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Carbohydrates and Their Free Radical Scavenging Capability: A Theoretical Study

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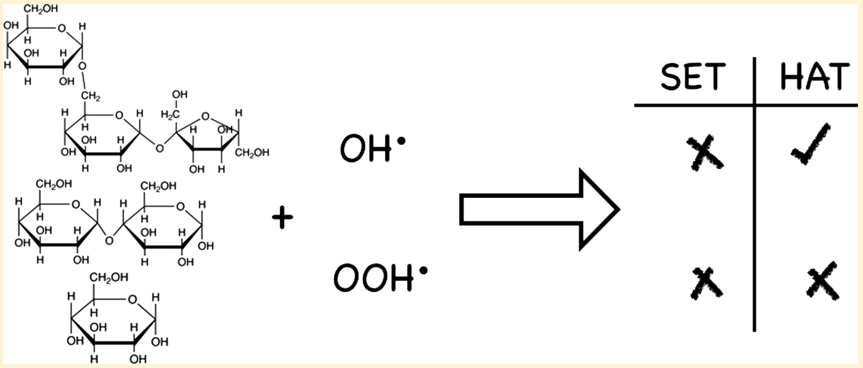
Supporting Information



ABSTRACT: A density functional theory (DFT) study on the free radical (OH• and OOH•) scavenging properties of some mono- and polysaccharides is presented. Two mechanisms, single electron transfer (SET) and hydrogen atom transfer (HAT), are considered. The former mechanism is studied by making use of the vertical ionization energy and vertical electron affinity of the radicals and carbohydrates. It is confirmed that the SET mechanism is not plausible to occur. With respect to the HAT, not only does the OH• radical react preferably with one hydrogen atom bonded to one carbon atom, but also the reaction with a hydrogen atom bonded to an oxygen is possible. Finally, it is suggested that the carbohydrates are not able to directly scavenge OOH•.

INTRODUCTION

Carbohydrates are one of the three most important components of living cells (the other two being amino acids and lipids).[1] Those carbohydrates such as mono- and disaccharides have a low molecular weight in comparison with other biomolecules such as more complex carbohydrate polymers, proteins, DNA, etc. In general, low molecular weight carbohydrates and their derivatives such as sugar alcohols are soluble in water and rather poorly soluble in most organic solvents.[2] These soluble sugars, such as glucose (a monosaccharide) and sucrose (a disaccharide) display a major role in the structure and function of all living cells. Fructans are water-soluble carbohydrate polymers consisting of a sucrose molecule that is elongated by a chain of fructosyl units connected through β–(1→2) or β–(6→2) linkages (Figure 1). Depending on the linkage type, they are called inulin or levan, respectively.[3] The smallest inulin-type fructan is the 1-kestose and the smallest levan-type fructan is the 6-kestose,[4] and both are trisaccharides. Fructans are regarded as one of the principal stored form of energy occurring in plants.[3] In addition, another characteristic of carbohydrates is their capability to react with some reactive oxygen species (ROS).[5−7]

ROS are molecules that contain oxygen, such as superoxide ($^{\bullet}O_2^-$), hydrogen peroxide (H_2O_2), organic peroxides (ROOR′), hydroxyl radical (OH•), and peroxynitrite ($ONOO^-$),[8] and they appear to be responsible of oxidative damage. In an environment of molecular oxygen there are some situations where the ROS production rate may increase in such a way that their levels can overwhelm those of the antioxidants (carotenoids, glutathione, α-tocopherol, catalase, superoxide dismutase, among others) within the cell.[9] This imbalance between ROS and antioxidants leads to some cellular damage.[10] It has been stated that chronic accumulation of ROS in the brain poses the onset and progression of Alzheimer's disease.[11,12] Molecules that may act as direct free radical scavengers constitute one mechanism of defense to avoid the possible damage due to the presence of ROS. Other mechanisms include the use of enzymes such as catalases, superoxide dismutases,[13] hexokinases,[14] etc. to maintain the ROS homeostasis.

There are three main mechanisms devoted to the direct scavenging of free radicals, namely, electron transfer reaction, hydrogen atom transfer, and radical addition.

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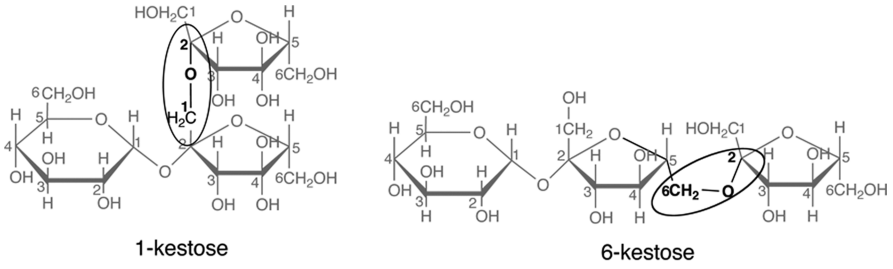

Figure 1. 1-Kestose (showing a β−(1→2) linkage) and 6-kestose (with a β−(6→2) linkage), the smallest inulin- and levan-type fructans.

