Plasma-Induced Free Radicals of Polycrystalline Monocarbohydrates Studied by Electron Spin Resonance

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We report here special features of plasma-induced free radicals of monocarbohydrates such as α- and β-glucose on its comparison with those of amylose and cellulose. The simulated spectra disclosed that the observed spectra of α-glucose consist of four kinds of spectral components, two isotropic spectra [doublet (I) and (II)], assigned to a hydroxylalkyl radicals and an anisotropic spectrum [anisotropic doublet (a-D) (III)], assigned to an acylalkyl radical, and a large amount of dangling-bond site (DBS) (V) with an intermediate level of conversion which includes a mixture of ring-opened and/or conjugated structure of no structural significance. On the other hand, those of β-glucose consist of four kinds of spectral components, I, III, V and an anisotropic doublet of doublets (a-DD) spectrum (IV) assignable to the acylalkyl radicals.

Key words plasma-induced free radical; electron spin resonance; monocarbohydrate; computer simulation; hydrogen-bonding network

The plasma-induced surface crosslink reaction can be considered to be a thin film formation by plasma polymerization starting from solid state materials. In view of the fact that all the surface reactions of plasma-induced polymer modification are initiated by plasma-induced surface radicals, we have often emphasized that the study of the radicals formed is of utmost importance for full understanding of the nature of plasma treatment. Thus, we reported a detailed electron spin resonance (ESR) study on plasma-induced radicals of several synthetic vinyl polymers[1] and fibrous polypeptides such as silk, wool and collagen.[2]

As part of our continuing work on elucidation of plasma-induced surface radicals, we have recently reported an ESR study on monocarbohydrate[3] (*myo*-inositol), dicarbohydrates[4] (maltose, cellobiose) and polycarbohydrates[5] (cellulose, amylose).

Several authors have reported studies on plasma treatment of several polycarbohydrates[6] such as cotton fibers (cellulose) and starch (amylose). But, research has been directed mainly toward characterization of the surface properties of the plasma-treated materials. The detailed ESR studies on the elucidation of the plasma-induced surface radicals have not been worked out.

In this study, we report the special features of plasma-induced free radicals of monocarbohydrates such as α- and β-glucose, on its comparison with those of amylose and cellulose studied by ESR coupled with systematic computer simulations.

Experimental

Materials Powdered α- and β-glucose were purchased from Sigma-Aldrich Japan. They were screened with a 100—140 mesh sieve, and dried at 70 °C for 3 d *in vacuo*.

Method of Plasma Irradiation Powdered samples (50 mg) were placed in a specially designed ampule (30 mm i.d., 100 mm long) with a capillary tube (2 mm i.d.) at the uppermost part. The ampule was filled with argon gas for plasmolysis (0.3 Torr) and sealed. Then the plasma state was sustained for the prescribed period of time with agitation of samples at room temperature by a radio frequency discharge of inductive coupling using a four-loop antenna at 13.56 MHz with the supplied power (50 W). Then, the ESR measurements were performed while turning the ampule upside down after plasma irradiation at appropriate intervals, which is fundamentally the same procedure as that reported earlier.[1] A schematic representation is shown in Fig. 1.

ESR Spectral Measurement ESR spectra were recorded by a JES-RE1X (JEOL) spectrometer with X-band and 100 KHz field modulation. Care was taken to ensure that no saturation occurred and that the line shape was not distorted by excessive modulation amplitude. From a plot of the square root of the microwave power *versus* the signal peak height, a mi-

