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Relevance of the capacity of phosphorylated fructose to scavenge the hydroxyl radical

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ABSTRACT

The hydroxyl radical (·OH) has detrimental biological activity due to its very high reactivity. Our experiments were designed to determine the effects of equimolar concentrations of glucose, fructose and mannitol and three phosphorylated forms of fructose (fructose-1-phosphate (F1P); fructose-6-phosphate (F6P); and fructose-1,6-bis(phosphate) (F16BP)) on ·OH radical production via the Fenton reaction. EPR spectroscopy using spin-trap DEPMPO was applied to detect radical production. We found that the percentage inhibition of ·OH radical formation decreased in the order F16BP > F1P > F6P > fructose > mannitol = glucose. As ketoses can sequester redox-active iron thus preventing the Fenton reaction, the Haber–Weiss-like system was also employed to generate ·OH, so that the effect of iron sequestration could be distinguished from direct ·OH radical scavenging. In the latter system, the rank order of ·OH scavenging activity was F16BP > F1P > F6P > fructose = mannitol = glucose. Our results clearly demonstrate that intracellular phosphorylated forms of fructose have more scavenging properties than fructose or glucose, leading us to conclude that the acute administration of fructose could overcome the body's reaction to exogenous antioxidants during appropriate therapy in certain pathophysiological conditions related to oxidative stress, such as sepsis, neurodegenerative diseases, atherosclerosis, malignancy, and some complications of pregnancy.

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1. Introduction

Increased fructose use by the food industry, both in its crystalline form and as high-fructose corn syrup, has aroused concern that frequent consumption and high body concentration of fructose can promote potentially deleterious metabolic changes such as hyperlipidemia, hyperuricemia, non-enzymatic fructosylation of macromolecules, lactacidemia, and disturbance in copper metabolism.[1] Recent evidence suggests that increased dietary consumption of fructose in Western societies may be a potentially important factor in the growing rates of obesity and the metabolic syndrome,[1] as well as the notion that fructose intake is independently associated with an increased risk of kidney stone formation.[2] Contrary to the above-mentioned concerns, it has been shown that fructose protects tissues against anoxia and hypoxia.[3] Fructose-1,6-bis(phosphate) (F16BP) prevents reperfusion injury,[4] protects against septic shock,[5] and provides protection against the cell damage associated with mitochondrial poisons and prooxidants. A protective effect of adding exogenous F16BP to preservation solution (University of Wisconsin storage solution) used during an experimental procedure of small bowel transplantation in rats has also been reported.[6] Furthermore, an increased intracellular concentration of fructose represents an important non-enzymatic defense mechanism in cold-provoked stress in plants.[7] Some of the known mechanisms of the protective effects of fructose are (i) increased energy production through formation of glycolytic ATP; (ii) sequestration of intracellular redox-active iron thus preventing the Fenton reaction; (iii) stabilization of the glutathione pool; and (iv) up-regulation of the pentose phosphate pathway producing NADPH.

A long-term study showed that after 17 months of exposure of mice to fructose the activity of manganese superoxide dismutase was significantly decreased in all the tested tissues.[8] According to Lane's double-agent theory of aging and development of age-related diseases,[9,10] this could lead to a lower threshold for activation of genes involved in the commencement of inflammation. In other words, 'chronic' consumption of excessive fructose could dictate

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long-term negative consequences such as the development of autoimmune and age-related diseases. It appears that fructose exhibits negative effects when frequently consumed under physiological conditions in the absence of oxidative stress. Therefore, any excess should be eliminated from normal diets. On the other hand, fructose exhibits various protective actions when an organ or a tissue is exposed to oxidative stress. Therefore, acute temporary ingestion of fructose could be very beneficial in some pathophysiological conditions connected with oxidative stress.

