 in α-cyclodextrin and 96.6 ± 0.2 in methyl-β-cyclodextrin (Fig. 5).

TABLE IV
Activation parameters for preD$_3$ ⇌ vitamin D$_3$ isomerization in an aqueous solution of β-cyclodextrin (A) and in n-hexane (B)

	$\Delta G_1^{\ddagger}$	$\Delta G_2^{\ddagger}$	$\Delta H_1^{\ddagger}$	$\Delta H_2^{\ddagger}$	$\Delta S_1^{\ddagger}$	$\Delta S_2^{\ddagger}$
	kJ mol^{-1}				*J K^{-1} mol^{-1}*	
A	96.05	96.97	79.89	66.86	−54.20	−101.0
B	105.7	110.9	82.42	98.02	−78.12	−43.19

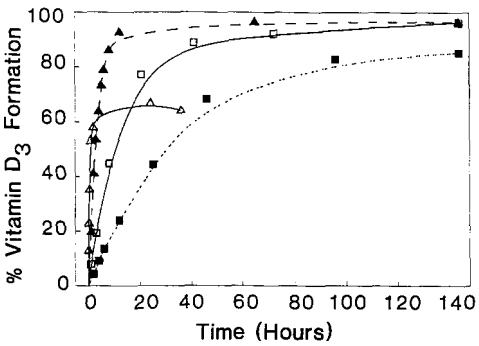

FIG. 5. **Thermal isomerization of preD$_3$ to vitamin D$_3$ as a function of time in *n*-hexane (■), and in aqueous solutions of α-cyclodextrin (□), methyl-β-cyclodextrin (▲) as well as β-cyclodextrin (△) at 37 °C.**

DISCUSSION

A change in the reaction medium such as polarity, viscosity, *etc.*, can have a substantial influence on the kinetics of chemical reactions. However, from a chemical point of view, these parameters usually do not have a major impact on an intramolecular concerted process. It has been assumed that the rate and equilibrium of preD$_3$ ⇌ vitamin D$_3$ interconversion was only affected by temperature (Hanewald *et al.*, 1961; Schlatmann *et al.*, 1964; Sanders *et al.*, 1969). However, in a biological system, the conventional chemical media have been replaced with diversified physiological environments, such as lipid bilayers, micelles, proteins, nucleic acids, and polysaccharides. In contrast to isotropic solutions, these organized and constrained media have the unusual ability to dramatically modulate the conformational equilibrium of guest molecules that may ultimately lead to catalysis or inhibition by favoring or disfavoring particular conformations. PreD$_3$ is conformationally flexible and undergoes rotation around C_5-C_6 single carbon bond to create cZc (s-*cis*,s-*cis*) and tZc (s-*trans*,s-*cis*) conformations (Fig. 1) (Dauben and Funhoff, 1988a, 1988b; Norman *et al.*, 1993). In isotropic solutions cZc conformation is energetically less stable due to steric interactions between C_{19} methyl group and C/D rings. The cZc conformers are able to undergo alternative reaction pathways. They can either thermally isomerize to vitamin D$_3$ or photochemically convert to lumisterol, whereas the tZc conformers are the precursors solely responsible for the photoproduction of tachysterol (Dauben and Funhoff, 1988a, 1988b; Terenetskaya *et al.*, 1992). We hypothesize that the complexation of preD$_3$ with β-cyclodextrin shifts its conformational equilibrium in favor of formation of cZc conformation, and therefore the rate constant is increased. This hypothesis is supported by the finding that irradiation of 7-DHC·β-cyclodextrin complex results in marked increase in the formation of lumisterol with a concomitant decrease in the amount of tachysterol compared with the reaction carried out in isotropic solutions.[2]