The free radical scavenging properties of polysaccharides from several sources such as mushrooms, plants, fruits, and algae have been studied in vitro.[6,15−18] For example, the scavenging activity of some mono- and disaccharides, and raffinose (a trisaccharide), as well as the food additives inulin and stevioside against hydroxyl radicals has been successfully tested.[5−7] Moreover, it has been suggested that sucrose, as well as some fructans, at high concentrations in the vacuoles may function directly as a protective agent against oxidative stress.[19] Thus, Bolouri-Moghaddam et al.[20] hypothesized that vacuolar sugars or sugar-like compounds, possibly in combination with phenolic compounds, form an additional antioxidant mechanism, along with the well-established cytoplastic ones, by directly scavenging the $OH^{\bullet}$ and $OOH^{\bullet}$ radicals that are produced in the vicinity of the membranes.

The very reactive hydroxyl radical ($OH^{\bullet}$) can be formed via the decomposition of hydrogen peroxide in the presence of transition metals such as Fe(II), Cr(IV), and Cu(I) according to a Fenton-type reaction.[21] $OH^{\bullet}$ is known to damage almost all biomolecules at very fast rates, with values as high as those of diffusion-controlled reactions. With most organic and biological molecules the rate constants are in the range 10^8 to 10^{10} $M^{-1}s^{-1}$.[22−24] In several reactions, mainly with organic compounds having double bonds or with aromatic compounds, $OH^{\bullet}$ prefers to add to the aromatic ring or to the double bonds yielding an addition product. The hydroxyl radical is one of the most powerful oxidants in aqueous solution (E° = 1.83 V),[22] and electron transfer reactions of the type $OH^{\bullet} + R \rightarrow OH^{-} + R^{+}$ can occur provided that, for example, the standard reduction potential of R^{+} is smaller than 1.83 V to favor the redox reaction. Moreover, $OH^{\bullet}$ reacts with most C−H bonds, forming an organic free radical:[22] $RH + OH^{\bullet} \rightarrow R^{\bullet} + H_2O$.

In spite of the experimental studies concerning the free radical scavenging capacity of polysaccharides, there are few investigations concerning the mechanism of action. For this reason, the main goal of this work is to investigate two of the free radical scavenging mechanisms that might occur between small carbohydrates and reactive oxygen species such as $OH^{\bullet}$ and $OOH^{\bullet}$. The carbohydrates used here are the monosaccharides D-glucose and D-fructose (and some of their cyclic isomers); the disaccharides sucrose and maltose; and the trisaccharides 1-kestose, 6-kestose, and raffinose. The four isomers of the D-glucose are considered: α-D-glucopyranose, β-D-glucopyranose, α-D-glucofuranose, and β-D-glucofuranose. The cyclic molecules α-D-fructofuranose and β-D-fructofuranose are the two D-fructose isomers taken into account. The schematic representations of the molecular structures are presented in Figure 2.

The vertical ionization energies and the vertical electron affinities of the free radicals and the carbohydrates allowed us to rule out the single electron transfer (SET) mechanism. On the other hand, the hydrogen atom transfer (HAT) between $OH^{\bullet}$ and carbohydrates is found to be an exergonic process, whereas the reaction with $OOH^{\bullet}$ appeared to be an endergonic process. The radical adduct formation (RAF) is not considered because the carbohydrates do not have aromatic or double bonds within their structure; therefore, the addition product is not expected.

■ COMPUTATIONAL DETAILS

All the calculations were performed using density functional theory (DFT) as implemented in the Gaussian09 program[25] using the Becke−Perdew exchange−correlation functional (BP86)[26−28] and the 6-311+g(d) basis functions for all atoms. Spin unrestricted calculations were used for all the open-shell systems. When full geometry optimizations were carried out, a frequency analysis allowed for the verification of the optimized minima. To investigate the SET mechanism, further single-point calculations based on the neutral optimized geometries were needed to estimate the vertical ionization energy (VIE) and vertical electron affinity (VEA) of the different carbohydrates. DFT methods have been used successfully to calculate reliable values of molecular gas-phase electron affinities and ionization energies.[29−32] Thus, the VIE was calculated as the difference between the energy of the cation and the energy of the neutral molecule. On the other hand, the VEA was calculated as the energy difference of the neutral and the anionic species. The solvent effect was included by making use of a polarizable continuum model (PCM), the integral-equation-formalism (IEF-PCM),[33,34] with water as the solvent.

