An *in situ* monitoring of biologically active solar UV-B radiation: A new biosensor of vitamin D synthetic capacity

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ABSTRACT

The new biosensor of vitamin D synthetic capacity of solar/artificial UV-B radiation is based on liquid crystal with provitamin D dopant. Nematic liquid crystals (LC-805, ZLI-1695) are converted into induced cholesteric phase using photosensitive steroid biomolecules of provitamin D_3 (7-dehydrocholesterol). During UV exposure remarkable decrease in the number of the Cano-Grandjean stripes has been observed in the wedge-like cell as a result of UV initiated photoisomerization of provitamin D_3 that changed helical twisting power of the dopant molecules.

Key words: solar UV-B radiation, vitamin D synthesis, personal UV-B biosensor, nematic LC and chiral dopant.

1. INTRODUCTION

Concerns of the environmental and health effects of solar UV radiation penetrating into the biosphere through the depleted ozone layer have greatly emphasized the urge for reliable measurements of biologically effective ultraviolet (UV) radiation. Although decreases in stratospheric ozone are well documented, much less is known about the effects of resulting increases in UV-B (280-315nm) radiation on human health and ecosystem.

Humans are usually not thought of as photosynthetic organisms, however, there are a number of UV sensitive molecules in human skin such as urocanic acid, 7-dehydrocholesterol (provitamin D_3), DNA, RNA and proteins that absorb solar UV radiation (UVR). High-energy UV photons initiate photochemical alterations in the molecular structure that may be responsible for a number of subsequent biological effects.

One can now appreciate that UV radiation is capable of producing both positive and negative biological effects on human health, and just the UV dose is a decisive factor. Excessive UV exposures are generally associated with acute and chronic health effects, such as erythema, skin cancer, immune system suppression, cataract. However, in proper dose UV radiation is beneficial for people, specifically due to generation of vitamin D_3 in skin. Vitamin D_3 is absolutely essential for the maintenance of healthy skeleton and bones. It is now acknowledged that traditionally perceived as an "antirachitic vitamin", vitamin D is responsible for a wide array of biological processes in human organism[1]. Besides, recent epidemiological studies indicate that vitamin D can significantly lower the risk of breast and colorectal cancer. Hence with respect to the importance of vitamin D_3 synthesis for human health and in view of wide spreading of endogenous synthesis of vitamin D in biosphere, an *in situ* monitoring of "antirachitic" UVR demands particular care.

A number of personal UVR detectors had been designed to measure ambient solar UV levels and give an indication of one's accumulated UV dose. For the most part their spectral sensitivity corresponds to the CIE erythema action spectrum[2], and biological activity of solar UVR is most commonly evaluated in minimal erythema doses (1 MED = 200 J/m^2). In view of considerable difference between the CIE erythema and "Vitamin D" action spectra an "erythemal" UV dosimetry can not provide correct evaluation of "antirachitic" UV doses. For this purpose Holick pioneered the application of an *in vitro* model of vitamin D synthesis (ethanol solution of 7-dehydrocholesterol, provitamin D_3)[3]. The ability of solar UV-B to provide the human requirements for vitamin D was investigated, and dramatic influence of seasonal and latitudinal changes in solar UVR on the vitamin D synthetic capacity (*in vivo* and *in vitro*) has been revealed[3].

As is known, the UV-B portion of sunlight (280-315nm) converts provitamin D into previtamin D which, in turn, undergoes a thermally induced isomerization into vitamin D[4]. Thus, the "antirachitic" UV dose is conveniently can be

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determined by measuring previtamin D accumulation as a function of the exposure time. However, the concentration analysis is significantly complicated by the side photoconversions of previtamin D that give rise to a multicomponent photoisomer mixture[4]. High performance liquid chromatography (HPLC) is a commonly accepted method to determine the concentration of accumulated previtamin D[3,4]. However, HPLC is labor and time-consuming, and thus is unable to provide "antirachitic" UV dosimetry *in situ*.

