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Two methods for direct assessment of the Vitamin D synthetic capacity of sunlight and artificial UV sources☆

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Abstract

Excessive UV exposures are commonly associated with adverse health effects, but proper amounts of UV are beneficial for people and essential in the natural production of Vitamin D_3 in skin. Two methods have been developed for direct evaluation of the Vitamin D synthetic capacity of sunlight (and artificial UV sources). The first one uses an in vitro model of Vitamin D_3 synthesis (ethanol solution of 7-dehydrocholesterol, 7-DHC), and concentration of previtamin D_3 accumulated during an UV exposure is determined using specially designed spectrophotometric analysis. The second method utilizes photoisomerization of provitamin D in nematic liquid crystalline (LC) matrix, and visual estimation of accumulated previtamin D becomes possible due to special design of a LC cell. This user-friendly method is appropriate for personal UV dosimetry and may have wide application in tanning saloons, in clinical dermatology and UV therapy.

Keywords: Provitamin D photoisomerization; UV biodosimeter; Spectral analysis; Steroid chirality; Cholesteric LC phase

1. Introduction

It is a matter of general experience that natural exposure to sunlight is responsible for adequate Vitamin D nutrition for most of the population in the world. In addition to the well-established effects of Vitamin D on the maintenance of mineral homeostasis, numerous evidences suggest a wider biologic role of Vitamin D not primarily related to mineral metabolism [1–3]. Besides, recent epidemiologic studies [4] demonstrate that cancer mortality rates are correlated inversely with local solar UV-B (280–315 nm) doses for 13 types of cancer, and the most likely mechanism whereby solar UV-B radiation provides protection against cancer is natural production of Vitamin D. All these data are evidence for the importance of permanent direct monitoring of the Vitamin D synthetic capacity of sunlight.

During the last decades concerns of the environmental and health effects of solar UV radiation penetrating into the biosphere through the depleted stratospheric ozone layer generated a need for reliable instrumentation and accurate measurements of increased amount of solar UV-B radiation reaching the earth surface. Although a number of UV radiometers have been designed for the UV monitoring, in most cases their spectral sensitivity corresponds to the CIE erythema action spectrum [5] that differs noticeably from the action spectrum of Vitamin D[1] synthesis [6]. As a result, the ratio between erythemal and 'antirachitic' doses is not constant with the wavelength [7] and may vary over a wide range with the constantly changing spectral composition of solar UV-B radiation. Hence, standard broadband radiometers are unable to ensure quantitative estimation of the Vitamin D_3 synthetic capacity of sunlight from the solar UV radiometric data.

As is well known, the complex network of Vitamin D synthesis consists of the two stages of monomolecular isomerizations (see Fig. 1) [8]. At the first stage UV irradiation of provitamin D yields previtamin D that is further converted into Vitamin D at body temperature. Obviously, previtamin D photosynthesis during UV exposure is intimately related to the Vitamin D synthetic capacity of sunlight, and the amount of accumulated previtamin D (which is the immediate precursor of Vitamin D) can be taken as a measure of biologically active 'antirachitic' UV dose.

For the first time Webb and Holick [9] used the photochemical stage of Vitamin D_3 synthesis in vitro for direct

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[1] The terminology "Vitamin D" is employed here in general sense although two principal chemical species are available. Vitamins D_2, or ergocalciferol ($C_{28}H_{44}O$) and D_3, or cholecalciferol ($C_{27}H_{44}O$) are produced from their precursors ergosterol and 7-dehydrocholesterol (7-DHC), respectively. It is significant to notice that basic monomolecular isomerizations of the two steroid species occur in perfect analogy.