The initial geometries for the monosaccharides (D-glucose, D-fructose and their cyclic isomers) were prepared with the Gaussview 5 visualization software.[35] In the case of the disaccharides maltose and sucrose, the initial structures were taken from the Chemistry, Structures & 3D Molecules Web site.[36] The Crystallographic Information Files of the trisaccharides 1-kestose (kestos.cif),[37] 6-kestose (celgij.cif),[38] and raffinose (rafino.cif)[39] from the Cambridge Structural Database were used to construct their initial geometries. The conversion to Cartesian coordinates was made with the visualization software Mercury CSD 2.3.[40]

While high-level calculations (CCSD(T)) on the hydrogen abstraction by $OH^{\bullet}$ from small molecules such as substituted formaldehyde afford a good agreement with experimental results,[41] some hydrogen abstraction reactions between small molecules have been successfully studied making use of the BP86 functional.[42]

■ RESULTS AND DISCUSSION

1. Single Electron Transfer. The concepts of vertical ionization energy (VIE) and vertical electron affinity (VEA) have been applied successfully in the study of the electron

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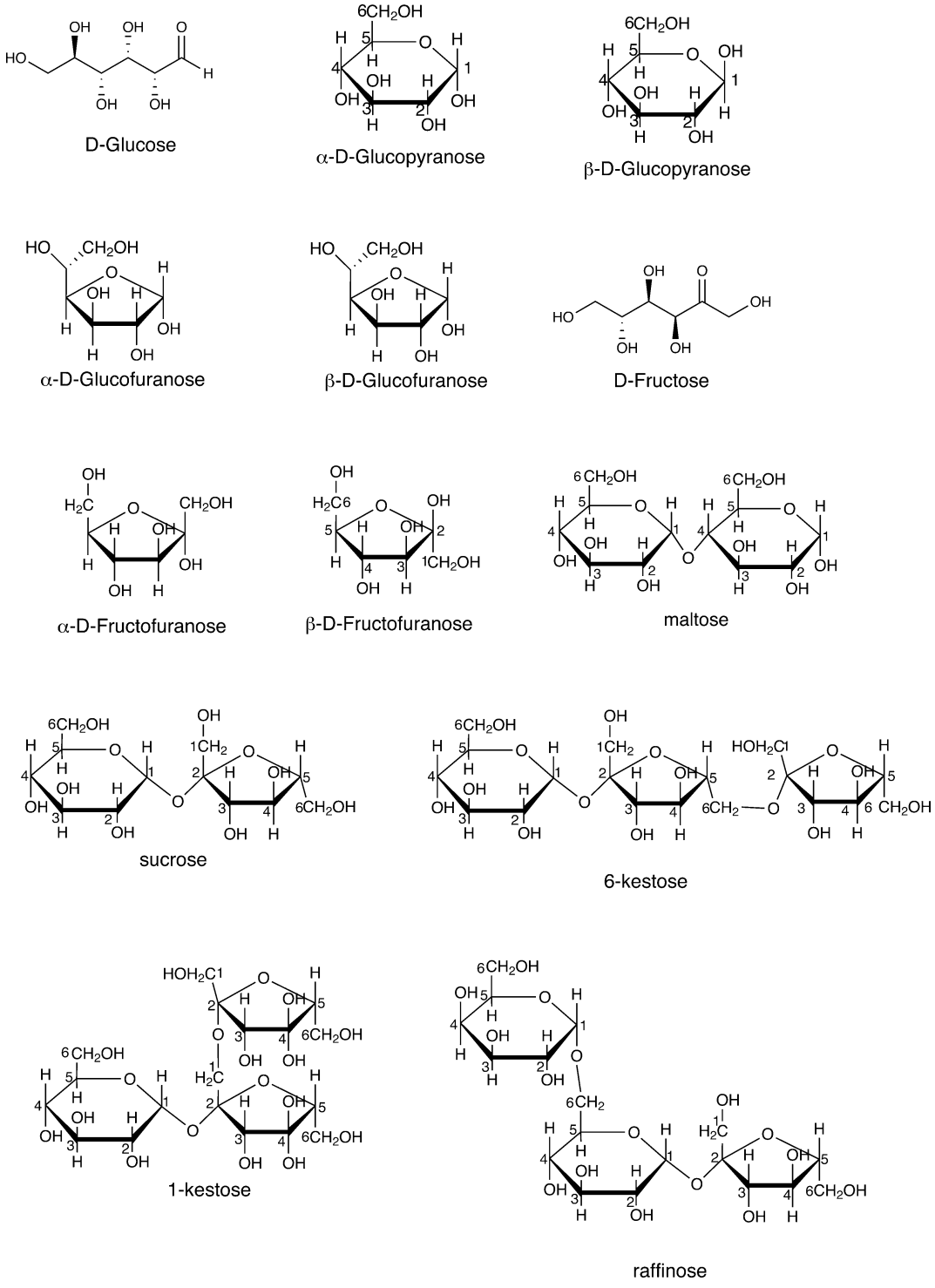

Figure 2. Schematic representations of the carbohydrates considered in this study.

transfer reactions between free radicals and molecules such as carotenoids and vitamins.[43−45] It was mentioned in the Introduction that the $OH^{\bullet}$ radical is one of the most powerful oxidants. Thus, it would be expected that this radical species should have a higher value of VEA with respect to another species such as the hydroperoxyl radical ($OOH^{\bullet}$) and the carbohydrates under study. To have an electron transfer, the carbohydrates should have a low VIE. Table 1 reports the calculated VIE and VEA. These values were obtained using the calculations including the solvent (water) effect as described in the Computational Details.