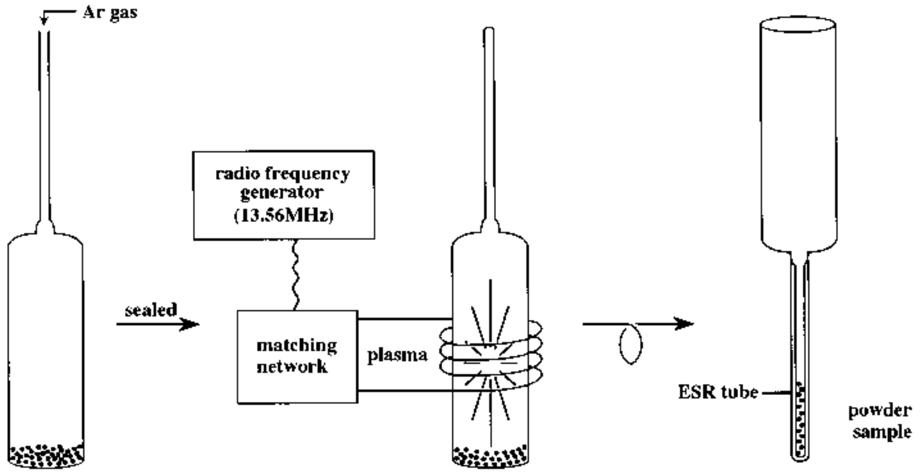

Fig. 1. Schematic Representation for Plasma-Irradiation and ESR Spectral Measurement

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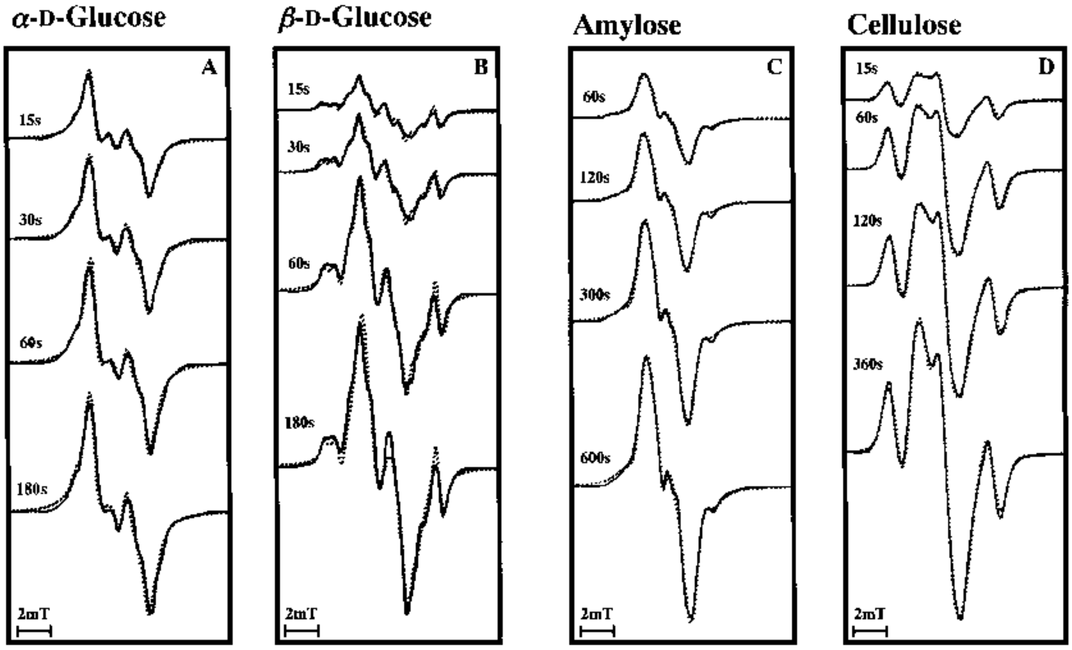

Fig. 2. Progressive Changes in Observed ESR Spectra of Plasma-Irradiated α-, β-Glucose, Amylose and Cellulose, Respectively, Together with the Simulated Spectra Shown as Dotted Lines

