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ELECTROMAGNETIC APPLICATIONS IN BIOLOGY AND MEDICINE

Application of Time-Domain THz Spectroscopy for Studying Blood Plasma of Rats with Experimental Diabetes

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Abstract—The absorption spectra of rat blood plasma have been studied by time-domain THz spectroscopy in the frequency range from 0.1 to 2.0 THz. The absorption coefficient of blood plasma is found to be significantly reduced, depending on the diabetes severity.

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1. INTRODUCTION

Many human diseases are caused by complex violation of metabolism, and the diagnostics based on a single marker is often spurious [1]. For example, development of diabetes mellitus leads to violation of carbohydrate, protein, and lipid exchange and is accompanied by a significant increase in the content of glucose, corticosteroid hormones, and some other metabolites in blood [2]. This disease is characterized by the development of severe complications, which lead to rapid disability and life span reduction [3]. In this context, the development of new rapid diagnostic methods for this disease and its complications is an urgent problem.

Time-domain THz spectroscopy has not yet found wide application in this field. A distinctive feature of this method is the possibility of measuring directly the refractive index, absorption coefficient, and permittivity spectrum of a sample under study. This circumstance makes it possible to obtain a detailed spectral characteristic of a sample during one measurement, due to which rapid diagnostics can be developed. There are only few studies on THz spectroscopy of blood components, in which the blood of healthy humans and animals was investigated [4, 5]. In this paper, we report the results of studying the absorption spectra of the blood plasma of rats with experimental diabetes in the frequency range from 0.2 to 2.0 THz.

2. EXPERIMENTAL

The study was performed on the blood plasma of male Wistar rats. The animals were subjected to alloxan diabetes (which leads to the development of stable hyperglycemia), using the technique described in detail in [6, 7]. Blood was collected in test tubes containing heparin. The test tubes were centrifugated for 15 min with a rotational speed of 4000 rpm at 4°C. Plasma was separated and stored at −20°C up to the analysis day. The concentrations of glucose, cholesterol, triglycerides, protein, and corticosterone in the samples were determined using diagnostic sets [6]. The experiments were performed with double distilled water and a 40% glucose solution.

The transmission of plasma samples was measured on a time-domain THz spectrometer; the principle of operation and the optical scheme of this instrument were described in detail in [8, 9]. A femtosecond Ti:sapphire laser with a central wavelength of 790 nm, a pulse-repetition frequency of 80 MHz, and a pulse width of 120 fs was used as a pump source for a THz oscillator. THz radiation was generated on a tilted surface of gallium arsenide (GaAs) semicon-

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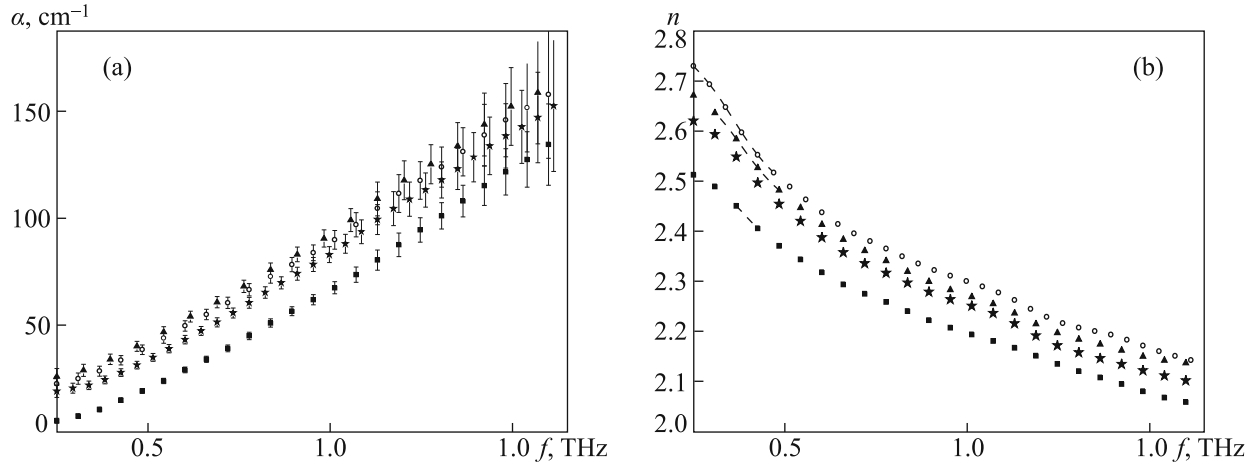

Spectra of (a) absorption coefficients and (b) refractive indices of (▲) water, (○) blood plasma of control rats, (⋆) blood plasma of rats with diabetes, and (■) glucose solution.

ductor and recorded using the electro-optical effect in a nonlinear 1-mm-thick zinc telluride (ZnTe) crystal. A THz beam was produced by a system of parabolic mirrors; they formed a focused-radiation region, into which a sample under study was placed. The samples did not degrade during measurements because the THz power incident on them was obviously below the limiting allowable level [10]. The spectral range of reliable measurements was from 0.2 to 2.5 THz, within which the transmitted beam was attenuated only slightly.