Original spectrophotometric analysis had been specially designed to ensure *in situ* measurements of accumulated UV doses[5-7]. To measure an "antirachitic" UV dose, a quartz cuvette with ethanol solution of provitamin D is exposed, and the absorption spectra of the solution are recorded within 230-330nm before and after UV exposure. Concentration of accumulated previtamin D is derived from the spectra by computer processing using original program. This so-called "D-dosimeter" permits to perform "antirachitic" UV dosimetry *in situ* (both day profile and daily-accumulated dose) if portable spectrophotometer is available on site. However, necessity of UV spectrophotometer and computer processing of the absorption spectra hinders the use of D-dosimeter for personal UV dosimetry.

In what follows we will describe further development of the method towards more simple registration of accumulated previtamin D that might be useful for elaboration of personal D-dosimeter. It is based on nematic liquid crystal doped with chiral biomolecules of provitamin D_3[8] that induce the cholesteric phase. It is anticipated that under UV irradiation the photochemical transformations of 7-DHC molecule (ring-opening and subsequent *cis-trans* isomerization) will cause its helical twisting power to increase and, as a result, the cholesteric pitch length to decrease.

2. MATERIALS AND METHODS

To comply with the requirements, the LC matrix should be transparent in the UV range, thermally stable over the temperature interval at least 10-40 ^{0}C, be a good solvent for 7-DHC and be stable relative to visible light. Two nematic liquid crystals LC-805 (1:1 mixture of 4-n-butyl- and 4-n-hexyl-trans-cyclohexancarbon acids) (CHCA, NIOPIK, Russia) and ZLI-1695 (the mixture of cyclohexylcyclohexanes) (E.Merck) have been selected as host matrices, and 7-DHC (Sigma, Germany) was dissolved in the LC material in concentration 5 ÷10 wt.%.

The wedge-like cell was prepared using two quartz plates (15 mm x 20 mm) as substrates. The cells thickness with Mylar spacer was measured by interferometer (L = 63 μm). The quartz substrates were spin coated with polydimethylsiloxane annealed on the hot plate at 200-250 ^{0}C over 4 hours and mechanically rubbed that ensured planar alignment of the LCs with chiral dopant. To avoid oxidation of 7-DHC the cells were carefully stuck along the perimeter. With these cells it was possible to follow the photoreaction course of provitamin D photoisomerization by recording the UV absorption spectra and, in parallel, by visual observations of the cells using polarized microscope.

The samples were irradiated with fluorescent lamp (EL-30) that delivered integral intensity of 0.3 - 0.6 mW/cm^2 within the spectral range of 250-350 nm depending on the distance 8-12 cm (Fig.1). To study the effect of solar irradiation we simultaneously exposed the suprasil cuvette (Hellma) with solution of 7-DHC in ethanol (d = 0.5cm, C = 2μg/ml) and the LC cell with 7-DHC dopant. Both samples were positioned at black paper normally to the solar beams.

UV absorption spectra of the samples were recorded by KSVU-23 spectrometer (LOMO, St.-Petersburg) before UV irradiation and after certain exposures within the spectral range of 230 -330 nm. In parallel, the UV effects on the induced cholesteric phase were investigated using polarizing microscope.

3. RESULTS AND DISCUSSION

3.1. UV lamp irradiation

Transformation of the nematic LCs into cholesteric phase due to chiral dopant was evidenced by observation of the Cano-Grandjean stripes in the wedge-shaped cell using polarizing microscope. Well known dependence of the number of Cano disclinations (N_C) on the dopant concentration (C) has been observed. N_C =10 was found at C =10 wt.% that corresponded to maximum solubility of 7-DHC. The cholesteric pitch length (P) was calculated from the number of the disclination lines ($P = 2L/N_C$)[9]. It depended on the dopant concentration and fell within the range of 9 – 24 μm. By this is meant that the selective reflection band lies outside the visible spectrum.

