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Solvent effect on previtamin D conformational equilibrium and photoreactions

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Abstract

The effect of solvent polarity on the conformational equilibrium of previtamin D has been discussed in terms of the abundance (%) of its conformers calculated by molecular mechanics in conjunction with nuclear magnetic resonance experimental data available in the literature. It has been assumed that polar reaction media shift the conformational equilibrium towards the most non-strained cZc conformations with the OH group in pseudo-equatorial orientation. 7-Dehydrocholesterol (provitamin D) photoisomerization on irradiation at $\lambda = 254$ nm has been studied in ethanol and hexane by UV spectroscopy. A consideration of the observed differences in combination with the calculations of the reaction kinetics provides an estimation of the quantum yields of previtamin D cis–trans isomerization and additional experimental evidence for the solvent-induced changes in the conformational equilibrium of previtamin D in favour of cZc forms in polar environments. © 1997 Elsevier Science S.A.

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1. Introduction

The manufacturing process of vitamin D and its synthesis in vivo consist of two basic stages: photosynthesis of previtamin D (provitamin D photoisomerization) and its thermochemical conversion into vitamin D [1,2].

Previtamin D Z/E isomerization into tachysterol is the most efficient undesirable side photoprocess in this complex network of isomerization reactions. Therefore a detailed knowledge of the sensitivity of this reaction, in particular to the action of various external physicochemical factors, is essential.

It has been shown that, in heterogeneous reaction media, the restriction of the intramolecular rotation of previtamin D, and therefore a significant inhibition of E isomer formation, can be achieved [3,4]. The same effect has been observed for provitamin D photolysis under high-intensity pulsed laser irradiation when first-formed non-equilibrated conformers of previtamin D are excited [5]. From studies on A-ring analogues [6,7], Z/E isomerization is sensitive, to a certain extent, to the structural modification of the previtamin D skeleton.

These studies and many others [8] (and the references cited therein) lead to convincing evidence of the dominant role of the previtamin D ground state geometry on its photochemical conversions. In Ref. [9], it has been proposed that the cZc conformers of previtamin D are the precursors of the ring-closure photoproducts, whereas the tZc conformers are the intermediates of Z/E isomerization (previtamin D → tachysterol).[1] This assumption is schematically illustrated in Fig. 1. Recent model analysis of the corresponding photoreaction sequence [11] has clearly demonstrated the different possibilities of influencing the reaction kinetics and product distribution and, in particular, has shown theoretically that a decrease in the Z/E isomerization efficiency can be achieved by the creation of a low tZc conformer population.

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[1] Z and E denote the cis and trans geometries respectively in relation to the C6=C7 double bond. The letters c and t refer to the cis and trans conformations of the C5–C6 and C7–C8 single bonds. The sign of the torsion angle follows the rules given in Ref. [10].

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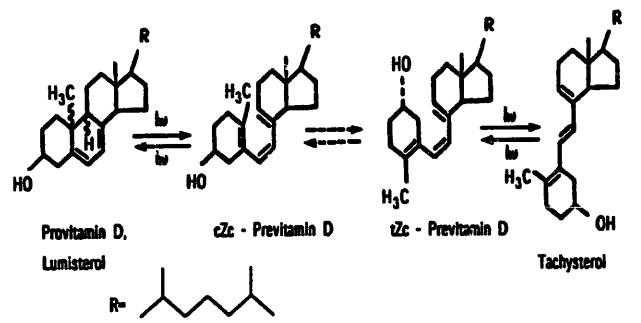

Fig. 1. Scheme of conformation-dependent previtamin D photoconversions.

In this work, we focus on the effect of the solvent polarity as one of the possible ways to control the photoisomerization reactions.

2. Experimental details

2.1. Molecular modelling

For the conformational analysis of previtamin D, various force field programs were used [12]. For simplicity, the side chains in all previtamins were substituted by a methyl group. The abundances (%) of the conformers according to a Boltzmann distribution at 298 K were calculated using their relative steric energies.

2.2. General procedures

7-Dehydrocholesterol (provitamin D) of 99% purity was kindly supplied by Dr. N.A. Bogoslovsky from the Institute of Vitamins (Moscow, Russia). All solvents employed were of spectroscopic grade. The irradiations of provitamin D (ethanol and hexane solutions, 0.00006 M) were carried out simultaneously in quartz cuvettes equipped with a magnetic stirring bar at a sufficiently low temperature such that the previtamin D→vitamin D transformation was negligible. Solutions were purged with nitrogen for 10 min prior to irradiation. As light source, a germicide lamp (BUV-60; wavelength emission, 254 nm) was used. The photoreaction kinetics were controlled by periodical recording of the UV absorption spectra on a KSVU-23 (LOMO, Russia) spectrometer.