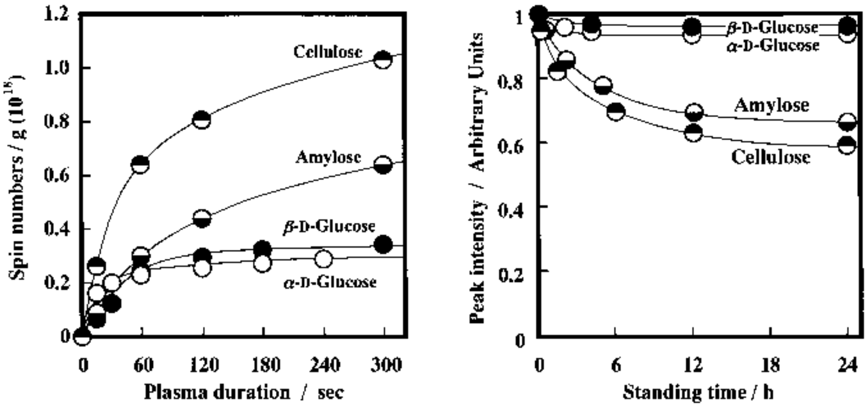

Fig. 3. Progressive Changes in Spectral Intensities Determined by Double Integration: (A) on Plasma-Duration, (B) on Standing at Room Temperature

crowave power level of 0.01 mW was chosen.

Computer Simulation of ESR Spectra Computer simulations were performed on a 32-bit microcomputer (NEC PC9821C×3). The simulated spectra were obtained from Lorentzian functions by fitting iteratively the spectroscopic parameters (*g*-value, line width at half-height, hyperfine splitting constant (HSC), and relative intensity) with the observed digitized spectra using nonlinear least squares method.[1] The simulation programs were fabricated so as to include the effect of *g*-factor anisotropy and/or α-hydrogen anisotropy on the line shape of powder spectra according to Kneubühl's equation[7a] and Cochran's equation,[7b] respectively.

Results and Discussion

Observed ESR Spectra of Plasma-Irradiated α- and β-Glucose The progressive changes of the room-temperature ESR spectral pattern of plasma-irradiated powdered α- and β-glucose with various plasma duration (A, B) are shown in Fig. 2, together with those of amylose and cellulose for comparison purposes (C, D).

It is seen from Fig. 2A and 2B that the spectral features vary with the materials, α- and β-glucose, but remain nearly unchanged in the course of plasma irradiation in each case. These spectra appear to be an outline of multicomponent spectra and also apparently differ from those of amylose and cellulose as shown in Fig. 2C and 2D, although α- and β-glucose are monomeric units obtained from the hydrolyses of the polycarbohydrates, amylose and cellulose, respectively.

Figure 3 shows the progressive changes in the total spectral intensities on plasma duration (A) and standing at room temperature (B) which were determined by double integration. It was shown that plasma-induced radical formation in α- and β-glucose is much lower than those in polycarbohydrates. The decay rate of both glucose radicals on standing at room temperature was also much lower than those of amylose and cellulose, their ESR spectra remaining unchanged in both spectral shape and intensity on standing for a long period of time at room temperature.

Corresponding Simulated Spectra We have systematically conducted computer simulations of these progressive changes in the complicated spectra in an interrelated manner.

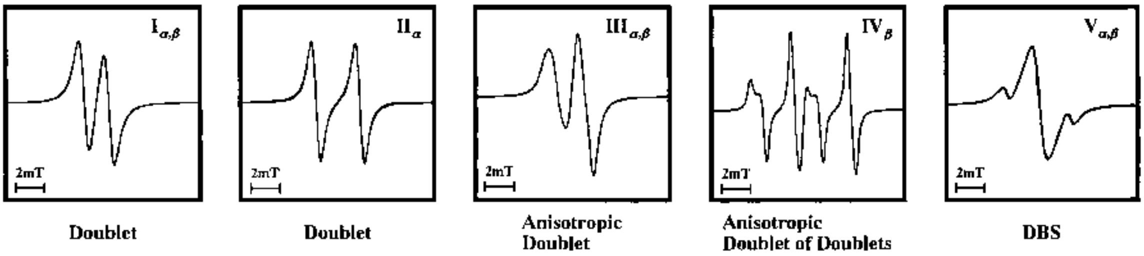

Fig. 4. Representative Selection of Component Spectra (I—V) for the Simulated ESR Spectra

Table 1. ESR Spectral Data for Component Radicals in Simulated Spectra of α- and β-Glucose[a)]