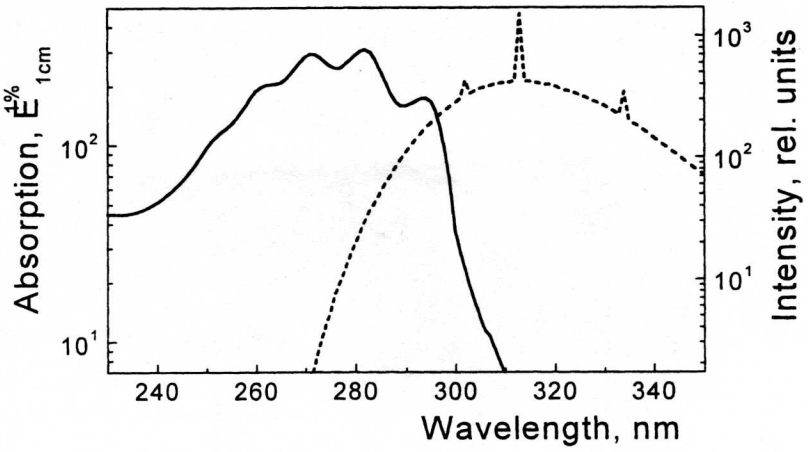

Fig.1. The EL-30 lamp radiation spectrum (dash line) and the absorption spectrum of provitamin D_3 (solid line).

Hence the twisting power of 7-DHC is inadequate to provide the selective reflection in visible spectral region that according to our assumption could enable the UVR monitoring by mere color change in the LC cell depending on the UV dose. However, well-defined dependence of the Cano stripes number on the UV exposure has been revealed using polarizing microscope (Fig.2).

The decrease in the N_C with increasing irradiation time indicates that the chiral dopant undergoes transformation into a photoisomer possessing considerably lesser helical twisting power (HTP). Obviously, HTP of 7-DHC molecule with its rigid steroid skeleton should be significantly decreased by hexadiene ring-opening into conformationally flexible molecule of previtamin D. This gives rise to the increase in the cholesteric pitch length and, as a result, is accompanied by the N_C decrease. By this means our findings hold promise for dosimetry of biologically active UV-B solar/artificial radiation (280 – 315nm) by visual observation of the Cano stripes number as a function of the accumulated UV dose. This distinctly simplifies previously suggested method of UV dosimetry based on the spectral monitoring of previtamin D photosynthesis in ethanol solution (*in vitro* model)[6,7].

3.1. Solar irradiation

It was important to convince that the decrease in the Cano stripes number in the LC cell closely corresponds to accumulation of previtamin D in the solution. For this purpose the LC cell (ZLI-1695 with 7-DHC dopant) and the cuvette with ethanol solution of 7-DHC were simultaneously exposed to solar light around noon (clear day). Before the exposure the UV absorption spectra of both samples had been recorded by the spectrophotometer, and the initial number of the Cano stripes in the LC cell had been fixed (N_C = 10 at t = 0). In the course of solar irradiation the number of the Cano stripes in the LC cell was checked time by time by placing the cell between crossed polarizers. At the moment when one Cano stripe was disappeared the absorption spectra of both samples had been recorded again. The same procedure was repeated to the total exposure time of 200 min. During the night the samples were stored at the refrigerator, and on the next day were exposed as long as the Cano stripes number reduced to N_C = 5 (t = 120 min).

To derive the concentrations of accumulated previtamin D the absorption spectra of the solution were processed with computer according to the original program in which known individual spectra of the photoisomers in solution were used as the reference ones[7]. However, the spectral analysis of the LC samples was impeded because of small shift and distortion of the 7-DHC spectrum in the LC matrix. Nevertheless, earlier HPLC analyses of 7-DHC photoisomerization in LC and in polymer film[10] showed that a reaction medium did not affect the photoreaction course and the same photoproducts were formed as in solution.

