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GLYCATION OF BOVINE SERUM ALBUMIN INVESTIGATED BY TERAHERTZ SPECTROSCOPY

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***Key words**: glycation, bovine serum albumin, terahertz spectroscopy*

INTRODUCTION

Terahertz time-domain spectroscopy (THz-TDS), provides a new method for study of structural changes and conformational flexibility of molecules by using collective vibrational modes in the terahertz frequency range of 0.1-3 THz. The protein motion is represented through the position of all normal modes of vibration that link the function of a protein to its structure (Rui L. *et all.* 2010).

Many studies showed that THz spectroscopy has a high sensitivity to protein flexibility and structure , hydration states (K. Shiraga *et all*. 2016), different species of proteins, evolution of the conformational state (L. Wei *et all.* 2019, Markelz, A. *et all.* 2002). In this context terahertz spectroscopy can be used to study details of reactions at the molecular level of the glycation process (Cherkasova *et all.,* 2018, Gusev *et all*. 2018).

Bovine serum albumin (BSA) is the most abundant protein in cows' plasma (0.6 mM), and therefore the main component of colloid osmotic pressure. BSA is a multifunctional protein with an extraordinary ligand binding capacity. Its main functions involve the binding and transport of various metal ions, metabolites, nutrients and drugs (Wolf, F.A. *and* Brett G.M 2008).

It is a large (~66 kDa) protein that is negatively charged at the physiological pH. BSA is heart-shaped and comprise three helical domains (I, II and III) each comprising two subdomains (A and B) (Kamila J. *et all.* 2012).

MATERIAL AND METHODS

Sample preparation

For this study we prepared three types of solutions. The first one was made using BSA (66 mg), fructose (72 mg), NaN_3 0,015 % (5 µl), distil water (757 µl) and buffer 100 µl. The composition of the second solution was: BSA (66 mg), glucose (79,2 mg), NaN_3 0,015 % (5 µl), pure water (750 µl) and buffer 100 µl.

The third solution contained: BSA 66 mg, NaN_3 0,015 %(5 µl), pure water, 829 µl, buffer (100 µl). The utilized buffer (pH 5) was made of: Na_2HPO_4 (190.82 mg), citric acid (101.85 mg), NaCl (742.13 mg) and the second (pH 7.4) was made of: Na_2HPO_4 (323.43 mg), citric acid (19.22 mg), NaCl (582.88 mg).

After that, we pour pure water into these solutions to the sign (10 ml). The buffer we obtained had a 10x concentration, this fact resulted in ten-fold dillution (1 part solution and 9 parts pure water) before using it. 1x dilluted sollutions had a buffer concentration of 10-20mM and a final ionic strenght of 150mM. After preparing them we placed the solution in the thermostat to incubate at a temperature of 37 ºC.

The reagents used in preparing the samples were purchased as follows:

(a) Sigma-Aldrich (USA): lyophilized BSA, sodium azide
(b) Carl Roth GmbH and Co. KG (Germany): fructose (>99.5 %) + glucose (>99.5 %).

Terahertz spectroscopy

Every four days, there were extracted 100 µl from each and they were analyzed using TPS Spectra 300 Spectometer (TeraView Limited, Cambridge)-ATR mode (attenuated total reflection) at an angle of incidence of 35°(M. Mernea *et all.* 2015).

THz spectra of non-glycated BSA solutions and BSA glycated with fructose and glucose were registered during 28 days in order to notice the changes occured due to the glycation process. The sample (25 µl of the solution) was placed directly on the ATR crystal. For each spectrum, 1800 scans were performed (30 scans/second) with a 1.2 cm^{-1} resolution, under constant N_2 purge.

The spectrum was processed by applying the Blackman-Harris apodization function. The apodization and absorbance calculation was performed using the TPS Spectra 3000 software.

RESULTS AND DISSCUTIONS

Absorbance was calculated at a value of 0.6 – 1.5 THz.

Figure 1 shows the absorbance at pH 5, Day 1 of experiment of BSA and fructose, BSA and glucose and BSA unglycated.

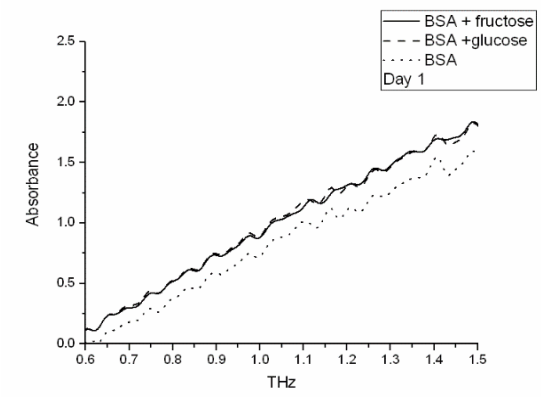

Figure 1. Day 1, pH 5 Values

BSA solution with fructose reaches a THz absorbance value of 1.80032, with glucose 1.7888 and BSA simple 1.55169.

Figure 2 shows the absorbance at pH 5, Day 28 of experiment and we have the values: BSA with fructose 1.7444, with glucose 1.09662 and BSA unglycated 0.51122.

