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NON-ENZYMATIC GLYCOSYLATION OF GUANOSINE-5'-TRIPHOSPHATE – A SHORT REPORT

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The influence of the non-enzymatic glycosylation (glycation) of guanosine-5'-triphosphate (GTP) by glucose on its physicochemical properties was investigated. The progress of glycation was monitored by the ionic-exchange chromatography. The results of analysis of the glycated mixture enabled identifying its three components: free GTP, glycated GTP and GTP-glycated complex. These results were also confirmed by spectral analysis and thin layer chromatography. Moreover, it was suggested that the glycation of nucleic acids could change their nutritive value, *e.g.* immunogenic and allergic potential.

INTRODUCTION

Non-enzymatic glycosylation (glycation) of protein, peptides amino acids, nucleic acids and phospholipids is a spontaneous reaction occurring in weakly alkaline medium in the presence of reducing sugars. This reaction occurs both *in vivo* and *in vitro*. The products of protein modifications by glycation referred to as "advanced glycation end products" (AGEs), which are formed *in vivo* during aging, diabetes and in renal failure *via* equivalent pathways like in food items, nowadays are generally accepted to play a pivotal pathophysiological role in several diseases [Henle *et al.*, 2003]. Glycation occurs also in stored and dried food and during the thermal processing of food. Glycation of food components can decrease their nutritional value and can be the source of the immunogenic, allergic and toxic substances [Hiramoto *et al.*, 1994; Krawczuk *et al.*, 2000; Kostyra *et al.*, 2002]. The protein isolates and concentrates obtained from plants are in fact the nucleo-glyco-metallo--phenol-protein complexes. This means that nucleic acids or products of their degradation – nucleotides – may also take part in the reaction of glycation. It is likely that the products formed may influence the nutrition or be the source of the immunogenic, allergic or toxic substances.

For this reason, the aim of these investigations was to determine the influence of the non-enzymatic glycosylation of guanosine-5'-phosphates (GTP) on its chemical nature and ability to form complexes with the non-glycated one.

MATERIALS AND METHODS

Reagents. GTP was purchased from Sigma, whereas glucose and phosphoric acid – from POCH-Poland.

Glycosylation. For GTP glycosylation, there were used 0.025 g of GTP and 0.220 g of glucose in 25 mL of deionized water (pH 8.0, obtained by 0.1 mol/L NaOH). The growth of microorganisms was inhibited by sodium azide (0.04%). A GTP-glucose mixture was incubated at 37°C for 72 h. The glycation progress of GTP was followed after 1, 2, 12, 24, 48, and 72 h by ion-exchange chromatography.

TEAE ion-exchange chromatography. 20 g of TEAE Sephadex was suspended in 1 mol/L NaOH, mixed and stored at room temperature for 1 h. Next, the supernatant was decanted and the gel was suspended in 0.2 mol/L NH_4HCO_3 and stored at room temperature for 0.5 h. After this time, the gel was injected onto a column (30×2 cm) and stabilized with 20 mL of 0.05 mol/L NH_4HCO_3. The column was applied with 1 mL of solutions containing non-glycated or glycated GTP. The elution was made with 0.05 NH_4HCO_3 (4 mL/10 min). Absorbance of the eluted fractions was measured at 260 nm.

Thin layer chromatography. For the thin layer chromatography there were used the plates Kieselgel 60 F_{254} (Merck). Chromatographic analyses were carried out on 0.04 mL of GTP solutions, before and after glycation. Chromatograms were developed by a solvent mixture: isopropanol:water (70:30 v/v) at a room temperature. The chromatograms were visualized with the fluorescence method using a quartz lamp (Emita VP-60).

UV spectral analysis of the non-glycated and glycated GTP. The non-glycated and glycated GTP were dissolved in deionized water (1.0 mg/3 mL). UV spectra were measured with a spectrophotometer.