		I	II	III		IV		V
α-D-Glucose	g	2.0027	2.0021	$\bar{g}=2.0027$	$g_1=2.0019$ $g_2=2.0022$ $g_3=2.0041$			2.0034
	A_α			$\bar{A}_\alpha=1.88$	$A_1=1.32$ $A_2=1.69$ $A_3=2.63$			
	$A_\beta(1)$	1.74	3.04	$\bar{A}_\beta=0.52$	$A_1=0.52$ $A_2=0.52$ $A_3=0.52$			
	$A_\beta(2)$	—	—					
β-D-Glucose	g	2.0056		$\bar{g}=2.0028$	$g_1=2.0020$ $g_2=2.0024$ $g_3=2.0041$	$g_1=2.0028$	$g_1=2.0021$ $g_2=2.0024$ $g_3=2.0038$	2.0036
	A_α			$\bar{A}_\alpha=2.09$	$A_1=1.35$ $A_2=1.97$ $A_3=2.95$	$\bar{A}_\alpha=2.41$	$A_1=1.73$ $A_2=2.12$ $A_3=3.39$	
	$A_\beta(1)$	1.74		$\bar{A}_\beta=0.20$	$A_1=0.20$ $A_2=0.20$ $A_3=0.20$	$\bar{A}_\beta=3.89$	$A_1=3.89$ $A_2=3.89$ $A_3=3.89$	
	$A_\beta(2)$	—						

a) Values of HSC are given in mT.

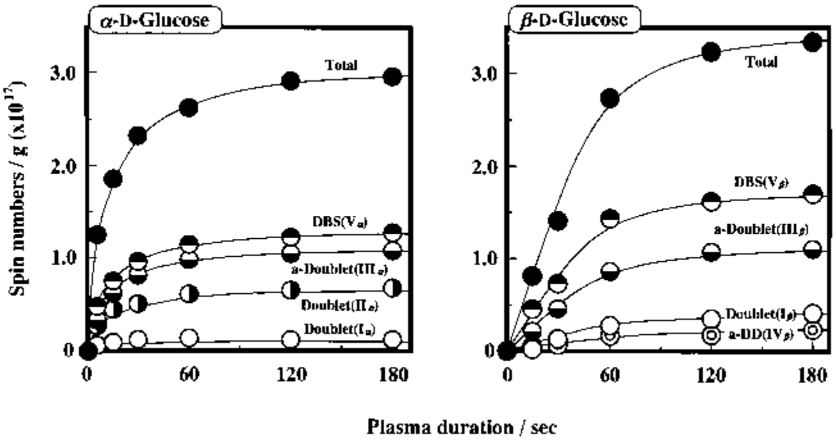

Fig. 5. Progressive Changes in Spectral Intensities of Component Spectra Corresponding to the Simulated Spectra of Plasma-Irradiated α- and β-Glucose on Plasma-Duration

The simulated spectra corresponding to the observed spectra are shown as dotted lines in Fig. 2. It can be seen that all the observed spectral features have been satisfactorily reproduced by the present simulations. The computer simulations disclosed that in the observed spectra there exist as many as four component spectra in α- and β-glucose, respectively. All the simulated spectra are obtained from admixtures of such component spectra with different ratios.

Figure 4 shows the representative component spectra of the simulated spectra of these monocarbohydrates: two isotropic spectra [doublet (I_α) and (II_α)], one anisotropic spectrum [anisotropic doublet (a-D) (III_α)] and a smeared-out broad line (V_α) for α-glucose. All these component radicals are essentially identical to those of plasma-irradiated amylose. On the other hand, those of β-glucose consist of four kinds of discrete spectral components, I_β, III_β, V_β and an anisotropic doublet of doublets (a-DD) spectrum (IV_β).

The ESR spectroscopic parameters for a representative selection of these component spectra deduced from the systematic simulations are summarized in Table 1. The smeared-out broad line (V_α) (g=2.0034) and (V_β) (g=2.0036) were approximated by a singlet (ΣH=11.3, 12.3 mT) with a small amount of a triplet (2.20, 2.21 mT). The principal anisotropic parameters vary somewhat with the spectra so that their values are only of semiquantitative significance. It should be noted that all these component spectra are similar in nature to those in amylose and cellulose, respectively.