It can be seen that the highest VEA corresponds to $OH^{\bullet}$ (5.34 eV). Also, the carbohydrates have smaller values of VIE (between 6 and 7 eV) with respect to the free radical species (VIE ∼ 9 eV). It should be noted that the size of a carbohydrate does not have a notable influence on the VIE (with all the calculated values ranging from 6.51 to 7.02 eV) nor on the VEA (1.00 to 1.85 eV). The $OOH^{\bullet}$ radical appears to be a worse

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Table 1. Calculated Vertical Ionization Energy and Vertical Electron Affinity (eV) in Water for the Carbohydrates under Study and the $OH^{\bullet}$ and $OOH^{\bullet}$ Radicals

molecule	VIE (eV)	VEA (eV)
D-glucose	6.86	1.85
α-D-glucopyranose	6.89	1.13
β-D-glucopyranose	6.98	1.14
α-D-glucofuranose	6.88	1.16
β-D-glucofuranose	6.86	1.16
D-fructose	6.88	1.57
α-D-fructofuranose	7.02	1.13
β-D-fructofuranose	6.81	1.12
sucrose	6.60	1.13
maltose	6.69	1.16
1-kestose	6.52	1.23
6-kestose	6.51	1.20
raffinose	6.66	1.00
$OH^{\bullet}$	9.79	5.34
$OOH^{\bullet}$	9.02	3.71

electron acceptor and a slightly better electron donator than $OH^{\bullet}$.

A useful tool for the identification of the relative electron-donor or electron-acceptor capability of a molecule is the graphic representation of the (x, y) coordinate pair (VEA,VIE). This coordinate system has been defined as the full electron donator acceptor map (FEDAM).[44] In the FEDAM, the molecules located in the lower left corner are considered to be good electron donors and poor electron acceptors. Those situated in the upper right corner are good electron acceptors and poor electron donors.

Although the FEDAM is a useful tool for a qualitative comparison among substances,[44] it is not adequate for quantitative predictions. However, when we are dealing with the transfer of one electron, as in the case of the SET mechanism, a better criterion appears to be that the following condition should be satisfied:[46]

$$\mathrm{VIE(donor)} < \mathrm{VEA(acceptor)} \quad (1)$$

The corresponding FEDAM constructed in terms of the calculated VIEs and VEAs is shown in Figure 3. From the results in Table 1, it is possible to see that $OH^{\bullet}$ has the largest value of VEA (5.34 eV) and VIE (9.79 eV). Therefore, for the SET between $OH^{\bullet}$ and another molecule (that will act as electron donor) to take place, the VIE of the electron donor should be smaller than 5.34 eV, as shown by the shaded area on the FEDAM (Figure 3). Thus, the condition in (1) is not satisfied for any carbohydrate in this study because the smallest VIE corresponds to 6-kestose and is equal to 6.51 eV. To verify the prediction obtained with the condition in (1), Table 2

Table 2. Vertical ΔE_{SET} and Adiabatic ΔH_{SET} and ΔG_{SET} at 298 K in Water for the Process Carbohydrate + $OH^{\bullet}$ → $^{\bullet}Carbohydrate^{+}$ + OH^{-} for Some Carbohydrates

carbohydrate	vertical ΔE_{SET} (kcal/mol)	adiabatic ΔH_{SET} (kcal/mol)	adiabatic ΔG_{SET} (kcal/mol)
α-D-glucopyranose	35.8	27.1	27.7
β-D-fructofuranose	33.9	23.4	24.5
sucrose	29.0	24.7	24.2
maltose	31.0	25.5	24.2