The controlled production of reactive oxygen species (ROS) plays an important role in various physiological processes such as receptor-mediated signaling pathways, the immune response, apoptosis, and plant growth.[11] In contrast, unbalanced radical generation is highly correlated with many pathophysiological events such as those occurring in neurodegenerative diseases, atherosclerosis, malignancy,[11] sepsis,[12] and complications of pregnancy related with thrombophilia.[13] Both positive and negative biological functions have been ascribed to both superoxide and hydrogen peroxide (H_2O_2). However, the hydroxyl radical's behavior is exclusively negative due to its very high reactivity. The main route of ·OH production is via the disproportionation of H_2O_2 during which H_2O_2 causes the oxidation of Fe^{2+} (or another transition metal) in a process termed the Fenton reaction. Both reactants (Fe^{2+} and H_2O_2) are habitually present in both physiological and pathophysiological settings. We have previously reported that fructose could significantly decrease the generation of ·OH in the Fenton reaction under physiological conditions.[14] However, the report failed to evaluate the antioxidative capacity of fructose, and no mention of the antioxidative capacity of related monosaccharides was made.

The aim of our current study was to compare direct effects of glucose, mannitol, and fructose, which are employed as infusion sugars, and three phosphorylated forms of fructose on ·OH radical production by the Fenton and the Haber–Weiss-like reaction.

2. Results

Using a modified experimental set-up, the effect of F1P, F6P, and F16BP (3 mM) on ·OH production by the Fenton system was compared with the effects of fructose, glucose, and mannitol. The rank order of ·OH scavenging activity of the monosaccharides was F16BP > F1P > F6P > fructose > mannitol = glucose. The results are shown in Figure 1. RI values obtained for glucose and mannitol