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Conductivity and pH of non-glycated and glycated GTP solutions. Electrolytic conductivity and pH of the non-glycated and glycated GTP solutions were measured with a conductometer (Hanna EC 215) and pH-meter (Radiometer PHM 95).

RESULTS AND DISCUSSION

The elution profiles of the glycated mixtures (GTP + glucose) after 1, 2, 12, 24 and 48 h incubation are presented in Figure 1. As a result of the chromatographic separation of the glycated mixture, three fractions eluted in the volume of *ca.* 5–20, 20–30 and 30–70 mL, respectively, were obtained. A comparative analysis of these separations indicates that three components are present in the glycated mixtures. From the theoretical point of view, these compounds are GTP, glycated GTP, free glucose and the complex formed between GTP and glycated GTP. The partial experimental confirmation of this assumption is the decrease of GTP fraction during glycation of the GTP-glucose mixture. The volume elution of GTP fraction decreased from 30–59 mL after 1 h of glycation to 30–60 mL one after 48 h. To prove the progress of the glycation, UV spectra (Figure 2) and thin layer chromatography (Figure 3) of the glycated mixture were made. The absorption spectrum of the fraction 3 was similar to the spectrum of GTP, known from literature [Lehniger *et al.*, 1993], which proves the presence of two absorption bands at about 250 and 275 nm. However, the spectra of the fractions 1 and 2 looked quite different.

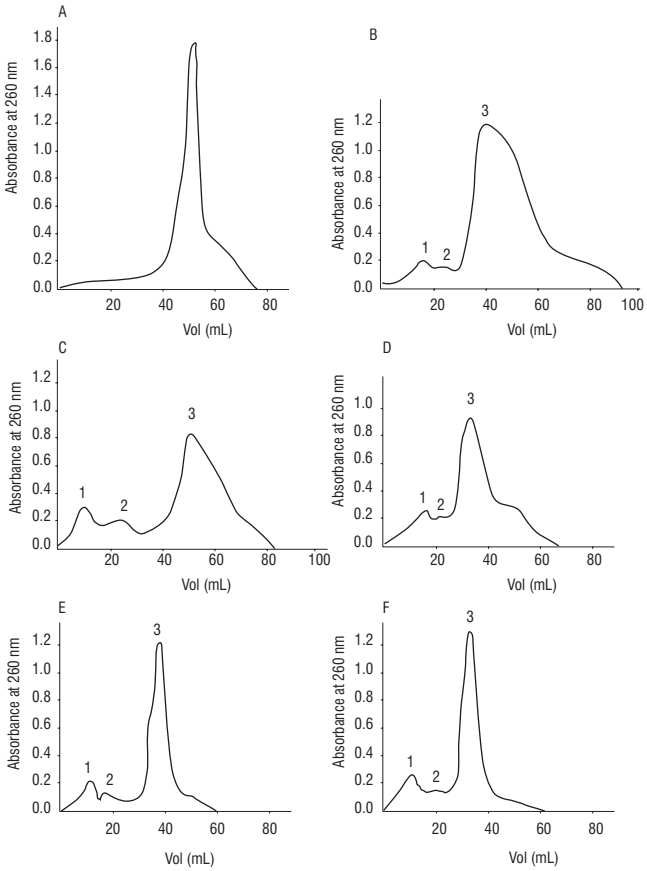

FIGURE 1. Elution profiles of the GTP standard (A) and the glycated GTP mixtures after : (B) 1 h, (C) 2 h, (D) 12 h, (E) 24 h and (F) 72 h of incubation.

