

Spectrofluorimetric Characterization of Serum Pentosidine and Retinol Binding Protein in Healthy Rats and Rats with Streptozotocin-Induced Diabetes

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Abstract

Recently, the fluorescence techniques have become increasingly important in medical diagnostics. Moreover, there is a growing need to introduce cost-effective and no time-consuming techniques for the investigation of various fluorophores in humans and animals with diabetes mellitus. In the studied literature, the newly diagnosis of diabetes mellitus and, subsequently, the risk of developing diabetes complications are reported to be correlated with the production of serum fluorophores pentosidine and retinol binding protein. As far as we are aware, there has been no study on the simultaneous fluorescence evaluation of pentosidine and retinol binding protein in biological fluids obtained from animals. In the present study, the emission intensity and levels of serum pentosidine and retinol binding protein were monitored in both healthy rats and rats with streptozotocin-induced diabetes. The results showed that the height of the peak at ~ 382 nm attributed to the presence of pentosidine in the serum, and the height of the peak at ~ 465 nm attributed to retinol binding protein in the serum were significantly higher in rats with streptozotocin-induced diabetes compared to healthy control rats. Also, their contributions to the total fluorescence of serum were significantly higher in rats with streptozotocin-induced diabetes compared to healthy control rats. Thus, fluorescence spectroscopy might be a reliable and useful technique that can be successfully applied in the evaluation and monitoring of serum pentosidine and retinol binding protein in both healthy rats and rats with streptozotocin-induced diabetes.

Keywords

Pentosidine • Retinol binding protein • Diabetes mellitus • Rats • Fluorescence spectroscopy

1 Introduction

Due to the increased number of animals and humans diagnosed with diabetes mellitus worldwide [1, 2], finding new methods and protocols for diabetes' biomarkers evaluation is mandatory. Pentosidine and retinol binding protein represent two of the recent studied compounds in diabetes mellitus [3–6]. Their increased levels in different body fluids and tissues were associated with the newly diagnosis of diabetes mellitus and, subsequently, with a higher risk of developing diabetes complications [3, 5, 7, 8]. However, as far as we are aware, there are no studies that have described the simultaneous fluorescence of both serum pentosidine and serum retinol binding protein in animals with diabetes mellitus compared to healthy controls. Thus, we decided to conduct a study in order to evaluate the levels of both pentosidine and retinol binding protein using spectrofluorimetry in the serum samples obtained from healthy rats and rats with streptozotocin-induced diabetes.

Pentosidine is formed through the reaction of glucose with lysine, arginine and ribose in the serum [9]. Increased pentosidine formation was described in various diseases associated with oxidative stress, including diabetes mellitus [10]. The evaluation of pentosidine in patients with diabetes may give us a valuable marker of long-term glycemic control which could seriously impact glycated hemoglobin levels [11]. Pentosidine is a fluorescent product which has been quantified and evaluated through various techniques such as: spectrofluorimetry, enzyme-linked immunosorbent assay, high-performance liquid chromatography and mass spectrometry [12]. However, there are limited data regarding the serum measurement of pentosidine in rats with streptozotocin-induced diabetes compared to controls using

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a simple and rapid method for quantitative determination such as the fluorescence technique followed by the Gaussian deconvolution of the obtained spectra.

Retinol is one on the most used parameters for the evaluation of serum vitamin A concentration in living organisms [13]. In the systemic circulation, there are three classes of specific transport proteins for vitamin A and its metabolites, called retinol binding proteins [14, 15]. The cellular retinol binding protein is the most abundant retinol binding protein in most tissues, such as: the liver, kidney and lung [15]. In fact, in order to prevent the disappearance of this retinol binding protein from blood by filtrating in the renal glomeruli, in the systemic circulation appears another protein called transthyretin [16, 17]. It was suggested that the complex formed between retinol and retinol binding protein is more fluorescent than free retinol [16, 18, 19]. The results of several investigations suggest an association of retinol binding protein with the newly diagnosis and presence of insulin resistance, diabetes mellitus and metabolic syndrome [5, 7, 20, 21].

In this paper, we wanted to simultaneously investigate the serum levels of pentosidine and retinol binding protein using fluorescence spectroscopy followed by the deconvolution method of the obtained spectra in an animal model of diabetes mellitus disease.

Therefore, we assessed the heights of the peaks attributed to these serum constituents and their contributions to the total serum fluorescence in rats with streptozotocin-induced diabetes compared to healthy control rats. Thus, eventual changes in the condition of these fluorophores could help the monitoring of animals' physiological response to diabetes mellitus diagnosis and to specific diabetes therapies.