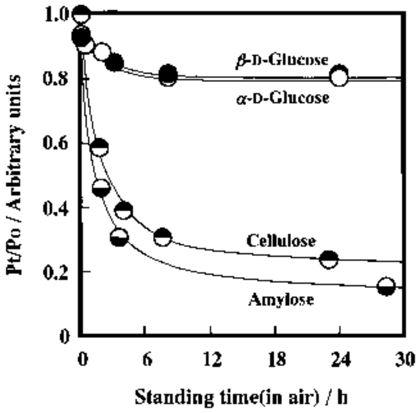

Fig. 6. Progressive Changes in Spectral Intensities Determined by Double Integration on Standing in Air of Carbohydrates Plasma-Irradiated for 3 min

Progressive Changes in Component Spectra Figure 5 shows the progressive changes of the spectral intensities of each component radical corresponding to the simulated spectra in the course of plasma irradiation shown in Fig. 2. The total spectral intensities were very compatible with the intensities determined by double integration of the observed spectra.

It is apparent that the rate of increase of each spectral intensity does not vary much with the component spectra (Fig. 5), accounting for rather small spectral changes in the pattern in the course of plasma irradiation in both cases.

Exposure of plasma-irradiated amylose and cellulose to air caused dissipation of the radicals in both cases, indicating the facile reaction of surface radicals of powders with oxygen. But the radicals formed in monocarbohydrates, α- and β-glucose (I_{24}/I_0=0.81 and 0.82 (total)), were very stable in dry air at room temperature for a long period of time (Fig. 6), unlike the case of the polycarbohydrates. It is apparent that oxygen under atmospheric pressure cannot diffuse through the tight hydrogen-bonding network of the monocarbohydrates as in the case of the recently reported *myo*-inositol.[3)]

Structural Assignments Both simulated spectra, in α- and β-glucose, are primarily characterized by the presence of a large amount of the smeared-out broad line ($V_{\alpha,\beta}$) as a major component, which was assigned to an immobilized dangling-bond site (DBS) with an intermediate level of conversion which includes a mixture of ring-opened and/or conjugated structures and of no structural significance.