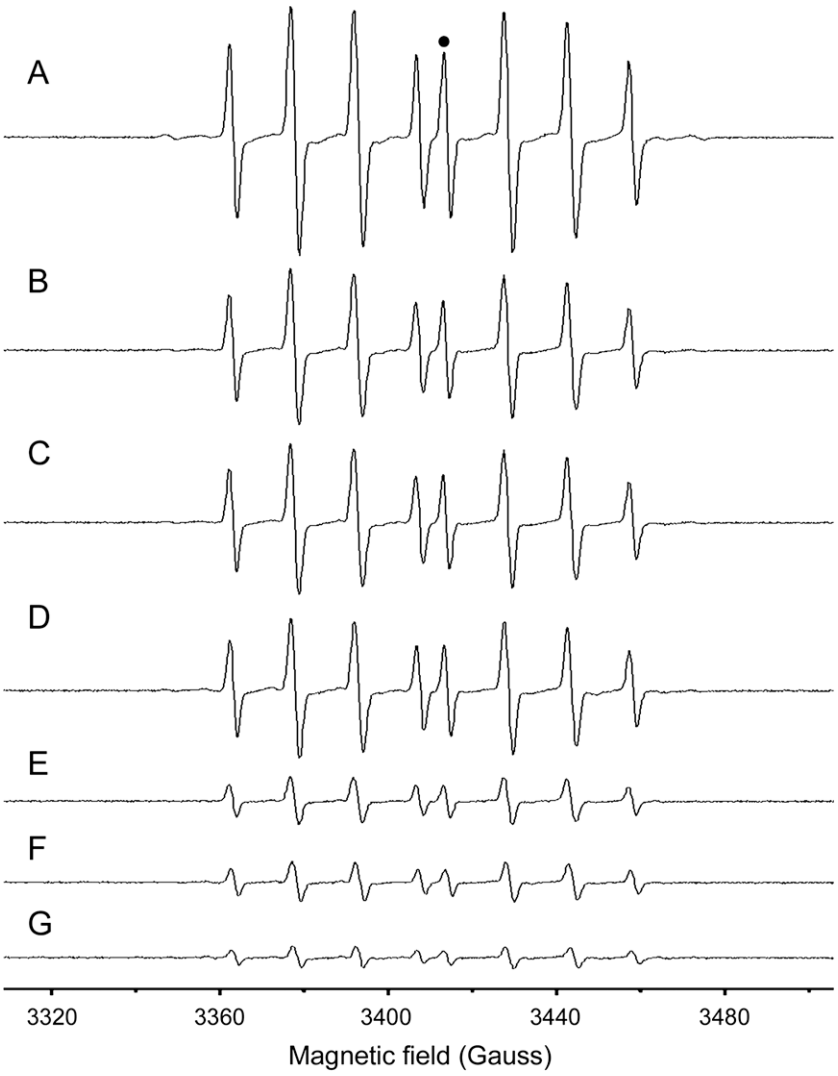

Figure 1. A comparison of the radical scavenging effects of phosphorylated fructose with those of other monosaccharides. EPR spectra represent the signal of the DEPMPO adduct of ·OH generated in the Fenton reaction (Fe^{2+} 0.3 mM; H_2O_2 1.2 mM). Panel (A) Fenton reaction. Panel (B) Fenton reaction + 3 mM glucose; RI = 38 ± 3%. Panel (C) Fenton reaction + 3 mM mannitol; RI = 41 ± 2%. Panel (D) Fenton reaction + 3 mM fructose; RI = 50 ± 3%. Panel (E) Fenton reaction + 3 mM F6P; RI = 80 ± 2%. Panel (F) Fenton reaction + 3 mM F1P; RI = 85 ± 1%. Panel (G) Fenton reaction + 3 mM F16BP; RI = 91 ± 1%. The filled circle marks the DEPMPO/OH peak whose amplitude was measured.