2.3. Simulation of the photoreaction kinetics

The simulation of the photoreaction kinetics and evolution curves of the relative optical density at 282 nm was performed on the basis of a differential equations system (Eqs. (1a–e)) which describes a reaction model incorporating the irreversible photoconversion of previtamin D [10,13]

$$dC_1/dt = A(\varphi_{12}\epsilon_2 C_2 - \varphi_{21}\epsilon_1 C_1) \quad (1a)$$

$$dC_2/dt = A\{\varphi_{21}\epsilon_1 C_1 + \varphi_{23}\epsilon_3 C_3 + \varphi_{24}\epsilon_4 C_4 - (\varphi_{12} + \varphi_{32} + \varphi_{42} + \varphi_{52})\epsilon_2 C_2\} \quad (1b)$$

$$dC_3/dt = A(\varphi_{32}\epsilon_2 C_2 - \varphi_{23}\epsilon_3 C_3) \quad (1c)$$

$$dC_4/dt = A(\varphi_{42}\epsilon_2 C_2 - \varphi_{24}\epsilon_4 C_4) \quad (1d)$$

$$dC_5/dt = A\varphi_{52}\epsilon_2 C_2 \quad (1e)$$

where $A = Jl(1-10^{-D})/VD$, J is the incident radiation, $D = l\sum_{i=1}^{5} C_i\epsilon_i$ is the optical density of the sample at the irradiation wavelength, ϵ_i and C_i are the extinction coefficient and concentration of component i, φ_{ij} is the quantum yield of the reaction $j \rightarrow i$, subscripts 1–5 correspond to Pro, Pre, T, L and Tox respectively and V and l are the volume and path length of the cell.

The system was solved numerically. Molar extinction coefficients were taken from Ref. [14]. On the basis of the calculated kinetic data, the evolution curves of the relative optical density (OD(t)/OD(0)) were simulated and compared with the experimental curves at the reference points $t = t_{max}/4$, $t = t_{max}/2$, $t = t_{max}$, $t = 2t_{max}$, etc. in order to determine the best fitting parameters.

3. Results and discussion

3.1. Ground state conformations of previtamin D and UV absorbance

Previtamin D may adopt a number of different conformations due to its exceptional internal flexibility caused by oscillations around the C5–C6 and C7–C8 single bonds and the chair–chair interconversion of the A-ring. A recent molecular mechanics-based re-investigation of the previtamin D ground state geometry has been reported in detail in Ref. [7]. It has been revealed (Table 1) that the global minimum among the conformations with a pseudo-equatorial OH group position is the (−)cZ(−)c form, whereas in the pseudo-axial series, it is the (+)tZ(−)c conformation[2] (Fig. 2).

[2] Both conformations are non-planar [7], so that the values of the torsion angles C10–C5–C6–C7 are close to the corresponding values in the so-called conical intersections [8] (molecular structures $CI_{c/t}$) through which the excited molecule returns to the ground state.

Table 1
Abundance (%) of previtamin D calculated conformers according to a Boltzmann distribution at 298 K

Conformation of previtamin D	(−)cZ(−)c	(+)cZ(+)c	(−)tZ(+)c	(+)tZ(−)c	(+)tZ(+)t	(−)cZ(+)t
OH-eq	20	6	2	9	2	8
OH-ax	11	13	3	19	4	3

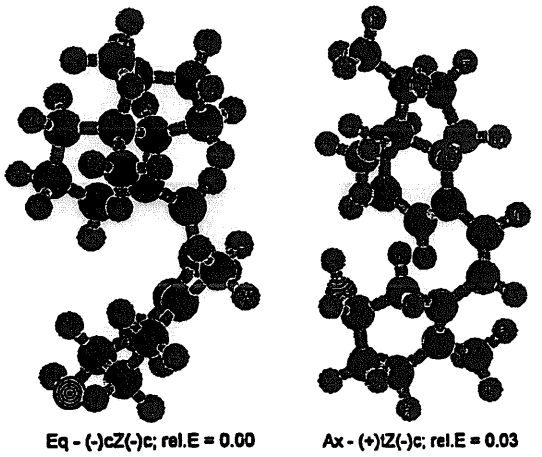

Fig. 2. Ball and stick representation of the two most stable conformations of previtamin D.