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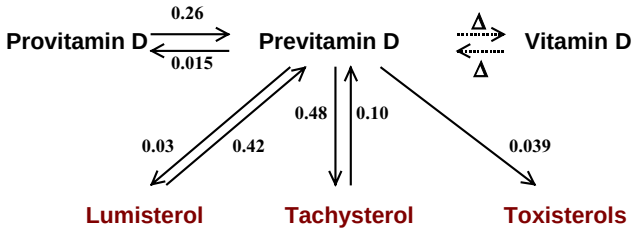

Fig. 1. Schematic representation of the photochemical transformation of provitamin D into previtamin D and its subsequent thermoconversion into Vitamin D. Numbers with arrows label the quantum yields of the reversible and irreversible side previtamin D photoreactions.

measurements of previtamin D accumulation under sunlight irradiation. With this aim they exposed solution of 7-DHC in ethanol, and high performance liquid chromatography (HPLC) was used for concentration analysis of the multicomponent photoisomer mixture that was formed during an exposure due to side photoconversions of previtamin D. They found that the Vitamin D synthetic capacity of sunlight is dramatically influenced by seasonal and latitudinal changes in solar UV-B radiation [10]. Besides, stratospheric ozone, clouds, aerosols and air pollution also hinder penetration of UV-B flux to the earth surface.

Hence, with due regard to the importance of Vitamin D synthesis for human health, there is a need to detect previtamin D formed in the course of UV exposure in the real time scale. Evidently, the labor- and time-consuming HPLC analysis does not meet the demands, and more rapid method that uses spectrophotometric analysis ('D-dosimeter') has been specially designed to measure previtamin D accumulation in situ without disturbing the irradiated solution [6,11].

In addition, a parallel effort went into the development of simpler personal biodosimeter with a capability of visual detection of previtamin D photosynthesis. It is based on provitamin D photoisomerization in the nematic liquid crystalline (LC) matrix [12]. It was suggested that dissolution of 7-DHC in nematic LC would be accompanied by formation of a cholesteric macrohelix due to chirality of steroid molecules. In this case the photoinduced transformations of 7-DHC molecules will change their helical twisting power and influence the cholesteric macrohelix pitch depending on the accepted UV dose.

2. Materials and methods

'D-dosimeter' uses the first—photochemical—step of Vitamin D synthesis. With this aim ethanol solution of 7-DHC ($C = 20$ mkg/ml) is exposed to sunlight in standard rectangular quartz cuvette of 0.5 cm thickness. As a result of UV irradiation, a multicomponent photoisomer mixture is formed from the initial provitamin D [8]. The UV absorption spectra of the solution are recorded by a double-beam UV spectrophotometer before and after the exposure within spectral range 230–330 nm with a 1 nm step, and the photoisomer concentrations are derived from the spectra by computer processing using specially designed computer program [11].

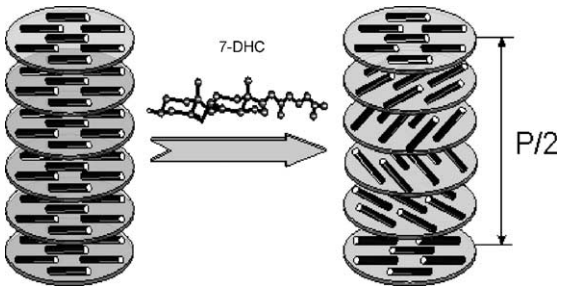

Fig. 2. Schematic representation of a nematic LC transformation into cholesteric phase induced by chiral dopant of 7-DHC. Each layer in induced cholesteric LC exhibits nematic-like order and the layers are twisted with respect to one another to form a macrohelix. The long axes of solute molecules are parallel to the axis of solvent molecules. The distance it takes the director to complete a turn of 360° is called the cholesteric pitch P of cholesteric LC.

As a measure of the accepted UV dose, quantities of decomposed provitamin D and accumulated previtamin D are to be used. The decay of starting provitamin D is analogous to biological response of many biodosimeters in the form of some damage caused by UV radiation, but 'antirachitic' dose is determined by measuring previtamin D accumulation depending on the exposure time.

Radiometric characterization of the 'D-dosimeter' has been performed at the Belgian Institute for Space Aeronomy (IASB) [7]. UV irradiation from the xenon arc lamp with a number of the broadband and narrow-band ($\Delta\lambda = 2$ nm) filters was used to link physical and erythemal UV doses with concentrations of accumulated previtamin D.