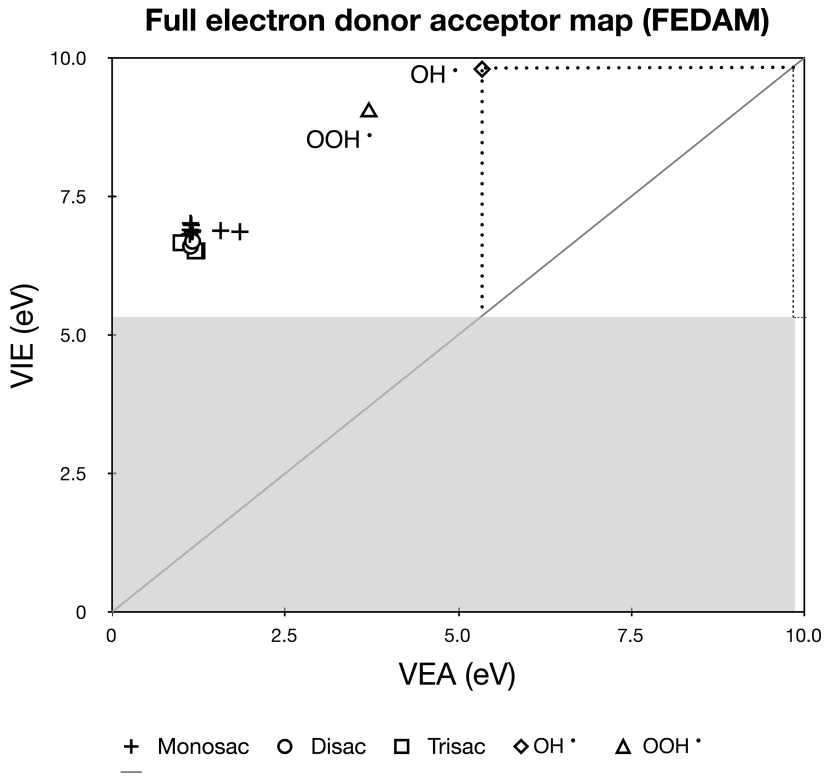

Figure 3. Full electron donor−acceptor map, FEDAM. The shaded area indicates the zone in which a species should be located to be able to donate one electron to $OH^{\bullet}$ in water.

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contains the estimated vertical ΔE_{SET} and the adiabatic (i.e., optimizing the geometry of all the involved species) ΔH_{SET} and ΔG_{SET} at 298 K in water for some carbohydrates considering the following process:

$$\text{carbohydrate} + OH^{\bullet} \rightarrow {}^{\bullet}\text{carbohydrate}^{+} + OH^{-}$$

The calculated vertical ΔE_{SET} corresponds to the difference VIE(donor) − VEA(acceptor). For example, with data taken from Table 1, VIE(α-D-glucopyranose) − VEA($OH^{\bullet}$) = 6.89 − 5.34 eV = 1.55 eV ≈ 35.8 kcal/mol (Table 2). Considering that the entropic contribution is deemed to be small in the SET process, solving the condition (1) for the difference VIE(donor) − VEA(acceptor) may give a good approximation to the enthalpy and free energy of the process.

It can also be seen in Table 2 that the electron transfer between $OH^{\bullet}$ and carbohydrates does not occur spontaneously because the adiabatic SET is an endergonic process. The adiabatic electron affinity of $OH^{\bullet}$ was calculated to be also 5.34 eV. Thus, according to the results shown in Table 2, the difference between the vertical ΔE_{SET} and adiabatic ΔH_{SET} is not larger than 10.5 kcal/mol, or 0.45 eV (this value calculated for β-D-fructofuranose). This means that the adiabatic ionization energies (AIEs) do not differ significantly from the vertical ones. In fact, for the saccharides in Table 2, the difference is always smaller than 0.5 eV when compared to the corresponding data in Table 1. Therefore, the use of a FEDAM (based in VEIs and VEAs) is useful to obtain the correct trend in reactivity toward the single electron transfer mechanism. Moreover, according to the data in Table 2, the entropic contribution to the adiabatic value of ΔG_{SET} is relatively small (around ±1 kcal/mol). Following this idea, it is possible to say that because the VEA of $OOH^{\bullet}$ is 3.71 eV, this species cannot be involved in a SET mechanism with the carbohydrates considered here. Further, given the small influence of the size of the carbohydrate on the VIE value, it is not expected that polysaccharides of larger molecular weight than those studied here will be able to scavenge $OH^{\bullet}$ nor $OOH^{\bullet}$ via the SET mechanism. It should be noted that some theoretical calculations on pyrimidine nucleosides within the polarizable continuum solvent model tend to underestimate (about 0.5 eV) the ionization energies in solution.[47] Consequently, the theoretical ΔE_{SET} values in Table 2 maybe considered as a lower limit, making the SET mechanism even less plausible to occur experimentally.

2. Hydrogen Atom Transfer. The hydrogen atom transfer (HAT) mechanism is probably the way in which the hydroxyl radical and the carbohydrates react. On the basis of model studies on D-glucose[48] and cellobiose (a disaccharide),[49] and from further investigations on polysaccharides such as xyloglucanes,[50,51] it has been proposed that the damage to polysaccharides in an aerobic environment due to $OH^{\bullet}$ may follow a sequence of reactions: $OH^{\bullet}$ abstracts a carbon-bonded H atom forming a carbon-centered radical and H_2O. Then, if available, a subsequent addition of O_2 and the elimination of the hydroperoxyl radical $HO_2^{\bullet}$ takes place. For the α-D-glucopyranose, it was found that there is no pronounced regioselectivity for the abstraction of the hydrogen atom.[48]

The hydrogen abstraction from the C−H bond is expected due to the lower bond dissociation energy (ca. 100 kcal/mol in $CH_3CH_2CH_2$−H, or 80 kcal/mol in the diatomic molecule C−H) compared to the O−H dissociation energy (around 105 kcal/mol in CH_3O−H or ∼103 kcal/mol in the diatomic molecule O−H).[52,53]