The thermodynamics and kinetics of preD$_3$ ⇌ vitamin D$_3$ reaction in β-cyclodextrin solution medium showed striking similarities to those in the sea urchin. First, the equilibrium of the reaction was greatly shifted to preD$_3$, *i.e.* from 8% in *n*-hexane to 48% in β-cyclodextrin solution at 10 °C, which agrees well with the reported value in the sea urchin (more than 45%) (Hobbs *et al.*, 1987). Second, like the reaction in sea urchin, the rate of the isomerization in β-cyclodextrin solution is among the fastest ever known, *i.e.* more than 40- and 600-fold increases in k_1 and k_2, respectively (Table I). Third, the percentage of vitamin D$_3$ at equilibrium in β-cyclodextrin solution is increased as the temperature is raised (Fig. 4), which is determined by the negative slope of the van't Hoff plot ($\Delta H^\circ > 0$) (Equations 4 and 5). And this is in contrast to all other reported reaction media to date (Schlatmann *et al.*, 1964; Cassis and Weiss, 1982; Yamamoto and Borch, 1985; Tian *et al.*, 1993, 1994). Since $\Delta H^\circ = E_{a1} - E_{a2}$, the mechanism responsible for the positive ΔH° is that the activation energy for the reverse reaction (E_{a2}) is markedly reduced and becomes smaller than E_{a1} (Table III), *i.e.* $E_{a1} - E_{a2} = \Delta H^\circ > 0$.

Based on Arrhenius' equation (Equation 3) it is evident that the rate constant can be increased either by lowering the activation energy (E_a) through the formation of inclusion complex, or by increasing frequency factor (A) through properly orienting C_{19} and C_9 of the preD$_3$ and vitamin D$_3$ molecules, or a combination of both. For the forward reaction, preD$_3$ → vitamin D$_3$ both effects exist and are additive, with E_{a1} being lowered by 2.5 kJ mol^{-1} and A_1 being increased by 17-fold. By this mechanism, the forward rate constant k_1 for the reaction carried out in β-cyclodextrin solution was increased more than 40 times compared to that in *n*-hexane at 5 °C (Table I). However, the dramatically increased k_2 is a consequence of a significantly lowered E_{a2} being offset by a smaller A_2 (Table III). If A_2 were not decreased, we could have expected that at 5 °C, k_2 for the vitamin D$_3$ → preD$_3$ isomerization in β-cyclodextrin would be increased by a million fold.

To examine the effects of cavity size of cyclodextrin on the reaction rate of preD$_3$ ⇌ vitamin D$_3$ isomerization, kinetic studies were carried out in α-cyclodextrin. We found that when the cavity diameter of cyclodextrin was decreased from 6.2 Å (β-cyclodextrin) to 4.9 Å (α-cyclodextrin), the rate constant was decreased by more than 20 times. These results indicate that similar to an enzymatic reaction, the size of the hydrophobic cavity had a great influence on the reaction rate. To assess the influence of outer surface hydroxyl groups of cyclodextrin on the reaction rate, comparisons were made between the reactions in β-cyclodextrin and in methyl-β-cyclodextrin. It was found that reaction rate in β-cyclodextrin was six times faster than that in methyl-β-cyclodextrin. Since partial permethylation had no effect on the cavity size and basic conformation of the cyclodextrin (Myles *et al.*, 1994), these data suggest for the first time that the host hydroxyl groups can accelerate the reaction rate of the [1,7]-sigmatropic hydrogen shift between preD$_3$ and vitamin D$_3$. It is interesting to note that the degree of the acceleration of the reaction rate by intermolecular hydroxyl groups is similar to the reported values for intramolecular hydroxyl-directing effects (π-facial selectivity) on the reactions involving [1,7]-sigmatropic hydrogen shift (Hoeger *et al.*, 1987, Wu and Okamura, 1990; Curtin and Okamura, 1991). The observation that either changing cavity size or masking host hydroxyl groups resulted in a dramatic increase in the percentage of vitamin D$_3$ at equilibrium revealed a novel mechanism by which the equilibrium of preD$_3$ ⇌ vitamin D$_3$ isomerization can be modulated by constrained media. A similar mechanism may exist *in vivo* by which the preD$_3$ ⇌ vitamin D$_3$ endocrine system is modulated to meet various physiological requirements.

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[2] X. Q. Tian and M. F. Holick, unpublished results.

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