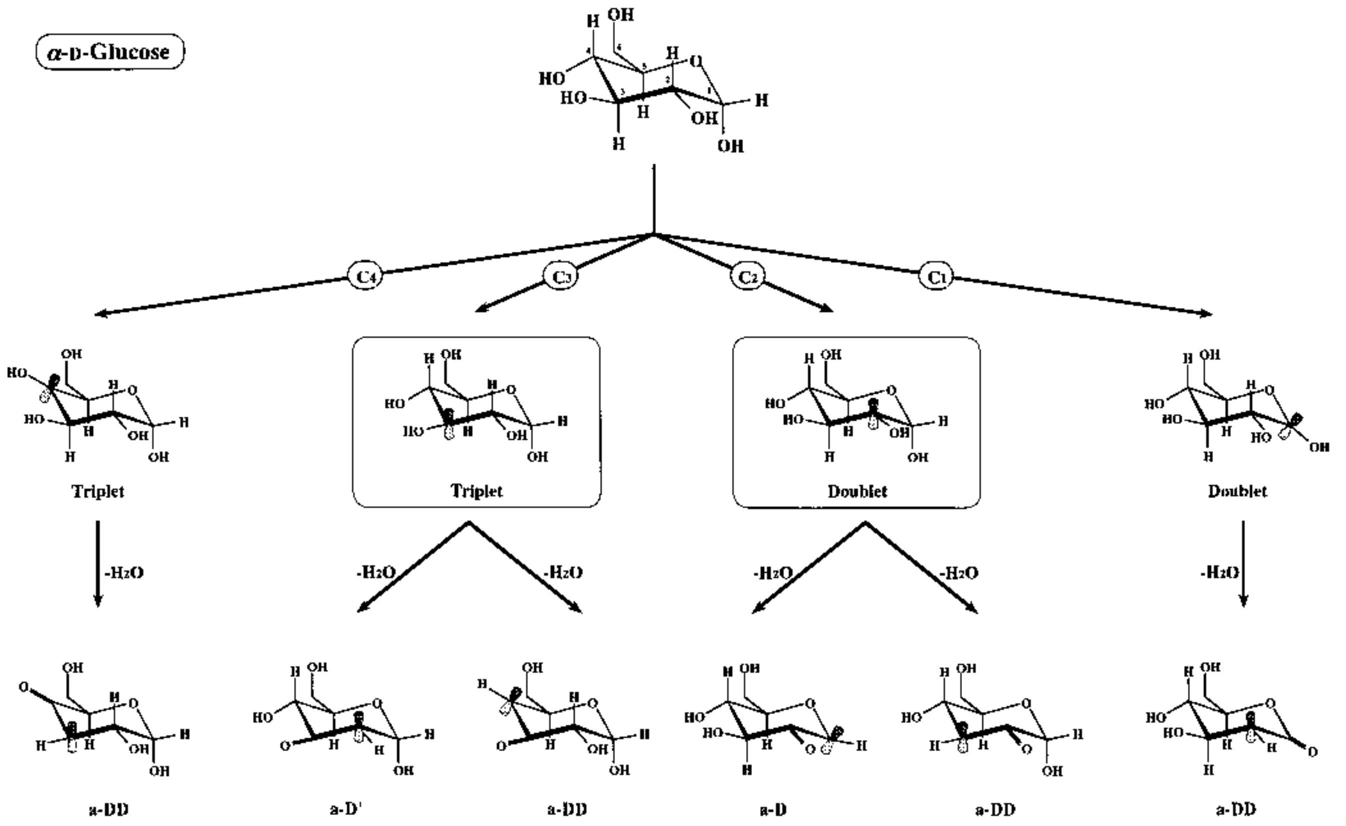

Fig. 7. Structures of Plasma-Induced Radicals in α-Glucose and Subsequent Reaction Pathways