In this study we have not considered the addition of the oxygen because the HAT is the first step, and probably the main one, of the whole process of the direct radical scavenging by carbohydrates. Therefore, the only reaction considered here is (where carbohydrate ≠ $H^{\bullet}$ corresponds to the carbohydrate free radical, without one hydrogen atom with respect to the original molecule)

$$\text{carbohydrate} + OH^{\bullet} \rightarrow \text{carbohydrate} \neq H^{\bullet} + H_2O$$

The calculated ΔH_{HAT} and ΔG_{HAT} for the abstraction of the hydrogen atom in different positions of α-D-glucopyranose are presented in Table 3. The entropic contribution is within ±2 kcal/mol, and it can be seen that not only the HAT from the C−H bond to the radical but also the transfer from any O−H group in α-D-glucopyranose can be considered as an exergonic process. The less negative ΔG_{HAT} (−20.4 kcal/mol) corresponds to the transfer from the O−H located on the C3 of the α-D-glucopyranose molecule. This represents a difference of almost 8 kcal/mol when compared to the most exergonic transfer of the hydrogen from the C2, C6, C4, C3, and C5 positions ($\Delta G_{HAT} \sim -28$ kcal/mol).

Table 3. Calculated ΔH_{HAT} and ΔG_{HAT} in Water for the Abstraction of the Hydrogen Atom in Different Positions[a] of α-D-Glucopyranose

position	ΔH_{HAT} (kcal/mol)	ΔG_{HAT} (kcal/mol)
C2	−26.0	−27.9
C6	−26.6	−27.8
C4	−26.3	−27.7
C3	−26.0	−27.7
C5	−27.2	−27.5
O1	−26.6	−27.3
C1	−24.5	−25.6
O2	−21.7	−22.3
O6	−20.6	−21.5
O3	−20.4	−21.3
O4	−20.6	−20.4

[a]The labels correspond to the atom (carbon or oxygen) to which the hydrogen is bonded. The numbering for carbon atoms is found in Figure 2. The labels for oxygen atoms correspond to the carbon to which they are bonded.

Not surprisingly, for the other carbohydrates, it was also found that the abstraction of a hydrogen atom from an OH group is exergonic. However, the most exergonic HAT is from a C−H position. Table 4 presents the two most negative values of the calculated ΔG_{HAT} for the other mono-, di-, and trisaccharides. Figure S1 (Supporting Information) shows the positions corresponding to the hydrogens that gives rise to the ΔG_{HAT} in Table 4.

With the exception of sucrose, which has the most negative ΔG_{HAT} (−37.8 kcal/mol), the remaining values are very similar and around −30 kcal/mol. Thus, a correlation between the size of the carbohydrate and the exergonicity of the HAT is not evident. Comparing α-D-glucopyranose and β-D-fructofuranose in Table 4, the reaction with the latter is slightly more exergonic. Moreover, it can be seen that for the di- and trisaccharides the positions that give rise to the largest negative ΔG values are essentially the same as in the corresponding monosaccharide. For example, the positions C3 and C5 in sucrose (β-D-fructofuranosyl ring) and in β-D-fructofuranose result in the two most exergonic $-\Delta G$ values (Table 4). In the

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Table 4. Two Most Exergonic ΔG_{HAT} Calculated in Water for the Abstraction of the Hydrogen Atom by OH• in Different Carbohydrates

molecule	position	ΔG_{HAT} (kcal/mol)
α-D-glucopyranose	C2	−27.9
	C6	−27.8
β-D-fructofuranose	C3	−30.2
	C5	−29.2
sucrose	C3 F[a]	−37.8
	C5 F	−32.3
maltose	C4 Gnr[b]	−31.6
	C3 Gnr	−29.9
1-kestose	C3 F[a]	−32.2
	C5 GS[c]	−32.1
6-kestose	C6 FS[d]	−31.8
	C3 FS	−31.4
raffinose	C3 F[a]	−33.5
	C3 G[e]	−32.3

[a]F refers to the D-fructose fragment. [b]Gnr refers to the nonreducing glucosyl fragment. [c]GS refers to the glucose fragment in the saccharose moiety. [d]FS refers to the fructose fragment in the saccharose moiety. [e]G refers to the glucose fragment added to the sucrose moiety. See also Figure S1 in the Supporting Information.

Table 5. Two Least Endergonic ΔG_{HAT} Calculated in Water for the Abstraction of the Hydrogen Atom by OOH• in Different Carbohydrates

molecule	position	ΔG_{HAT} (kcal/mol)
α-D-glucopyranose	C2	9.5
	C6	9.4
β-D-fructofuranose	C3	7.1
	C5	8.1
sucrose	C3 F[a]	−0.5
	C5 F	5.0
maltose	C4 Gnr[b]	5.7
	C3 Gnr	7.4
1-kestose	C3 F[a]	5.0
	C5 GS[c]	5.1
6-kestose	C6 FS[d]	5.5
	C3 FS	5.9
raffinose	C3 F[a]	3.8
	C3 G[e]	5.0