However, taking into account the small steric energy differences between the individual conformers, the ground state equilibrium of previtamin D is accessible to change. Changes in different equilibrium conformer populations may well cause shifts in the UV absorption spectrum. It has been shown [15] that such shifts can be predicted sufficiently well on the basis of force field calculation results. The observation of a red shift in the low-temperature UV and circular dichroism (CD) spectra of previtamin D [16,17] is in good accordance with the outcome of our conformational search. This shift has been attributed to the preference of cZc conformations that absorb in the longer wavelength range. Indeed, a decrease in temperature should lead to a further stabilization of the most unstrained (−)cZ(−)c conformation which is the most populated at room temperature.

In Ref. [16], it has been observed that the absorption maximum of previtamin D is solvent dependent and undergoes a red shift as the solvent polarity increases. This has also been explained by the redistribution of the conformational population in favour of cZc forms.

The comparison of our calculation results with nuclear magnetic resonance (NMR) studies on previtamin D [16] leads to the same conclusion. It has been revealed in Ref. [16] that the pseudo-equatorial C3 OH orientation is dominant in polar media, whereas it is a minority component in non-polar solvents.

Table 1 shows that (−)cZ(−)c is the most unstrained form in the pseudo-equatorial series. Therefore this conformer should be prevalent in polar microenvironments. The (+)tZ(−)c conformer should be more stable in non-polar media.

Thus the conformational equilibrium of the previtamin D molecule should be amenable to modulation by changes in the solvent polarity. Since the increased polarity of the solvent may exert a depopulating effect on the tZc conformers, a decrease in the previtamin D Z/E photoisomerization efficiency in polar media may be expected.

3.2. Reaction kinetics of provitamin D photoisomerization in solution

The main four photoisomers in the provitamin D photoisomerization reaction network (Fig. 3(a)) have absorption bands between 250 and 300 nm (Fig. 3(b)); therefore UV irradiation of provitamin D under conditions excluding the accumulation of vitamin D results in the formation of a photoisomeric mixture and the establishment of a dynamic equilibrium between the photoisomers at a certain stage. The composition of the mixture in such a quasi-photostationary state depends on the irradiation wavelength [18] (and references cited therein). On prolonged irradiation, all these compounds gradually disappear in favour of toxisterols, whose absorption bands are shifted to the shorter wavelength region. Therefore Fig. 4 demonstrates the calculated kinetics of provitamin D photoisomerization on irradiation at $\lambda = 254$ nm using the set of quantum yields determined in ether [10]: 0.31, 0.0092, 0.11, 0.42, 0.41, 0.021 and 0.039 for Pro → Pre, Pre → Pro, T → Pre, Pre → T, L → Pre, Pre → L and Pre → Tox photoconversions respectively.

As Fig. 4 illustrates, tachysterol (E isomer of previtamin D) is a dominant component of the quasi-photostationary mixture on short-wavelength irradiation. This E isomer, due to its high extinction coefficient, causes a remarkable increase in the optical density (OD) of the solution until a quasi-photostationary state is reached. This increase then stops and,

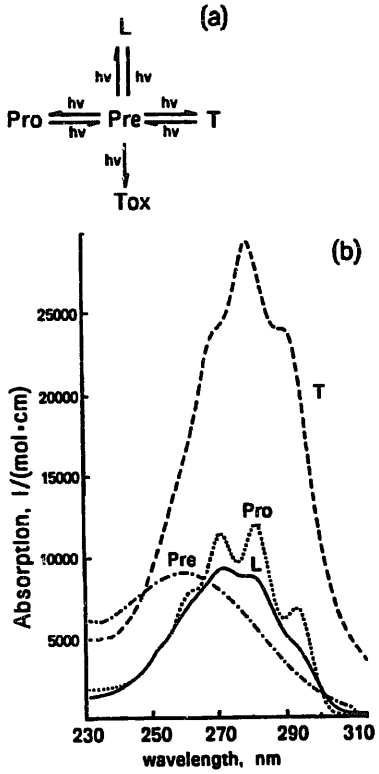

Fig. 3. (a) Reaction network scheme of provitamin D photoisomerization: Pro, provitamin D; Pre, previtamin D; T, tachysterol; L, lumisterol; Tox, toxisterols (products of prolonged irradiation). (b) UV absorption spectra of provitamin D and its main photoisomers.