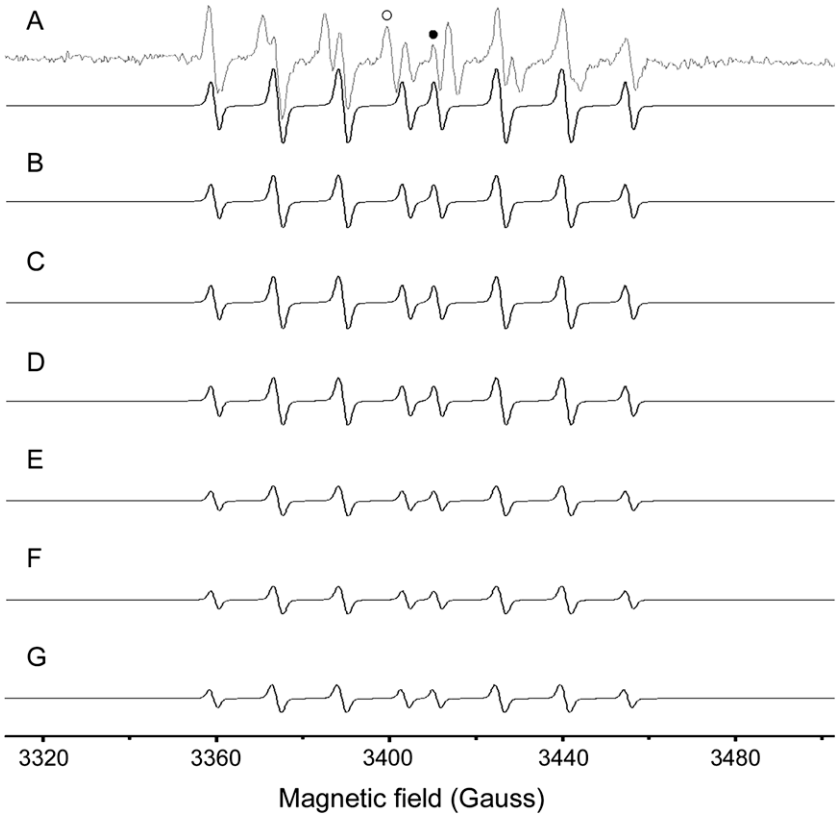

Figure 2. A comparison of the radical scavenging effects of phosphorylated fructose with those of other monosaccharides in the Haber–Weiss-like ·OH-generating system (KO_2 1.4 mM; H_2O_2 5 mM). Panel (A) Signal obtained in the Haber–Weiss reaction (pale line) consisting of spectra of DEPMPO adduct with $\cdot O_2^-$ (generated by KO_2) and DEPMPO adduct with ·OH (generated in Haber–Weiss reaction). Characteristic lines of DEPMPO/OH and DEPMPO/OOH adduct[14] are marked with a filled and an open circle. Based on the characteristic spectrum obtained in each system, spectral simulation of the signal of DEPMPO/OH adduct was performed (black line in panel A and in subsequent panels). Panel (B) Haber–Weiss reaction + 3 mM glucose; RI = 20 ± 5%. Panel (C) Haber–Weiss reaction + 3 mM mannitol; RI = 21 ± 3%. Panel (D) Haber–Weiss reaction + 3 mM fructose; RI = 17 ± 5%. Panel (E) Haber–Weiss reaction + 3 mM F6P; RI = 30 ± 5%. Panel (F) Haber–Weiss reaction + 3 mM F1P; RI = 33 ± 4%. Panel (G) Haber–Weiss reaction + 3 mM F16BP; RI = 40 ± 5%. Spectral simulations of DEPMPO/OH adduct of the representative signal from repeated experiments (n = 3) are illustrated.

were not significantly different ($P > 0.05$). The RI value for fructose was significantly higher than the RI values obtained for glucose and mannitol ($P = 0.035$ and $P = 0.041$, respectively). The RI values for F1P, F6P, and F16BP were statistically different from values obtained for infusion sugars. Finally, the rank order of phosphorylated sugars presented here is based on statistically significant differences between the RI values (P was less than 0.05 in statistical comparison of each of the following sample pairs: F1P and F6P; F1P and F16BP; and F6P and F16BP). The concentration (3 mM) of monosaccharide employed is that which is present in a physiological setting.

The ·OH radical scavenging effects of phosphorylated fructose with other monosaccharides in the Haber–Weiss-like ·OH-generating system (KO_2 1.4 mM; H_2O_2 5 mM) are shown in Figure 2. The rank order was F16BP > F1P ⩾ F6P > fructose = mannitol = glucose. The RI values obtained for glucose, fructose, and mannitol were not significantly different from each other. Although F1P showed slightly higher scavenging effects in the Haber–Weiss-like system, relative to the F6P, RI values were not significantly different. The RI value obtained for F16BP was significantly higher when compared to those obtained for each of other five sugars investigated in the study.

3. Discussion

Our comparison of the radical scavenging effects of phosphorylated fructose with those of other monosaccharides in the Fenton ·OH-generating system indicated the rank order of antioxidative activity: F16BP > F1P > F6P > fructose > glucose = mannitol. Significantly lower RI values obtained in Haber–Weiss-like reaction as an iron-free ·OH-generating systems show that fructose and its phosphorylated forms perform antioxidative actions in the biologically relevant Fenton system via two mechanisms: the iron sequestration activity of the ketose and the ·OH scavenging. In both ·OH-generating systems, phosphorylated forms of fructose had the same order of activity: F16BP > F1P > F6P > non-phosphorylated monosaccharides, so it is obvious that sequestration of intracellular redox-active iron does not influence the radical scavenging capacity of the phosphorylated forms of fructose. From this we conclude that intracellularly generated forms of monosaccharides have greater scavenging properties. Conditions for both free-radical formation and chemical reactions with various carbohydrates are widely present in organisms. Reactions between carbohydrates and Fenton-like reactions under physiological conditions have been previously investigated in vitro. It has been suggested that DNA cleavage is mediated via the generation of ·OH by a combination of the peroxidase reaction of cytochrome *c* and the Fenton-like reaction of free iron ions released from oxidatively damaged cytochrome *c* in the cytochrome *c*/H_2O_2 system.[15] During the incubation of deoxyribose with cytochrome *c* and H_2O_2, deoxyribose degradation increases in a time-dependent manner, suggesting that the released iron ions may participate in a Fenton-like reaction to produce ·OH radicals that could cause DNA cleavage. Carbohydrates can be degraded to glycolate through both reactions with H_2O_2 and with ·OH being produced from H_2O_2 in the Fenton reaction, the latter in situations of intense degradation.[16] Systematic analysis of glyoxal formation has been performed from a range of