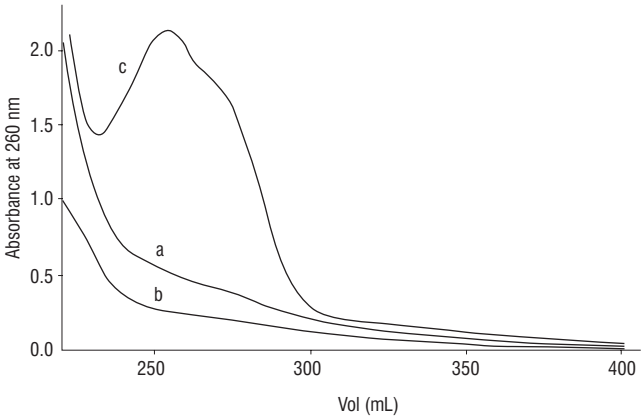

FIGURE 2. UV spectra of fractions after TEAE ion-exchange chromatography of glycated GTP mixtures after 24 h of incubation: (a) –fraction 1, (b) – fraction 2, (c) – fraction 3.

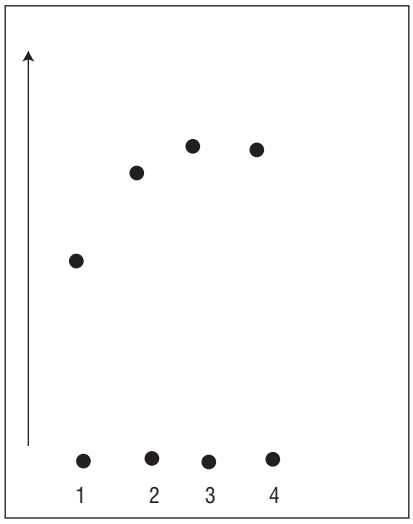

FIGURE 3. Thin layer chromatography of the fractions 1, 2 and 3 after 24 h (24 h of incubation) obtained from TEAE ion-exchange chromatography and GTP standard.

Their profiles were very similar but differed in the absorption intensity. Fraction 1 showed higher absorption intensity. Generally, it can be said that glycation caused a change in the spectrum profile and lowered the absorbance. This proves different exposition of the group of chromophore GTP to UV radiation in the changed structural arrangements of glycated GTP. In addition, the differences in the absorption spectra of the fractions 1, 2, 3 confirm the results of the thin layer chromatography. All three fractions had different R_f coefficients. Taking into consideration the hydrophilic nature of the mobile phase and molecular weights of GTP, glycated GTP and the complex formed between them, GTP should be characterized by the highest R_f coefficient. The results obtained prove this assumption. Assuming the same criteria for the fraction 1 and 2 it can be predicted that fraction 1 contains the GTP-glycated GTP complex, whereas fraction 2 only glycated GTP. In this context, there appears an interesting question about the nature of the interactions stabilizing the GTP-glycated GTP complex. This complex is stabilized mainly by hydrophobic interactions and hydrogen bonds. The purine ring of GTP is

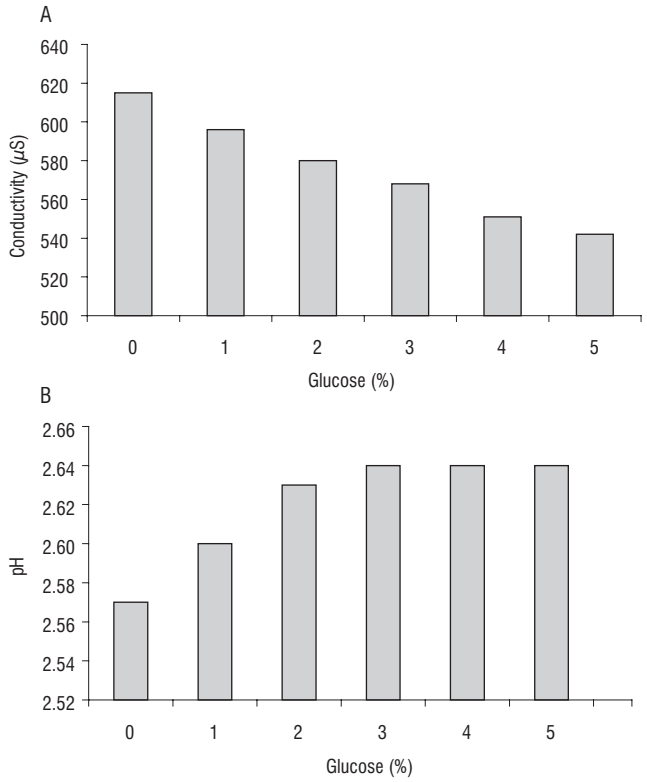

FIGURE 4. Relationships between (A) conductivity of phosphoric acid and concentration of glucose and (B) pH of phosphoric acid and concentration of glucose.