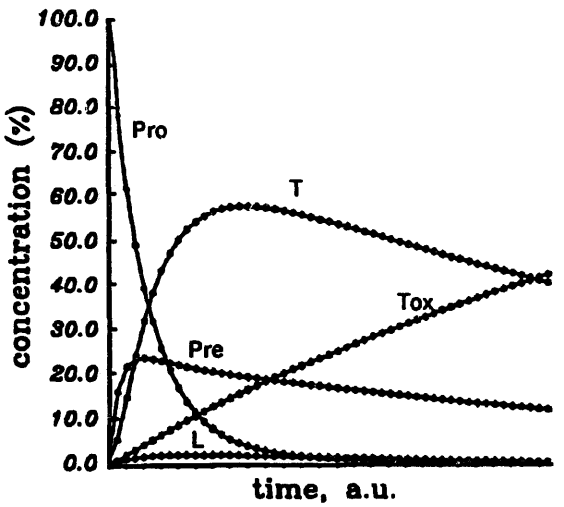

Fig. 4. Calculated reaction kinetics of provitamin D photoisomerization at $\lambda = 254$ nm on the basis of the quantum yields in ether [10].

on further irradiation, the OD gradually decreases as a result of the irreversible photoconversion to toxisterols [1,2]. The accumulation of tachysterol is well represented by the kinetic dependence of the relative optical density (OD(t)/OD(0)) at $\lambda = 282$ nm. This can be used for the indirect evaluation of the Pre → T and Pre → Tox efficiencies [4].

3.3. UV spectroscopic control of provitamin D photoisomerization on irradiation at $\lambda = 254$ nm

Fig. 5 presents the experimental kinetic dependence of the relative optical density at 282 nm obtained for irradiation of provitamin D solutions in ethanol (curve 1) and hexane (curve 2). The differences are marked: curve 2 has a higher maximum and a more pronounced slope at the stage of irreversible photoconversion relative to curve 1.

To draw conclusions about the reaction quantum yields in both cases, calculations of the kinetic dependence have been performed on the basis of numerical solutions of the kinetic equations given above (Eqs. (1a–e)).

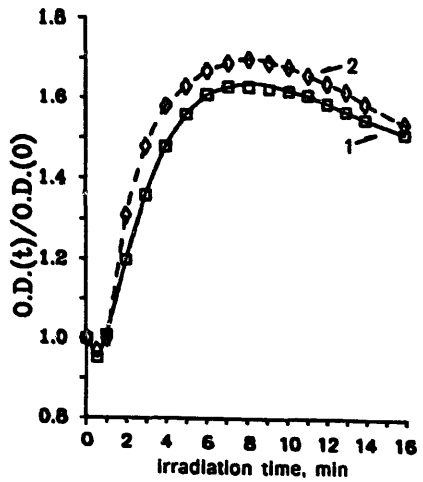

Fig. 5. Experimental kinetic dependence of the relative optical density at $\lambda = 282$ nm for provitamin D photolysis ($\lambda = 254$ nm) in ethanol (1) and hexane (2).

Initially, we used in our calculations the values of the quantum yields from Ref. [16]. They were obtained in ether taking into account the irreversible Pre → Tox transformations. To achieve a good fitting of the calculated kinetic dependence to the experimental dependence for ethanol (curve 1, Fig. 5), we used the value of the Pre → Tox quantum yield for ethanol at $\lambda_{irr} = 266$ nm from Ref. [18] and varied the value of Pre → T. These simulations have revealed that the set of quantum yields chosen for ethanol (0.43 for Pre → T and 0.026 for Pre → Tox) describes the experimental situation well.

To obtain an estimation of the reaction quantum yields in hexane, the logarithmic dependence of the experimentally measured relative optical densities at 282 nm vs. the irradiation time has been plotted and shows a linear dependence at the stage of irreversible photoconversion (Fig. 6). From previous work [10,18], it is known that the slope of the analogous dependence on the irradiation dose is proportional to the rate of the Pre → Tox photoprocess. Since the irradiation doses were not measured in the present study, we may use this approach as a first approximation. Therefore the slopes of the lines (Fig. 6) provide an estimation of the ratio of the rates of the irreversible photoprocess in ethanol and hexane of 0.73. From this, the estimated quantum yield of the Pre → Tox photoreaction channel is of the order of 0.036. The more efficient formation of toxisterols in hexane can be understood in view of the conformational equilibrium of previtamin D. According to the NEER principle [1], tZc conformers may give a wider spectrum of "overirradiation" products than cZc conformers. Therefore it may be speculated that, by depopulating the tZc forms using more polar solvents, a decrease in previtamin D degradation via the irreversible reaction channel should result.