monosaccharides and related compounds to determine their potential role as sources of this alpha-oxoaldehyde in vivo.[17] The rank order of monosaccharides as glyoxal sources was found to be fructose > glucose = mannose = galactose > glucose-6-phosphate > mannitol. We found here that fructose was a far more efficient inhibitor of ·OH generation (via the Fenton reaction) than glucose and mannitol. However, in one previous study using high, non-physiological concentrations of fructose and glucose (8 mM), similar antioxidative activity against ·OH generation in the Fenton reaction was found.[18] In addition to glycolate generation, monosaccharides can be transformed into enediols, which are known sources of ·OH and can facilitate iron redox-cycling.[19,20] We propose that under such high levels, antioxidative activity of monosaccharides in the Fenton system preferentially goes via ·OH scavenging, for which glucose and fructose have similar capacities as we have shown using the iron-free Haber–Weiss reaction as an ·OH-generating system. It has been shown previously that ·OH radicals can react with carbonate ions to produce carbonate radicals with an amplified oxidative effect. For this reason, we chose to measure radical production in a non-buffered system.[21]

ROS and the antioxidative defense system are just two intertwined components present within global physiological mechanisms of homeostasis.[22] Therefore, the administration of various exogenous antioxidants in an attempt to prevent consequences of oxidative stress at the level of the whole organism is generally ineffective. The body as a whole prevents the intracellular redox state from being 'swamped' by antioxidant supplements. The double-agent theory[9,10] makes a clear prediction even if long-term supplementation raises blood levels of antioxidants as this will have a limited effect on intracellular levels or redox status because homeostatic mechanisms will correct for the rise at the physiological level. Our results clearly demonstrated that phosphorylated forms of fructose (F1P, F6P, and F16BP), key intracellular metabolites, had even more scavenging properties against generated radicals than fructose. So, we propose that if the organism in times of crisis is presented with the 'energy' in the form of fructose, it will not reject it. Rather, fructose would be introduced into the cells and involved in pathways generating phosphorylated forms of fructose, thus increasing the intracellular oxidative status.

Although excessive fructose uptake may induce the metabolic syndrome associated with glomerular hypertension and renal microvascular damage in rats,[23] the application of fructose and/or its phosphorylated derivatives to affected cells or tissues under severe oxidative stress should not be restricted. Although intravenous fructose infusion has also been associated with lactic acidosis, we speculate that via fructose infusion, among other beneficial effects, the body's natural reaction to the administration of exogenous antioxidants may be overcome. In the light of other data concerning the metabolic consequences of glucose, mannitol, and fructose infusion, our current results could assist in making decisions regarding the choice of clinical intravenous infusions used in situations characterized by intense ROS production such as those found in septic patients whose intestinal fructose transport is impaired by sepsis.[24] We provide compelling evidence for a novel mechanism of protection against oxidative stress involving fructose and its phosphorylated forms that can be incorporated into cell metabolism that could be applied to treat oxidative stress-related pathophysiological conditions.

4. Experimental

4.1. Chemicals

Chemicals were obtained from commercial providers: $FeSO_4$ (E. Merck, Darmstadt, Germany); H_2O_2 (Renal, Budapest, Hungary); DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-*N*-oxide) spin-trap (Alexis Biochemicals, Lausen, Switzerland). We used 20% w/v commercial monosaccharide infusion solutions: Fructose (Mayrhofer Pharmazeutika GmbH, Linz, Austria), Mannitol (C.D.M. Lavoisier, Paris, France) and Glucose (Leopold Pharma, Graz, Austria). F1P, F6P, and F16BP were purchased from Sigma–Aldrich (St. Louis, MO, USA).