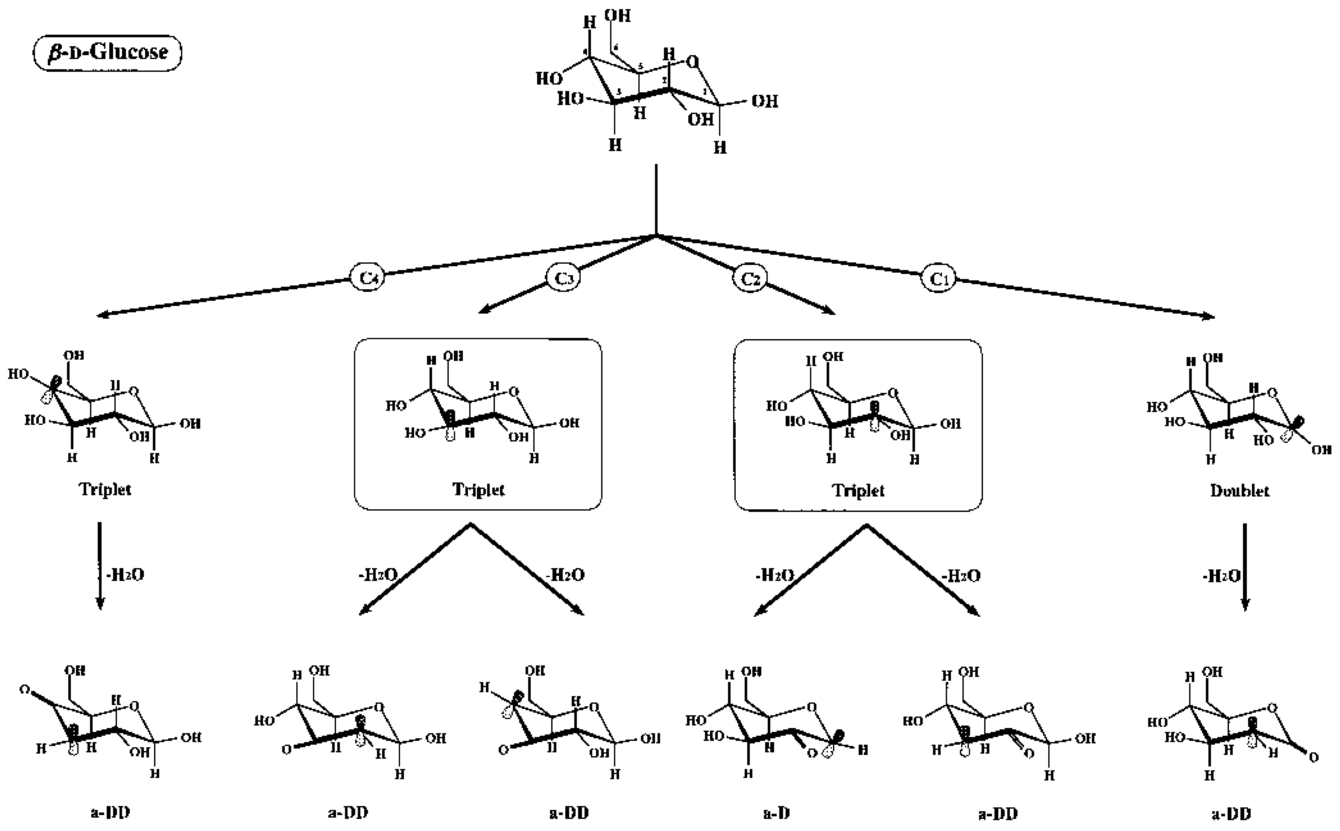

Fig. 8. Structures of Plasma-Induced Radicals in β-Glucose and Subsequent Reaction Pathways

A nearly isotropic doublet ($I_{\alpha,\beta}$) with *ca.* 1.7 mT of HSC can be assigned to an alkoxylalkyl radical at C_1 (1) of the glucose unit formed by a hydrogen abstraction. Based on the cosine square rule, the rather large *g*-values and the smaller HSC of the doublet for axial β-hydrogen at C_2 may stem from the influence of the two oxygens bonded to the radical center.

And we can assign a anisotropic doublet (a-D) ($III_{\alpha,\beta}$) to the acylalkyl radical at C_1, which resulted from the facile dehydration of the hydroxylalkyl radical at C_2.

On the other hand, we can assign a doublet of doublets (a-DD) (IV_{β}) to the acylalkyl radical at C_2, C_3 and/or C_4, which has resulted from the facile dehydration of the hydroxylalkyl radical at C_1, C_2, C_3 and/or C_4. The ESR spectra can not discriminate among these alternatives.

The most interesting contrast in the ESR spectra between α- and β-glucose is that the spectra of plasma-irradiated α-glucose contained an doublet spectrum (II_{α}) different in HSC from I_{α}, but assignable to the hydroxylalkyl radical at C_2, since the C_1 hydrogen is equatorial, while those of plasma-irradiated β-glucose don't contain such a doublet. The discrepancy in the radical formation could be ascribed to the difference in the crystalline structure between the two anomeric monocarbohydrates, α- and β-glucose. Thus, the nature of radical formation is affected by the configuration at the C_1 anomeric carbon, in a sensitive manner.

All these radical structures and the reaction sequence of α-glucose and β-glucose are summarized in Figs. 7 and 8, respectively.

Conclusion

We have presented a detailed analysis of plasma-induced radicals of α- and β-glucose based on ESR coupled with systematic computer simulations. From the present study, the radicals formed in plasma-irradiated α- and β-glucose were elucidated. The special feature of the radical formation in monocarbohydrates is the fact that a major component is a smeared-out broad line ($V_{\alpha,\beta}$) which was assigned to an immobilized DBS with an intermediate level of conversion which includes a mixture of ring-opened and/or conjugated structures of no structural significance. It was also shown that the decay rate of monocarbohydrate radicals was much lower than that of polycarbohydrates even on prolonged standing in air at room temperature. It is apparent that oxygen under atmosphere pressure cannot diffuse through the tight hydrogen-bonding network of the crystalline state of monocarbohydrates.

Acknowledgments This work was financially supported in part by a Grant in Aid of Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Grant No. 09672191), which is gratefully acknowledged.

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