To determine the approximate value of the Pre → T quantum yield in hexane, the family of curves representing the dependence of the relative optical density at $t = t_{max}$ on the

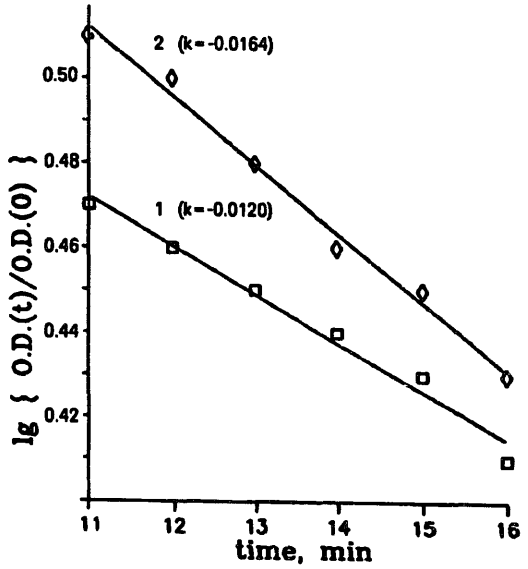

Fig. 6. Comparison of the logarithmic dependences of the relative optical density at 282 nm vs. the irradiation time in ethanol (1) and hexane (2).

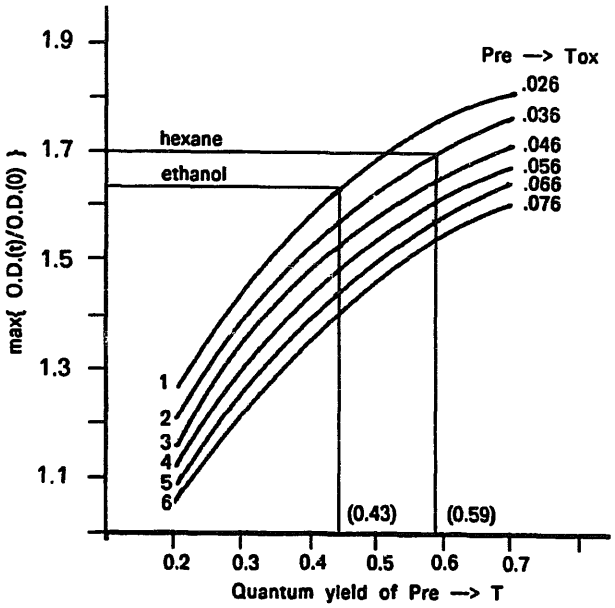

Fig. 7. Family of calculated curves representing the dependence of the maximum relative optical density on the Pre → T quantum yield for different values of Pre → Tox: 0.026 (1); 0.036 (2); 0.046 (3); 0.056 (4); 0.066 (5); 0.076 (6).

Pre → T quantum yield has been calculated by varying the values of Pre → Tox (Fig. 7). From curve 2, the value $\varphi(\text{Pre} \rightarrow \text{T}) = 0.59$ is obtained. A comparison of the calculated data, using the new values of 0.59 and 0.036 for Pre → T and Pre → Tox, with the experimental data (curve 2, Fig. 5) reveals a satisfactory agreement and, therefore, supports our estimation of the quantum yields for irradiation in hexane. Thus we may deduce that the efficiency of Z/E photoisomerization is approximately 1.4 times higher in hexane than in ethanol.

It should be noted that all the quantum yields of the reversible reactions starting from previtamin D must be changed in accord with the conformational shift which occurs in hexane. Nevertheless, a number of simulations obtained by varying each quantum yield separately have shown that the reaction Pre → T is responsible for the increase in the optical density. This justifies its value estimated in this work.

4. Conclusions

The results presented here demonstrate the effect of solvent-induced changes in the previtamin D conformational equilibrium on the photoisomerization. The polarity of the reaction medium can be used to alter the A-ring and polyene geometries in previtamin D which, in turn, influence the photoisomerization reaction course. This may be of particular importance for a better understanding of the role of the previtamin D conformational distribution in its photoconversions and thermoconversions in vitro and in vivo

Indirect spectrophotometric evaluation of the reaction quantum yields can be applied to search for optimal solvents or solvent systems for the manufacture of vitamin D and for the development of an in vitro model of previtamin D photosynthesis for UVB dosimetry.

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