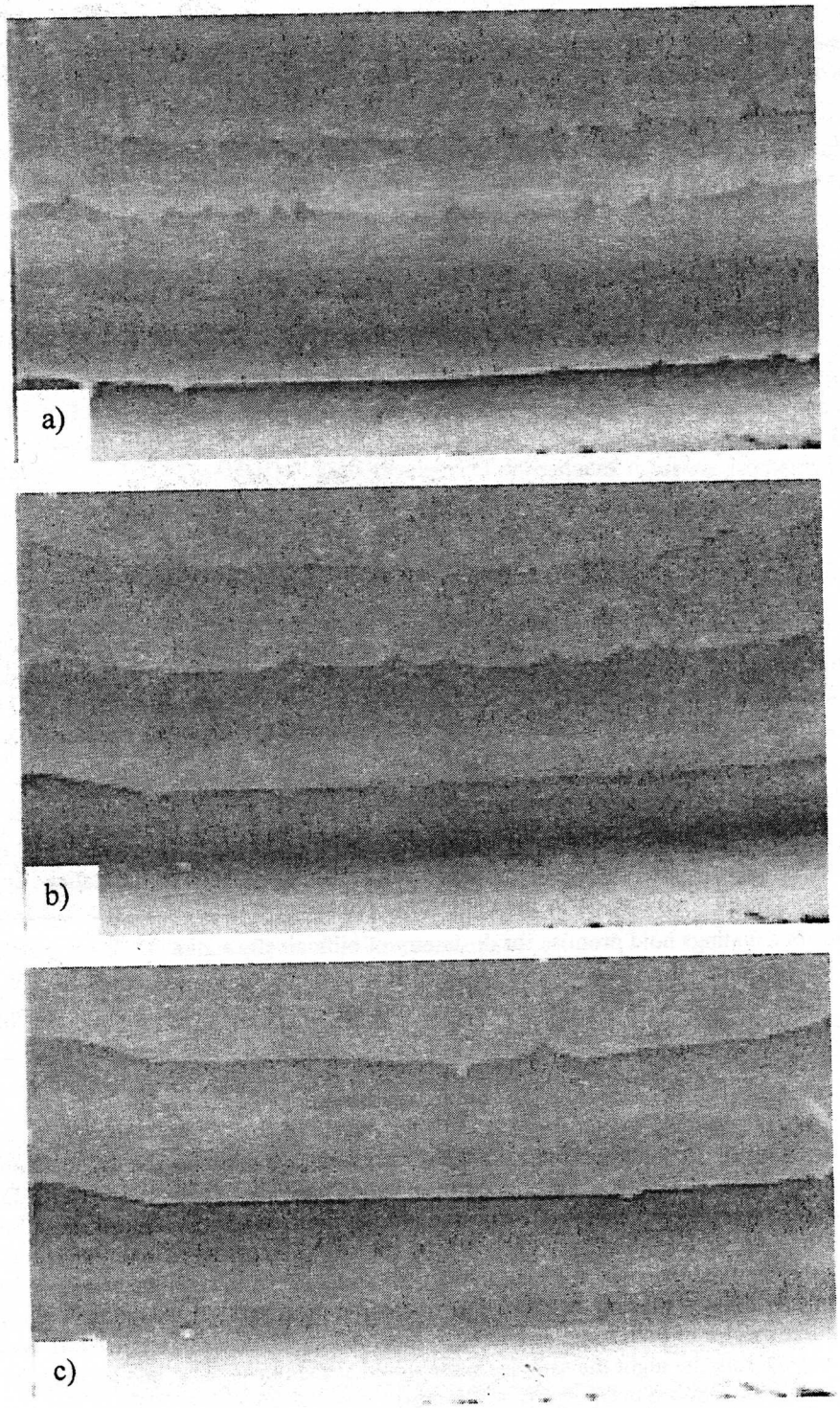

Fig.2. Decline of the Cano stripes number under the UV lamp irradiation:
a) $N_C = 5$ at $t = 0$; b) $N_C = 4$ at $t_{irr} = 25$min; c) $N_C = 3$ at $t_{irr} = 60$min.

Accumulation of previtamin D in the ethanol solution during the total exposure to solar light is shown in Fig.3a), and the associated changes of the Cano stripes number in the simultaneously exposed LC cell are presented in Fig.3b). From

the data obtained it may be deduced that dependence of the Cano stripes number on the accumulated UV dose proceeds in complete agreement with photosynthesis of previtamin D in solution.

