In: XVIth IUPAC Symposium on Photochemistry (Helsinki, Finland, July 21-26, 1996), Book of Abstracts.-P. 305-306.

ON THE OPTICAL INVESTIGATIONS OF SOME COMPONENTS OF PHOTOCHEMICAL MODEL SYSTEM OF HUMAN PLASMA

A.M. Aukhadeev, A.V. Barmasov and V.E. Kholmogorov

Institute of Physics, St.Petersburg State University, St.Petersburg, 198904 Russia

Process of interaction of near ultraviolet (UV) light with human blood, resulting in photobiological and photochemical changes of blood components, is still not well investigated. Erythrocyte suspensions are partly investigated from this point of view, but there are no reliable demonstrations of the role of light-induced modifications of human plasma in therapeutic effect of photomodified blood. We used the method of creating of photochemical and spectral model of human plasma to investigate photochemical processes in plasma under the influence of UV light.

As the first step of such investigation we have studied photoinduced spectral changes of such human plasma components as tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe) and albumin.

In this work among others chemicals from Chemapol and Reanal were used. For the irradiation of samples high-pressure mercury lamps DRSh-250 and DRT-240 with water infrared filters were used. Absorption spectra were recorded with Specord Uv Vis, fluorescence and excitation spectra - with Hitachi-850.

As a result of UV-irradiation the intensities of short-wave (220 nm) and long-wave (280 nm) absorption bands of Trp aqueous solutions (2.2 mg/100 ml and 0.55 mg/100 ml) decreased (structure of long-wave band disappeared) and the total absorption increased. UV-irradiation of Trp water solution (4.2 mg/100 ml) also resulted in the decreasing of the intensity of 360 nm fluorescence band (excitation at 230 nm) and its shift to 357 nm. In the excitation spectrum intensity of 275-280 nm band also decreased as the result of UV-irradiation.

UV-induced changes of absorption of Tyr water solutions (5.0 mg/100 ml, 2.8 mg/100 ml and 2.2 mg/100 ml) resided in decreasing of the intensities of short-wave (225 nm) and long-wave (272 nm) absorption bands (275 nm band held its structure) and in the total increasing of absorption. Photoirradiation of Tyr aqueous solution (2.4 mg/100 ml) also resulted in the decreasing of the intensity of 301 nm and other fluorescence bands (excitation at 230 nm) and the shift of 301 nm band to 303 nm. In the excitation spectrum intensity of 272 nm band also decreased and moved to 275 nm as the result of irradiation by UV-light.

As a result of photoirradiation of Phe aqueous solutions (8.6 mg/100 ml, 3.22 mg/100 ml and 2.15 mg/100 ml) new absorption band (309 nm) appeared and its intensity was comparable with the intensity of 208 nm band.

We also studied the UV-induced changes in the absorption spectra of albumin water solutions (100 mg/100 ml and other concentrations). The increasing of absorption was observed in the whole registration region (200 -800 nm). The most intensive increasing of absorption was observed in the regions of 240-280 nm and 300-350 nm. However the shape of absorption spectrum remained unchanged. It must be emphasised that the continuation of irradiation leaded to spasmodic increasing of absorption intensity. Photoirradiation of albumin aqueous solution (25 mg/100 ml) also resulted in the decreasing of the intensity of 320 nm (excitation at 230 nm) and its shift to 310 nm. In the excitation spectrum intensity of 270 nm band also decreased without shift as the result of irradiation by UV-light.

Then we have studied UV-induced changes in the absorption and fluorescence spectra of human blood plasma.

We used human blood from different donors (including stabilised with sodium nitrate). Blood plasma was obtained by centrifuging the stabilised and fresh non-stabilised (clotted) blood at 1000 g for up to 30 min at room temperature.

With the aim to obtain plausible optical spectra the procedure of "thin layer" has been taken [2] for the measurements of absorption and fluorescence of human blood plasma.

As a result of UV-irradiation the smooth fall of intensity of absorption of human blood plasma was observed in the region of 240-350 nm. As this took place, a shape of spectrum was unchanged in the whole region except 305-350 nm, where low-intensive band was increasing.

From the analysis of photoinduced changes in the spectra of aromatic amino acids it is possible to suppose that the result of UV-irradiation is the process of photodegradation with the formation of final stable molecular products.

Changes in the absorption spectrum of albumin showed that in the result of UV-irradiation albumin was subject of some changes including conformational changes in the albumin molecule. These changes were induced by disulphide bonds splitting with Trp and Tyr destruction. Thus albumin molecule obtained less polar environment. Fluorescence and excitation spectra and their UV-induced changes showed that only one aromatic amino acid - Trp, was in fluorescent state. Maxima of Trp fluorescence in albumin were shifted to short-wave region in comparison with fluorescence of Trp in aqueous solution, that could be explained by the influence of microenvironment on its spectral properties.

Analysis and comparison of absorption, fluorescence and their behaviour under the influence of UV−irradiation of albumin and human blood plasma showed that albumin was the main "spectral" component of plasma.

With the purpose of investigation of influence of UV-irradiation of blood on the properties of plasma obtained from that irradiated blood a special series of experiments was conducted. Blood from one donor was divided into 2 equal parts and one of those parts was irradiated by UV-light. After it both parts of the same blood (irradiated and not irradiated) were centrifuged simultaneously in the same centrifuge (OPn-3UKhL4.2) and plasma samples were obtained. This way obtained samples of plasma were used for various comparative experiments and optical spectra registration.

Absorption spectra of plasma obtained from irradiated and not irradiated blood differed mainly in intensity (absorption of plasma obtained from the preliminary irradiated blood was higher). Band of low intensity with the maximum at 238 nm determined the shape of the differential absorption spectrum of differently obtained plasma samples. The luminescence spectrum of not irradiated plasma sample (excitation at 230 nm) had a band with maximum at 335 nm. This band in the similar spectrum of previously irradiated sample had lower intensity.

[1] V.Ye. Kholmogorov, *Biophysics*, **39** (1994), 923-928

[2] E.V. Aksyonova, *Graduation work, St.Petersburg State University*, (1995) (Russ)