2 Materials and Methods

2.1 Animals

The experiment was conducted in accordance with Romanian laws regarding correct manipulation of laboratory animals and was approved by the local Ethical Committee of University of Agricultural Sciences and Veterinary Medicine in Cluj-Napoca, Romania.

A number of 10 Wistar rats with the age of 6–7 weeks old, weighing between 250 and 300 g were used in this experiment. They were housed in two stainless steel cages with free access to food and water and under standard environmental conditions: temperature 24 ± 5 °C, relative humidity $60 \pm 4\%$, light/dark cycle (12 h/12 h). The same rats were used in the first and second part of the study (after the administration of streptozotocin and, consequently, the induction of experimental diabetes mellitus).

2.2 Induction of Diabetes

Diabetes mellitus was induced with a single intraperitoneal dose of 60 mg/kg streptozotocin (Sigma, Aldrich). The experimental diabetes was induced in a number of 1–4 days after streptozotocin administration. Rats with blood glucose levels >300 mg/dl were considered as having diabetes mellitus [22].

2.3 Blood Sampling

The venous blood (0.2 ml) was collected from the orbital sinus of healthy rats and rats with streptozotocin-induced diabetes in 0.5 ml Eppendorf tubes. Further, the blood was centrifuged at 1000 g for 10 min. The levels of glycemia were measured using commercially available methods (Hitachi, Roche Diagnostics).

For the spectrofluorimetric analyses, further dilutions of serum (4 μ l) were performed (1:500) using physiological serum (NaCl 0.9%).

2.4 Spectrofluorimetric Determination of Pentosidine and Retinol Binding Protein

The spectrofluorimetric analysis was made using a FP-8200 spectrofluorometer (Jasco, Japan) (Fig. 1). The measurements were performed at room temperature using a 1 cm quartz cell. The serum spectra were recorded in a region from 360 to 600 nm using an excitation of 335 nm [23, 24] at a medium sensitivity.

The fluorescence intensity was recorded at the emission maximum of ~ 380 nm for pentosidine [24] and at ~ 465 nm [19] for the retinol binding protein, with a wavelength accuracy of ± 2 nm.



Fig. 1 Spectrofluorometer FP-8200 (Jasco, Japan)

The interpretation of obtained data was done using Origin Pro 8.1 software. Further, we performed the deconvolution of the obtained spectra using the peak analyzer option of Origin Pro 8.1 software in order to have a better quantification of the serum levels of pentosidine and retinol binding protein in rats with and without diabetes [24, 25]. Therefore, utilizing a combination of six Gaussian bands, we established the peaks position and full width at half maximum while the intensity was allowed to vary in order to match the line shape of the experimental spectra (Fig. 2).