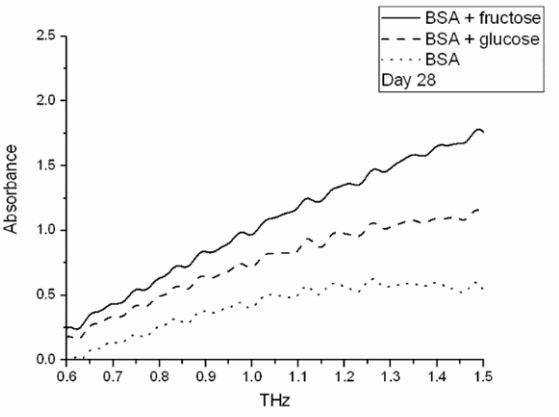

Figure 2. Day 28, pH 5 Values

The absorbance differences between the two moments of the experiment, Day 1 and Day 28, at pH 5 are: BSA with fructose 0.05592, BSA with glucoze 0.69218 and BSA simple 1.04047.

Figure 3 shows the absorbance at pH 7.4, Day 1 of experiment of BSA and fructose, BSA and glucose and BSA unglycated.

BSA solution with fructose reaches a THz absorbance value of 1.86078, with glucose 1.68684and BSA simple 1.58862.

Figure 4 shows the absorbance at pH 7.4, Day 28 of experiment and we have the values: BSA with fructose 1.42162, with glucose 0.85934 and BSA unglycated 0.30155. The absorbance differences between the two moments of the experiment, Day 1 and Day 28, at pH 7.5 are: BSA with fructose 0.43916, BSA with glucoze 0.8275 and BSA simple 1.28707.

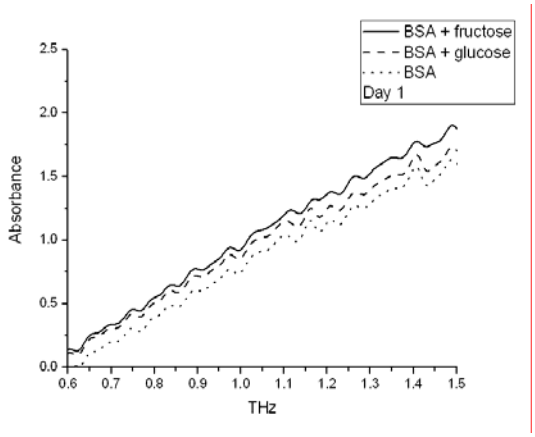

Figure 3. Day 1, pH 7 Values

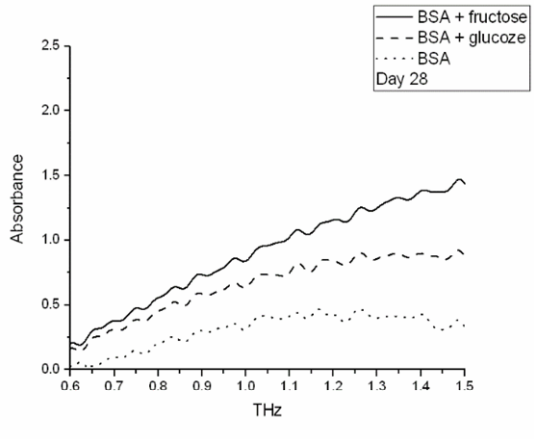

Figure 4. Day 28, pH 7.4 Values

Following the study of protein solutions (BSA) glycated with fructose and glucose, we observed several aspects. The novelty of this study was also given by the use of terahertz spectroscopy to study the time evolution of the glycation process and to observe how the pH value can influence this process.

As a result of the analyzed results, we found that terahertz spectroscopy is sensitive both in terms of the environmental pH issues and in the changes induced by the glycation process. More significant results were obtained in the case of glucose glycation compared to the samples that were glycated with fructose, suggesting that glycation process involving glucose presents a continuous progression over the times.

We show that the THz absorption decreases with the incubation time of HSA. The most spectacular results were obtained in the case of HSA samples glycated using glucose,

The THz absorption of HSA samples incubated with fructose for 28 days show that

glycation by fructose is a faster process. The differences between glucose glycation and fructose are closely related to the pH value of the solution.

The order of absorbances of the three protein solutions is maintained throughout the experiment as at the time of the first test analysis performed (day 1). On the last day of the experiment the absorbance values decrease

CONCLUSIONS

Also we could see that the differences between glycation made with glucose and fructose is closely related to the pH value of the solution in which the glycation process is carried out. In the range 0,6 to 1.5 terahertz, it has been observed that glycosylated protein with glucose, fructose or non-glycosylated interacts with THz radiation differently.

At the molecular level during the transport process carried out by BSA, seems to develop areas with different pH, which is why the glycation process was carried out under different conditions.

All these results show that THz spectroscopy is a useful tool for monitoring the progression of glycation in time.

Future experimental work will focus to investigate the changes induced by a wide range of pH and concentration values on the THz spectra of Bovine Serum Albumine (BSA) and Human Serum Albumine (HSA) as well as other protein entities, using Thz spectroscopy supplemented with other interdisciplinary tools: Molecular Modeling, Molecular Dynamics Simulations, as well as with other spectroscopy techniques: Infrared (FTIR), Small-angle X-ray scattering (SAXS), fluorescence spectroscopy.

ABSTRACT

The process of glycation induces conformational changes that alter serum albumin function. This study aims to investigate the evolution of the glycation process by analyzing the Terahertz (THz) spectra of Bovine Serum Albumin unglycated and glicated with glucose and fructose.

After analyzing the results we obtained and comparing the spectra of the three types of solutions we concluded that glycation of BSA by glucose produces more significant changes in the THz spectrum than the glycation by fructose.

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