[a]F refers to the D-fructosyl fragment. [b]Gnr refers to the nonreducing glucosyl fragment. [c]GS refers to the glucose fragment in the saccharose moiety. [d]FS refers to the fructose fragment in the saccharose moiety. [e]G refers to the glucose fragment added to the sucrose moiety. See also Figure S1 in the Supporting Information.

case of 1-kestose the HAT from the position C3 of the β-D-fructofuranosyl ring gives rise to the largest $-\Delta G$ value. Conversely, this position gives the most negative ΔG for the corresponding monosaccharide (Table 4). For maltose, the positions C4 and C3 from the nonreducing glucosyl fragment have the largest $-\Delta G$. From Table 3, it can be seen that those positions are only 0.2 kcal/mol below the C2 position in α-D-glucopyranose. In 6-kestose, the positions that result in the largest $-\Delta G$ are also located in one of the β-D-fructofuranosyl ring. In 6-kestose, the abstraction of a hydrogen from the (C6,O2) linkage is preferred due to the stability provided by an intramolecular hydrogen-bonding network generated by the geometry of the resulting carbohydrate radical (Supporting Information, Figure S2). In contrast, when the hydrogen is abstracted from the fructosyl (C2,O2) linkage in 1-kestose, the geometry of the resulting radical generates a molecule that is slightly less stable than the one from 6-kestose. Nevertheless, the ΔG for the hydrogen atom transfer from the (C2,O2) linkage in 1-kestose is still exergonic (−25.9 kcal/mol).

In contrast to the reaction with the OH• radical, the calculations made for the process

$$\text{carbohydrate} + \text{OOH}\bullet \rightarrow \text{carbohydrate} \neq \text{H}^\bullet + \text{H}_2\text{O}_2$$

is rather endergonic, with the exception of sucrose which has one value of ΔG = −0.5 kcal/mol as shown in Table 5. Consequently, a direct scavenging of OOH• by the carbohydrates is not plausible.

Returning to the OH• radical, it is known that its reactions with most molecules occur at very fast rates, in the range 10^8 to 10^{10} M^{-1} s^{-1}.[23−25] Some of those values are as high as those of diffusion-controlled reactions. Diffusion controlled reactions of most solutes in water have activation energies between 3 and 4 kcal/mol.[24]

It was attempted to calculate the activation energy of the HAT process for the C−H bond that gave the most exergonic reaction with the hydroxyl radical for the mono and disaccharides in Table 4. However, in all cases a zero-energy barrier was found. It might be possible that DFT fails to predict the activation energy of this mechanism. During the study of the reaction between fluoroformaldehyde (FCHO) with OH•, Jursic stated that DFT methods cannot generate transition state structures for radical abstraction reactions if the activation energy is very low (∼5 kcal/mol).[54] Thus, our results point out to the possibility that the scavenging of OH• by carbohydrates is also a diffusion-controlled reaction with an activation energy around 5 kcal/mol.

Although the activation energy for the HAT process was not possible to be calculated, two α-D-glucopyranose structures with one imaginary harmonic frequency (IF) and displacement vectors pointing to/from one H atom from/to the oxygen of the hydroxyl radical were found and are shown in Figure 4. A figure showing the displacement vectors only can be found in the Supporting Information (Figure S3).

In Figure 4a (IF = −351 cm^{-1}), it can be seen that the distance between the H bonded to the O1 of the carbohydrate and the oxygen of the radical is 1.42 Å, and the distance between O1 and the hydrogen atom is 1.07 Å, which is larger than the average OH bond distance of 0.98 Å. Figure 4b (IF = −214 cm^{-1}) displays a structure where the distance between the H bonded to the C5 of the carbohydrate and the oxygen of the radical is 1.47 Å, and the distance between C5 and the hydrogen atom is 1.18 Å, which is slightly larger than the average CH bond distance (1.09 Å). The energy of both structures in Figure 4 is higher than the separate products (H_2O and carbohydrate ≠ H•) but lower than the energy of the separate reactants (OH• and carbohydrate). This points out the possibility that prior to the hydrogen atom transfer, a carbohydrate−radical adduct is formed. The energy of the structure in Figure 4a is slightly higher (1.7 kcal/mol) than one radical−carbohydrate adduct with a H(O1)−O(radical) distance of 1.8 Å. In the case of Figure 4b, its energy is smaller than that of one radical−carbohydrate adduct with a H(C5)−O(radical) distance of 2.4 Å.