In addition, mathematical model has been developed that enables calculation of the photoreaction course under irradiation by any mono- or polychromatic UV source [6]. It is significant that laboratory and field tests showed close agreement of the experimentally measured and calculated concentrations of previtamin D when the spectroradiometric data of a UV source were used at the model input [6,7].

For development of personal UV biodosimeter 7-DHC steroid was used as chiral dopant. As is known, chiral dopant is optically active substance that induces cholesteric phase when added in small amount into nematic liquid crystal [13]. The director of the LC molecules adopts a helically twisted orientation under the action of chiral dopant (Fig. 2). It was shown [14] that the same photoproducts were formed in the LC matrix as in organic solvents.

Two nematic LCs LC-805 (NIOPIK, Russia) and ZLI-1695 (Merck) have been selected as host matrices, and 7-DHC (Sigma) was dissolved in the LC material in concentration $5 \div 10$ wt.%. The wedge-like cell with Mylar spacer of 60 μm thickness was prepared using two quartz plates (15 mm × 20 mm) as substrates that were specially treated to provide planar boundary conditions. Transformation of

the nematic LC into cholesteric phase was evidenced by observation of the Cano-Grandjean stripes when the cell was sandwiched between crossed polarizers. To avoid the 7-DHC oxidation, the cell was carefully glued up along the perimeter.

Using the UV lamp it was found that the Cano-Grandjean stripes number (N_C) gradually decreased under UV irradiation as a result of provitamin D phototransformations [14]. To link the stripes number N_C with concentration of previtamin D which is accumulated in ethanol solution, the wedge-like LC cell was exposed to sunlight simultaneously with the cuvette with ethanol solution of 7-DHC (C = 20 μg/ml). Both samples were positioned at black paper in horizontal position. Before the exposure the initial number of the Cano-Grandjean stripes in the LC cell had been fixed ($N_C = 10$ at $t = 0$) and the UV absorption spectrum of 7-DHC in ethanol solution was recorded by Perkin-Elmer Lambda 25 spectrophotometer. In the course of solar irradiation the stripes number in the LC cell was checked time by time by placing the cell between crossed polarizers. At the moment when the stripe number decreased just for one stripe, the absorption spectrum of the ethanol solution was recorded again. Both samples were exposed in total 100 min around noon until the Cano-Grandjean stripes number reduced to $N_C = 7$.

3. Results and discussion

The results of the laboratory test on the UV irradiation of ethanol solution of 7-DHC are shown in Fig. 3. Transformation of the initial absorption spectrum depending on the UV exposure is shown in Fig. 3a), and corresponding concentration kinetic obtained by computer processing of these spectra are presented in Fig. 3b). These figures clearly demonstrate high sensitivity of the spectrophotometric analysis to the least changes in the solution absorption spectrum. Moreover, high accuracy (±1%) and reproducibility of the analysis and good correspondence of the experimentally measured and calculated kinetic were confirmed by numerous of laboratory and field tests [6,11,16].

The results of simultaneous exposure of 7-DHC in ethanol solution and in the LC cell are presented in Fig. 4. Accumulation of previtamin D in ethanol is shown in Fig. 4b) and the associated changes in the Cano-Grandjean stripes (N_C) number are shown in Fig. 4a).

From the result obtained it may be deduced that both dependencies have non-linear pattern of change with accumulated UV dose. However decrease in the Cano-Grandjean stripes number proceeds in close agreement with photosynthesis of previtamin D in solution, and the linear relationship between the N_C in the LC cell and previtamin D_3 concentration in ethanol solution has been obtained [12,15]. It was found that disappearance of one stripe in the LC cell corresponds to accumulation of 6% of previtamin D in vitro.