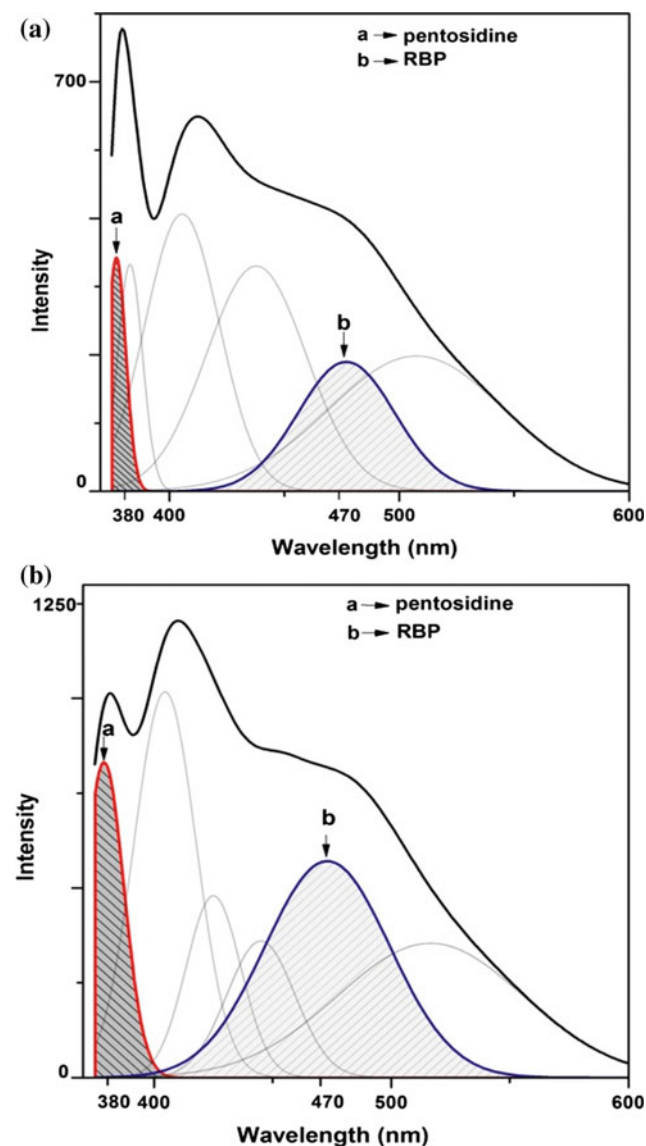


Fig. 2 The deconvolution of fluorescence spectrum of serum collected from rats before (a) and after the administration of streptozotocin (b) (excitation wavelength of 335 nm)

2.5 Statistical Analysis

Data analyses were performed using R commander version 3.0.1 for Windows. Peak heights and areas under the peak were normally distributed when evaluated using Kolmogorov–Smirnov test, and were expressed as means \pm standard deviation. Group comparisons of the mentioned variables were performed using t-test. A p value <0.05 was considered statistically significant.

3 Results

Figure 3 shows the emission intensities of pentosidine and retinol binding protein in the serum of healthy rats and rats with streptozotocin-induced diabetes.

The height of the peak from ~ 380 nm attributed to the presence of pentosidine in the serum was significantly higher in rats with streptozotocin-induced diabetes compared to healthy rats ($p < 0.001$). Also, significant differences between the rats with streptozotocin-induced diabetes and healthy controls were noticed for the height of the peak near 470 nm attributed to the presence of retinol binding protein in the serum ($p < 0.001$). Moreover, when comparing the average contribution (area under the peak) of pentosidine and retinol binding protein to the total fluorescence of serum in rats with streptozotocin induced diabetes and healthy rats, the results showed that a statistically significant difference exists between the two study groups ($p < 0.001$) (Table 1).

4 Discussions

We evaluated the heights of the fluorescence signals attributed to pentosidine and retinol binding protein and their contributions to the total fluorescence of serum in order to study these biochemical compounds in the serum of healthy rats and how they change with the induction of diabetes mellitus. Concerning the existing connection between an increased pentosidine formation and the presence of diabetes mellitus, it was declared that hyperglycemia promotes the accumulation of pentosidine [10]. In our study, the fluorescence evaluation of pentosidine in rats with streptozotocin-induced diabetes revealed significantly higher peaks and contributions to the total fluorescence of serum compared to healthy control rats.

Therefore, we can declare that the levels of pentosidine in rats with diabetes mellitus were statistically significant higher compared to healthy controls. Similarly, previous studies reported significant difference in pentosidine formation between the patients with diabetes and controls [26]. In the same study, it was mentioned that the presence of complications of diabetes such as retinopathy and chronic