4.2. The Fenton system

For a comparative study of the ·OH scavenging capacities of glucose, mannitol, fructose, F1P, F6P, and F16BP (final concentrations 3 mM), the Fenton reaction was initiated using H_2O_2 and $FeSO_4$ at final concentrations of 1.2 mM and 0.3 mM, respectively. Samples were prepared by adding $FeSO_4$ to solutions containing monosaccharide (final pH 4.2). H_2O_2 was added after 2 min incubation, and electron paramagnetic resonance (EPR) measurements were performed. In all experiments the DEPMPO spin-trap (final concentration 28 mM) was added prior to the addition of H_2O_2. In all experiments ultra-pure MilliQ (18 M) water was used.

4.3. The Haber–Weiss-like system

In order to evaluate the antioxidative capacity of sugars in a ·OH-generating system free of metal ions that are readily chelated by ketoses (fructose), the Haber–Weiss reaction ($\cdot O_2^- + H_2O_2 \rightarrow \cdot OH + OH^- + O_2$) was used. Each of six monosaccharides (final concentration 3 mM) was mixed with H_2O_2 (final concentration 5 mM) and DEPMPO (final concentration 28 mM), all dissolved in ultrapure (18 MΩ) water. Thereafter, KO_2 (a $\cdot O_2^-$ generator) dissolved in anhydrous DMSO was added to obtain a final concentration of 1.4 mM, and EPR spectra were obtained (as detailed below). Spectral simulations were performed using the computer program WINEPR SimFonia (Bruker Analytische Messtechnik GmbH, Karlsruhe, Germany). Based on the spectra obtained in each experimental system, which represented a combination of signals of DEPMPO adducts with $\cdot O_2^-$ and ·OH (DEPMPO/OOH and DEPMPO/OH, respectively), spectral simulations of the DEPMPO/OH adduct were performed using the following parameters: $a^P = 46.7$; $a^N = 13.9$; $a^H(1H) = 13.5$.[25]

4.4. EPR spectroscopy

EPR spectra were recorded at room temperature using a Varian E104-A EPR spectrometer operating at X-band (9.51 GHz) using the following settings: modulation amplitude, 2 G; modulation frequency, 100 kHz and microwave power, 10 mW; scanning time, 4 min. Spectra were recorded using EW software (Scientific Software Inc, Bloomington, IL, USA). Samples were drawn into 10-cm long, gas-permeable Teflon tubes (wall thickness 0.025 mm and internal diameter 0.6 mm; Zeus industries, Orangeburg, SC, USA). Measurements were performed using quartz capillaries into which Teflon tubes were placed 2 min after the addition of H_2O_2 or KO_2.

4.5. Statistical analysis

All experiments were performed three times (on three separate days). The relative inhibition (RI) of ·OH formation was calculated according to the following formula: RI = [(peak amplitude (Fenton reaction) − peak amplitude (Fenton reaction + monosaccharide)]/[peak amplitude (Fenton reaction)]. RI of ·OH formation in the Haber–Weiss system was calculated in the same fashion: RI = [peak intensity (Haber–Weiss reaction) − peak intensity (Haber–Weiss reaction + monosaccharide)]/[peak intensity (Haber–Weiss reaction)]. RI values obtained for each monosaccharide were compared with values obtained for every other studied sugar, in order to

evaluate statistical difference by means of the non-parametric two-tailed Mann–Whitney test using STATISTICA 6.0 (StatSoft Inc, Tulsa, OK, USA). Results are presented as means ± S.D. (standard deviation) and were taken to be statistically different if $P < 0.05$.

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