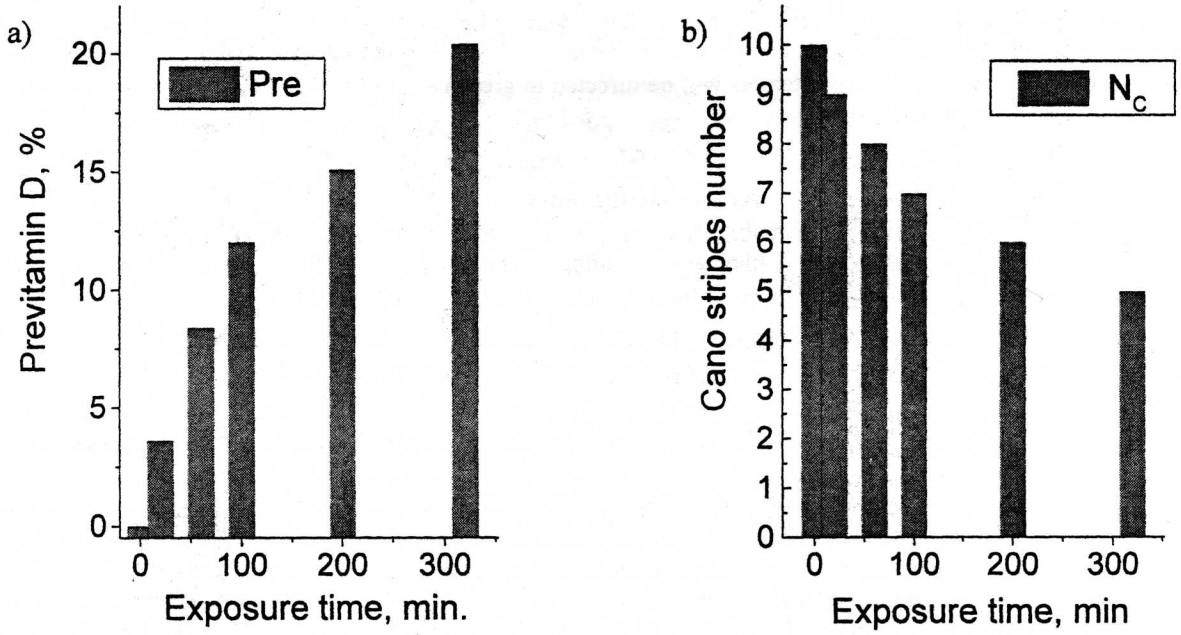

Fig.3. Accumulation of previtamin D_3 in the ethanol solution (a) and changes of the Cano stripes number in the LC cell (b) during simultaneous exposure to solar light
(August 21, 2000, local time 12.00-15.20 and August 22, 2000, local time 12.40-14.40)

This conclusion is more convincingly demonstrated in Fig. 4 where one can see wholly satisfactory linear fit between the N_C and previtamin D_3 concentration.

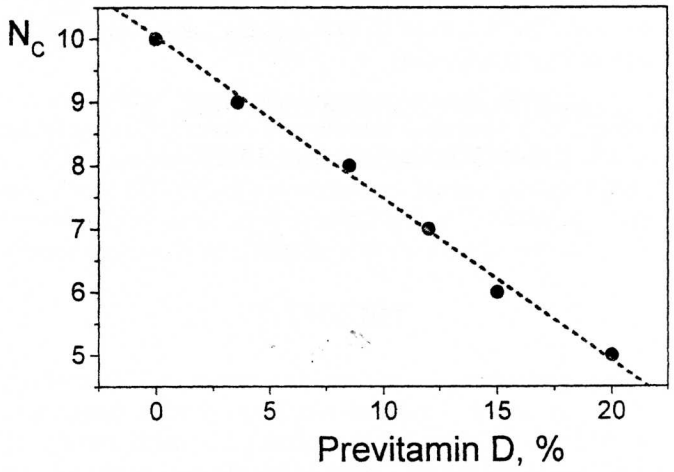

Fig.4. Correlation between the Cano stripes number in the LC cell and previtamin D_3 accumulation in solution.

4. CONCLUSIONS

For the first time it has been experimentally demonstrated that the nematic liquid crystal doped with chiral molecules of 7-DHC can be used as personal UV-B biodosimeter. Clearly defined dependence of the Cano stripes number in a wedge-like cell on the exposure time is well representative of the accumulated dose of biologically active "antirachitic" solar/artificial UV radiation. The doped LC film can ensure easy monitoring of vitamin D synthetic capacity of sunlight by visual observation using crossed polarizers. Further attempts will be directed to greater simplification of the procedure by shifting the selective reflection band into visible spectral region.

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