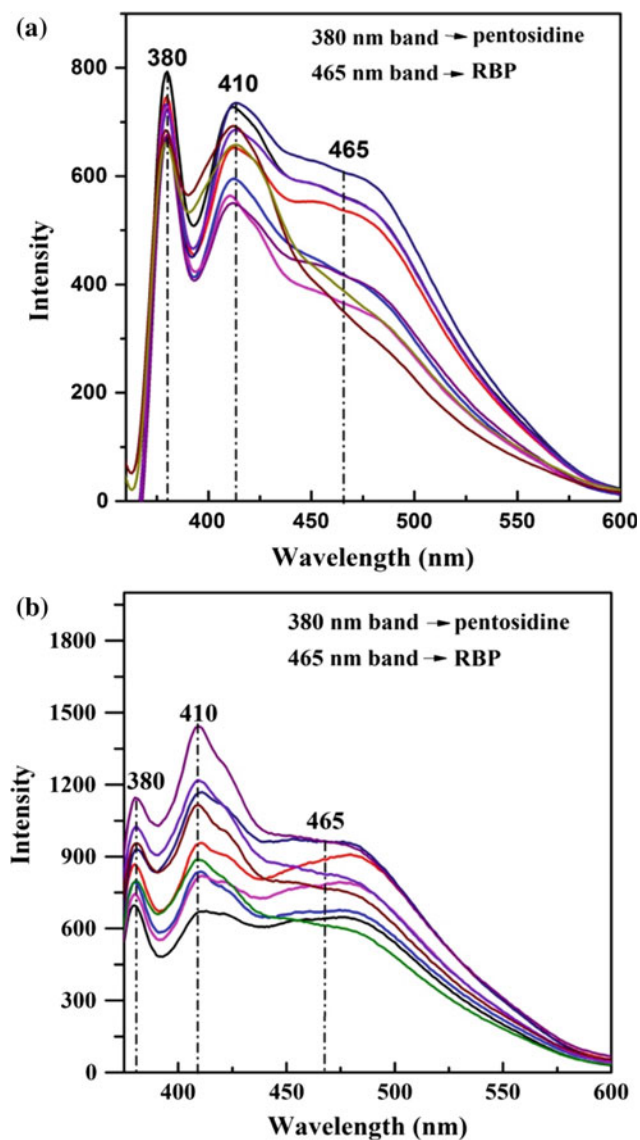


Fig. 3 The emission spectrum of serum pentosidine and retinol binding protein (RBP) in healthy controls (a) and rats with streptozotocin-induced diabetes (b)

Table 1 The characterization parameters of pentosidine and retinol binding protein that significantly ($p < 0.001$) differentiate between healthy rats and rats with streptozotocin-induced diabetes

	Characterization parameters		
	Peak center (nm)	Peak height (a.u)	Average of the area under the peak (cm^{-1})
Control rats	~380	708.6 ± 44.5	2.3 ± 0.4
	~470	465 ± 95.3	13.3 ± 0.9
Rats with streptozotocin induced diabetes	~380	877.1 ± 135.7	8.0 ± 1.2
	~470	789.4 ± 126.3	24.2 ± 2.7

nm, nanometers; a.u., arbitrary units

kidney disease led to significantly higher pentosidine levels [26]. It has been reported that an increased concentration of pentosidine measured by reverse-phase high performance liquid chromatography might be an independent predictor of all-cause and cardiovascular mortality and that it may represent a useful biomarker for monitoring clinical outcome in patients with end-stage renal disease [27]. In our article, we used a cost-efficient and no time-consuming fluorescence spectroscopic method with a high sensitivity and selectivity of detection for the investigation of pentosidine in the serum of rats with streptozotocin-induced diabetes.

We compared the contributions and the heights of the serum peak near 465 nm, attributed to retinol binding protein in healthy control rats and rats with streptozotocin-induced diabetes. Our results showed that both the heights of the peak attributed to retinol binding protein and its contribution to the total fluorescence of the serum were significantly higher in rats with streptozotocin-induced diabetes compared to controls, suggesting higher levels of this product in the presence of diabetes. Our results are somewhat in concordance with a study that reported higher serum and urine concentrations of retinol binding protein in type 2 diabetes mellitus patients compared to healthy controls [28]. Our study showing increased serum levels of retinol binding protein in rats with streptozotocin-induced diabetes highlights the similarities between these two species concerning the retinol metabolism in diabetes mellitus, and therefore, the possibility of using these animals as models in studying the pathogenesis of human diabetes mellitus. According to other studies, the levels of retinol in insulin treated diabetes mellitus patients and in streptozotocin induced diabetes mellitus rats were significantly lower than the levels of control subjects [29]. This finding was supported by numerous observations, such as: lower transthyretin levels or the presence of some problems in the synthesis of retinol binding protein due to altered renal function [30]. As can be observed, at this time, in the literature isn't established whether or not the levels of retinol binding protein are actually increased in patients with diabetes mellitus. Our study brings an improvement to the existing literature and claims an increased level of retinol binding protein in rats with streptozotocin induced diabetes mellitus when compared to healthy control rats. Also, we describe an increased serum retinol level in rats with streptozotocin induced diabetes.

5 Conclusions

In conclusion, the contributions of both pentosidine and retinol binding protein to the total serum fluorescence and also the heights of these peaks attributed to the presence of these biochemical compounds in serum are significantly

higher in rats with streptozotocin-induced diabetes mellitus compared to healthy control rats. On the basis of these data, we could claim the fact that the levels of pentosidine and retinol binding protein are significantly higher in the serum of rats with streptozotocin-induced diabetes compared to controls.

In addition, fluorescence spectroscopy represents a reliable and no time-consuming technique that can be applied successfully in the evaluation and monitoring of both serum pentosidine and retinol binding protein in animals diagnosed with diabetes mellitus.

Future studies should better investigate the correlation of pentosidine and retinol binding protein as determined by fluorescence spectroscopy with others biochemical compounds such as hyperglycemia and glycated hemoglobin and with the presence of diabetic complications in order to better understand the pathophysiology of these compounds in diabetes mellitus in both humans and animals.

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Conflict of Interest The authors declare that they have no potential conflict of interest relevant to this article.

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