responsible for the hydrophobic interactions. However, the hydrogen bonds can be formed between hydroxyl groups of ribose, glucose and phosphoric residues. One theoretical model of such complex is presented in Figure 5. The formation of the hydrogen bonds between hydroxyl groups of sugars and phosphoric residues should evoke an increase of basicity of phosphoric acid. To prove this assumption experimentally, determinations were carried out for the influence of the concentration of glucose on the conductivity and pH of phosphoric acid. The results are presented in Figure 4. They show that the conductivity of phosphoric acid decreased together with the increase in the concentration of glucose. Likewise, pH increased together with the concentration of glucose. These facts prove that the active acidity of phosphoric acid decreases due to the formation of the hydrogen bonds between hydroxyl groups of glucose and phosphoric residues. Generalizing these results in relation to nucleic acids, it can be said that the glycation process changes their chemical nature, which can influence their enzymatic degradation. This fact is worth considering while evaluating the nutritional and immunogenic/allergic properties of food.

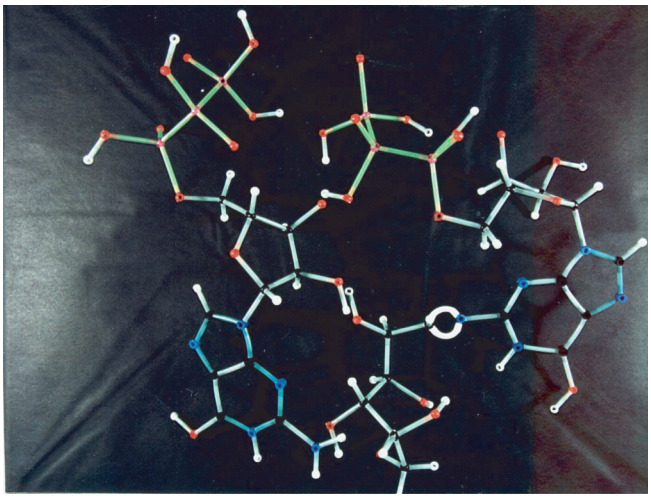

FIGURE 5. Theoretical model of GTP – glycated GTP complex.

CONCLUSIONS

1. As a result of the non-enzymatic glycosylation (glycation) of GTP by glucose, the mixture consists of free GTP, glycated GTP and GTP-glycated GTP complex.
2. The glycation of GTP changes its spectral and chromatographic properties, which proves its different conformation in comparison with the non-glycated molecule.
3. The results obtained suggest the influence of the glycation process of nucleic acids on their chemical and nutritional properties, *e.g.* immunogenic and allergic potential.

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NIEENZYMATYCZNA GLIKOZYLACJA GUANOZYNO-5'-TRIFOSFORANU – KRÓTKI KOMUNIKAT

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Badano wpływ nieenzymatycznej glikozylacji (glikacji) guanozynotrifosforanu (GTP) za pomocą glukozy na jego właściwości fizykochemiczne. Reakcję glikacji monitorowano za pomocą chromatografii jonowymiennej. W mieszaninie glikacyjnej stwierdzono obecność wolnego i zglikolizowanego guanozynotrifosforanu oraz kompleksu guanozynotrifosforanu z zglikolizowanym guanozynotrifosforanem.W oparciu o uzyskane wyniki zasugerowano, że glikacja kwasów nukleinowych może zmieniać ich właściwości żywieniowe, tj. potencjał immuno- i alergenny.