We recorded the temporal shape of a pulse transmitted through blood plasma samples (200 μm thick), placed in a cell with polystyrene windows. These samples provided a transmittance of 0.2 to 0.5 and a tenfold excess above the signal-to-noise ratio. A Fourier transform of a measured temporal shape of THz pulses made it possible to obtain a complex radiation spectrum $E(f)$, i.e., the amplitude $|E(f)|$ and the phase $\arg(E(f))$ of the spectrum. Here, E and f are, respectively, the electromagnetic-wave-field strength and the wave frequency. The information about the refractive index and absorption of the medium through which a THz pulse passed was derived from the spectrum of a pulse transmitted through either an empty cell ($E_0(f)$) or a cell filled with distilled water ($E_{\text{water}}(f)$). To recalculate the transmission spectrum into the absorption and refraction spectra, we used the Fresnel formulas and the methods described in [8]. In the simplified form the absorption coefficient spectrum was calculated as $\alpha(f) = \ln[|E(f)|/|E_0(f)|]/d$, where d is the sample thickness.

3. RESULTS AND DISCUSSION

The water content in blood plasma exceeds 90%. Water is known to have high THz absorption [8]. We found that the shape and amplitude of the absorption spectra of the blood plasma of healthy animals differs little from the absorption spectrum of water (the figure (a)). This fact was previously reported by some researchers who studied human [4] and rat [5] blood plasmas. However, the absorption spectrum of the blood plasma of rats with experimental diabetes, which is characterized by a high glucose content (30 mmol l^{-1}), as well as the spectrum of a glucose solution, has a smaller (as compared with water) amplitude. Note also that the amplitude of the glucose spectrum is much smaller than that for the blood plasma of rats with diabetes. We used a glucose solution with a concentration of 55.5 mmol l^{-1}, which is much higher than the glucose content in the blood plasma of rats with diabetes. It was shown in [11] that the THz absorption of solution decreases with an increase in the glucose concentration. This is caused by the fact that some of water molecules, which strongly absorb THz radiation, are replaced by components (in particular, glucose) less absorbing in this frequency range. The refractive index of glucose solution, as well as that of rat blood plasma with a high glucose content, is smaller than the refractive index of water (the figure (b)).

To make the differences between the absorption spectra of blood plasma and the absorption spectrum of water more illustrative, we used differential spectra, in which the measured values are normalized to the absorption of distilled water. The differential absorption coefficient is determined as

$$\alpha_{\text{diff}} = \frac{\ln(|E_{\text{sample}}|/|E_{\text{water}}|)}{d}, \qquad (1)$$

where E_{sample} and E_{water} are the spectra of radiation transmitted through a cell of thickness d, filled with blood plasma and water, respectively. The differential refractive index of blood plasma is determined as

Characteristics of animals in hormonal, metabolic, and spectral parameters

Group	α_{diff} at 1.0 THz, cm^{-1}	n_{diff} at 1.0 THz	Glucose, mmol l^{-1}	Weight index of kidney mg/100 g	Corticosterone in blood plasma, ng ml^{-1}
Control ($n=4$)	-2.62 ± 0.02	1.005 ± 0.005	6.6 ± 0.4	0.30 ± 0.01	133.2 ± 26.5
Group 1 ($n=3$)	-2.48 ± 0.42	1.023 ± 0.012	20.4 ± 2.7^{a}	0.460 ± 0.005^{a}	134.2 ± 44.5
Group 2 ($n=5$)	$-5.11 \pm 1.55^{a,b}$	$0.989 \pm 0.009^{a,b}$	24.4 ± 1.9^{a}	$0.570 \pm 0.008^{a,b}$	196.4 ± 17.0^{a}

Notes: n is the number of animals; (a) $p < 0.05$ statistically significant differences from control rats; (b) $p < 0.05$ statistically significant differences from animals of group 1.

$$n_{\text{diff}} = \frac{\arg(E_{\text{sample}}/E_{\text{water}})2\pi c}{df}, \qquad (2)$$

where c is the speed of light and f is the frequency.

An analysis of the differential absorption and refraction spectra of the blood plasma of experimental animals showed that these characteristics can be divided into two groups with respect to the changes occurring in them. In group 1, the α_{diff} and n_{diff} values barely differ from the corresponding parameters for control healthy rats but reliably exceed those for the animals from group 2 (the table). The animals from group 2 differed by a reliably high level of corticosteroid hormones in blood and adrenal glands, a large weight index of kidney, and a higher level of glucose in blood in comparison with the control animals and animals from group 1 (see the table). Concerning the contents of protein, triglycerides, and cholesterol in blood plasma, no reliable distinctions between the groups were revealed.

The found differences in the hormonal metabolic indices are indicative of severity of experimental diabetes for animals of group 2 [7, 12]. Groups of animals 1 and 2 do not exhibit significant differences in the contents of glucose and protein in blood plasma (i.e., the components that may contribute to the absorption of the blood plasma of experimental rats in the frequency range under study [8, 13, 14]). The content of corticosteroid hormones, which can also contribute to absorption [9, 15], increased on average by half. However, α_{diff} in group 2 differs by a factor of more than two from the corresponding value in group 1. This fact indicates that some components secreted into the blood plasma under experimental diabetes complications may contribute to the total absorption. An example of such complications is the change in kidney functioning, which leads to a significant increase in the weight index of kidney. In particular, the blood of patients with diabetes mellitus and diabetic nephropatia is known to have much higher contents of glycated hemoglobin, peptides, adhesion molecules, and chemokines [16]. Additional diagnostic procedures must be applied to determine each of these components. It was demonstrated in this study that a decrease in the amplitudes of absorption and refraction spectra of the blood plasma of rats with diabetes complications can be considered as an integral estimate of the existence of such changes.

4. CONCLUSIONS

We showed that diagnostics based on time-domain THz spectroscopy makes it possible to make an integral estimation of the content of metabolites in blood plasma and distinguish blood plasmas of healthy rats and rats with experimental diabetes of different degrees of severity.

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