Additionally, the respective singly occupied molecular orbitals (SOMO) are included in Figure 4. Although the vector O(C)···H···O is nonlinear, the orbitals located on that

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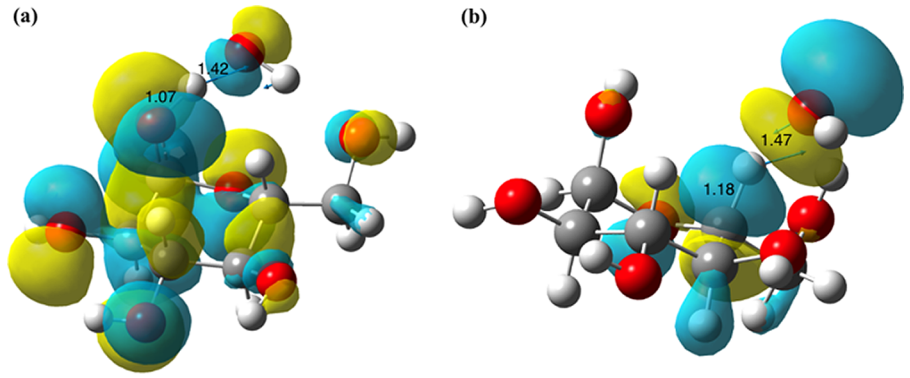

Figure 4. Geometry, displacement vectors of the imaginary frequency, and SOMO of two transition state-like structures. (a) The distances (Å) for O1−H and H−O(radical) are shown. (b) The distances (Å) for C5−H and H−O(radical) are indicated.

interaction cannot be considered to be perpendicular to the vector. Therefore, the structures in Figure 4 better describe a HAT process because if the orbitals were orthogonal to the O(C)−H−O vector, then the process would follow a proton-coupled electron transfer (PCET) mechanism.[55,56]

Morelli et al.[5] reported indirect measurements (based on data for deoxyribose) of the rate constants for the reaction between sucrose, maltose, glucose, and fructose with $OH^{\bullet}$. They estimated k to range between 1.5×10^8 M^{-1} s^{-1} for fructose and 1.22×10^9 M^{-1} s^{-1} for sucrose.[5] Although it has been suggested that the calculations of rate constant employing deoxyribose and Fenton reagents should be reexamined,[57] the available data can still be used to judge the order of magnitude of the expected activation energy. $\Delta G^{\ddagger}$, the free energy of activation, can be estimated by applying the simplest expression of the transition state theory, in which the rate constant k for a reaction is given by $k = (k_B T/h) \exp(-(\Delta G^{\ddagger}/RT))$, where k_B is the Boltzmann constant, h is the Planck constant, R is the gas constant, and T is the temperature. Using the rate constants reported by Morelli et al., the activation energies are found to be 6.3 and 5.1 kcal/mol for fructose and sucrose, respectively. Recall that, when it was possible to calculate an activation energy for α-D-glucopyranose, the estimated value was 1.6 kcal/mol (difference between the structure in Figure 4a and one adduct carbohydrate−radical). We see that in such a case the activation energy has been underestimated. However, this activation energy is small enough to consider this process as a diffusion-controlled reaction. Unfortunately, a straightforward comparison between the experimental and the theoretical data is not possible because the available experimental data are not a direct measurement of the reaction between the carbohydrates and the hydroxyl radical.

CONCLUDING REMARKS

It has been pointed out elsewhere that the direct ROS scavenging by carbohydrates may occur in vivo in plants (at vacuolar membrane tissues)[19] or in higher aerobic organisms (for example, at gastrointestinal tract cells).[58] Therefore, the results exposed here represent more a fair approximation to the in vitro conditions. With this in mind, the application of DFT computational methods allowed us to definitely rule out the SET mechanism for the carbohydrates in solution to scavenge $OH^{\bullet}$ and $OOH^{\bullet}$

Likewise, the theoretical calculations indicate that a direct scavenging of $OH^{\bullet}$ by carbohydrates via HAT is plausible. However, the scavenging of $OOH^{\bullet}$ might not be possible because all the reactions with this radical were calculated to be endergonic.

Although it is already established that the hydroxyl radical reacts preferably with a hydrogen atom bonded to a carbon, the reaction with a hydrogen bonded to an oxygen is also exergonic. The energy of some transition state-like structures were found to be higher than the separate products but smaller than the energy of the separate reactants. Thus, it is possible that prior to the HAT, a carbohydrate−radical adduct is formed. The activation energy, with respect to a carbohydrate−radical adduct, is 1.6 kcal/mol for the abstraction of a hydrogen from an O−H bond. It was not possible to locate a transition state for the HAT on a C−H bond. Thus, it is possible that the scavenging of $OH^{\bullet}$ by carbohydrates is a diffusion-controlled reaction with an activation energy of approximately 5 kcal/mol. The kinetics experimental data available do not allow for a direct comparison and more investigations are needed.

It might be possible to mimic, as a first approximation, the physiological conditions by the use of a continuum model (as we did in this investigation to account for the solvent). In such a case, a suitable value of dielectric constant able to represent the polarizability of the tissue should be used. However, if the same level of theory is used, the reactivity trends (if not the energetics) are expected to be preserved.

ASSOCIATED CONTENT

Supporting Information

Figures showing the positions that give rise to the two most exergonic ΔG_{HAT} for some of the carbohydrates considered here, the intramolecular hydrogen-bonding interactions from some 1-kestose and 6-kestose radicals, and the displacement vectors of the imaginary frequency of two TS structures. This information is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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