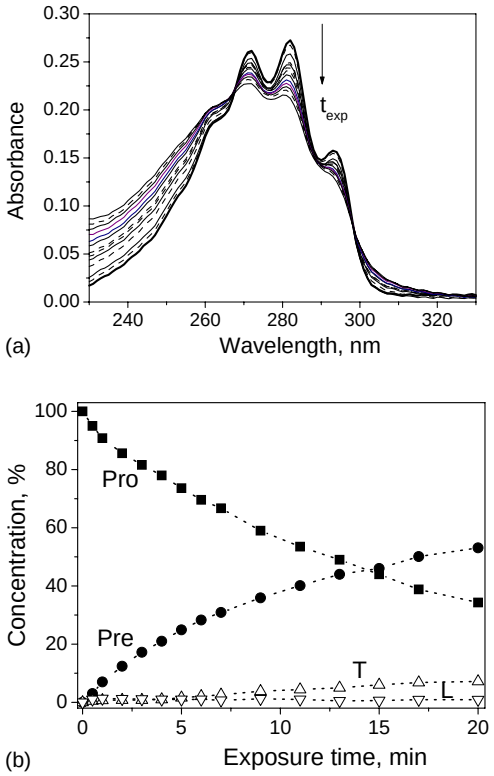

Fig. 3. Spectral transformation of 7-DHC in ethanol solution depending on the exposure time under UV irradiation using xenon arc lamp with broadband UV filter (a) and corresponding concentration kinetics (b). Pro: provitamin D; Pre: previtamin D; T: tachysterol; L: lumisterol.

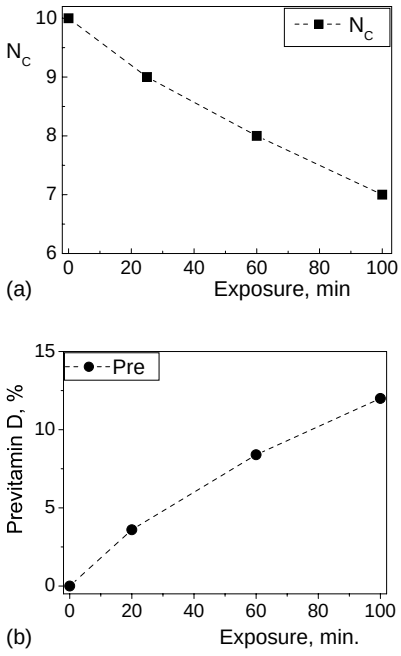

Fig. 4. Reduction of the Cano-Grandjean stripes number in the wedge-like LC cell (a) and corresponding accumulation of previtamin D in ethanol solution (b) during simultaneous exposure to sunlight.

4. Conclusions

Recent years the problem of potentially harmful UV effects on human health was extensively investigated in view of expected increase in the surface level of solar UV-B caused by stratospheric ozone depletion. Little attention was given to determination of the lowest healthy UV doses that are necessary to provide the Vitamin D requirement for most of the world's population despite recent findings on the protective role of UV-B sunlight against many types of cancers associated with Vitamin D synthesis.

Special-purpose 'D-dosimeter' has been designed for routine inspection of the Vitamin D synthetic capacity of sunlight and artificial UV sources. Its workability was clearly demonstrated in the course of laboratory and field tests. The availability of a mathematical model for previtamin D photosynthesis (from measured or modeled solar spectra) will allow prediction of profiles of antirachitic solar UV radiation over the globe.

Additionally, it was shown that the wedge-like LC cell filled with nematic LC doped by chiral 7-DHC molecules can be used for personal UV biodosimetry, and clearly defined dependence of the Cano-Grandjean stripe number on the exposure time is well representative of the accumulated dose of biologically active "antirachitic" UV radiation. The doped LC film can ensure easy detection of the Vitamin D synthetic capacity of sunlight by visual fixing the disclination stripes reduction when the LC cell is sandwiched between crossed polarizers. Such a personal UV biodosimeter can be used to evaluate UV exposure in realistic conditions of changing orientation and surface position, e.g. for investigating exposure of different body parts during various activities.

It is believed that the results obtained generate the basis for quantitative assessments of Vitamin D_3 production per square centimeter from the radiometric and spectroradiometric solar UV measurements. The exposure time and actual irradiated skin area to satisfy daily requirement for Vitamin D_3 can be calculated using the photoreaction model [6], 7-DHC concentrations in human skin [10] and the skin transmission [17].

Further studies are required to establish the correspondence between the D-dosimeter readings in vitro and previtamin D accumulation in vivo.

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